Chapter 21 Genital evolution

R. Brian Langerhans

21.1 Introduction

Genital morphology, particularly in males, is strikingly variable in animals with internal fertilization (Eberhard 1985; Edwards 1993; Sirot 2003; Hosken & Stockley 2004; Evans & Meisner 2009; Eberhard 2010). Indeed, genitalia may experience more rapid, divergent evolution than any other animal character—but why? Several hypotheses have been proposed to explain this remarkable degree of variation (table 21.1), with those involving postmating sexual selection currently enjoying the strongest empirical support (mostly from insects and spiders). Here, I review and synthesize existing data to assess the possible importance of each hypothesis for genital evolution in poeciliid fishes. Each hypothesis proposes an important role for a distinct process in genital evolution; the hypotheses are conceptually distinct but not mutually exclusive.

Poeciliids display conspicuous variability in genital morphology, with gonopodium diversity being particularly well studied (fig. 21.1; see also fig. 1.5 in Greven, **chapter 1**). As with many internal fertilizers, male genital characters are critically important for distinguishing among close relatives (Eigenmann 1907; Regan 1913; Henn 1916; Rosen & Bailey 1963). Owing to the dramatic diversity of genital morphology, range of mating strategies employed, breadth of habitats occupied, ease of laboratory experimentation, and the existence of some fairly well-resolved phylogenies, poeciliid fishes represent a model system for studies of genital evolution. So far, we have only scratched the surface—much future work is needed to gain a strong understanding of the causes and consequences of genital diversification in poeciliid fishes.

This chapter assumes a basic familiarity with poeciliid reproductive biology (see Greven, **chapter 1**, for details) and focuses on the mechanisms potentially responsible for genital evolution. I particularly focus on male gonopodium morphology and female urogenital aperture (and surrounding integument) morphology. However, nonmorphological traits associated with genitalia, such as sperm properties (mobility, chemistry), accessory fluids, or pheromones might exhibit similar levels of variability and experience similar forms of selection (Aspbury & Gabor 2004b; Mendez & Cordoba-Aguilar 2004; see also Evans and Pilastro, **chapter 18**). In this chapter, I inevitably focus on genitalic structures that possess the most available data.

Many previous tests of hypotheses of genital evolution in poeciliids either lacked statistical analyses, used small sample sizes, ignored phylogenetic relationships, or used indirect evidence rather than direct observations for basing functional conclusions. Thus, I conduct a number of new analyses here to provide at least crude tests of both old and new hypotheses. These tests (presented in online appendices; see online supplementary material [OSM] at http://www.press.uchicago.edu/books/Evans) were all performed within a phylogenetic context and are meant to guide future work. Family-wide analyses used the molecular phylogeny of Hrbek et al. (2007). Because resolution of much of these data is currently available only at the generic level, data were collected and analyzed at this scale. The phylogeny was pruned to yield a genus-level topology (28 genera) with all branch lengths equal to 1 (see appendix 21.1,

Table 21.1 Primary hypotheses for the evolution of genital diversity

Category of explanation	Hypothesis
Premating sexual selection	Male contest competition: intermale competition over access to mates
	Mate choice: selection of mates based on phenotypic values Premating sexual conflict: sexually antagonistic selection over control of mating
Postmating sexual selection	Sperm competition: competition between sperm of different males over fertilization
	Cryptic female choice: postmating ability of females to bias fertilization success among males
	Postmating sexual conflict: sexually antagonistic selection over control of fertilization
Natural selection	Lock-and-key: selection against hybridization favors species-specific complementarity in male (key) and female (lock) genitalia
	Nonmating natural selection: selection on genitalia via agents independent of mating
Neutrality	Pleiotropy: genitalia not under direct selection but genetically correlated with such traits

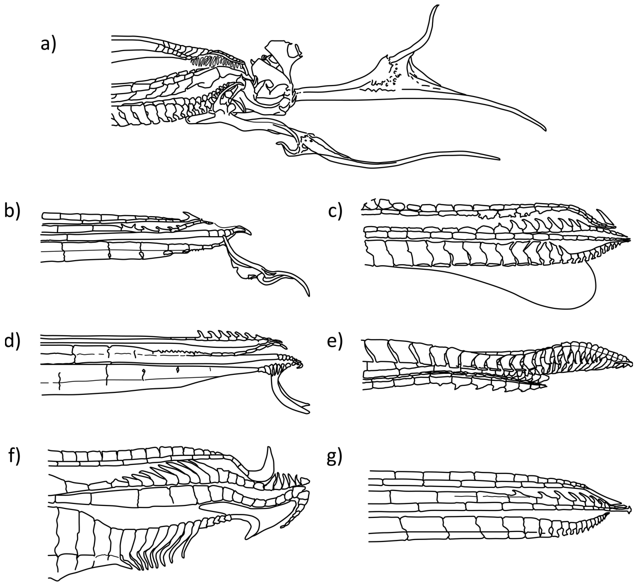


Figure 21.1 Lateral depiction of gonopodial distal tips at rest, with the fish facing left (i.e., tip pointing caudally). (a) *Tomeurus gracilis*, (b) *Cnesterodon decemmaculatus* (ten-spotted livebearer), (c) *Poecilia elegans* (elegant molly), (d) *Girardinus cubensis* (Cuban topminnow), (e) *Carlhubbsia kidderi* (Champoton gambusia; note the asymmetry), (f) *Xiphophorus hellerii* (green swordtail), (g) *Priapichthys nigroventralis*. Adapted from Rosen and Tucker 1961 and Rosen and Bailey 1963.

OSM). For analyses performed within the genus *Gambusia*, I generated a topological hypothesis based on molecular and morphological data and set all branch lengths equal to 1 (appendix 21.2, OSM). When testing for correlated evolution between two discrete binary traits, I used Pagel's (1994) Discrete method using maximum likelihood (Pagel 2000). To test for correlated evolution between continuous traits and between a continuous trait and a binary trait, I used the PDAP module of the Mesquite package (Maddison & Maddison 2008; Midford et al. 2008) to examine phylogenetically independent contrasts (PIC; Felsenstein 1985), with correlations forced through the origin. Character data sets are provided in appendices 21.3 and 21.4 (OSM). In all cases where a priori one-sided hypotheses exist, I use one-tailed *P*-values.

21.2 Basics of poeciliid copulation

All poeciliids possess internal fertilization, whereby males use their gonopodium to transfer spermatozeugmata onto or into female genitalia. Prior interpretations of how this process occurs were largely based on deductions from genital morphology, actions of gonopodia forcibly manipulated on preserved or anesthetized fish, and photographic glimpses of extremely rapid, complex copulatory behaviors of a few species (e.g., Rosen & Gordon 1953; Warburton et al. 1957; Rosen & Tucker 1961; Peden 1975). Put simply, we have a very incomplete functional understanding of how spermatozeugmata are released and travel along the gonopodium, how sperm reach the female reproductive tract, and how accessory structures (e.g., pectoral and pelvic fins), gonopodium size (e.g., length, surface area), and gonopodial distal-tip morphology influence this process. To accurately and thoroughly assess alternative hypotheses for genital diversification, we would ideally begin with a strong functional knowledge of the mechanics of copulation—thus, future work on this topic is paramount. In the meantime, I will briefly evaluate what we do know so that we may use this knowledge in our appraisals of the hypotheses discussed throughout the chapter.

During a successful copulation (i.e., one in which insemination occurs), a male locates a female, circumducts the gonopodium, and transfers spermatozeugmata to the female genitalia. It has been suggested that spermatozeugmata travel along the gonopodium by unknown means (e.g., cilia, centrifugal force) through either a permanent groove in species with bilaterally asymmetric gonopodia or a transitory groove formed by the folding of anal-fin rays during circumduction in species with bilaterally symmetric gonopodia. This suggestion is primarily based on deductions from morphology of asymmetric gonopodia (Rosen & Bailey 1959; Chambers 1987) and direct manipulations of anesthetized specimens of *Gambusia affinis* (western mosquitofish; Kuntz 1914) and *Xiphophorus hellerii* (green swordtail; Rosen & Gordon 1953). However, recent work has demonstrated that the groove is actually permanent, not temporary, in at least two genera exhibiting bilaterally symmetric gonopodia—*Gambusia* (Rivera-Rivera et al. 2010) and *Belonesox* (R. B. Langerhans, unpublished data)—calling into question the accuracy and generality of these previous descriptions. Prior work suggested that pelvic fins sometimes aid in guiding spermatozeugmata along the gonopodium (Rosen & Gordon 1953; Rosen & Tucker 1961), but detailed descriptions of direct observations are lacking. In *Gambusia*, pectoral fins have long been thought to support the gonopodium during copulation (Hubbs & Reynolds 1957; Warburton et al. 1957; Peden 1972b, 1975; Rosa-Molinar et al. 1994; Rosa-Molinar et al. 1996). Yet, recent work produced what is probably the most detailed observations of copulatory behaviors ever reported for poeciliid fish—simultaneous ventral, lateral, and frontal digital recordings of copulation attempts (1000 Hz, 1024- × 1024-pixel resolution)—and failed to confirm this purported behavior in *G. affinis* (Rivera-Rivera et al. 2010). Finally, functions of the distal-tip elements of gonopodia (e.g., hooks, spines, serrae) are less than obvious (Rosen & Gordon 1953; Clark et al. 1954). Clearly, we need a more detailed knowledge of the biomechanics of poeciliid copulation.

21.3 Sexual selection

To begin evaluating the possible mechanisms underlying genital diversity in Poeciliidae, let us first consider sexual selection. The mating process in poeciliids comprises mate acquisition, copulation, and fertilization—sexual selection can act on genital morphology at any of these stages. First, conspecifics of the opposite sex must locate one another: premating sexual selection on genitalia can occur at this stage (sections 21.3.1–3). Once copulation is initiated, insemination of sperm and fertilization of ova must occur if embryos are to result from mating: postmating sexual selection on genitalia can occur at this stage (sections 21.3.4–6; see also Evans & Pilastro, **chapter 18**).

21.3.1 Male contest competition

Although male poeciliids exhibit considerable variation in the degree of male-male agonistic interactions (Farr 1989; Bisazza 1993a; Earley & Dugatkin 2005; see also Rios-Cardenas & Morris, **chapter 17**), the role of male contest competition in genital evolution is virtually unexplored. Fighting among males for mating rights appears common in several poeciliid species and might influence gonopodium evolution in two ways: (1) gonopodia functioning as male-combat weapons, and (2) gonopodia serving as badges of status. Since gonopodia reach very large sizes in some species (e.g., >70% of standard length in *Cnesterodon*) and sometimes exhibit conspicuous pigmentation, and since males of some species exhibit gonopodial swinging during male-male interactions, it seems that the opportunity for male contest competition to drive gonopodial evolution exists—partially analogous to the use of swords (elaborate, ventral-ray elongations of the caudal fin) in male-male competition in *X. hellerii* (Benson & Basolo 2006; Prenter et al. 2008).

If males used gonopodia either as weapons or as honest status signals, we might expect larger or more colorful gonopodia in species with a greater intensity of intermale competition. Yet this prediction would be naive, as it assumes that costs associated with gonopodium size and color are similar across species, which could obviously be violated if costs in aggressive species were higher than in nonaggressive species (i.e., traits can have costs of both somatic growth and aggression toward the trait bearer). Furthermore, both scenarios (i.e., weapons, badges of status) might result in strong, directional selection favoring larger gonopodia, which should generate hyperallometry of gonopodia (Green 1992), assuming that selection from other sources, like the braking effects of natural selection (section 21.4.2), is negligible (Bonduriansky & Day 2003; Bertin & Fairbairn 2007; Bonduriansky 2007). Gonopodial allometry has been examined in only a few species (Kelly et al. 2000; Jennions & Kelly 2002). Nevertheless, other mechanisms (e.g., female preference, sexually antagonistic selection) can also produce positive allometry of gonopodia (Bonduriansky 2007; Eberhard 2009). Thus, the importance of male contest competition will unlikely be deduced from comparative trends or allometric patterns but instead necessitates experimental approaches. No study has yet demonstrated the use of gonopodia as either male-combat weapons or badges of status.

Male contest competition could also influence the evolution of female genitalia. First, this could occur as a simple by-product of accommodating gonopodium modification resulting from male contest competition (e.g., enlarged gonopodia might favor enlarged urogenital apertures). Alternatively, a female might advertise her fertility to intensify inter-male competition and thus enhance her probability of mating with a more aggressive, socially dominant male (Farr 1989). I will describe here how this might be accomplished via genital evolution. Females often produce chemicals that stimulate male mating behaviors (Constantz 1989; see Greven, **chapter 1**). These chemicals likely derive from the urogenital aperture and could conceivably intensify male contest competition, although such a possibility has not been tested. Additionally, some female poeciliids (especially *Gambusia* species) exhibit contrasting coloration of the genital region (e.g., yellow spots in *G. affinis*) and/or conspicuous anal spots (darkened pigmentation of the urogenital aperture or nearby integument). In some species, these spots exhibit cyclic expression, intensifying during ovulation, and have been suggested to influence male mating behavior (Peden 1973; Kodama et al. 2008). If male contest competition has been important in female genital evolution, we might expect to find genital modifications for fertility advertisement more prevalent in species where male mating success is greatly influenced by male contest competition. This test has not yet been conducted.

21.3.2 Mate choice

Mating preferences based on visual and olfactory cues are known to occur in both male and female poeciliids (see Rios-Cardenas & Morris, **chapter 17**). If genitalia provide mating cues during precopulatory behaviors, mating preferences could influence the evolution of genital diversity. The male gonopodium is often relatively conspicuous, exhibits wide variability among species in size and color, and is sometimes extended or abducted during mating displays (Rosen & Gordon 1953; Rosen & Tucker 1961; Hughes 1985; Basolo 1995b; Langerhans et al. 2005). For these reasons, it seems plausible that female mating preferences might influence gonopodial diversity. Female preference for males with larger gonopodia has been demonstrated using an experimental approach in *G. affinis* (Langerhans et al. 2005) and *Gambusia holbrooki* (Kahn et al. 2010) and with correlational observations in *Poecilia reticulata* (guppy; gonopodium length; Brooks & Caithness 1995). Tests of female preferences for gonopodium size in other species, or for gonopodium color in any species, do not yet exist to my knowledge. If female preferences for larger, more colorful gonopodia have played a major role in gonopodial evolution not just within species but across species, we might predict that species with mating displays would exhibit larger, more colorful gonopodia. For gonopodium size, previous work and new analyses suggest that the opposite pattern actually exists (see section 21.3.3). For gonopodium color, new results cannot rule out the role of female mate choice in influencing its evolution (appendix 21.5, OSM).

Female genitalia are often relatively inconspicuous and therefore at first glance would seem ill-suited for evolution via male mate choice. Nevertheless, there are a couple of possibilities that deserve further attention. First, males might exhibit preferences for particular chemical cues associated with the urogenital aperture, possibly driving the evolution of pheromone diversity (e.g., McLennan & Ryan 1999; Hankison & Morris 2003; Shohet & Watt 2004; Plath et al. 2006). Second, the aforementioned anal spots possessed by some female poeciliids vary considerably among species in size, intensity, location, and shape (Peden 1973). It is possible that male mate choice could influence the evolution of anal-spot diversity, but no study has yet examined this. In sum, the importance of mate choice in the evolution of genital diversity appears variable across hierarchical levels for male gonopodium size, has some cautious support for gonopodium color, and is unclear for females.

21.3.3 Premating sexual conflict

Sexual conflict over control of mating and fertilization is a common phenomenon and can result in numerous forms of sexual selection (Arnqvist & Rowe 2005). The premating sexual conflict hypothesis of genital evolution is explicitly concerned with the role of sexually antagonistic selection on traits that function prior to copulation. Under this scenario, trait values that are beneficial to one sex impose direct costs on the other sex. Sexually antagonistic selection could be due to the fact that males typically benefit from increasing mating frequency, while females often benefit from rejecting some males, which reduces costs associated with superfluous male mating attempts. Female poeciliids are likely to suffer considerable costs due to harassment by males, including lost feeding opportunities, exposure to predators, and physical injury from aggressive interactions (Plath et al. 2007b; see Magurran, **chapter 19**). Because sexually antagonistic selection might involve nongenitalic traits, coevolution of male and female genitalia is not necessarily predicted under this scenario. However, genital morphology certainly might evolve as a response to selection on either side of this conflict of interests between the sexes.

Males might circumvent female mating preference/resistance by evolving a longer gonopodium to achieve mating attempts from longer distances or otherwise enhance mating success against the female's wishes. Whether longer gonopodia function to facilitate mating from longer distances is unknown, but prior work based on indirect evidence has suggested that a longer gonopodium somehow increases mating success during gonopodial thrusting (i.e., copulation attempts not preceded by any form of display) (Rosen & Gordon 1953; Rosen & Tucker 1961; Reynolds et al. 1993; Greven 2005). If longer gonopodia effectively prevent female choice, then we would predict that species with longer gonopodia would not exhibit mating displays. While Ptacek and Travis (1998) found the opposite trend across three *Poecilia* species, a recent analysis conducted within a phylogenetic context across 65 species found confirmatory evidence (Martin et al. 2010). Indeed, examining this question across genera in a phylogenetic context yields confirmatory results, as shorter gonopodia tend to evolve in concert with male display (appendix 21.6, OSM). Although this work grossly categorizes taxa as having either short or long gonopodia, Martin et al. (2010) demonstrated that poeciliids appear to exhibit a bimodal distribution of relative gonopodium length (fig. 21.2), indicating that such a categorization will likely capture the major trends at this scale of analysis. Furthermore, intraspecific studies sometimes report that males with relatively longer gonopodia exhibit higher rates of gonopodial thrusting (Farr et al. 1986; Reynolds et al. 1993; Travis 1994; but see Schröder et al. 1996; Ptacek & Travis 1998), and that males in environments characterized by higher predation risk, where female choice is expected to be less important than in low-risk environments, tend to exhibit longer gonopodia (Kelly et al. 2000; Jennions & Kelly 2002; but see Cheng 2004; Langerhans et al. 2005). While these findings alone do not reveal causation (we need functional approaches that directly address the hypotheses), they do provide supporting evidence for a possible role of premating sexual conflict in the evolution of gonopodium size.

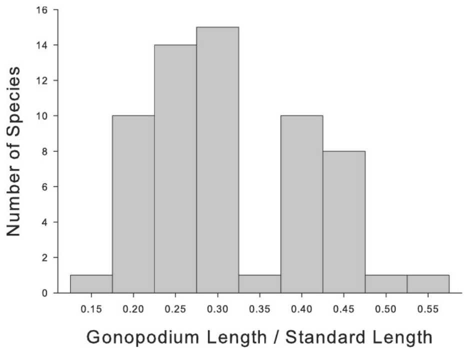


Figure 21.2 Gonopodium lengths in poeciliids. Frequency distribution of poeciliid fish species ($n = 61$) sharing common relative gonopodial lengths. Adapted from Martin et al. 2010.

21.3.4 Sperm competition

Because poeciliid females often mate with multiple males and can store sperm, postmating sexual selection plays an important role in poeciliid evolution, with sperm competition receiving much attention in the group (see Evans & Pilastro, **chapter 18**). However, the role of sperm competition in genital diversification is largely unknown. With respect to genital evolution, sperm competition will generally favor male genital traits that (1) increase insemination success (e.g., number of spermatozeugmata delivered per copulation), (2) increase postinsemination fertilization success, or (3) decrease insemination or postinsemination fertilization success of other males. In poeciliids, male gonopodial morphology may be a target for such selection. Although little evidence so far exists regarding the influence of variation in gonopodial morphology on insemination or postinsemination fertilization success (e.g., Clark & Aronson 1951; Kadow 1954), there are many reasons to believe this is a promising arena for future investigation.

Two obvious ways that males might enhance insemination or postinsemination fertilization success are by exhibiting gonopodial traits that increase the duration of copulation (which is known to increase the number of sperm inseminated in guppies; Pilastro et al. 2007) or that place spermatozeugmata in more favorable locations on or in female genitalia. Four general male reproductive traits that appear to exhibit wide diversity among poeciliids provide intuitive candidates for such traits: gonopodium size (e.g., length, surface area), gonopodial armament (e.g., hooks, serrae, acuteness of tip), accessory mating structures (e.g., gonopodial hood/palp, gonopodial bony extensions, modified paired fins), and the type of gonopodial groove (i.e., permanent asymmetrical folding, permanent dorsal groove, transient folding) (see Greven, **chapter 1**, for details on these structures). It is possible that males with longer gonopodia are capable of achieving deeper penetration or can better position the gonopodium during copulation with the aid of visual cues. Some types of gonopodial armament might function as holdfasts during copulation (Clark & Aronson 1951; Rosen & Gordon 1953; Clark et al. 1954; Cheng 2004) and subsequently increase the duration of copulation. Armament might also facilitate the release of spermatozeugmata in more favorable locations, such as providing deeper penetration or altering trajectories of spermatozeugmata release. Accessory structures might help guide and stabilize the gonopodium during copulation in order to place the gonopodial tip in a position that enhances insemination or fertilization success (e.g., palps might limit depth of insertion; Rosen & Bailey 1963; Greven 2005) or perhaps reduce the loss of spermatozeugmata after copulation (Leo

& Greven 1999). Variation in the gonopodial groove might influence the efficiency of spermatozeugmata transfer from the urogenital pore to the gonopodial tip. No direct tests of any of these hypotheses have yet been performed.

Seven genera of poeciliids exhibit bilaterally asymmetric gonopodia (see appendix 21.3, OSM; and see also fig. 21.1e; Greven, **chapter 1**, fig. 1.5), which possess a permanent groove in either the dextral or the sinistral position. A permanent groove might enhance insemination success relative to a temporary one, but asymmetry is not a prerequisite for permanent groove formation, and it is unclear what selective effects related to insemination/fertilization success bilateral symmetry per se might confer. Previous studies have discussed an interesting pattern in which all asymmetric gonopodia are also relatively long (>35% of standard length; Rosen & Tucker 1961; Rosen & Bailey 1963; Greven 2005), suggesting the correlated evolution of gonopodium symmetry and length due to a functional integration of the two traits where certain combinations improve insemination/fertilization success. A test of this association within a phylogenetic context suggests that it does indeed represent a fairly robust pattern of correlated evolution, at least at the level of genera (appendix 21.7, OSM), although whether this pattern is the result of sperm competition is unknown. Moreover, it is not clear why symmetric gonopodia evolved in the first place. Based on ancestral-state reconstruction using maximum parsimony, it now appears that gonopodial asymmetry was likely the ancestral condition of the family—both the sister lineage to all other poeciliids (*Xenodexia*) and the family Anablepidae contain species with asymmetric gonopodia—and was lost early in poeciliid evolution but regained four or five times. Perhaps gonopodial symmetry evolved in concert with holdfast devices, as the two might be functionally integrated—that is, selection for holdfasts might lead to bilateral symmetry since they might be more effective when symmetric than when asymmetric. Across poeciliid genera, the evolution of symmetry is indeed significantly associated with the evolution of potential holdfast devices (appendix 21.7, OSM). Thus, it is plausible that symmetric gonopodia may sometimes evolve as a means of enhancing the effect of holdfasts, perhaps in response to selection via sperm competition.

Gonopodial morphology might also serve to decrease the insemination or fertilization success of rival males. One previously proposed function of larger gonopodia and increased armament is to cause injuries to female genitalia that tend to keep females chaste (Constantz 1984). Copulations in some species are known to at least occasionally injure females (Clark et al. 1954; Peters & Mäder 1964; Constantz 1984; Horth 2003), and the tearing and subsequent swelling of the urogenital sinus could reduce insemination or fertilization success of later matings. This hypothesis can be tested easily by examining the effects of gonopodial morphology on injuries and the effects of injuries on the success of subsequent matings, but no such tests have been performed. An alternative means of decreasing the fertilization success of rival males is to remove sperm from the urogenital sinus or reproductive tract of females. Perhaps some hooks, spines, or gonopodial extensions (palps, bony processes) sometimes serve this role. Again, the ability of such traits to remove sperm from females has not yet been tested.

Previous studies suggest that some of these male reproductive traits evolve in a correlated fashion, with one group of species exhibiting relatively long gonopodia that have few holdfasts and that are guided visually during copulation without the aid of accessory structures, and another group of species exhibiting short gonopodia that have numerous holdfasts and that are aided by several accessory structures (Rosen & Tucker 1961; Rosen & Bailey 1963; Greven 2005). Sperm competition could have driven this correlated evolution to enhance postmating insemination/fertilization success via functionally integrated traits. New analyses performed across poeciliid genera within a phylogenetic context were only moderately consistent with these previous claims (appendix 21.8, OSM). First, it seems that once adjusted for phylogenetic relatedness, gonopodium length and holdfasts do not exhibit as tight an association as previously suggested. Second, while a suggestive, positive relationship between holdfasts and accessory structures was found, the pattern is not indicative of a major evolutionary association, and holdfasts are also known to exhibit a modest level of variation within several genera. In contrast, it is quite clear that gonopodium length and accessory structures have indeed evolved in a correlated manner among poeciliid genera. This suggests that functional explanations by previous researchers may be correct, in that longer gonopodia that reach to or beyond the eye are effectively guided by visual cues, while shorter gonopodia that cannot be seen by the bearer are generally aided in positioning by accessory structures (Rosen & Tucker 1961; Rosen & Bailey 1963; Chambers 1987). Such correlated evolution suggests that these patterns have resulted from selection via sperm competition as a means of enhancing insemination and fertilization success or perhaps via premating sexual conflict to increase mating frequency.

21.3.5 Cryptic female choice

After copulation is initiated, females might influence the probability of insemination or fertilization by a given male—and this bias in paternity might depend on the male's genital morphology. This female-driven influence on male reproductive success may derive from a number of sources, including female genital morphology or chemistry, and essentially represents a challenge to males to find the optimal means of stimulating a female. Under this scenario, females indirectly benefit from rejecting some males based on their genital morphology via increased offspring quality. Recent work suggests that cryptic female choice is important in poeciliid evolution (see Evans and Pilastro, **chapter 18**), although we have virtually no knowledge so far of its role in genital evolution.

Cryptic female choice has received considerable attention and support in the study of genital evolution in insects and spiders, where the hallmark of cryptic female choice is the evolution of highly unusual male genital morphologies (Eberhard 1985, 1996). The distal tip of poeciliid gonopodia would seem to meet the criterion of peculiarity, as some distal-tip elements are so bizarre (see figs. 21.1 and 21.3) that it would be surprising if cryptic female choice did not play some role in their evolution. To date, however, no direct evidence exists in support of this hypothesis of genital evolution, and well-designed experiments are needed to assess its potential importance.

21.3.6 Postmating sexual conflict

Poeciliid females might suffer a direct cost from copulation, such as copulatory injuries (see section 21.3.4). Thus, selection might favor traits that allow females to gain control of insemination or fertilization, reducing such costs. The postmating sexual conflict hypothesis of genital evolution focuses on sexually antagonistic selection resulting from direct costs to females after the initiation of copulation (rather than indirect costs of offspring quality, as in cryptic female choice).

The postmating sexual conflict hypothesis of genital evolution makes three predictions: (1) the male genital traits that enhance male fitness reduce female fitness, causing females to directly benefit from rejecting some conspecific males by reducing direct costs of unwanted inseminations, (2) female genital morphology directly influences the insemination or fertilization success of males, sometimes conferring the ability to completely exclude some conspecific males, and (3) a tight coevolutionary arms race of male and female genitalia occurs. In support of the first prediction, copulatory injuries have been shown to occur (see section 21.3.4), suggesting that females might directly benefit from rejecting at least some males for this reason. However, we do not know injury frequencies within species, their variability among species, or the extent of their possible negative consequences. Moreover, no studies have yet examined whether such copulatory injuries to females represent male adaptations (direct selection favoring injuries; "adaptive harm") or negative side effects of traits that evolved because of other selective advantages to males (selection favoring other performance attributes incidentally resulting in injuries; "collateral harm") (Lessells 2006). Here, I assume that substantial costs may be incurred from copulation, and I evaluate the possible ways that postmating sexual conflict might drive genital diversification; clearly, this assumption should be tested in the future. Related to the second prediction, some species exhibit urogenital apertures and sinuses that appear to contain defensive structures (Constantz 1984), although no tests have yet been conducted to determine their function. Finally, previous work has suggested that coevolution of male and female genital morphology may occur in some poeciliid groups (Peden 1972a; Constantz 1984), but detailed tests of how these structures are associated and their possible offensive and defensive functions have not yet been conducted. Extending the work of Peden (1972a), I have performed the first quantitative test of genital coevolution among the sexes in poeciliids (for results, see appendix 21.9, OSM).

What genital structures might we expect to evolve by postmating sexually antagonistic selection? We might envision a range of possible solutions for minimizing injury in females, such as placing obstructions in the urogenital aperture or sinus (i.e., genital papillae), covering the aperture with tissue, shifting the location of the sinus or reproductive tract, enlarging the sinus to minimize contact with tissue, and reinforcing the region with strengthened tissue to absorb gonopodial blows. Variation in all these traits exists in poeciliids, although whether they function as defensive structures is unknown (Peden 1972a; Constantz 1984; Greven 2005). It is also possible that these features provide a means of exerting cryptic female choice rather than, or in addition to, serving as defensive structures to reduce injury. For males, a host of possible offensive structures are known in poeciliids, such as large tips, highly acute tips, and large and numerous hooks, spines, and serrae. Yet, a greater diversity of solutions is possible. For instance, rather than attempt to break through a defensive structure, males might circumvent intromission and simply deposit spermatozeugmata on the exterior of the female genitalia, with sperm only later traveling into the reproductive tract (external depositors do occur, e.g., *Tomeurus*; Rosen & Tucker 1961). The occurrence of such a diversity of apparently offensive and defensive genital structures is certainly suggestive of sexual conflict, although functionally oriented studies are needed to elucidate how these structures work, and comparative studies are needed to test whether patterns of genital evolution are consistent with predictions from sexual conflict.

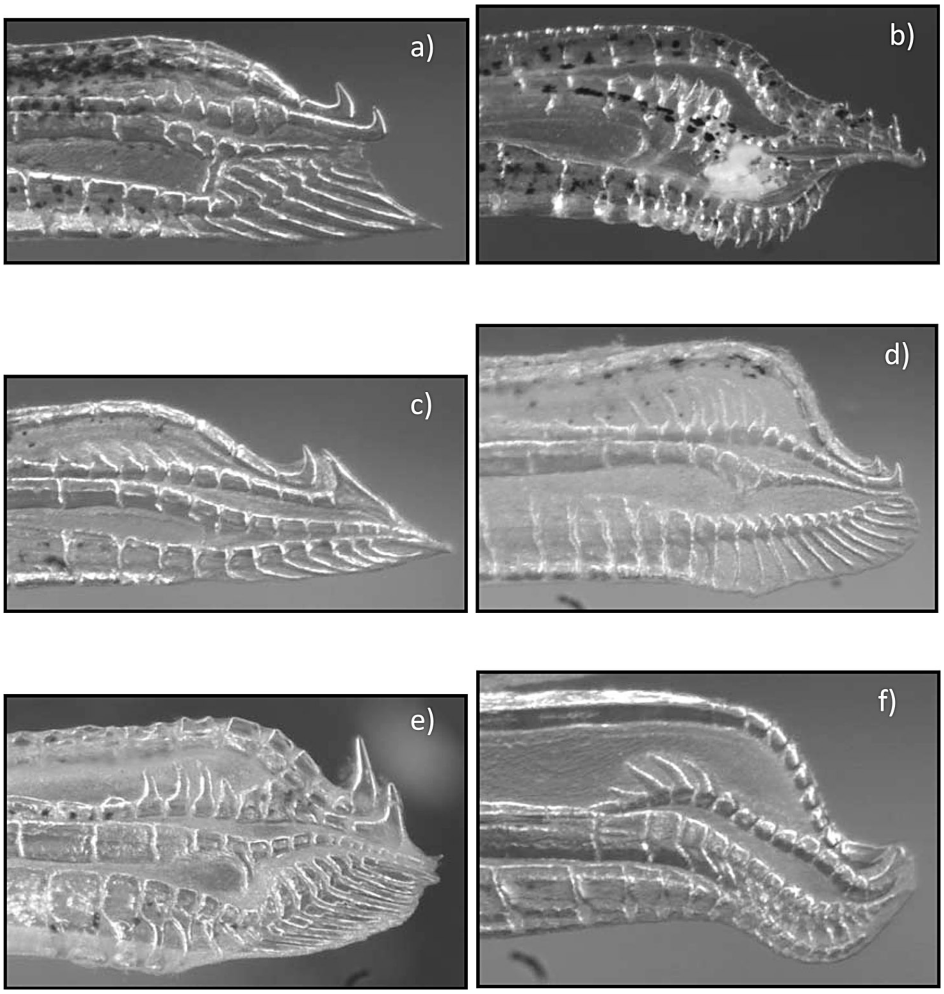


Figure 21.3 Representative examples of gonopodial tips of *Gambusia* species, illustrating the high degree of interspecific variation. (a) *G. atrora*, (b) *G. echeagarayi*, (c) *G. nicaraguensis*, (d) *G. panuco*, (e) *G. punctata*, and (f) *G. vittata*.

Gambusia males exhibit obvious, dramatic variation in the distal-tip morphology of the gonopodium (fig. 21.3), and Peden (1972a) demonstrated a correspondingly high degree of variation in female genitalia among *Gambusia* species, suggesting that male and female genital morphology coevolved in *Gambusia*. Constantz (1984) suggested that some female genital structures represent defensive traits resulting from sexually antagonistic selection. While the functions of these structures have not yet been examined, we can now address more thoroughly the question of whether a tight coevolutionary relationship truly exists among male and female genital morphology in *Gambusia*. Although coevolution of genitalia is a possible outcome of other processes, no other hypothesis of *sexual* selection explicitly predicts strong, genital coevolution.

I found striking evidence for coevolution of external genital morphology among the sexes in *Gambusia* (appendix 21.9, OSM; fig. 21.4). Thus, Peden's suggestion of

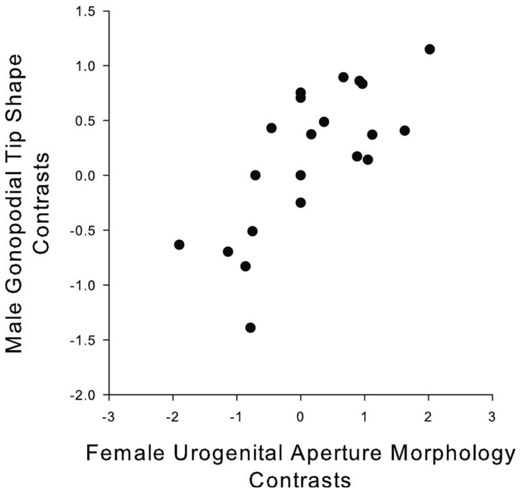


Figure 21.4 Coevolution of external genital morphology among the sexes in *Gambusia*. Each axis depicts phylogenetically independent contrasts.

coevolution is strongly supported after greatly expanding the sample size and placing the analysis within a quantitative, phylogenetic context. This suggests that male and female genitalia evolve in concert—when males evolve blunt/rounded gonopodial tips, females evolve reduced or absent genital papillae within small or enclosed apertures; whereas in species with males exhibiting sharply pointed tips, females tend to exhibit a large papilla within a large aperture. While consistent with the hypothesis of postmating sexual conflict, without corroborating evidence regarding function of the structures we cannot be sure of the underlying process. Moreover, such a pattern is also predicted by the lock-and-key hypothesis, which additionally has further support in this case (section 21.4.1).

21.4 Natural selection

During the past several decades, the role of natural selection has received far less attention and support than sexual selection in the evolution of genital diversity (Eberhard 1985; Arnqvist 1998; Hosken & Stockley 2004; House & Lewis 2007). However, a long-standing hypothesis of taxonomists is that genitalia evolve via natural selection against hybridization (lock-and-key), and previous work in poeciliids suggests it is a viable hypothesis in need of future work (see below). Moreover, unlike genitalia in taxa traditionally studied in this research arena, gonopodia are external, nonretractable, and sometimes quite large relative to body size. Although genitalia are often viewed as doubtful targets of natural selection (Eberhard 1985; 1993; Andersson 1994), gonopodia might often be subjected to various forms of natural selection, such as through their effects on locomotion. While little research has investigated these questions in poeciliids so far, I demonstrate here that numerous lines of evidence suggest that natural selection likely plays an important role in genital evolution of poeciliids.

21.4.1 The lock-and-key hypothesis

The lock-and-key hypothesis has received little convincing support despite a long history of investigation (Shapiro & Porter 1989; Arnqvist 1998). It describes the scenario where selection favoring hybridization avoidance leads to patterns of morphological (or chemical) complementarity of genitalia among the sexes. This hypothesis makes several predictions: (1) female genitalia should reduce mating, insemination, or fertilization success of sympatric, heterospecific males compared with conspecific males, (2) there should be tight coevolution of male and female genitalia, and (3) reproductive character displacement in genitalia should occur, where genital differences are greater between sympatric populations/species than between allopatric populations/species. Although strong coevolution of genitalia between the sexes might also result from other processes (see above), the other two predictions are unique to the lock-and-key hypothesis. Although it seems possible for genitalic traits to experience such selection in poeciliids, none of these predictions have previously received significant attention. Experimental work could easily test the first prediction, but no such work has yet been conducted. The second prediction now enjoys strong support in *Gambusia* (see above), and some anecdotal evidence indicates that broader trends might exist in the family (see Greven, **chapter** 1, fig. 1.5). The third prediction has never been investigated in detail to my knowledge in poeciliid fishes, and thus in appendix 21.10 (OSM) I provide a first test in the genus *Gambusia*.

In poeciliids, early work suggested that differences in genital morphology provided a poor means of reducing hybridization among species (Sengün 1949; Clark et al. 1954; Liley 1966). However, Peden's (1972a, 1973, 1975) work with *Gambusia* species, which represents the most thorough set of comparative studies of male and female genitalia across poeciliid species to date, resurrected the viability of the lock-and-key hypothesis. Peden suggested that the observed covariance in copulatory behaviors, gonopodial morphology, and female genital morphology observed among species was indicative of their acting together in a lock-and-key fashion, producing "more efficient sperm transfer in conspecific than in heterospecific copulation" (1975, 1296). Although it is true that the lock-and-key hypothesis could have generated the apparent associations described by Peden, other mechanisms could also have generated such patterns (see above). Building from Peden's findings, in appendix 21.10 (OSM) I provide the strongest test to date of the lock-and-key hypothesis in poeciliids, testing for reproductive character displacement in *Gambusia* species.

Support for the lock-and-key hypothesis in *Gambusia* is strong for male gonopodial tip shape and female urogenital aperture morphology and is suggestive for female anal-spot location (fig. 21.5). While this pattern of reproductive character displacement in both male and female genitalia provides strong support for the lock-and-key hypothesis, it does not necessarily indicate the exact process by which the pattern emerged; the trend could reflect either evolutionary adjustments to minimize heterospecific insemination or community-level assortment of preexisting differences between species. Yet in either case, the same underlying mechanism is at work—selection against hybridization—merely

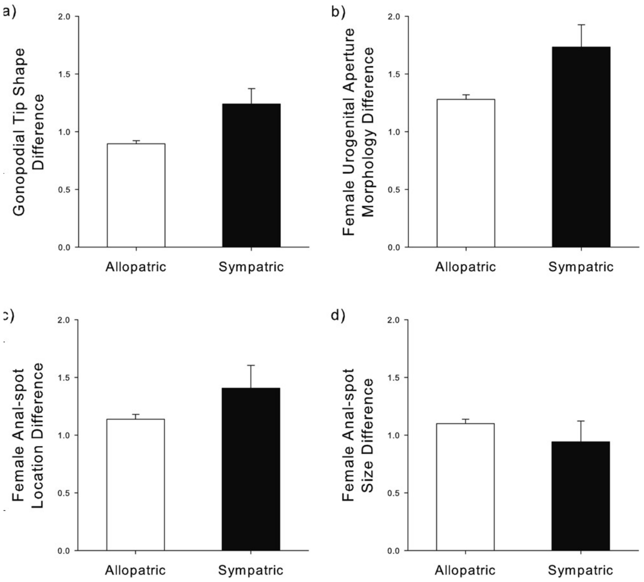


Figure 21.5 Reproductive character displacement in *Gambusia*. Divergence between allopatric and sympatric species pairs in (a) male gonopodial tip shape, (b) female urogenital aperture morphology, (c) female anal-spot location, and (d) female anal-spot size, controlling for phylogenetic relatedness. See appendix 21.10 (OSM) for methodology. Means ± 1 SE presented.

acting at different hierarchical levels. Thus, combining two lines of evidence from *Gambusia* presented in this chapter (tight genitalic coevolution and reproductive character displacement), the lock-and-key hypothesis appears to play a significant role in genital evolution in the genus and deserves future consideration in other poeciliids. Importantly, these findings do not exclude the role of postmating sexual conflict, as both processes might operate simultaneously (or at different times during the evolutionary history of the lineages).

21.4.2 Nonmating natural selection

Natural selection on genital form might also arise from selection independent of mating. There are three obvious sources from which such selection could occur: (1) costs of somatic growth, (2) conspicuousness of genitalia, and (3) effects of gonopodia on locomotor abilities. First, gonopodial development requires resources, and growing a large gonopodium requires more resources than growing a small one. It is unclear exactly how costly gonopodium growth might be relative to other sources of selection on gonopodium size, but it is doubtful that costs of somatic growth have a large influence on selection on female genitalia. Second, both male gonopodia and female genitalia (especially anal spots) are sometimes visually conspicuous features of poeciliid fish. Larger, more colorful, or otherwise more conspicuous genitalia (e.g., potent pheromones could increase female conspicuousness) could draw the attention of predators or aggressive heterospecifics, similar to the known costs of increased attention by predators for poeciliids with bright coloration, larger size, large fins, or elaborate swords (e.g., Rosenthal et al. 2001; Basolo & Wagner 2004; Johansson et al. 2004; see also Kelley & Brown, **chapter 16**). This hypothesis seems plausible but has not yet been tested. Finally, gonopodium size and stability can affect locomotor performance, and thus any source of selection on locomotion might affect gonopodium size. Because strong selection on swimming abilities appears widespread in poeciliid fish (O'Steen et al. 2002; Ghalambor et al. 2004; Langerhans & DeWitt 2004; Langerhans et al. 2004; Walker et al. 2005; Hendry et al. 2006; Langerhans et al. 2007; Zúñiga-Vega et al. 2007; Langerhans & Reznick 2010), this source of selection seems like a particularly promising area for future research. It is also the only component of nonmating natural selection on gonopodia that has so far received much attention in poeciliids.

Gonopodia are expected to influence locomotion primarily through costs of drag, as it is unlikely they contribute much useful thrust. Poeciliids employ steady swimming (constant-speed cruising) for a variety of important activities, such as searching for food, courtship chases, male-male agonistic interactions, and seeking favorable abiotic conditions. Selection should generally favor various means of reducing the energetic costs of movement. We might thus expect natural selection to favor reduced gonopodium size (relative to body size) and increased gonopodial stability, particularly in environments where steady swimming is of paramount importance. This is because a gonopodium with a larger surface area should incur greater drag, and an unstable gonopodium that freely swings about during steady swimming should also incur greater drag than a sturdy gonopodium (Lighthill 1970; Beamish 1978; Webb & Gerstner 2000). To maintain stability and reduce surface area of the gonopodium, we might expect males to often press the gonopodium against their body during cruising. However, it might actually be possible for larger gonopodia to reduce energetic costs of steady swimming by delaying the separation of the boundary layer (Anderson et al. 2001; Fish & Lauder 2006), especially when males depress the gonopodium to the ventral surface of their body. No previous study has directly examined the link between gonopodium size and steady-swimming performance, although Basolo and Alcaraz (2003) showed that larger swords—which are superficially similar to gonopodia—do incur energetic costs. Of course, swords are not gonopodia, and recent results from a swim-tunnel experiment with *G. affinis* suggest that males with relatively larger gonopodia actually tend to exhibit higher endurance (time before fatigue) during steady swimming (R. B. Langerhans, unpublished data). The relationship between steady-swimming performance and gonopodium size requires further investigation.

In addition to steady swimming, poeciliids also frequently employ unsteady locomotor behaviors, such as rapid acceleration and turning. One of the most important unsteady swimming activities routinely performed by poeciliids is the C-start escape burst used when avoiding a predator strike (Domenici 2010). As expected, relative gonopodium surface area has been shown to exhibit a negative association with burst-swimming speed (*G. affinis*; Langerhans et al. 2005). Based on this locomotor cost of gonopodium size, combined with its possible effects on conspicuousness, we would expect selection via predation to favor smaller gonopodia. I recently tested this hypothesis by measuring selection on gonopodium length in *Gambusia hubbsi* (Bahamas mosquitofish) males in the presence of a predatory fish (*Gobiomorus dormitor*, bigmouth sleeper) within large experimental tanks (400 L) (R. B. Langerhans, unpublished data). Consistent with the prediction, I found strong evidence for selection against gonopodium length (fig. 21.6; logistic regression of survival on relative gonopodium length: one-tailed $P = 0.016$) after allowing overnight predation to occur (~8 hours). Thus, predators can generate selection for smaller gonopodium size, but whether this form of selection exists in the wild is unknown.

If selection via predation generally drives smaller gonopodia, then we might predict divergence in gonopodium size between environments differing in predation intensity. Indeed, some poeciliids exhibit relatively smaller gonopodia in populations experiencing higher levels of predation from piscivorous fish (*P. reticulata*: Cheng 2004; *G. affinis*, *G. hubbsi*: Langerhans et al. 2005). However, other studies have found the opposite pattern, where males possessed longer gonopodia in environments with higher predation intensity (*P. reticulata*: Kelly et al. 2000; *Brachyrhaphis episcopi*: Jennions & Kelly 2002). Two possible explanations for these latter findings are that either (1) selection for longer gonopodia via increased mating frequency (section 21.3.3) or insemination success (section 21.3.4) is stronger in high-predation environments and outweighs any potential locomotor costs, or (2) confounding effects of increased water velocity (likely leading to stronger selection favoring increased steady-swimming performance) in high-predation localities led to longer gonopodia. These studies, combined with the possibility that gonopodia influence conspicuousness to predators, suggest that further investigations in the context of divergent predator regimes might elucidate some complex interactions between natural selection and sexual selection in poeciliid genital evolution.

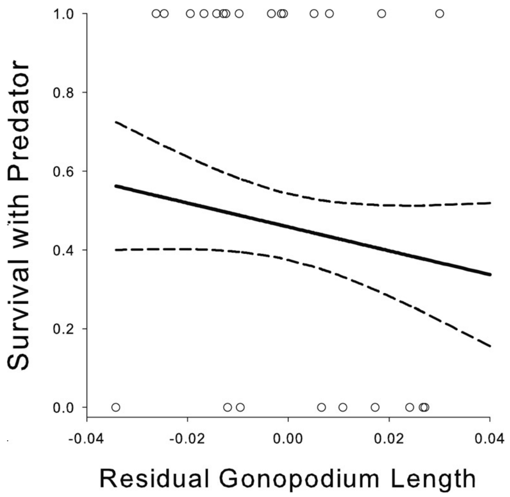


Figure 21.6 Selection in *Gambusia hubbsi* favoring smaller gonopodium length in the presence of a predatory fish (*Gobiomorus dormitor*). Relative gonopodium length measured as residuals from regression of log-transformed gonopodium length on log-transformed standard length. Fitness function estimated using the nonparametric cubic-spline regression technique. The solid line represents mean survival probability, and the dashed lines indicate ± 1 SE of predicted values from 1000 bootstrap replicates of the fitness function. R. B. Langerhans, unpublished data.

21.5 Pleiotropy

Some researchers have contended that genital diversity arises as pleiotropic effects of selection on other traits, with genitalia being selectively neutral (Mayr 1963; Eberhard 1985; Arnqvist 1997; Arnqvist & Thornhill 1998). This hypothesis is contentious, however, as it is unclear how such a process could produce the observed rapid and divergent evolution of genitalia unless genital traits were disproportionately affected by pleiotropic effects of functionally important genes—an assumption for which we have no reason a priori to suspect is true. Yet, if genitalia do not experience direct selection, and instead diversify via pleiotropy, four testable consequences are predicted: (1) genitalia should not experience direct, contemporary selection, (2) genitalia should not exhibit strong signatures of past selection, (3) genes under selection should tend to pleiotropically affect genital organs more frequently than other traits, and (4) genitalia should often exhibit high intraspecific variability.

Based on the results discussed above, considerable evidence exists that at least some features of poeciliid genitalia either experienced strong selection in the past or continue to experience such selection today. These findings directly contradict the first two predictions of the pleiotropy hypothesis. The third prediction could be confirmed either by demonstrating that genes underlying functionally important traits tend to influence genitalia more often than nongenital traits or by showing stronger genetic correlations between functionally important traits and genital traits relative to other traits. Recent work has demonstrated a genetic association between gonopodial development and the growth of swords in some *Xiphophorus* species (Zauner et al. 2003; Offen et al. 2009). Specifically, gonopodium and sword development are both associated with expression of the *msxC*, *rack1*, *dusp1*, *klf2*, and *tmsβ-like* genes, some of which may influence the growth of long anal-fin rays in general. If so, selection on fin size, such as selection for larger fins via predation or by mate choice, could indirectly affect gonopodium size. However, if gonopodium size has evolved more rapidly than other fin sizes, this finding would be insufficient to explain its diversity. One could test the consequences of this pleiotropic link by examining genetic correlations among gonopodium length and unpaired fin-ray lengths, as well as by testing for correlated evolution of these traits among species. Of course, genetic correlations can arise for other reasons, including correlational selection, and are not exclusive to the pleiotropy hypothesis. In the data I have personally collected, I have never observed phenotypic or genetic correlations involving gonopodium size that are stronger than correlations involving other traits (including unpaired fin lengths)—indeed, correlations involving gonopodium size are typically smaller than other trait correlations (R. B. Langerhans, unpublished data). Finally, the fourth prediction derives from the fact that neutral traits can accumulate considerable amounts of variation within species. Evidence to date does not suggest that variation in gonopodium size (length, surface area) exhibits higher intraspecific variation than other traits (Kelly et al. 2000; Jennions & Kelly 2002; R. B. Langerhans, unpublished data), although other genital traits have not yet been examined. Overall, predictions of the pleiotropy hypothesis have not fared well in the face of empirical evidence in poeciliid fishes, suggesting that pleiotropy is unlikely to play a major role in driving genital diversification.

21.6 Consequences of genital evolution

Has genital diversification played a major role in lineage proliferation of poeciliids? It is certainly plausible that rapid divergence of genitalia might lead to speciation more readily than similar levels of divergence in nonreproductive traits. Considering the numerous sources of natural and sexual selection that might act on genitalia, and the remarkable diversity in genital form, an important role for genital diversity in promoting speciation is a reasonable expectation. Future work could test for a relationship between rates of genital evolution and speciation rates within lineages with well-resolved phylogenies. The finding in *Gambusia* that genital differences are greater between sympatric species than between allopatric species is consistent with a role of genital evolution in speciation but does not rule out postspeciation processes. One means of addressing the role of genital evolution in speciation is to conduct empirical investigations at scales where the process of speciation is more easily observed or inferred: sister species, incipient species, and populations within species.

21.7 Conclusions

This chapter provides the first review of the evidence for all major hypotheses of genital evolution in poeciliids. There are many unanswered questions, yet it is now clear that genital evolution in poeciliids is complex, resulting from multiple processes (table 21.2). Many genital traits are likely to be shaped by multiple selective processes, serve multiple, simultaneous functions, and differ in functionality among species. Moreover, major causes of genital evolution in poeciliids may not match those in other taxa. For instance, the lock-and-key hypothesis has gained little previous support in most taxa and yet appears important in poeciliids. Additionally, an important gap in our understanding of poeciliid genital evolution is that no study has yet directly tested for cryptic female choice, despite the fact that this hypothesis has garnered much empirical support in other taxa (Eberhard 1985, 1996; Cordero & Eberhard 2003). Furthermore, we badly need a stronger, functional

Table 21.2 Summary of the existing evidence for each hypothesis of genital diversification in poeciliid fishes

Hypothesis	Importance for poeciliid genital evolution
Male contest competition	Largely untested
Mate choice	Some role confirmed
Premating sexual conflict	Suggestive evidence
Sperm competition	Suggestive evidence
Cryptic female choice	Largely untested
Postmating sexual conflict	Highly suggestive evidence
Lock-and-key	Some role confirmed
Nonmating natural selection	Some role confirmed
Pleiotropy	Unlikely

understanding of the mechanics of copulation. We also have little knowledge of the importance of genital divergence in the speciation process. Clearly, we have much work ahead of us. Understanding the causes and consequences of genital diversification in poeciliid fishes will require integrative approaches, and I am optimistic about the advancement in this understanding that will be described in the next review of this field.

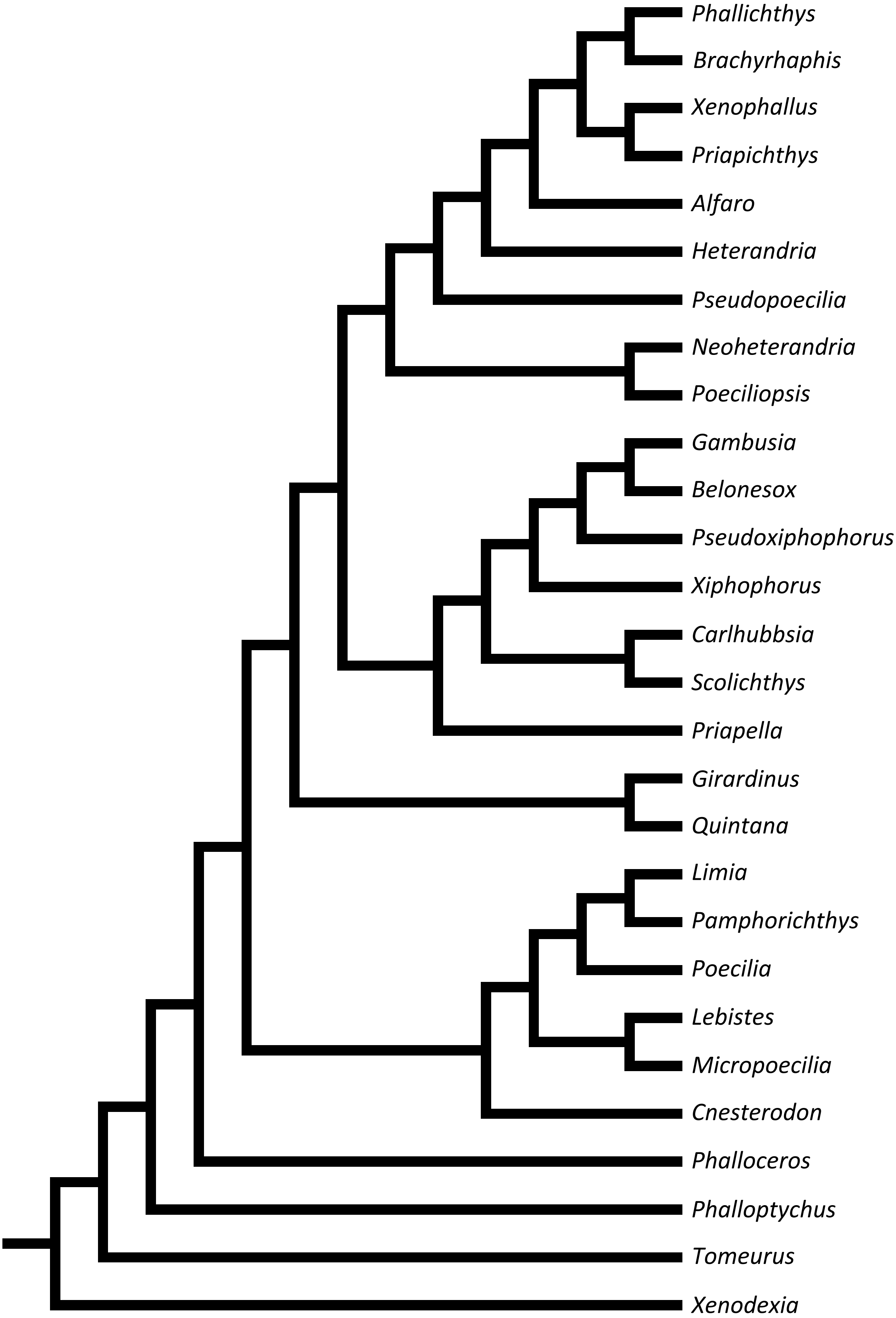
Acknowledgments

I am grateful to the editors for inviting me to contribute to this book. I thank J. Evans, M. Jennions, B. Mautz, A. Pilastro, and an anonymous reviewer for comments that improved the chapter.

Online Supplementary Material for Chapter 21

Genital evolution, *R. Brian Langerhans*

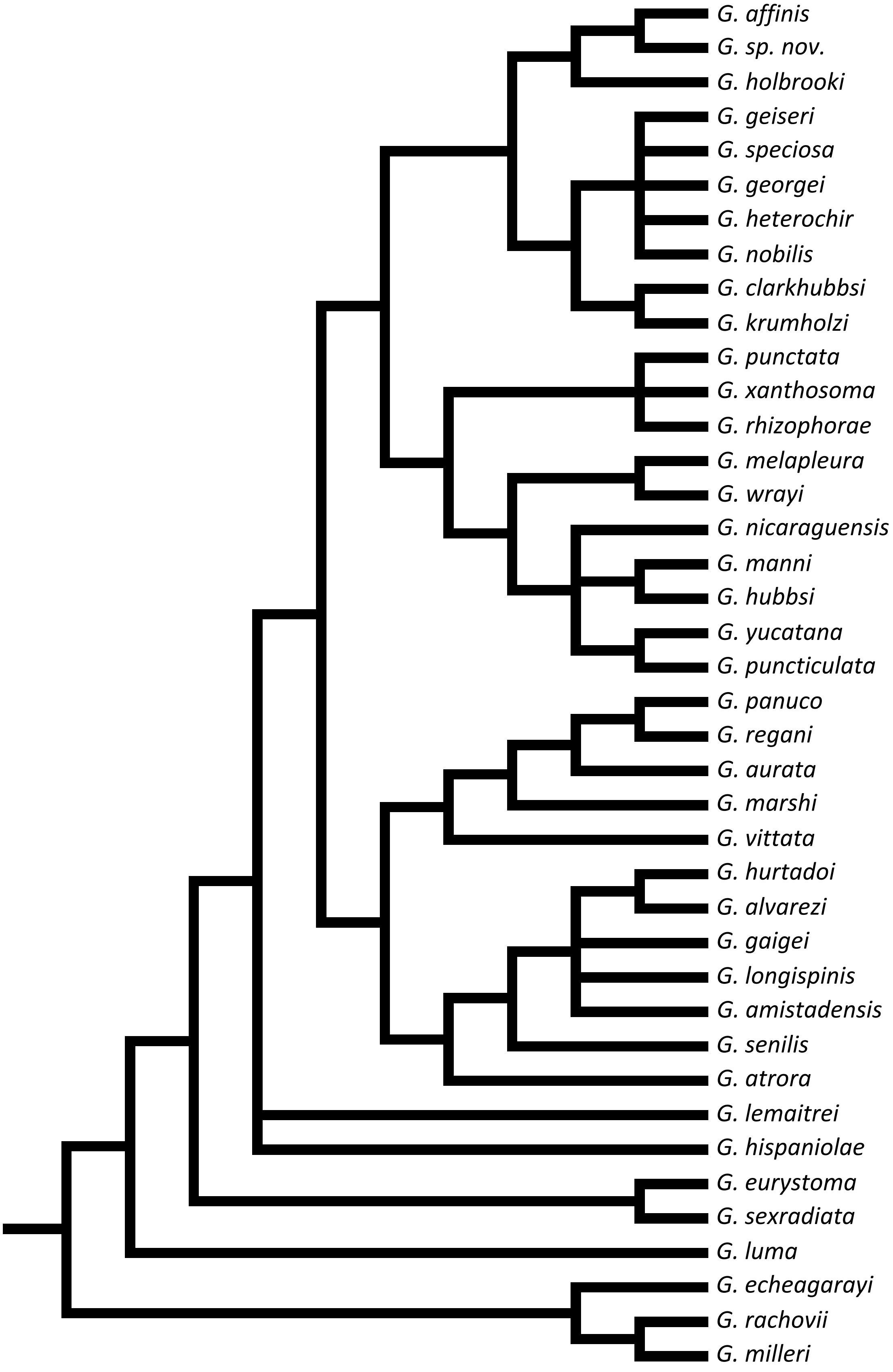
Appendix 21.1 Phylogenetic hypothesis of Poeciliidae used in genera-scale analyses (from Hrbek et al. 2007). *Lebistes* represents the *Poecilia reticulata* lineage, which is genetically distinct from the rest of *Poecilia*. *Pseudoxiphophorus* represents the *Heterandria* lineages from Mexico and Central America, which are genetically distinct from *H. formosa*, found in the United States.



Online Supplementary Material for Chapter 21

Genital evolution, *R. Brian Langerhans*

Appendix 21.2 Phylogenetic hypothesis of *Gambusia* used in analyses (from Lydeard et al. 1995; Rauchenberger 1989; R.B. Langerhans, M.E. Gifford, O. Domínguez-Domínguez, I. Doadrio unpubl. data).



Online Supplementary Material for Chapter 21

Genital evolution, *R. Brian Langerhans*

Appendix 21.3 Character data examined in the genera-scale analyses. See text for descriptions of character values (appendices 21.5 – 21.8).

Genus	Courtship Display	Gonopodium Color	Gonopodium Length	Gonopodium Symmetry	Holdfasts	Accessory Structures
Alfaro	0	0	0	1	1.5	2
Belonesox	1	2	0	1	4	1.5
Brachyrhaphis	1	3	0	1	1.5	0.5
Carlhubbsia	0	1	1	0	2	1
Cnesterodon	0	0	1	1	3	1
Gambusia	1	2	0	1	4.5	2
Girardinus	0	1	1	1	4	1.5
Heterandria	0	0	1	1	2	0
Lebistes	1	0	0	1	4	3
Limia	1	1	0	1	2.5	2.5
Micropoecilia	1	1	0	1	2	3
Neoheterandria	0	0	1	1	2.5	0.5
Pamphorichthys	1	-	0	1	2	3
Phallichthys	0	0	1	0	2	0
Phalloceros	0	1	1	1	2	1
Phalloptychus	0	1	1	0	2	0
Poecilia	1	0	0	1	2	3
Poeciliopsis	0	1	1	0	1.5	0
Priapella	0	0	0	1	3	1
Priapickthys	0	0	1	1	3	0.5
Pseudopoecilia	0	0	1	1	2	1
Pseudoxiphophorus	0	-	1	1	2	0
Quintana	0	0	1	0	3	0
Scolichthys	0	0	0	1	3	1
Tomeurus	0	0	1	1	3	2
Xenodexia	0	-	1	0	2	2
Xenophallus	0	3	1	0	0	0.5
Xiphophorus	1	0	0	1	5.5	2

Appendix 21.4 Character data examined in *Gambusia* analyses. See text for descriptions of character values (appendices 21.9 – 21.10).

Species	Gonopodial Tip Shape	Female Genital Morphology	Female Anal Spot Location	Female Anal Spot Size
G. affinis	2	4	3	1
G. alvarezi	1	2	1	3
G. amistadensis	1	2	1	3
G. atrora	1	2	3	1
G. aurata	1	4	1	1
G. clarkhubbsi	0	2	1	1
G. echeagarayi	3	4	0	0
G. eurystoma	1	4	1	2
G. gaigei	1	2	1	1
G. geiseri	2	4	0	0
G. georgei	1	2	2	1
G. heterochir	0	1	2	3
G. hispaniolae	2	4	0	0
G. holbrooki	2	4	3	1
G. hubbsi	1	4	0	0
G. hurtadoi	1	2	1	3
G. krumholzi	0	2	1	1
G. lemaitrei	2	3	0	0
G. longispinis	1	2	1	2
G. luma	0	2	0	0
G. manni	1	4	0	0
G. marshi	0	1	1	1
G. melapleura	1	4	0	0
G. milleri	3	4	-	-
G. nicaraguensis	2	4	3	1
G. nobilis	0	2	3	3
G. panuco	0	1	1	1
G. punctata	0	2	0	0
G. puncticulata	1	4	1	1
G. rachovii	3	4	0	0
G. regani	1	1	-	-
G. rhizophorae	0	2	0	0
G. senilis	1	2	-	-
G. sexradiata	1	4	0	0
G. sp. nov.	2	4	3	2
G. speciosa	1	4	0	0
G. vittata	0	0	0	0
G. wrayi	1	4	0	0
G. xanthosoma	0	2	0	0
G. yucatana	1	4	-	-

Online Supplementary Material for Chapter 21

Genital evolution, *R. Brian Langerhans*

Appendix 21.5 A comparative test for the role of female mate choice in the evolution of gonopodium color.

To test the hypothesis that species with mating displays have evolved more colorful gonopodia due to female mating preference, I collected relevant data at the level of genera. Genera were assigned one of two mating strategies following prior work (Rosen & Tucker 1961; Bisazza 1993; Martin et al. In press), in which the genus either did not comprise any species that exhibit courtship displays (0) or did comprise at least some species that exhibit courtship displays (1). I collected genus-level data on gonopodium color using photographs, personal observations, and prior descriptions, scored on a scale of 0-3: 0 = no color, 1 = dark pigmentation only, 2 = bright coloration, 3 = strong, contrasting coloration, such as black and orange. Some genera contain species varying in the presence of gonopodial coloration, and these genera were scored according to the species that do exhibit coloration. Results suggest that gonopodial coloration has evolved in association with male display, as genera with bright or contrasting coloration also tend to include species exhibiting mating displays (25 genera, PIC: $r = 0.41$, one-tailed $P = 0.021$). This admittedly crude test provides cautious support for the hypothesis that female mate choice plays an important role in the evolution of gonopodium color. However, an alternative explanation is that males are generally more colorful in species with courtship displays, as female preference might have driven a general increase in male color, not in gonopodia *per se*. This would be consistent with the previous finding that sexual dichromatism may be associated with display behavior in poeciliids (Farr 1989; Bisazza 1993). While a trend between genera with sexual dichromatism and those exhibiting more brightly colored gonopodia does not seem apparent, the possible confounding role of correlated traits (i.e. brightly colored fins and bodies) deserves further examination.

Appendix 21.6 A comparative test for the role premating sexual conflict in the evolution of gonopodium size.

Gathering generic-level data, I tested the hypothesis that longer gonopodia have evolved to effectively prevent/overcome female choice. Specifically, I examined the association between gonopodium length and mating display, where species with relatively long gonopodia are not expected to exhibit courtship. Genera were assigned to a mating strategy as described earlier (see appendix 21.5). Genera were classified as either having short (< ~35% of SL) or long (> ~35% of SL) gonopodia based on previous work (Rosen & Tucker 1961; Martin et al. In press). I found a significant evolutionary relationship between the two characters, as genera with longer gonopodia tend to also exhibit a lack of mating displays (28 genera, Pagel's 1994 test: one-tailed $P = 0.0003$).

Appendix 21.7 Comparative analyses for the evolution of gonopodial symmetry/asymmetry.

Rosen and Tucker (1961) suggested that gonopodial asymmetry should be disfavored in species with short gonopodia because it would interfere with the function of bilaterally symmetric accessory structures, such as pelvic and pectoral fins, effectively reducing insemination and fertilization success. Presumably, this selection for enhanced insemination/fertilization success results from sperm competition (but could additionally result from sexually antagonistic selection to thwart female control of mating), and predicts that gonopodial asymmetry should primarily evolve in species with long gonopodia. While prior work has suggested this association indeed exists (Rosen & Tucker 1961; Rosen & Bailey 1963; Greven 2005), no explicit test performed within a phylogenetic context has yet been performed. Here I provide such a test. Genera were assigned as either exhibiting asymmetric (0) or symmetric (1) gonopodia, and either exhibiting short or long gonopodia as described earlier (see appendix 21.6). The two characters exhibited a significant evolutionary association (28 genera, Pagel's 1994 test: one-tailed P = 0.020). While this association points to a possible factor responsible for the evolution of gonopodial asymmetry (functionally enhancing insemination/fertilization success for long gonopodia), it does not explain the existence of symmetric gonopodia.

One hypothesis for the evolution of gonopodial symmetry is that it is a byproduct of selection via sperm competition favoring holdfast devices at the gonopodium tip. That is, symmetric holdfasts might be more effective at enhancing insemination or fertilization than asymmetric ones, incidentally leading to the evolution of bilaterally symmetric gonopodia to ensure increased functionality. If accurate, gonopodial symmetry should evolve in concert with holdfasts. To test this prediction, I estimated holdfasts as the total number of potential types of holdfast structures (hooks, spines, and serrae) present on each fin ray (data from Rosen & Gordon 1953; Rosen 1967). If a holdfast type was invariably absent or present within a genus, it was scored as 0 or 1, respectively; if a type was variable in its presence within a genus, it was scored as 0.5. The sum of these values was used as the overall holdfast score for each genus. I found significant correlated evolution between gonopodial symmetry and holdfasts (28 genera, PIC: r = 0.45, one-tailed P = 0.008). Because gonopodial symmetry and gonopodium length also exhibit correlated evolution, it is possible that the relationship between symmetry and holdfasts is a spurious result of both characters evolving in concert with gonopodium length. However, this does not appear to be the case, as holdfasts and gonopodium length are not strongly associated (see appendix 21.8) and the relationship remains significant when examined within a multiple regression context (one-tailed P = 0.023).

Appendix 21.8 Comparative tests for correlated evolution of gonopodium length, holdfasts, and accessory structures.

To test the oft-suggested correlated evolution of gonopodium length, holdfasts, and accessory structures (Rosen & Tucker 1961; Rosen & Bailey 1963; Greven 2005), I examined these relationships across poeciliid genera within a phylogenetic context. Gonopodium length and holdfasts were estimated as described earlier (see appendices 21.6, 21.7 respectively). Accessory structures were estimated for each genera as the sum of the number of structures present that are thought to aid the gonopodium during copulation through tactile or kinesthetic means: hood/palp, bony extension, modified paired fins, and ventral spines. First, I did not find a particularly strong relationship between gonopodium length and holdfasts (28 genera, PIC: r = -0.27, P = 0.17). Second, a moderate, positive relationship between holdfasts and accessories was suggested by the data (28 genera, PIC: r = 0.35, P = 0.069). Finally, a strong association between gonopodium length and accessory structures was uncovered (28 genera, PIC: r = -0.59, P = 0.0009).

Appendix 21.9 A comparative test of coevolution of male and female genitalia in *Gambusia*.

Extending Peden's (1972a) findings, I recorded a crude score for male and female genital morphology for most species of *Gambusia*. For males, I recorded gonopodial tip shape using a range of 0-3: 0 = blunt/rounded, 1 = broadly acute, 2 = acute, 3 = strongly and narrowly acute (data for 18 species from Peden [1972b], data for 22 additional species gathered from previously published figures or personal examination of specimens). External female genital morphology was recorded using a range of 0-4: 0 = no papilla in aperture, aperture completely covered by tissue protuberance, 1 = no papilla in aperture, aperture partially enclosed by external tissue, 2 = small or absent papilla in small- to moderately-sized aperture, 3 = small to moderate papilla in large aperture, 4 = large papilla in large aperture (data for 18 species from Peden [1972b], data for 22 additional species gathered from personal examination of specimens). The phylogeny contained eight polytomies (Appendix 21.2), which I resolved by inserting branches of zero length. The degrees of freedom in the analysis was then adjusted for the number of polytomies (Purvis & Garland 1993; Garland & Diaz-Uriarte 1999). Because genital morphology was measured using ranks, the values were non-normally distributed, and their association was thus tested with the nonparametric Spearman rank correlation test. Strong evidence for coevolution among the sexes in genitalia was found (40 species, PIC: $\rho = 0.79$, one tailed $P < 0.0001$; Fig. 21.4).

Appendix 21.10 A comparative test of reproductive character displacement in *Gambusia*.

The genus *Gambusia* represents an excellent system to test the lock-and-key hypothesis, as gonopodial morphology is highly variable (see Fig. 21.3), we have a high degree of knowledge of female genital morphology compared to other poeciliid groups, *Gambusia* species exhibit a wide range of sympatry/allopatry status, and hybridization is known to occur in the wild (Hubbs 1955; Hubbs 1959; Scribner & Avise 1993). The pattern of reproductive character displacement might be observed at two scales, both of which could be examined in *Gambusia*: 1) populations of two species exhibit greater genitalic differences in sympatry than when in allopatry, and 2) species pairs found in sympatry exhibit greater genitalic differences than species pairs that are exclusively allopatric. Here I test for the latter pattern.

I estimated four external genitalic characters in *Gambusia*: male gonopodial-tip shape, female urogenital aperture morphology, female anal-spot location, and female anal-spot size. The first two traits were measured for 40 species as described earlier (see appendix 21.9), while anal-spot morphology was measured for 36 species (15 species from Peden [1973], 21 species from personal examination). Anal-spot location was scored on a scale of 0-3: 0 = absent, 1 = between anus and urogenital aperture, 2 = on or beside urogenital aperture, 3 = posterior to urogenital aperture. Anal-spot size was also scored on a scale of 0-3: 0 = absent, 1 = small, 2 = medium, 3 = large. I tested the prediction of greater differences in genital morphology between sympatric species than allopatric species, controlling for phylogenetic effects, by calculating matrices of pair-wise distances among species pairs (distances in genital morphology, phylogenetic distance), and testing for matrix association using one-tailed partial Mantel tests (using 9999 randomizations). Significant support for the prediction of reproductive character displacement was found for gonopodial tip shape (one-tailed $P = 0.021$) and urogenital aperture morphology (one-tailed $P = 0.036$), and suggestive support was found for anal-spot location (one-tailed $P = 0.113$); while the hypothesis was clearly rejected for anal-spot size (one-tailed $P = 0.794$) (Fig. 21.5). As a means of circumventing possible errors in the phylogeny, I also conducted analyses restricting the dataset to a well-supported clade of 12 *Gambusia* species in northern Mexico / southwest Texas that exhibit considerable variation in sympatry/allopatry status. Results are similar to that from the full dataset, regardless of whether phylogeny is included in the analyses (gonopodium tip: both one-tailed $P < 0.010$, urogenital aperture: both one-tailed $P < 0.028$, anal spot location: both one-tailed $P < 0.101$, anal spot size: both one-tailed $P > 0.79$). Thus, these results appear fairly robust.

Supplemental References

Bisazza, A. 1993. Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. Marine Behaviour and Physiology 13: 257-286

Farr, J. A. 1989. Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. In G. K. Meffe and

J. Snelson, F. F. (eds) *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*. New Jersey: Prentice Hall pp 91-123

Garland, T. and Diaz-Uriarte, R. 1999. Polytomies and phylogenetically independent contrasts: examination of the bounded degrees of freedom approach. Systematic Biology 48: 547-558

Greven, H. 2005. Structural and behavioral traits associated with sperm transfer in Poeciliinae. In M. C. Uribe and H. J. Grier. (eds) *Viviparous Fishes*. Homestead: New Life Publications pp 145-163

Hrbek, T., Seckinger, J. and Meyer, A. 2007. A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. Molecular Phylogenetics and Evolution 43: 986-998

Hubbs, C. 1959. Population analysis of a hybrid swarm between *Gambusia affinis* and *G. heterochir*. Evolution 13: 236-246

Hubbs, C. L. 1955. Hybridization between fish species in nature. Systematic Zoology 4: 1-20

Martin, S. B., Albert, J. S. and Leberg, P. L. In press. The evolution of the poeciliid gonopodium: integrating morphological and behavioral traits. In M. Uribe and H. Grier. (eds) *Viviparous Fishes II*. Homestead, FL: New Life Publications pp

Peden, A. E. 1972a. Differences in the external genitalia of female gambusiin fishes. Southwest Naturalist 17: 265-272

Peden, A. E. 1972b. The function of gonopodial parts and behavioural pattern during copulation by *Gambusia* (Poeciliidae). Canadian Journal of Zoology 50: 955-968

Peden, A. E. 1973. Variation in anal spot expression of Gambusiin females and its effect on male courtship. Copeia 1973: 250-263

Purvis, A. and Garland, T. 1993. Polytomies in comparative analyses of continuous characters. Systematic Biology 42: 569-575

Rauchenberger, M. 1989. Systematics and biogeography of the genus *Gambusia* (Cyprinodontiformes: Poeciliidae). American Museum Novitates 2951: 1-74

Rosen, D. E. and Gordon, M. 1953. Functional anatomy and evolution of male genitalia in poeciliid fishes. Zoologica 38: 1-47

Rosen, D. E. and Tucker, A. 1961. Evolution of secondary sexual characters and sexual behavior patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). Copeia 1961: 201-212

Rosen, D. E. and Bailey, R. M. 1963. The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. Bulletin of the American Museum of Natural History 126: 1-176

Rosen, D. E. 1967. New poeciliid fishes from Guatemala, with comments on the origins of some South and Central American forms. American Museum Novitates 2303: 1-15

Scribner, K. T. and Avise, J. C. 1993. Cytonuclear genetic architecture in mosquitofish populations and the possible roles of introgressive hybridization. Molecular Ecology 2: 139-149