

Male genital size reflects a tradeoff between attracting mates and avoiding predators in two live-bearing fish species

R. Brian Langerhans*[†], Craig A. Layman[‡], and Thomas J. DeWitt[§]

*Department of Biology, Campus Box 1137, Washington University, St. Louis, MO 63130-4899; [‡]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520-8106; and [§]Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258

Edited by David B. Wake, University of California, Berkeley, CA, and approved April 6, 2005 (received for review February 4, 2005)

Male genitalia may experience more rapid, divergent evolution than any other animal character, but why? Research during the past several decades has culminated in the view that genital diversification primarily results from postmating sexual selection (e.g., sperm competition or cryptic female choice). However, the potential roles of premating sexual selection (e.g., mate choice) and natural selection have received little attention. We examined the possible importance of these mechanisms by investigating divergence in male genitalia among populations differing in predator regime for two species of live-bearing fish (*Gambusia affinis* in Texas and *Gambusia hubbsi* in The Bahamas). When controlled for body size, males exhibited a larger gonopodium (sperm-transfer organ) in predator-free environments than in predatory environments, a trend that persisted across space (multiple populations), time (multiple years), and species. By conducting laboratory experiments with *G. affinis*, we found that premating sexual selection seems to favor larger male genitalia (females exhibited mating preference for males having larger gonopodia), but natural selection in the presence of predatory fishes seems to favor reduced genital size (larger gonopodium size was associated with reduced burst-swimming performance, an important antipredator behavior). Although postmating sexual selection is widely presumed to be the most important mechanism driving genital diversification, these findings suggest that alternative mechanisms, particularly for organisms that cannot retract their genitalia, may also prove important.

fitness tradeoff | genital evolution | mate choice | natural selection | sexual selection

The striking diversity of male genital morphology in animals with internal fertilization has long puzzled biologists (1). Several hypotheses have been developed to explain this remarkable diversity (e.g., lock-and-key, pleiotropy, and postmating sexual selection). Research accumulating over the past several decades has produced the widespread view that genital diversification primarily results from postmating sexual selection (1–4). Postmating sexual selection can result from several mechanisms, such as sperm competition, cryptic female choice, or sexual conflict over fertilization decisions (1–8). Although much of the current research in this field centers around the postmating sexual selection hypothesis, there are two alternative ideas that have received comparatively little investigation: premating sexual selection and natural selection on males (9–12).

The role of premating sexual selection in the diversification of male genitalia may have been traditionally discounted partly because of the longstanding dichotomy between primary and secondary sexual traits (only secondary sexual characters were presumably subject to sexual selection) (13). Further, male genitalia in some taxa are usually retracted and frequently exhibit subtle, complex differences among closely related species, characteristics that seem to reduce their probability of experiencing premating sexual selection (1). Although premating sexual selection (via sexual conflict over mating decisions)

may be important in the evolution of genital claspers in insects (14), the role of premating sexual selection in diversification of male intromittent genitalia (sperm-transfer organs) has rarely been investigated (11, 15, 16). Yet, it is possible that females of some taxa assess male genital morphology before copulation (e.g., during premating courtship), producing genital divergence via mate choice.

Lack of research on the effects of natural selection on males in genital diversification is likely due to the perception that male genitalia typically entail small costs (1, 6, 17). In Eberhard's (1) original description of the postmating sexual selection hypothesis, he stated that, "In the case of genitalia, the 'braking' effects of natural selection are probably very weak." Yet, in some taxa, particularly those where male genitalia cannot be retracted or hidden, costs of male genitalia might play a major role in shaping genital morphology through natural selection. Because many animal traits are believed to reflect an evolutionary balance between premating sexual selection and natural selection, it seems reasonable to predict that male genital traits might often reflect this balance. Here we provide a test of this hypothesis.

Elaborate male traits favored by female mate choice often increase susceptibility to predation (6, 18). Divergence is expected in such traits between environments dominated by sexual selection (predator-free environments) and those dominated by natural selection (predatory environments). We tested whether genital morphology might evolve in this manner by examining intraspecific divergence in size of a copulatory organ among populations differing in predator regime for two species of live-bearing fish in the family Poeciliidae (*Gambusia affinis* and *Gambusia hubbsi*). Poeciliid fishes exhibit internal fertilization in which males inseminate females using a nonretractable, modified anal fin called a gonopodium. This elongate intromittent organ is highly variable among taxa, ranging from <20% to >70% of the male's body length (19–22). Poeciliid fishes are common prey items for a variety of predators, particularly piscivorous fish (23), and represent ideal models for studies of predator-driven evolution, because these fish inhabit a wide range of environments that vary substantially in predator community (24–28).

We hypothesized that premating sexual selection might influence genital morphology through female mate choice, where females prefer to mate with males possessing larger gonopodia, similar to the female preference for elaborated fins of males in some poeciliid species (29–33). This hypothesis is supported by the fact that males of many poeciliid species (including both species examined in this study) display the gonopodium during courtship by swinging or lowering it approximately perpendic-

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: log SL, natural logarithm of standard length; log GA, natural logarithm of gonopodium area; ANCOVA, analysis of covariance.

[†]To whom correspondence should be addressed. E-mail: langerhans@wustl.edu.

© 2005 by The National Academy of Sciences of the USA

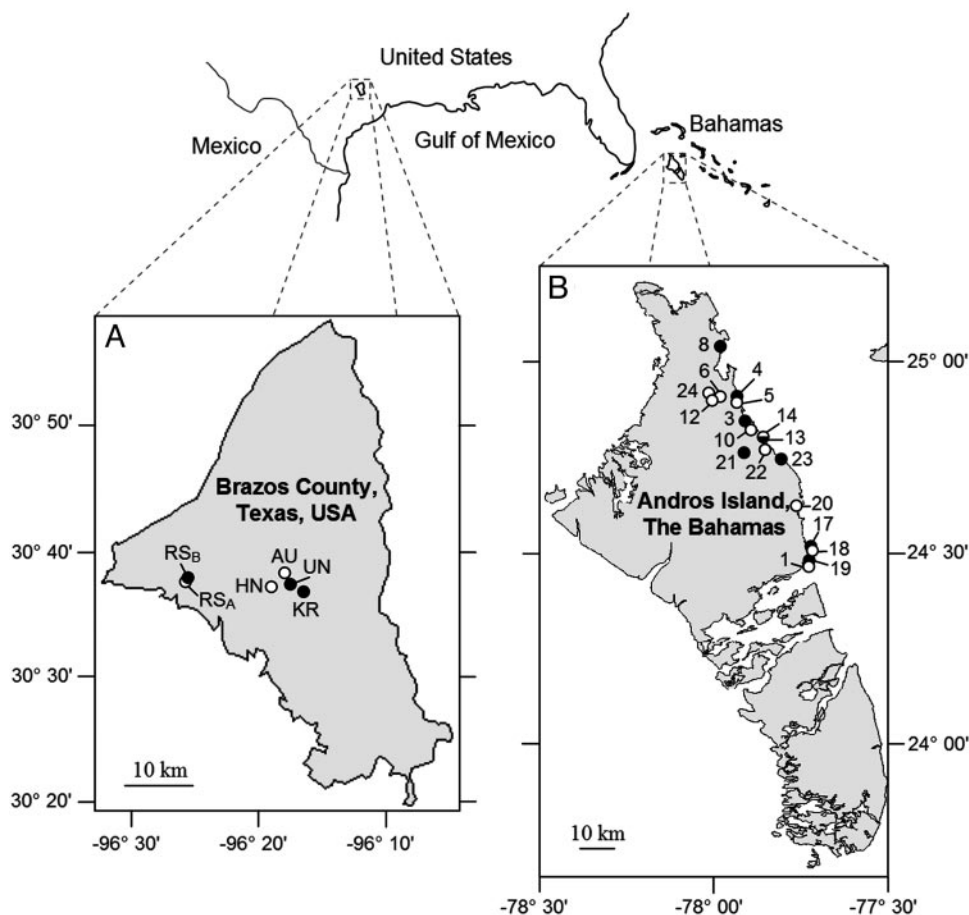


Fig. 1. Collection sites for *G. affinis* (A) and *G. hubbsi* (B). Open symbols represent predator-free populations, and filled symbols represent predator populations. Outer labels of maps represent latitude and longitude values. Labeling beside collection localities identifies populations and corresponds with those presented in Fig. 2.

ular to the body (29, 30). Predator-induced natural selection might play a role in gonopodium diversification through the effects of gonopodium size on swimming performance. Most fish, including poeciliids, produce a highly stereotyped escape response called a “c-start” when evading a predator strike (34). This swimming burst is very important in avoiding predation, and males possessing relatively large gonopodia might experience reduced burst-swimming speed owing to the enhanced drag generated by the large genitalia. To test these hypotheses, we conducted three laboratory experiments using *G. affinis*. (i) We reared progeny of wild-caught fish in a common-garden experiment to examine whether divergence in genital size reflected heritable variation. (ii) We conducted a mate-choice experiment to test whether females exhibited a mating preference for males with larger gonopodia. (iii) We tested for an association between gonopodium size and c-start burst-swimming speed to evaluate whether larger genitalia resulted in reduced burst speed.

Materials and Methods

Collections. We collected *G. affinis* (western mosquitofish) and *G. hubbsi* (Bahamas mosquitofish) from natural populations that differed in predator regime. Owing to the high dispersal and colonization ability of these small fishes relative to predatory fish species, they inhabit many environments that are devoid of piscivorous fish. Populations were classified “predator” or “predator-free” in relation to piscivorous fish presence, although other predators may have been present (e.g., invertebrates, turtles, and birds). Fish were collected by using seines, dip nets,

and minnow traps. We intensively surveyed populations for predatory fishes using visual observations, seines, cast nets, dip nets, and hook-and-line angling. All sites were surveyed on multiple occasions to ensure accurate assessment of predator presence. Detection and identification of all resident fish species were readily accomplished with our survey methods. For both *Gambusia* species, we attempted to collect from sites in a manner that avoided covariation between predator presence and other environmental parameters. Thus, alternative predator regimes did not differ systematically in any measured environmental characteristic (e.g., aquatic vegetation, depth, turbidity, temperature, dissolved oxygen, water velocity, salinity, and pH).

G. affinis is native to the southern United States and occupies a wide range of habitats varying in predator regime. We collected *G. affinis* from six freshwater ponds in Brazos County, TX (three with predators and three without; see Fig. 1A) over a 3-year period. A total of 349 *G. affinis* males were collected for analysis (138 in 2001, 86 in 2002, and 125 in 2003), with an average of 19.8 males per population in each year (range, 7–27 males). Common predatory fishes found with *G. affinis* were native sunfishes (family Centrarchidae), including largemouth bass (*Micropterus salmoides*), green sunfish (*Lepomis cyanellus*), warmouth (*Lepomis gulosus*), longear sunfish (*Lepomis megalotis*), bluegill sunfish (*Lepomis macrochirus*), and white crappie (*Pomoxis annularis*). Although the phylogenetic relationships of the populations are unknown, sites were selected so as to minimize the likelihood of predator populations being more closely related to each other than to any predator-free popula-

tion and vice versa. This was accomplished by collecting from isolated ponds across the landscape, where populations of alternative predator regimes were as close or closer geographically than populations of the same predator regime.

G. hubbsi is endemic to The Bahamas and inhabits a broad range of habitats varying in predator regime, including estuarine creeks, freshwater ponds, and blue holes (vertical solution caves penetrating the island's freshwater lens). We collected *G. hubbsi* from 18 populations on Andros Island, The Bahamas (8 with predators and 10 without; see Fig. 1*B*). Two populations (populations 6 and 23) were collected over a 2-year period, and the other populations were collected only once. A total of 342 *G. hubbsi* males were collected over the 2 years (23 in 2001 and 319 in 2002), with an average of 14.3 males per population in each year (range, 6–38 males). Common predatory fishes coexisting with *G. hubbsi* included the bigmouth sleeper (*Gobiomorus dormitor*), great barracuda (*Sphyrna barracuda*), redfin needlefish (*Strongylura notata*), Atlantic needlefish (*Strongylura marina*), tarpon (*Megalops atlanticus*), and snappers (*Lutjanus* species). Genetic evidence suggests marked divergence among the several populations used in this study and that populations of the same predator regime are not more closely related to each other than to populations of the alternative predator regime (35).

Measurements. A lateral photograph was taken of each adult male specimen. Standard length and the lateral area of the gonopodium were measured on these images by using TPSDIG software (36). Gonopodium area was measured by tracing the outline from its insertion into the body to its distal tip. The insertion base of the gonopodium was defined as a line connecting the anterior insertion of the gonopodium and the insertion of anal fin ray 5. R.B.L. performed all measurements. Before the study, we calculated repeatability of gonopodium area by taking two measurements for 20 fish of each species and conducting a model II ANOVA (see refs. 37–39). Repeatability was very high for both species (*G. affinis*: $r = 0.982$, $P < 0.0001$; *G. hubbsi*: $r = 0.969$, $P < 0.0001$). The natural logarithm of standard length (log SL) served as a measure of body size, and the natural logarithm of gonopodium area (log GA) served as a measure of gonopodium size in statistical analyses.

To test whether gonopodium size differed between predator regimes, controlling for body size, we conducted a nested analysis of covariance (ANCOVA) for each year, for each species. Because similar results were observed among years, we additionally pooled data across years and conducted the same statistical model to assess the overall magnitude of divergence in gonopodium size within each species. The ANCOVA model tested for effects of log SL (covariate), predator regime, and populations nested within predator regime on log GA. Heterogeneity of slopes was tested and never observed. Thus, interaction terms were not included in final analyses.

Common-Garden Experiment. To assess whether differences between predator regimes in gonopodium size had a heritable basis, *G. affinis* offspring from each of the six populations were reared in a common laboratory environment. We obtained offspring from six wild-caught females from each population. Offspring were raised in 15-liter aquaria (two tanks per population) at 22–25°C under a 14-h light/10-h dark photoperiod. Fish were fed newly hatched brine shrimp nauplii daily. Aquaria were arranged side-by-side in the laboratory, alternating between predator and predator-free populations, to reduce possible effects of microenvironmental factors. Further details concerning the common-garden experiment can be found in ref. 25. After 30 weeks of rearing, male morphology was measured and analyzed as described above.

Mate-Choice Experiment. We tested for an effect of male gonopodium size on mating preference of females by presenting female *G. affinis* (laboratory-reared F₁ progeny; $n = 48$) with two video recordings of males differing only in gonopodium size. Video playback of male courting behavior has been previously used to evaluate female mating preferences in poeciliid fishes and offers the advantage that male traits can be digitally altered to test effects of one trait while holding all other traits constant (40–42). We produced the videos by recording a displaying male (lateral display where the male lowers the gonopodium and erects dorsal and caudal fins) with an average gonopodium size and digitally altering the gonopodium (enlarging by 15%) to generate a second video. Thus, females were presented with videos showing identical males performing identical behaviors, with only the size of the gonopodium differing between videos. Females were isolated from males 24 h before experimentation. During each trial, one female was placed in a 25 × 15-cm staging arena of which two sides were opaque, one side displayed the two videos side-by-side (separated by 15 mm), and one side was transparent for recording behavioral observations. The female was allowed to acclimate for 10 min before the video was initiated. After the video played for 5 min, we recorded female mating preference for 10 min, switched the left–right presentation order of the two video males, allowed the female to acclimate with the new video presentation for 5 min, and recorded female mating behavior for another 10 min. Female mating response for each male was measured in two ways: (i) time spent by the female directly interacting with the respective male, and (ii) number of approaches by the female within one body length of the male. Both measurements of mate choice were summed across the two observation periods for each female. We tested for differences in female mating preference between large-gonopodium and small-gonopodium males using Wilcoxon's signed-ranks test, treating the data as randomized blocks, with females as blocks and gonopodium size as the treatment. This nonparametric test examined whether differences between males in interaction time or number of approaches significantly differed from zero (zero representing no preference).

Burst-Swimming Speed. We measured c-start burst speed for male *G. affinis* collected in 2003. Details of performance trials can be found in ref. 25. Briefly, fish were placed in a 12 × 12-cm staging arena with a 5-mm-square grid on the bottom. C-start responses were elicited by startling the fish with a sudden, downward thrust of a cylindrical wooden probe (5 mm in diameter and 100 mm in length), hitting the bottom of the stage within 3 cm of the fish. A video camera mounted above the arena recorded three c-starts for each fish, and the fastest response was retained for analysis. Burst-swimming speed was calculated by digitizing the center of mass for fish in each frame of the c-start response by using TPSDIG software (36). To examine the relationship between relative gonopodium size and locomotor performance, we calculated population means for burst-swimming speed, log SL, and log GA. We used these population means to conduct a multiple regression where average burst-swimming speed was tested for effects attributable to average log SL and average log GA. This analysis examined the relationship between gonopodium size and swimming performance, controlling for body size.

Results

Relative to body size, gonopodium size significantly differed between predator regimes in each year for each species (Table 1). For both species, males in predator-free populations exhibited relatively larger gonopodia than males in predator populations (Fig. 2). When using data pooled across years, fish in predator-free populations exhibited a gonopodium area that was 12.2% larger on average in *G. affinis* and 18.5% larger on average in *G. hubbsi* than conspecifics in predator populations.

Table 1. ANCOVA results examining differences in gonopodium size (log GA), controlling for body size, between predator regimes and populations nested within predator regimes for *G. affinis* and *G. hubbsi*

Species	Year	Predator regime			Population (predator regime)		
		<i>F</i>	<i>df</i>	<i>P</i>	<i>F</i>	<i>df</i>	<i>P</i>
<i>G. affinis</i>	2001	23.46	1, 131	<0.0001	4.84	4, 131	0.0011
	2002	16.25	1, 79	0.0001	4.18	4, 79	0.0040
	2003	19.17	1, 118	<0.0001	1.91	4, 118	0.1138
	Pooled	43.19	1, 342	<0.0001	2.67	4, 342	0.0322
	CG	6.72	1, 11	0.025	0.50	4, 11	0.7337
<i>G. hubbsi</i>	2001	4.18	1, 20	0.0543	—	—	—
	2002	82.10	1, 300	<0.0001	2.33	16, 300	0.0030
	Pooled	59.61	1, 323	<0.0001	2.07	16, 323	0.0093

The covariate (log SL) was significant in all cases. Pooled, analyses are based on data combined across years; CG, fish raised in the common-garden experiment.

In the common-garden experiment, *G. affinis* males derived from predator-free populations exhibited a larger gonopodium, when controlling for body size, than males derived from predator populations (Table 1 and Fig. 3). Thus, fish raised in a common laboratory environment retained the differences in gonopodium size observed in wild-caught fish. These results suggest that divergence in gonopodium size between predator regimes had a heritable component.

The mate-choice experiment found that females exhibited mating preference for the large-gonopodium male (Fig. 4). Females spent 81% more time directly interacting with the large-gonopodium male than the small-gonopodium male, and approached the large-gonopodium male within one body length 28% more times than the small-gonopodium male.

Burst-swimming speed was significantly associated with log SL (positive association, $F_{1,3} = 96.19$, $P = 0.002$) and log GA (negative association, $F_{1,3} = 88.21$, $P = 0.003$). Populations with larger fish, irrespective of gonopodium size, and smaller gonopodia, irrespective of body size, exhibited faster burst-swimming speeds. Thus, relatively large gonopodia seem to incur a cost of reduced burst-swimming speed.

Discussion

We found parallel divergence in size of male genitalia between predator regimes within two *Gambusia* species that inhabit

different geographic regions and are prey for different species of predatory fishes. Males of both species exhibited larger gonopodia in predator-free populations than in predator populations. For *G. affinis*, females preferred to mate with males having larger gonopodia, but burst-swimming speed was negatively associated with gonopodium size. These results suggest that within each species, gonopodium size reflects an evolutionary balance between premating sexual selection favoring larger gonopodia and natural selection via predation favoring smaller gonopodia.

Observed differences in gonopodium size between predator regimes might have resulted from genetic differences among populations, phenotypic plasticity, or a combination of both. In *G. affinis*, divergence in gonopodium size observed in the field was retained in offspring reared in a common laboratory environment. These results demonstrate that genetic differentiation among populations, or maternal special environmental effects that differ between predator regimes, underlie phenotypic differences observed in laboratory-reared fish (43–45). Either of these sources of phenotypic variation might reflect adaptive responses to selection.

Our results provide strong support for the role of premating sexual selection in promoting larger genitalia in *Gambusia*. For both measures of mating response, female *G. affinis* exhibited significant preference for the male with a larger gonopodium.

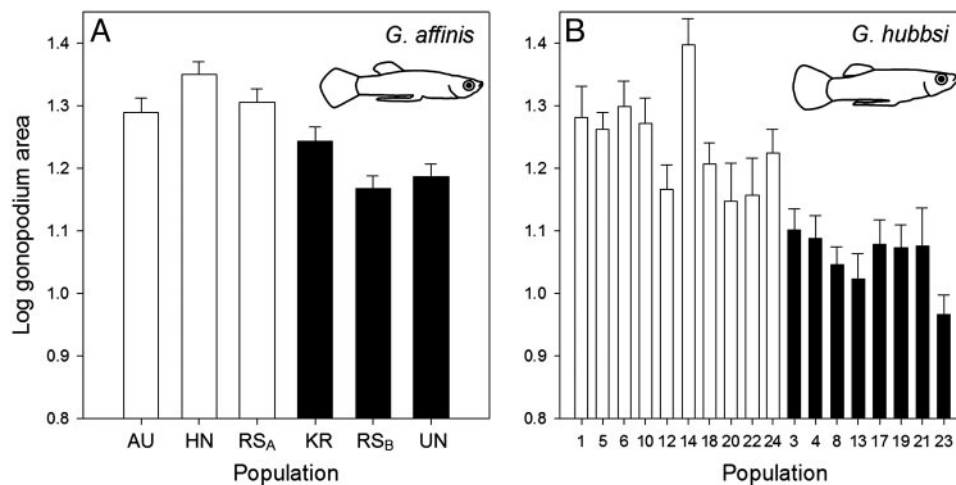


Fig. 2. Divergence in gonopodium size, controlling for body size, between predator regimes in *G. affinis* (A) and *G. hubbsi* (B). Data were pooled across years for both species. Least-squares means \pm 1 standard error from ANCOVAs are depicted. Open bars represent predator-free populations, and filled bars represent predator populations. Population names are as in Fig. 1.



Fig. 3. Representative laboratory-reared *G. affinis* males derived from predator-free (A) and predator (B) populations. Arrows indicate the gonopodia. Note the larger gonopodium in A. (Bar, 5 mm.)

Thus, larger gonopodia of males in predator-free populations seem to reflect, at least in part, the influence of female mate choice on the evolution of male genital size.

Whether female mating preference for males with large gonopodia evolved multiple times is unknown, but it is possible that the preference reflects a shared ancestral character in *Gambusia* species and is widespread within live-bearing fishes. That is, female mate choice might be evolutionarily conserved, driving repeated evolutionary responses by males. If true, the preference could serve as an important sensory bias responsible for various elaborations of fin characteristics other than gonopodia in male live-bearing fishes. For instance, female mating preference based on gonopodium size might help explain the evolution of swords (elongate, sword-like projections of the caudal fin) in male swordtail fishes. That is, swords, due to their resemblance to gonopodia in overall shape, might effectively represent gonopodium mimics and exploit a preexisting sensory bias in females. Support for this hypothesis comes from the fact that female preference for swords arose before the evolution of swords (30, 46), the gonopodium is evolutionarily older than the sword, and both gonopodium and sword development are associated with expression of the same gene (47). Further, males from predator-free environments exhibit larger swords than males from predatory environments in *Xiphophorus helleri* (48), a pattern parallel to that observed with gonopodium size in the present study. Thus, a better understanding of the mechanisms

driving genital diversification might provide insight into the evolution of other sexually selected traits.

If larger gonopodium size results in higher mating success, then why don't all males have large gonopodia? The reduced gonopodium size observed in predator populations of both species examined in this study may reflect a locomotor cost of the gonopodium. We found that populations of *G. affinis* with relatively larger gonopodia exhibited slower burst-swimming speeds. Because c-start bursts are important in evading predator strikes, the reduced burst speed suffered by males with larger gonopodia will presumably result in increased vulnerability to predation (34, 49, 50).

A recent study of an invertebrate (the spider *Tidarren sisyphoides*) with large, nonretractable copulatory organs also revealed a locomotor cost of male genitalia (10). Contrary to the perception that male genitalia suffer minimal costs (1, 6, 17), results in *Gambusia* and *Tidarren* suggest “braking” effects of natural selection might sometimes play a substantial role in genital evolution, particularly for organisms that cannot retract or hide their genitalia. For gonopodium size, strong braking effects seem likely because larger gonopodia might suffer several costs other than reduced burst speeds, such as increased energetic costs during swimming (as in the case of elaborated swords in swordtail fish; see ref. 51), reduced maneuverability, or enhanced conspicuousness to predators.

Although our results are consistent with the hypothesis that gonopodium size reflects a balance between premating sexual selection and natural selection, other mechanisms might also be important in the evolution of gonopodium morphology. For example, postmating sexual selection might influence gonopodium size through cryptic female choice, where females differentially use sperm based on gonopodium size of males (as appears to be the case for male coloration in guppies; see ref. 52), or sperm competition, where larger gonopodia effectively reduce insemination success of subsequent matings by temporarily traumatizing female genitalia, as suggested by Constantz (53).

Interestingly, the pattern observed in the two *Gambusia* species in the present study is opposite that observed in previous studies of intraspecific divergence in gonopodium size (9, 54). In these other studies, males exhibited larger gonopodia in predatory environments compared with low predation localities, indicating that predation's influence on gonopodium diversification may be complicated. However, as Jennions and Kelly (9) point out, several factors covaried with predation intensity in

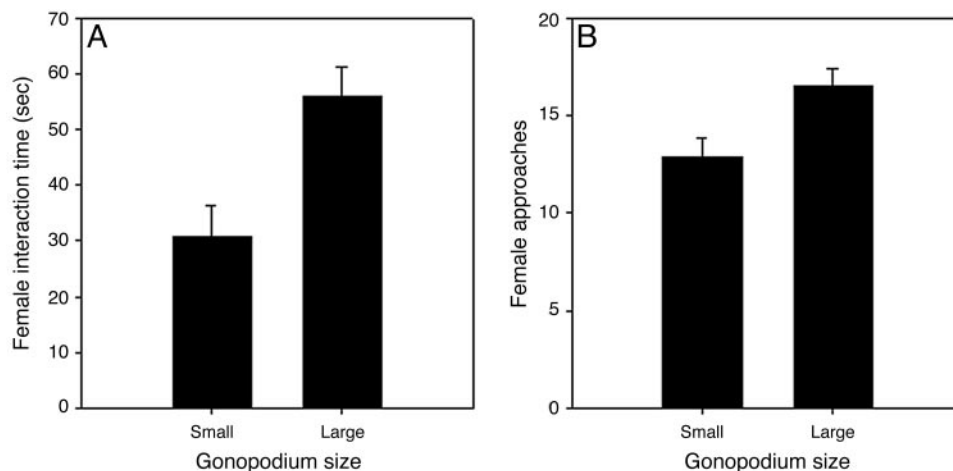


Fig. 4. Female mating responses to videos of a male with a small vs. large gonopodium. (A) Females spent more time directly interacting with the large-gonopodium male than the small-gonopodium male ($P < 0.0001$). (B) Females approached the large-gonopodium male within one body length more times than the small-gonopodium male ($P = 0.0002$). Means \pm 1 standard error are depicted.

those studies, such as food availability and water velocity, and were proposed as likely selective mechanism(s) responsible for gonopodium variation. In the present study, we specifically chose populations in an attempt to avoid such covariation between predator community and other environmental characteristics to examine the influence of predator regime *per se*. The marked association between predator community and other environmental parameters in the upstream–downstream systems examined in previous studies (9, 54) may explain the opposite nature of gonopodium divergence compared with the *Gambusia* species examined here. Thus, gonopodium size might reflect the action of sexual and natural selection in all of these species, but the nature or source of selection might vary among species.

Our findings suggest that similar selective pressures have resulted in similar phenotypic responses for multiple populations of two different species. Whether divergence in gonopodium size was achieved via similar genetic and developmental modifications is currently unknown. Because of the phylogenetic relatedness among the congeners examined in this study, it is plausible that most populations share similar genetic architectures that predispose them to respond in similar manners to common selective pressures (55). The genetic and developmental mechanisms underlying the divergence in genital size observed in this study require further investigation.

The current study suggests that premating sexual selection and natural selection on males may play important roles in the evolutionary diversification of male genitalia. This is not to say that postmating sexual selection is unimportant in genital evolution; indeed, it has garnered significant empirical support in many organisms (primarily insects), and might represent an important mechanism in gonopodium divergence. The general importance of postmating sexual selection in genital diversification is well established; however, a better understanding of genital divergence will likely arise from a more complete examination of the potential mechanisms responsible. Many diverse taxa exhibit genitalia that cannot be retracted, making them particularly susceptible to premating sexual selection and natural selection, suggesting that these heretofore underappreciated mechanisms may prove important in genital evolution.

We thank A. Langerhans, H. Prestridge, and M. Shokrollahi for field assistance in Texas; A. Arrington for assistance in The Bahamas; M. Blackwell of The Bahamas Environmental Research Center for valuable logistical support on Andros Island; and J. Losos, D. Irschick, D. Wake, and an anonymous reviewer for comments that improved the manuscript. This work was partially supported by a Sustainable Coastal Margins Program fellowship (to R.B.L.), a Texas Water Resources Institute Mills Scholarship (to R.B.L.), an Environmental Protection Agency Science to Achieve Results (STAR) fellowship (to R.B.L.), and National Science Foundation Grant DEB-9908528 (to T.J.D.).

- Eberhard, W. G. (1985) *Sexual Selection and Animal Genitalia* (Harvard Univ. Press, Cambridge, MA).
- Eberhard, W. G. (1996) *Female Control: Sexual Selection by Cryptic Female Choice* (Princeton Univ. Press, Princeton, NJ).
- Hosken, D. J. & Stockley, P. (2004) *Trends Ecol. Evol.* **19**, 87–93.
- Arnqvist, G. (1998) *Nature* **393**, 784–786.
- Birkhead, T. R. & Møller, A. P. (1998) *Sperm Competition and Sexual Selection* (Academic, San Diego).
- Andersson, M. (1994) *Sexual Selection* (Princeton Univ. Press, Princeton, NJ).
- Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. (2003) *Trends Ecol. Evol.* **18**, 41–47.
- Rice, W. R. (1996) *Nature* **381**, 232–234.
- Jennions, M. D. & Kelly, C. D. (2002) *Oikos* **97**, 79–86.
- Ramos, M., Irschick, D. J. & Christenson, T. E. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 4883–4887.
- Yang, L. H. & Wang, Q. (2004) *J. Insect Behav.* **17**, 695–707.
- Gack, C. & Peschke, K. (2005) *Biol. J. Linn. Soc.* **84**, 307–312.
- Arnqvist, G. (1997) *Biol. J. Linn. Soc.* **60**, 365–379.
- Arnqvist, G. & Rowe, L. (2002) *Nature* **415**, 787–789.
- Brooks, R. & Caithness, N. (1995) *Anim. Behav.* **50**, 301–307.
- Preziosi, R. F. & Fairbairn, D. J. (1996) *J. Evol. Biol.* **9**, 317–336.
- Eberhard, W. G. (1993) *Am. Nat.* **142**, 564–571.
- Rosenthal, G. G., Martinez, T. Y. F., de Leon, F. J. G. & Ryan, M. J. (2001) *Am. Nat.* **158**, 146–154.
- Rosen, D. E. & Gordon, M. (1953) *Zool. Sci. Contrib. N.Y. Zool. Soc.* **38**, 1–47.
- Rivas, L. R. (1963) *Copeia*, 331–347.
- Ghedotti, M. J. (2000) *Zool. J. Linn. Soc.* **130**, 1–53.
- Chambers, J. (1987) *J. Fish Biol.* **30**, 389–418.
- Meffe, G. K. & Snelson, F. F. (1989) in *Ecology and Evolution of Livebearing Fishes (Poeciliidae)*, eds. Meffe, G. K. & Snelson, F. F. (Prentice–Hall, Englewood Cliffs, NJ), pp. 13–31.
- Langerhans, R. B. & DeWitt, T. J. (2004) *Am. Nat.* **164**, 335–349.
- Langerhans, R. B., Layman, C. A., Shokrollahi, A. M. & DeWitt, T. J. (2004) *Evolution (Lawrence, Kans.)* **58**, 2305–2318.
- Endler, J. A. (1995) *Trends Ecol. Evol.* **10**, 22–29.
- Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. (1997) *Science* **275**, 1934–1937.
- Reznick, D. N. (1996) *Neth. J. Zool.* **46**, 172–190.
- Rosen, D. E. & Tucker, A. (1961) *Copeia*, 201–212.
- Basolo, A. L. (1995) *Proc. R. Soc. London Ser. B* **259**, 307–311.
- Ptacek, M. B. (1998) *Anim. Behav.* **56**, 1145–1154.
- Bischoff, R. J., Gould, J. L. & Rubenstein, D. J. (1985) *Behav. Ecol. Sociobiol.* **17**, 253–255.
- Gould, J. L., Elliott, S. L., Masters, C. M. & Mukerji, J. (1999) *Curr. Biol.* **9**, 497–500.
- Domenici, P. & Blake, R. W. (1997) *J. Exp. Biol.* **200**, 1165–1178.
- Schug, M. D., Downhower, J. F., Brown, L. P., Sears, D. B. & Fuerst, P. A. (1998) *Heredity* **80**, 336–346.
- Rohlf, F. J. (2003) TPSDIG (Dept. of Ecology and Evolution, State Univ. of New York, Stony Brook, NY), Version 1.38.
- Sokal, R. R. & Rohlf, F. J. (1995) *Biometry* (Freeman, New York).
- Lessells, C. M. & Boag, P. T. (1987) *Auk* **104**, 116–121.
- Yezerinac, S. M., Lougheed, S. C. & Handford, P. (1992) *Syst. Biol.* **41**, 471–482.
- Basolo, A. L. & Trainor, B. C. (2002) *Anim. Behav.* **63**, 469–474.
- Johnson, J. B. & Basolo, A. L. (2003) *Behav. Ecol.* **14**, 619–625.
- Trainor, B. C. & Basolo, A. L. (2000) *Anim. Behav.* **59**, 83–89.
- Falconer, D. S. & Mackay, T. F. C. (1996) *Introduction to Quantitative Genetics* (Pearson Education Limited, Essex, U.K.).
- Rossiter, M. (1996) *Annu. Rev. Ecol. Syst.* **27**, 451–476.
- Wolf, J. B., Brodie, E. D., Cheverud, J. M., Moore, A. J. & Wade, M. J. (1998) *Trends Ecol. Evol.* **13**, 64–69.
- Basolo, A. L. (1990) *Science* **250**, 808–810.
- Zauner, H., Begemann, G., Mari-Beffa, M. & Meyer, A. (2003) *Evol. Dev.* **5**, 466–477.
- Basolo, A. L. & Wagner, W. E. (2004) *Biol. J. Linn. Soc.* **83**, 87–100.
- Howland, H. C. (1974) *J. Theor. Biol.* **47**, 333–350.
- Webb, P. W. (1986) in *Predator–Prey Relationships*, eds. Lauder, G. V. & Feder, M. E. (Univ. of Chicago Press, Chicago), pp. 24–41.
- Basolo, A. L. & Alcaraz, G. (2003) *Proc. R. Soc. London Ser. B* **270**, 1631–1636.
- Pilastro, A., Simonato, M., Bisazza, A. & Evans, J. P. (2004) *Evolution (Lawrence, Kans.)* **58**, 665–669.
- Constantz, G. D. (1984) in *Sperm Competition and the Evolution of Animal Mating Systems*, ed. Smith, R. L. (Academic, Orlando, FL), pp. 465–485.
- Kelly, C. D., Godin, J. G. J. & Abdallah, G. (2000) *Can. J. Zool.* **78**, 1674–1680.
- Schluter, D. (1996) *Evolution (Lawrence, Kans.)* **50**, 1766–1774.