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MALE PREGNANCY AND THE EVOLUTION OF BODY SEGMENTATION IN SEAHORSES AND PIPEFISHES

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Abstract.—The evolution of complex traits, which are specified by the interplay of multiple genetic loci and environmental effects, is a topic of central importance in evolutionary biology. Here, we show that body and tail vertebral numbers in fishes of the pipefish and seahorse family (Syngnathidae) can serve as a model for studies of quantitative trait evolution. A quantitative genetic analysis of body and tail vertebrae from field-collected families of the Gulf pipefish, *Syngnathus scovelli*, shows that both traits exhibit significantly positive additive genetic variance, with heritabilities of 0.75 ± 0.13 (mean ± standard error) and 0.46 ± 0.18, respectively. We do not find any evidence for either phenotypic or genetic correlations between the two traits. Pipefish are characterized by male pregnancy, and phylogenetic consideration of body proportions suggests that the position of eggs on the pregnant male's body may have contributed to the evolution of vertebral counts. In terms of numbers of vertebrae, tail-brooding males have longer tails for a given trunk size than do trunk-brooding males. Overall, these results suggest that vertebral counts in pipefish are heritable traits, capable of a response to selection, and they may have experienced an interesting history of selection due to the phenomenon of male pregnancy. Given that these traits vary among populations within species as well as among species, they appear to provide an excellent model for further research on complex trait evolution. Body segmentation may thus afford excellent opportunities for comparative study of homologous complex traits among disparate vertebrate taxa.

Key words.—Axial skeleton, **G**-matrix, heritability, natural selection, quantitative genetics, *Syngnathus scovelli*, vertebral number.

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One of the most challenging areas of research in evolutionary biology over the last several decades has been the study of quantitative traits (Mackay 2001; Phillips 2005). These complex traits are determined by the interplay of multiple genetic loci and environmental factors, a situation that can result in extremely complicated evolutionary dynamics (Lynch and Walsh 1998). The vast majority of traits that contribute to the overall phenotypes of most multicellular organisms are quantitative, and phenotypic differences between populations or species almost always involve suites of complex characters (Arnold and Phillips 1999; Begin and Roff 2004). The central importance of quantitative traits to evolution has motivated a wide array of theoretical and empirical approaches directed toward a deeper understanding of these types of traits (Arnold et al. 2001; Mackay 2001). However, there are still significant gaps in our understanding of the genetic basis and evolutionary dynamics of complex traits (Phillips 2005).

The most serious shortcomings with respect to our comprehension of quantitative trait evolution arise from incomplete knowledge of their genetic architecture. The importance of genetic architecture can be appreciated by considering the multivariate breeders equation, $\Delta\bar{\mathbf{z}} = \mathbf{G}\boldsymbol{\beta}$ (Lande 1976). The change in $\bar{\mathbf{z}}$, the vector of trait means, is a function of $\boldsymbol{\beta}$, the vector of directional selection gradients on the traits, and **G**, the **G**-matrix. In this equation, the genetic architecture of a multivariate phenotype comprising a suite of quantitative traits is described by the **G**-matrix, which is a matrix of the additive genetic variances and covariances for the traits under consideration. The evolutionary dynamics of **G** can be quite complex, substantially complicating evolutionary inference based on quantitative genetics (Lande 1979; Turelli 1988; Jones et al. 2003, 2004). The nature of mutations at individual quantitative trait loci (QTLs) theoretically has major effects on the evolution of the **G**-matrix, yet we have almost no empirical data on this topic (Mackay 2001; Phillips 2005).

The paucity of data on the genetic architecture of quantitative traits has inspired two promising new research avenues. The first is the comparative analysis of **G**-matrices among populations and species (Steppan et al. 2002), and the second is the mapping of QTLs using genetic markers (Mackay 2004). Both of these techniques have led to insights about the genetic architecture of quantitative traits, but the meaningful synthesis of these endeavors is at a very early, albeit exciting, stage (e.g., Colosimo et al. 2005; Kroymann and Mitchell-Olds 2005). Comparative analyses of the **G**-matrix typically focus on distinct natural populations that exhibit differences with respect to quantitative traits (Steppan et al. 2002), whereas QTL mapping studies have been most successful in model organisms or agriculturally important species (Burke et al. 2002; Mackay 2004). For example, the garter snake has been extremely useful in the comparative study of **G**-matrices (Arnold and Phillips 1999), but its long generation time makes QTL mapping virtually impossible. In contrast, *Drosophila melanogaster* seems to offer the greatest potential among animal models for QTL mapping (Mackay 2001, 2004), but its cosmopolitan geographical distribution with extremely high gene flow (Dieringer et al. 2005) means that it may not produce generalizations that apply to species with more restricted dispersal. These systems and others that offer great promise will no doubt continue to provide useful insights, but we would benefit from additional

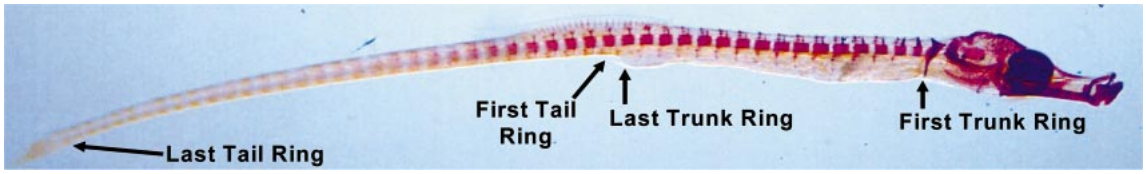

FIG. 1. A stained embryo showing the traits of interest. Trunk rings are counted from the ring bearing the base of the pectoral fin through the ring bearing the anus. The first tail ring is the first ring posterior to the ring bearing the anus, and the last tail ring is the final ring before the element that possesses the caudal fin. Note that each ring corresponds to a single vertebra (although not all of the anteriormost vertebrae are included in the trunk ring count).

model systems in which to study the genetic architecture and evolutionary dynamics of complex traits.

Our goal was to explore the fishes of the family Syngnathidae, which includes pipefishes, seahorses and sea dragons, as a model for the study of quantitative trait evolution. These fishes possess several characteristics that are favorable for the study of complex trait evolution. As is the case for garter snakes, the number of body segments can be scored by external examination at any age, because rings of bony plates (trunk and tail rings) encircle each individual (Dawson 1982, 1985) and each ring is associated with a single vertebra. Because the number of vertebrae in fishes is fixed early during development, trunk and tail ring counts at birth represent an individual's lifetime trait value (McDowall 2003). Another favorable feature of this system is that males carry offspring on their bodies and males have complete confidence of paternity (Jones and Avise 2001), so a father can be collected with his offspring, permitting the estimation of additive genetic variances and covariances from field collections (Lynch and Walsh 1998). In addition, advances in syngnathid aquaculture and the popularity of these fishes in the aquarium trade demonstrate that pipefishes and seahorses can be maintained in captivity, permitting multigenerational breeding studies (Job et al. 2002). One other interesting aspect of the family Syngnathidae is that early during its diversification, the group split into two major lineages, one of which carries the embryos on the trunk of the male and the other of which carries the embryos on the tail (Wilson et al. 2003). The placement of the embryos during pregnancy could provide a selective pressure on body proportions. Finally, substantial variation in numbers of trunk and tail rings has been documented within and among species (Dawson 1982). Thus, this system offers great potential for the investigation of the evolutionary processes that have led to divergence of these traits in separate lineages.

In the present study, we address several specific questions. First, we test the hypothesis that within-population phenotypic variation in trunk and tail ring counts has a heritable genetic component. Second, we estimate genetic and phenotypic correlations between the two traits to assess whether or not genetic constraints contribute to the patterns of evolution in this group. Finally, we look at the patterns of trunk and tail ring evolution among species within the family to address the hypothesis that the placement of the offspring on the male during pregnancy is related to the evolution of trunk and tail ring numbers.

MATERIALS AND METHODS

Sample Collection and Scoring of Phenotypic Traits

We collected 27 pregnant male Gulf pipefish, *Syngnathus scovelli*, from a site near Port St. Joe, Florida, in the Gulf of Mexico (29°47.765′N, 85°18.237′W) on 24–26 August 2003 by pulling a seine with a 3-mm mesh through shallow seagrass meadows. The pipefish were transported back to the lab in aerated buckets filled with water from the Gulf of Mexico. In the laboratory, we placed each male in a 10-gal aquarium filled with artificial salt water (Instant Ocean, Aquarium Systems, Inc., Mentor, OH) and heated to a constant temperature of 27°C. Males were housed individually until they gave birth to their offspring, at which time the males and offspring were sacrificed and preserved in 10% formalin.

After the fish had been in formalin for at least 24 h, we rinsed them for 1 h under running water and submerged them in 5% KOH for 1 h. We then added a small amount of alizarin red dye to the solution and allowed the fish to stain overnight. The stained fish were transferred to glycerol for destaining and long-term storage. We counted the number of trunk and tail rings for each father plus an average of 10.3 offspring per male. In the Gulf pipefish, the first trunk ring houses the base of the pectoral fin, and the last trunk ring is marked by the presence of the anus (Fig. 1, Dawson 1982). The first tail ring is the first complete ring posterior to the anus, and the last tail ring is the last complete ring anterior to the caudal fin (i.e., excluding the element possessing the caudal fin, Fig. 1).

Quantitative Genetic Analysis

We computed quantitative genetic parameters by using the techniques implemented in the H2boot software package (Phillips 1998; Arnold and Phillips 1999). We calculated the additive genetic variance as twice the parent-offspring covariance for each trait. The additive genetic covariance is given by the average of the two cross-trait covariances: the covariance between trunk ring counts in parents and tail ring counts in offspring and the covariance between tail ring counts in parents and trunk ring counts in offspring. Phenotypic variances and the phenotypic covariance were calculated by using the full-sibling analysis of variance (ANOVA) approach implemented in H2boot. We also used H2boot to calculate standard errors for all of the quantitative genetic estimates by bootstrapping across 10,000 replicates.

TABLE 1. Quantitative genetic estimates for trunk and tail ring characters in our Gulf pipefish focal population. Standard errors are shown in parentheses.

Parameter	Trunk rings	Tail rings	Trunk, tail covariances
Heritability	0.75 (±0.13)	0.45 (±0.18)	
Genetic variance	0.25 (±0.07)	0.21 (±0.09)	−0.01 (±0.07)
Phenotypic variance	0.34 (±0.05)	0.47 (±0.06)	−0.01 (±0.04)
Environmental variance	0.08 (±0.04)	0.26 (±0.09)	−0.01 (±0.05)

Comparative Analysis

We used the taxonomic literature (Lythgoe and Lythgoe 1975; Dawson 1982, 1985; Lourie et al. 1999) to compile trunk and tail ring counts from 51 of the 52 genera recognized by Dawson (1985) in the family Syngnathidae. In most cases, we used multiple species and generated an average across species for each genus. We did not obtain data for the genus *Enneacampus*, and we also followed Dawson (1985) in considering *Dunckerocampus* part of *Doryrhampus* and *Oostethus* part of *Microphis*. We tested the hypothesis that tail brooders had more tail rings for a given number of trunk rings by the use of an analysis of covariance, with number of trunk rings as the covariate. A formal comparative analysis correcting for shared ancestry (Felsenstein 1985) was not possible, because our current understanding of the phylogeny of Syngnathidae indicates that trunk breeding and tail breeding each originated only once in the history of this group, and only a third of the recognized genera have representatives in the current molecular phylogeny (Wilson et al. 2003).

RESULTS

The results of the quantitative genetic analysis clearly indicate that both traits—number of trunk rings and number of tail rings—in the Gulf pipefish exhibit significant additive genetic variance, despite a relatively low level of phenotypic variance. The number of trunk rings in our adult population varied from 15 to 16 (mean = 15.4), whereas the number of tail rings ranged from 30 to 32 (mean = 31.2). The phenotypic variances were 0.34 (± a standard error of 0.05) and 0.47 (±0.06) for trunk and tail rings, respectively. The phenotypic covariance and correlation were −0.01 (±0.04) and −0.04 (±0.10), respectively. Heritabilities and additive genetic variances for the two traits were significantly positive (Table 1), but the point estimate for the genetic covariance was very close to zero (Table 1). Consequently, the genetic correlation also did not differ significantly from zero, with a point estimate of 0.01 (±0.34).

Figure 2 shows a male of a typical tail-brooding pipefish species in comparison to a male of a trunk-brooding species. For a given number of trunk rings, tail brooders usually have more tail rings than do trunk brooders. Figure 3 explores this relationship in more detail. Figure 3A shows the current state of the phylogeny of Syngnathidae. Only 17 genera are currently represented in the DNA-based phylogeny. Nevertheless, the phylogeny suggests reciprocal monophyly of the trunk- and tail-brooding clades. The phylogeny also shows very strong support for the monophyly of all represented genera, but the exact relationships among genera within each of the two major syngnathid lineages are poorly supported for the most part (Wilson et al. 2003). The sticklebacks are a close outgroup to the family Syngnathidae. Figure 3B shows a scatter plot of average number of tail rings as a function of the number of trunk rings for the 51 syngnathid genera in our sample. We also plot for comparison the approximate number of body and tail vertebrae in the threespine stickleback (*Gasterosteus aculeatus*). An analysis of covariance shows that for a given number of trunk rings, tail brooders

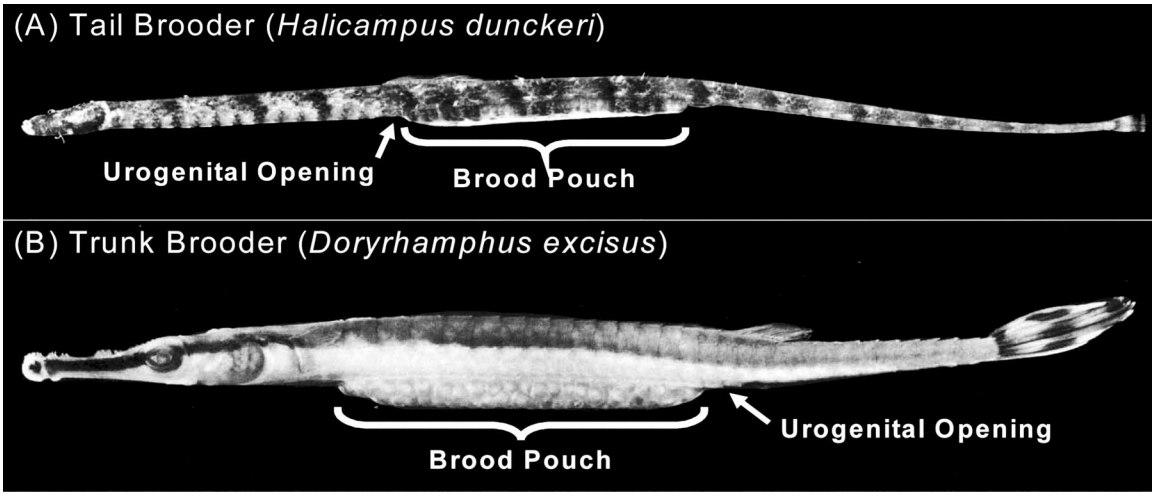

FIG. 2. A typical tail-brooding species (A) and a typical trunk-brooding species (B) of pipefish. The tail brooder has the brood pouch located posteriorly to the anus, whereas the trunk-brooding species has a brood pouch anterior to the anus. These images were reproduced from Dawson (1985) with permission from the Gulf Coast Research Laboratory.

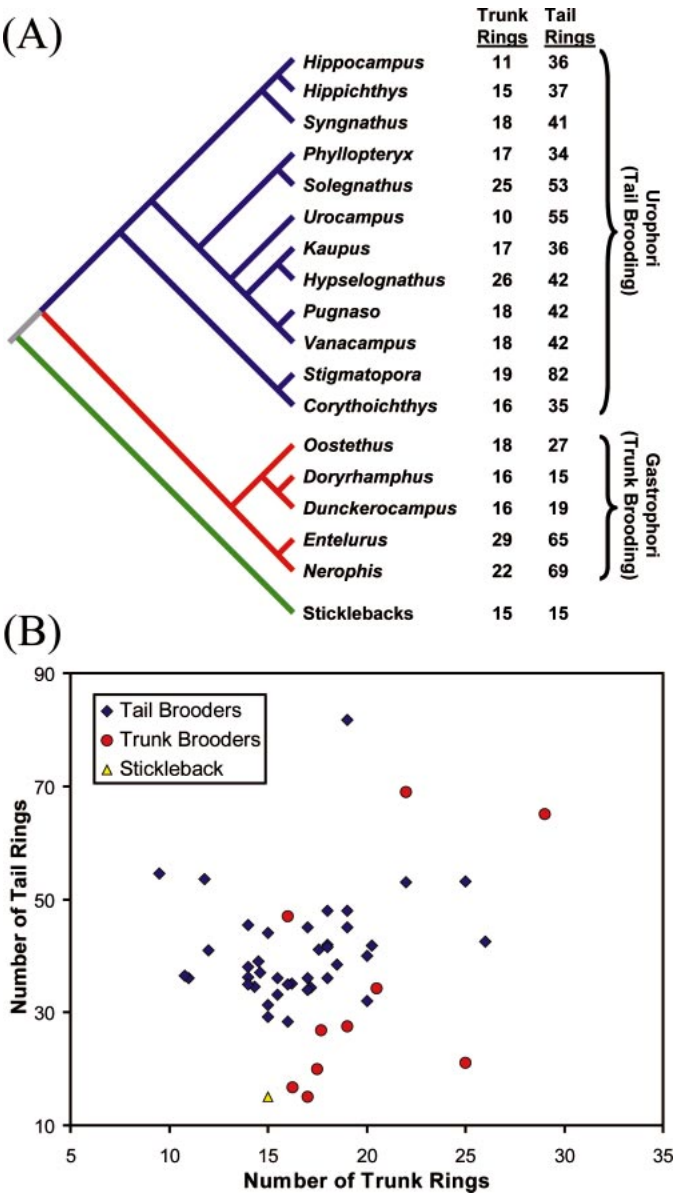

FIG. 3. (A) The current molecular phylogeny for the family Syngnathidae, redrawn from Wilson et al. (2003). Tail-brooding lineages (Urophori) are shown in black (blue online) and trunk-brooding lineages (Gastrophori) in dark gray (red online). We also show the mean numbers of trunk and tail rings among species in each genus. (B) A scatter plot of mean numbers of tail rings as a function of trunk rings for 51 syngnathid genera. Diamonds represent tail-brooding genera, circles represent trunk-brooding genera, and the triangle shows approximate vertebral counts in the threespine stickleback. For a given number of trunk rings, tail brooders have significantly more tail rings than do trunk brooders (ANCOVA, $N = 51$, $P = 0.004$). Note that Dawson (1985) considered *Oostethus* part of *Microphis* and *Dunckerocampus* part of *Doryrhamphus*. We present the mean for all of *Microphis* next to *Oostethus* on the phylogeny and combine *Dunckerocampus* and *Doryrhamphus* into one point on the graph (B). Figure appears in color in the online version.

have significantly more tail rings on average than do trunk brooders ($N = 51$, $P = 0.004$). There are a few notable exceptions to this pattern, the most striking of which are a few trunk-brooding taxa that have unexpectedly high numbers of tail rings given their number of trunk rings (Fig. 3B). Interestingly, these three outlying genera, *Nerophis*, *Entelurus*, and *Syngnathoides*, all have prehensile tails, which may provide an additional selective pressure for long tails.

DISCUSSION

Our investigation of trunk and tail ring counts as models for the study of complex traits in the family Syngnathidae results in several interesting insights. First, our within-population quantitative genetic analysis in the Gulf pipefish shows that variation in numbers of trunk rings and numbers of tail rings has a significant additive genetic component. The heritabilities were quite high, with values of 0.75 and 0.46, respectively, indicating that these traits are capable of a response to selection. Our second observation is that we found no evidence for either a genetic or a phenotypic correlation between number of trunk rings and number of tail rings. Third, we found that trunk-brooding species display fewer tail rings for a given number of trunk rings than do tail-brooding species. One interpretation of this pattern is that the position of the embryos on pregnant males provides a selective pressure on the body plan of syngnathid fishes. We might expect selection to favor males with longer tails in the tail brooders, because a longer tail can accommodate a larger brood pouch capable of holding more offspring. Trunk brooders, on the other hand, would experience selection for longer trunks without any obvious selection pressure on tail length. Overall, these results indicate that numbers of trunk and tail rings in the family Syngnathidae are quantitative traits with significant heritable variation and that they have experienced a potentially interesting history of selection.

Our results are also interesting in light of the developmental basis of axial skeleton evolution in vertebrates. The patterns of variation in pipefish segmentation probably involve two fundamental developmental processes, namely somitogenesis and patterning. The process of somitogenesis determines the number of body segments, whereas patterning specifies the positional identities of the segments. Numerous genes involved in somitogenesis have been identified in vertebrate model systems, including mice, chickens and zebra fish (Aulehla and Herrmann 2004; Dubrulle and Pourquié 2004). The number of segments in an organism is determined by the interplay of a segmentation clock and a gradient of signaling molecules. The relationship between the clock and gradient is too complex to describe in detail here (Aulehla and Herrmann 2004), but our current knowledge of somitogenesis provides numerous candidate genes that could be related to intra- or interpopulation variation in vertebral counts in pipefish. For example, genes in the Notch signaling pathway, including *lunatic fringe*, *her1*, and *her7*, among others, are related to the clock, whereas *fgf8*, *Wnt3a*, and associated genes seem to be responsible for the gradient (Dubrulle and Pourquié 2004). The increase in tail vertebrae in the tail-brooding pipefishes relative to a stickleback-like ancestor could be explained by a simple increase in the time period over which somitogenesis occurs during ontogeny.

However, the difference in body proportions between tail brooders and trunk brooders almost certainly also involves changes in patterning. In fish, *Hox* genes are the most im-

portant loci responsible for specifying positional identity (Hunt and Krumlauf 1992), so changes in *Hox* gene expression patterns are probably related to some of the differences in morphology among species of syngnathid fishes. Significant work has already been done on axial evolution and *Hox* gene expression in the threespine stickleback (Ahn and Gibson 1999a,b,c), setting the stage for additional comparative work in syngnathids. Furthermore, a QTL mapping approach has been used successfully in sticklebacks to identify genes involved in major morphological evolution among populations (Shapiro et al. 2004; Colosimo et al. 2005). The stickleback results provide a huge step toward a synthesis of traditional quantitative genetics and evolutionary developmental biology and suggest that similar approaches could be fruitful in other vertebrates such as syngnathid fishes.

Our results can be considered profitably in light of other models for quantitative trait evolution. In many ways, the trunk and tail ring traits in the family Syngnathidae mirror the classic scalation traits in garter snakes (Arnold and Phillips 1999). Like the trunk and tail rings in syngnathids, ventral and subcaudal scale counts in thamnophiine snakes directly correspond to vertebral counts in the body and tail, respectively. In addition, in both snakes and pipefish the collection of a gravid female or pregnant male yields an entire family of related individuals. Because vertebral counts are specified early in development (McDowall 2003), the traits can be scored at birth, a feature that facilitates the rapid collection of quantitative genetic data relevant to natural populations. This feature minimizes potential effects of the laboratory environment on phenotypic variation, because most individuals are returned to the laboratory after their trait values have already been determined.

The patterns of evolution that have been observed in garter snakes provide some perspective for our results. In *Thamnophis elegans*, both ventral and subcaudal counts exhibit positive additive genetic variance, with heritabilities of 0.41 to 0.54 for ventral counts and 0.45 to 0.66 for subcaudal counts (Arnold 1988; Arnold and Phillips 1999). These values are similar to those we observed for trunk and tail ring counts in the Gulf pipefish. The most interesting difference between the two groups is that ventral and subcaudal scale counts in garter snakes show a positive genetic correlation (Arnold and Phillips 1999), whereas trunk and tail ring numbers in the Gulf pipefish appear not to be genetically correlated. In both groups, the standard error for the genetic covariance is large. In our case, for example, the 95 percent confidence interval for the genetic correlation ranges from −0.66 to 0.68. However, the point estimate of the phenotypic correlation is also near zero with a considerably narrower 95 percent confidence interval (−0.23 to 0.16), suggesting that the genetic correlation actually is quite close to zero.

The genetic correlation between numbers of ventral and subcaudal vertebrae in garter snakes appears to be related to patterns of evolution between populations and species, as evolution generally has proceeded along genetic lines of least resistance (Schluter 1996) with respect to these characters (Arnold and Phillips 1999). This pattern is probably partially due to selection for proportional snakes, because snakes with relatively large numbers of body vertebrae show higher crawling speeds if they also have relatively large rather than relatively small numbers of tail vertebrae (Arnold and Bennett 1984). If we assume that our population of Gulf pipefish is representative of the syngnathids in general, then there are no genetic lines of least resistance in this group with respect to trunk and tail ring counts. Any direction of evolution is equally unconstrained from a quantitative genetic standpoint (Lande 1979). It is possible that this lack of constraint is related to the major evolutionary shift in body proportions that has occurred between the tail- and trunk-brooding groups in this family. This topic would be a fruitful area for future research.

One other interesting aspect of trunk and tail ring counts in fish of the family Syngnathidae is that surveys of phenotypic distributions among populations within species have revealed substantial variation in these traits among geographic locales (Dawson 1982). For example, the mean numbers of trunk rings and tail rings in our population of Gulf pipefishes were 15.4 and 31.2, respectively. However, Gulf pipefish are distributed in coastal waters from the Atlantic Coast of Florida, through the Gulf of Mexico, and along the east coast of Central and South America to Brazil (Dawson 1982). Populations in distinct locales sometimes have different distributions of trunk and tail ring counts. For example, the mean numbers of trunk and tail rings in populations from Brazil are 16.0 and 32.9, respectively. The absolute differences in means among populations, though small, are often statistically significant due to the low phenotypic variance within populations (see data in Dawson 1982). Given our observation that these traits are heritable, they provide a good opportunity to study the microevolutionary processes that contribute to the maintenance of within- and among-population genetic variation in complex traits. This interpopulation variation provides an opportunity for QTL mapping of genes involved in the evolution of morphological traits, such as body segmentation, fin ray counts (Dawson 1982), and other unexplored traits that vary among populations. Pipefish can be raised in the laboratory and have been amenable to microsatellite-based studies (Jones and Avise 1997), so it appears that QTL mapping would be a feasible enterprise.

There are several potential complexities that should be kept in mind in the interpretation of our data. First, our experimental design did not explicitly address maternal or paternal effects (Lynch and Walsh 1998). In pipefish, an added complication relative to most vertebrates is that paternal as well as maternal effects could be at work, because the males carry the embryos for an extended period in a brood pouch until they are born as miniature versions of the adults. Since we used a father-offspring design to estimate our quantitative genetic parameters, the paternal effects are the most important concern for the present study. However, it seems unlikely that maternal or paternal effects would play a major role in traits like vertebral counts, since it is hard to imagine a plausible scenario under which a male or a female with a large number of vertebrae, for example, would be more likely to produce a brood pouch or eggs predisposed to produce offspring with a large number of vertebrae. Indeed, maternal effects have not been a major issue in the studies of scalation traits in snakes (but see King et al. 2001). However, without careful experiments, the possible occurrence of maternal and paternal effects in pipefish should not be entirely discounted.

If paternal effects were important in this system, then they would artificially inflate our estimates of heritability, but would be interesting in their own right. A second phenomenon that could influence our estimates of heritability is multiple mating, which would result in underestimates (Arnold and Phillips 1999; King et al. 2001). However, in the two populations of Gulf pipefish in which parentage has been studied, one of which is the focal population of the present study, only one of 61 males (1.6 percent) carried a brood with more than one mother (Jones and Avise 1997; Jones et al. 2001).

Another potential intricacy is that vertebral counts in fishes have been documented to be sensitive to environmental parameters, the two most important of which are temperature and salinity (McDowall 2003). Given that our study involved pregnant males, most of which were collected after the offspring had probably finished somitogenesis, the environmental variance in our study should approximate the environmental variance in the field. In future comparisons among populations, however, it will be necessary to determine the effects of these environmental parameters on population means and variances, and the most direct way would be to perform common-garden experiments in the laboratory. However, experiments in sticklebacks (Ahn and Gibson 1999a) show that neither temperature nor salinity contributes meaningfully to phenotypic variation in vertebral counts, suggesting that these factors may not be particularly important in pipefishes either. Laboratory-based studies, similar to those of Ahn and Gibson (1999a), of the quantitative genetic architecture of these traits would definitively resolve this issue. Common-garden experiments involving fish from geographically disparate and morphologically distinct populations would also contribute to the resolution of the role of environmental variation in the ontogeny of these traits.

The final important consideration is that the comparative method cannot be rigorously used to test statistically whether selection has played a role in the evolution of differing body plans between the trunk-brooding and tail-brooding groups of pipefish. Because the two groups appear to be reciprocally monophyletic given our current understanding of their phylogeny, trunk brooding and tail brooding each evolved only once. Without convergent evolution, we cannot apply the method of independent contrasts (Felsenstein 1985). The incomplete representation of taxa in the phylogeny also contributes to this problem. Consequently, two viable explanations remain consistent with our data. One possibility is that selection favors males with longer tails (i.e., more tail vertebrae) in the tail-brooding species, and a correlated response to selection also results in the evolution of larger numbers of tail rings in females. In contrast, no such selective pressure is at work in the trunk-brooding species; thus, for a given number of body vertebrae, trunk-brooding species have fewer tail vertebrae than do tail-brooding species. The other possibility is simply that the tail-brooding species evolved from a common ancestor that had longer tails than the ancestor from which trunk-brooding species evolved, and the current patterns of trunk and tail rings among syngnathid taxa represent retention of the symplesiomorphic condition in each lineage. Additional detailed studies of patterns of contemporary selection within species will probably be needed to distinguish between these two hypotheses.

In summary, this initial investigation of the quantitative genetics of vertebral counts in the family Syngnathidae shows that this group has experienced an interesting history of evolution with respect to these complex traits. In the present study, we show that trunk and tail ring counts in the Gulf pipefish exhibit heritable variation. These traits possess similarities and differences with respect to scalation traits in snakes, so they should provide an excellent opportunity for comparative analysis. The similarities are that vertebral counts in both snakes and pipefish are heritable and exhibit among-population variation. The major difference is that number of body vertebrae is positively genetically correlated with number of tail vertebrae in snakes, but apparently not in pipefish. These differences may be related to the different patterns of evolution among taxa in these different groups, although the causal relationships are not yet clear. One other interesting pattern from the present study is that in syngnathids the tail-brooding taxa have more tail vertebrae than the trunk-brooding taxa, suggesting that selection may have played a role in the evolution of vertebral counts. Overall, these results indicate that syngnathid fishes can serve as a valuable new model system for the study of complex trait evolution in natural populations. Furthermore, the fact that vertebral counts are variable and heritable in pipefish and snakes suggests that these traits may serve as valuable tools for the comparison of the quantitative genetics of homologous complex traits across a wide range of vertebrate taxa.

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