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Authors

Jones, AG
Avisé, JC

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Mating Systems and Sexual Selection in Male-Pregnant Pipefishes and Seahorses: Insights from Microsatellite-Based Studies of Maternity

A. G. Jones and J. C. Avise

In pipefishes and seahorses (family Syngnathidae), the males provide all postzygotic care of offspring by brooding embryos on their ventral surfaces. In some species, this phenomenon of male “pregnancy” results in a reversal of the usual direction of sexual selection, such that females compete more than males for access to mates, and secondary sexual characteristics evolve in females. Thus the syngnathids can provide critical tests of theories related to the evolution of sex differences and sexual selection. Microsatellite-based studies of the genetic mating systems of several species of pipefishes and seahorses have provided insights into important aspects of the natural history and evolution of these fishes. First, males of species with completely enclosed pouches have complete confidence of paternity, as might be predicted from parental investment theory for species in which males invest so heavily in offspring. Second, a wide range of genetic mating systems have been documented in nature, including genetic monogamy in a seahorse, polygynandry in two species of pipefish, and polyandry in a third pipefish species. The genetic mating systems appear to be causally related to the intensity of sexual selection, with secondary sex characters evolving most often in females of the more polyandrous species. Third, genetic studies of captive-breeding pipefish suggest that the sexual selection gradient (or Bateman gradient) may be a substantially better method for characterizing the mating system than previously available techniques. Finally, these genetic studies of syngnathid mating systems have led to some general insights into the occurrence of clustered mutations at microsatellite loci, the utility of linked loci in studies of parentage, and the use of parentage data for direct estimation of adult population size.

In various classes of animals a few exceptional cases occur, in which the female instead of the male has acquired well pronounced secondary sexual characters, such as brighter colors, greater size, strength, or pugnacity.

Charles Darwin, 1871

One major theme in evolutionary biology has been the attempt to identify ultimate factors responsible for the differences between males and females (Andersson 1994; Darwin 1871). Many of the obvious external differences between the sexes result from the operation of sexual selection or from adaptations related to parental responsibilities. Fortunately, evolution has provided certain exceptional groups of taxa that, by virtue of unusual biological features, can facilitate comprehension of more orthodox conditions. With respect to the evolution of the sexes, species exhibiting “sex-role reversal” are especially important because they allow critical tests of theories that were derived primarily to explain patterns of variation in species

with typical sex roles (Williams 1966, 1975).

Male parental care is fairly common in the animal world, but reversals of the direction of sexual selection are not. For example, males of many bird species participate in the rearing of offspring (Lack 1968), and, in fishes with parental care, the male is more often the caregiver than the female (Breder and Rosen 1966). In most species with male parental care, males must nevertheless compete for access to mates, and males (more so than females) tend to be modified by sexual selection (Andersson 1994). These species are of interest with respect to parental investment theory, which predicts that males should apportion their investment in progeny as a function of their certainty of paternity (Clutton-Brock 1991). However, species of special interest to sexual selection theory are those in which females compete more intensely for access to mates and, conse-

From the Zoology Department, 3029 Cordley Hall, Oregon State University, Corvallis, OR 97331 (Jones), and Genetics Department, University of Georgia, Athens, Georgia (Avise). We thank A. Berglund, C. Kvarnemo, G. Moore, G. Rosenqvist, and L. Simmons for excellent collaborative interactions. A. Berglund, A. DeWoody, A. Fiumera, C. Kvarnemo, B. McCoy, D. Pearce, B. Porter, and D. Walker provided valuable comments on the manuscript. AGJ's work in the Avise lab was supported by the National Science Foundation, the Pew Foundation, funds from the University of Georgia, and by an NIH training grant. Address correspondence to Adam G. Jones at the address above or e-mail: jonesa@bcc.orst.edu. This paper was delivered at a symposium entitled “DNA-Based Profiling of Mating Systems and Reproductive Behaviors in Poikilothermic Vertebrates” sponsored by the American Genetic Association at Yale University, New Haven, CT, USA, June 17–20, 2000.

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Table 1. Definitions of genetic mating systems (following Searcy and Yasukawa 1995)

Term	Number of successful mates per mating male	Number of successful mates per mating female	Gender experiencing more intense sexual selection
Polygyny	Many	One	Males
Polyandry	One	Many	Females
Monogamy	One	One	Neither
Polygynandry	Many	Many	Either or neither

quently, the females are most modified by sexual selection.

Species that are sex role reversed (with respect to sexual selection intensity) have been described from only a handful of evolutionary lineages, including some insects (Gwynne 1991; Smith 1980), fishes (Berglund et al. 1986a,b; Vincent 1992), and birds (Jehl and Murray 1986; Oring and Lank 1986). Additional role-reversed taxa no doubt will be discovered, but for now, three groups stand out as most promising for continued study of sexual selection. In katydids (family Tettigonidae), the male invests in progeny by transferring an edible spermatophylax to the female during copulation (Brown and Gwynne 1997). Populations and species vary with respect to the amount of male investment, and in some situations females compete more intensely than males for access to mates (Gwynne and Simmons 1990). Shorebirds (Charadriiformes) are a second important group that includes sex-role-reversed taxa, and these too have had a large impact on the development of sexual selection theory. The best known of sex-role-reversed shorebirds are the spotted sandpiper (*Actitis macularia*), phalaropes (genus *Phalaropus*), and jacanas (family Jacanidae). The shorebirds are of particular interest, because, as in the katydids, they include species with conventional as well as reversed sex roles (Jehl and Murray 1986).

A third promising group, the fish family Syngnathidae with more than 200 species of pipefishes (Dawson 1985) and about 32 species of seahorses (Lourie et al. 1999), is one of the most unusual collections of animals with respect to paternal investment. During copulation the female transfers unfertilized eggs to the male's ventral surface, where fertilization takes place. Unlike other attentive fish fathers which may build nests, fan eggs, or carry developing young in their mouths (Breder and Rosen 1966), pipefish and seahorse males maintain a placenta-like connection that permits the transfer of nutrients from a male to his offspring (Berglund et al. 1986b; Haresign and Shumway 1981). Brood pouches vary considerably among species (Dawson 1985; Herald 1959). In

some species, eggs are glued to the male's ventral surface without any outer covering, whereas in others, such as the seahorse, the male possesses an elaborate pouch with a small opening through which eggs are deposited. Presumably the extent of energetic investment by a male in his offspring also varies among species (Berglund et al. 1986b), an important consideration for sexual selection theory (Trivers 1972).

Syngnathid lineages therefore provide excellent opportunities for comparative analysis. Numerous field and laboratory studies have shown that two pipefish species, *Syngnathus typhle* and *Nerophis ophidion*, are sex role reversed, with females competing more intensely for mates than males (Berglund et al. 1986a, 1989). Sexual selection appears to be especially intense in *N. ophidion*, and females possess secondary sexual characters that are important in male choice (Rosenqvist 1990). Females with intense blue coloration on the head and flank and with appearance-enlarging skin folds are preferred by males, demonstrating that these traits probably arose through sexual selection (Rosenqvist 1990). Similar sexually dimorphic traits can be seen in females of many other (thus far unstudied) pipefish species (Dawson 1985), suggesting that numerous sex-role-reversed pipefish species exist. In addition, other species in Syngnathidae appear not to be sex role reversed. In seahorses, for example, males appear to be the predominant competitors for mates (Vincent et al. 1992). Thus comparisons among syngnathid lineages, particularly in light of a molecular phylogeny, should provide insight into factors affecting sexual selection and sex role reversal.

In this article we review microsatellite data pertaining to the comparative analysis of sexual selection in pipefishes and seahorses. A primary goal of these studies has been to resolve possible relationships between the genetic mating system and sexual selection in sex role reversed taxa, so we begin with a short introduction to these topics. A brief description of genetic parentage analysis follows, and empirical examples are provided of genetic mating

systems in natural populations of four syngnathid species. Do pipefish and seahorse males have complete confidence of paternity, as might be expected given their involved parental care? Do genetic mating systems appear to be linked to the evolution of sexual dimorphism? Can improved quantitative methods be used to compare genetic mating systems? After answering these and related questions, we also summarize some unexpected genetic results from syngnathids that are relevant to molecular studies of parentage in general.

Sexual Selection and the Genetic Mating System

The same processes that cause a reversal in the direction of sexual selection across taxa probably also function on a smaller scale to affect the intensity of sexual selection within and among species with typical sex roles. Thus an enhanced understanding of the causes of sexual selection in sex-role-reversed taxa should shed light on the principles that govern this important evolutionary mechanism in all species.

A topic of special interest is the relationship between the mating system (definitions in Table 1) and the strength of sexual selection. Here we are concerned with the genetic mating system rather than the social mating system (Andersson 1994; Emlen and Oring 1977; Searcy and Yasukawa 1995). These two views of the mating system must be connected to some degree, of course, but whereas the social mating system is concerned with pair bonds and other observable interactions among individuals, the genetic mating system is concerned with the allocation of biological parentage. In principle, a complete description of the genetic mating system for a particular breeding assemblage would provide data on the number of mates and the number of offspring produced by each reproductively active adult during a well-defined breeding interval.

The genetic mating system is undoubtedly related to the operation of sexual selection and, indeed, should be seen as an integral component of the process of sexual selection. Darwin (1871) originally defined sexual selection as differential reproductive success of individuals caused by competition over mates (Andersson 1994). Hence the variance in fitness due to sexual selection comes mainly from differential mating success (although other factors such as mate quality also may contribute; Andersson 1994). A description of the ge-

Table 2. Comparison of microsatellite polymorphism in syngnathid species

Species	Loci used	Number of alleles per locus	Range of observed heterozygosities	Four-locus exclusion probability ^a
<i>S. scovelli</i>	<i>micro11.1, micro22.3, micro25.10, micro25.22</i>	19–29	0.88–0.95	.999
<i>S. floridae</i>	<i>micro11.1, micro22.3, micro25.10, micro25.22</i>	18–44	0.78–0.95	.999
<i>S. typhle</i>	<i>typh04, typh16, typh18, typh12</i>	15–39	0.55–0.95	.999
<i>H. angustus</i>	<i>Han03, Han05, Han06, Han15</i>	9–22	0.73–0.95	.997

Pipefish and seahorse primer sequences are available in Jones et al. (1998, 1999b).

^aThe expected proportion of individuals in a population that would be rejected as possible parents of a typical offspring, given that one of the offspring's parents is known.

netic mating system includes data regarding both mating and reproductive success, and therefore describes the majority of the variance in fitness resulting from sexual selection (Arnold 1983). Given the importance of the mating system to sexual selection, a more precise understanding of mating systems should be informative with regard to sexual selection (Arnold and Duvall 1994). The above argument applies only to the term genetic mating system as it is defined here. Broader uses of the term mating system typically do not possess so clear a relationship to sexual selection (Andersson 1994; Emlen and Oring 1977).

For species with typical gender roles, Darwin (1871) observed that highly polygynous species tend to be more sexually dimorphic than less polygynous ones, with secondary sexual characters evolving in males (Table 1). Numerous attempts to quantify this relationship have yielded mixed results (e.g., Björklund 1990; Clutton-Brock et al. 1977, 1980; Höglund 1989; Oakes 1992; Payne 1984), perhaps in part because, until recently, molecular markers were not available to assess genetic mating systems reliably. However, even now that scores of studies have quantified rates of extrapair fertilization (notably in birds), the data have yet to be integrated in ways that definitively address relationships between the genetic mating system and the strength of sexual selection.

While these issues are being sorted out in species with typical sex roles, we can also examine the predictions for species with reversed sex roles. In such organisms, stronger sexual selection is expected in species that are more polyandrous, with secondary sexual characters evolving in females (Jehl and Murray 1986). Thus the expectations regarding the strength of sexual selection for polygyny, polyandry, and monogamy are fairly simple (Table 1). Polygynandry is more problematic because

sexual selection under this mating system may affect either sex or may be nonexistent, depending on the relative distributions of mating success for each gender. Sexual selection in polygynandrous mating systems may occur, but in general is expected to be weaker than in strictly polygynous or polyandrous populations (because polygynandry will tend to reduce the ability of successful individuals to monopolize access to mates).

These simple hypotheses have motivated our genetic studies of mating systems in pipefishes and seahorses. Various species can be ranked a priori with respect to level of sexual dimorphism. Then the hypothesis to be tested is whether differences among species in sexual dimorphism are related to the underlying genetic mating systems, perhaps with the most dimorphic species also being the most polyandrous (Table 1).

Microsatellites and Mating Measures

Microsatellite markers (Goldstein and Schlötterer 1999; Hughes 1998; Tautz 1989) are particularly suited to studies of genetic parentage because they are highly polymorphic, codominant, single locus, and PCR based. Thus individuals in a population often exhibit unique DNA profiles, parental genotypes can be reconstructed from progeny array data, and the markers can be assayed from small tissue sources such as individual embryos.

One drawback of microsatellites is that the primers often are species or genus specific, and we have had to generate primers independently on several occasions for the three species of pipefish (genus *Syngnathus*) and one seahorse (*Hippocampus subelongatus*) assayed to date (Table 2). High levels of microsatellite polymorphism were observed for all species, with 9–44 alleles per locus and ob-

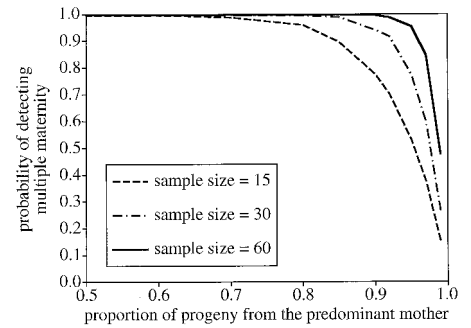


Figure 1. Results of computer simulations investigating the power of two seahorse microsatellite loci (*Han03* and *Han05*) to detect multiple maternity within a male's pouch (after Jones et al. 1998). Each simulated brood contained 458 embryos that were the progeny of two mothers. Each mother was assigned a genotype based on Hardy-Weinberg expectations, and the first mother contributed from 50 to 99% of the male's eggs. Samples of 15, 30, or 60 embryos were drawn at random from the brood and if three or more maternal alleles appeared in the progeny at either locus, then multiple maternity was deemed to have been detected. Almost all failures to detect multiple mating by males were because embryos from one of the females went unsampled rather than because of insufficient variation at the marker loci.

served heterozygosities ranging from 0.55–0.95 (Table 2). The power of these marker systems for parentage assessment can be gauged by the four-locus exclusion probabilities (Chakraborty et al. 1988), which always were greater than 0.997 (Table 2).

Available evidence suggests that most or all individuals even in large syngnathid populations have unique multilocus genotypes. For example, calculated expected frequencies of observed four-locus genotypes across all species ranged from 3.3×10^{-7} to 1.8×10^{-11} . We never observed two individuals that shared even a two-locus genotype in population samples of adult *S. scovelli* ($n = 79$), *S. floridae* ($n = 65$), or *H. angustus* ($n = 41$). Three pairs of individuals with identical two-locus genotypes were observed in a sample of 293 adult *S. typhle* (42,778 pairwise comparisons), but no two individuals were observed to share a three-locus genotype. These considerations indicate that the microsatellite markers were more than adequate for the parentage assessments. In fact, simulations by Jones et al. (1998) show that two seahorse microsatellite loci are sufficient to detect essentially all instances of multiple mating. With this level of genetic variation, multiple maternity is difficult to detect only when reproductive skew between two mates is high and the number of offspring sampled from a brood is low (Figure 1), but even in this case the failing is not in the markers but in the sampling strategy (Jones et al. 1998).

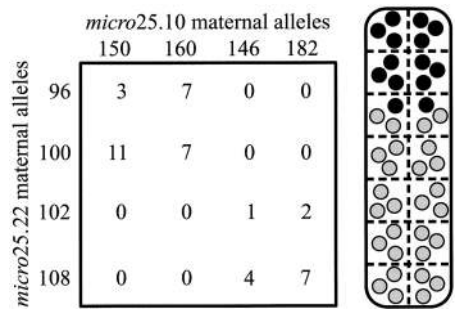


Figure 2. An example from *S. floridae* showing the logic behind reconstruction of maternal genotypes using two microsatellite loci. By examining a pregnant male and the embryos in his brood pouch, maternal alleles in each embryo are deduced by subtraction. Then a dilocus table is constructed, each cell of which reports the number of progeny with a particular combination of alleles at the two loci. The maternal dilocus genotype is evident from these nonrandom allelic associations. In this case, one mother had the genotype 150/160 at *micro25.10* and 96/100 at *micro25.22*, while the other mother's genotype was 146/182, 102/108. The diagram at right shows a spatial map of these maternal alleles in the male's brood pouch. In this case, the pouch was divided into 14 sections, with three embryos sampled from each. Embryos of the same pattern are full sibs and those of different patterns are half sibs (had different mothers).

Beyond the mere detection of multiple mating, the particular genotypes of parents contributing to a brood also can be reconstructed. Pipefish and seahorses are convenient in this regard because a pregnant male carries an entire brood that may include hundreds of offspring. Once the male is established as the true genetic sire of all progeny within his pouch (see below), the maternal contribution to each embryo can be deduced by subtraction. Then, with the maternal alleles known for each embryo, a table can be constructed that reveals the unique dilocus genotype of each mother. An example from *S. floridae* is shown in Figure 2. In this case, the male evidently mated with two females. Positions of the embryos were mapped during dissection of the brood pouch, and the reconstituted maternal genotypes were projected onto this map. This example reveals another convenient feature of *Syngnathus* brooding: In each of 43 multi-mated pipefish males genetically assayed, embryos invariably proved to be clumped by maternity within the brood pouch, a finding that lends additional support to the validity of the reconstructed maternal genotypes.

Deduced maternal genotypes also can be used to document instances of multiple mating by females, as well as to match particular collected specimens of adult females to their mates. If the genotype of an assayed female is the same as the inferred genotype from a male's brood, then given

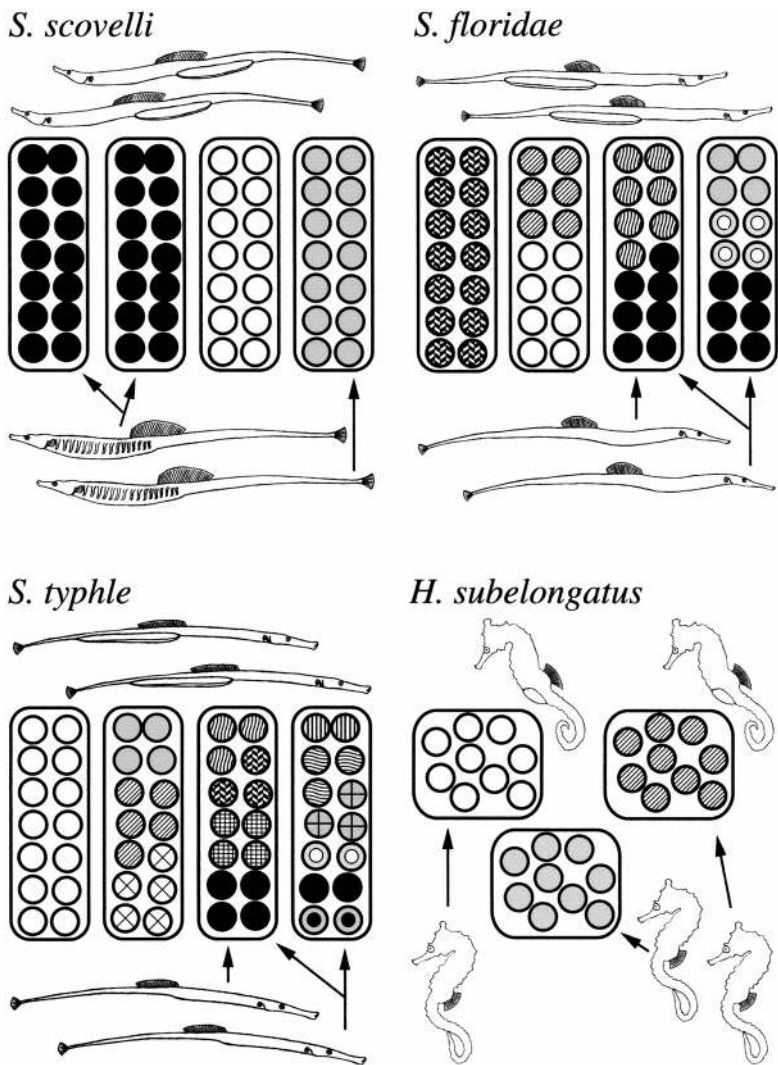


Figure 3. Diagrammatic representation of the patterns of parentage observed during microsatellite-based parentage studies of four syngnathid species. Males are shown above the brood pouch diagrams, females below. Notice the brood pouches on the ventral surfaces of depicted males. Also shown are spatial maps of full- and half-sib embryos (as in Figure 2) within males' pouches. These diagrams are intended to present a comparative overview of the patterns of mating in these species. Therefore they are not drawn to scale and only portray a small subset of the assayed progeny per pouch. Arrows indicate patterns of egg depositions (matings) by females. In *S. scovelli*, *S. floridae*, and *S. typhle*, females mate with multiple males, but not so in *H. subelongatus*. Males of *S. floridae* and *S. typhle* often receive eggs from multiple females, whereas *S. scovelli* and *H. subelongatus* males typically do not. As described in the text, available data indicate a positive relationship between polyandry and the evolution of sexual dimorphism in these taxa.

the high levels of polymorphism at the microsatellite loci (see above), that particular female most likely mothered the brood. Similarly if the same multilocus maternal genotype appeared in the progeny of more than one male, then it is highly likely that the identified female had mated with multiple males. In this way, multiple mating by females can be deduced solely from the contents of male brood pouches—the adult females need not be available for assay.

Parental Investment and Pipefish Paternity

Pipefish in the genus *Syngnathus* possess a pouch with a ventral seam that, when

closed, fully covers all embryos (see Figure 3). During courtship in *S. typhle*, receptive males present an opening at the anterior end of the pouch through which females deposit unfertilized eggs. Males release sperm directly into the pouch and then shake the zygotes posteriorly to make room for additional eggs. The ventral seam opens only when the male gives birth several weeks later. Seahorse males possess a pouch that is fully enclosed except for a small opening through which females deposit eggs, and the sequence of egg transfer resembles that of pipefish. Thus in these species it is difficult to imagine how rival males might steal any fertilizations.

Indeed, every pregnant syngnathid male whose brood has been genetically assayed to date has proved to be the true genetic father of all embryos within his pouch. Thus males with enclosed pouches appear to have complete confidence of paternity, a result consistent with expectations based on pouch configuration and parental investment theory, which predicts that a male's devotion to offspring should be positively related to paternity confidence (Clutton-Brock 1991). However, not all species of pipefish have enclosed pouches. In *Nerophis ophidion*, unfertilized eggs are loosely attached to the male's outer ventral surface where he fertilizes them by floating through a cloud of sperm that he releases into the water (Rosenqvist 1993). Of interest, males of this species invest less energy per zygote than females, whereas in *S. typhle*, a species with complete paternity confidence, males and females invest equally in progeny (Berglund et al. 1986b). These observations raise two interesting questions for future research: Do syngnathid species without enclosed pouches have complete confidence of paternity? And, did the brood pouch evolve as a structure to nurture and protect embryos, or as a barrier to prevent cuckoldry?

Genetic Mating Systems in Natural Populations of Syngnathids

For each syngnathid species genetically surveyed, field collections of pregnant males, their brooded embryos, and a sample of adult females were analyzed for microsatellite genotypes. Here we summarize the results species by species.

The Gulf Pipefish (*S. scovelli*)

This North American coastal species is the most sexually dimorphic of the syngnathids genetically examined thus far. Females are larger than males and possess a deeply keeled abdomen, silvery stripes on the trunk, and an enlarged dorsal fin (Figure 3) that probably are the result of sexual selection (Brown 1972). These characteristics are absent in other *Syngnathus* species, and hence *S. scovelli* appears to be the member of the genus most modified by sexual selection (Brown 1972; Dawson 1985).

Jones and Avise (1997a) collected specimens from one locale on Florida's northern Gulf coast. Despite high statistical power, the molecular appraisal found evidence for multiple maternity in only one brood among the 40 pregnant males as-

Table 3. Relevant reproductive information on three pipefish species (genus *Syngnathus*) and one seahorse species (genus *Hippocampus*) that have been the subject of molecular parentage analyses

	<i>S. scovelli</i>	<i>S. floridae</i>	<i>S. typhle</i>	<i>H. subelongatus</i>
Sexual dimorphism				
Sexual size dimorphism	Female > male	Female > male	Female > male	Female = male
Sexual color dimorphism	Permanent female stripes	Some yellow on female	Transient female ornament	Body color frequencies differ
Sexual shape dimorphism	Female keeled abdomen	Slightly larger female trunk	Slightly larger female trunk	Female tail:trunk ratio slightly less
Results of parentage studies				
Percent males multiply mated	<5	73	90	0
Average number of mates per male	1.0	1.9	3.1	1.0
Multiple mating by females	Common	Common	Common	None
Reproductive ecology				
Typical male brood size range	30–120	250–700	25–130	200–700
Egg maturation	Continuous	Continuous	Continuous	Batch
Length of breeding season	Year-round	Year-round	~3 months	~4 months
Site fidelity	No data	No data	None	Yes

sayed (Table 3, Figure 3). On the other hand, the genetic data documented multiple mating by females (Table 3, Figure 3). On two separate occasions, a single deduced female was the mother of embryos in more than one male's pouch. Thus the genetic data showed that females are able to split a batch of eggs among multiple males, and that multiple mating by females was common in this population. The logic behind the latter statement stems from the fact that only a small fraction of the breeding population was sampled from an extensive sea grass meadow. Thus females probably deposited eggs in the pouches of males not sampled, as well as in the males documented in our sample. Given the low power to detect multiple mating by a particular female, the fact that we detected the phenomenon at all suggests that multiple mating by females was common. Overall the Gulf pipefish mating system is best described as polyandrous, with a male typically receiving eggs from only one female, and a female often mating with multiple males during the time of male pregnancy.

The Dusky Pipefish (*S. floridae*)

This western Atlantic species also is sexually dimorphic, but to a much lesser extent than the Gulf pipefish (Brown 1972). Mature females are larger than males and possess a slightly deeper and more yellow abdomen, probably due to the presence of ripe ova (Brown 1972). This species does not possess obvious secondary sexual characters.

In a genetic analysis of brooded embry-

os in pregnant dusky pipefish (Jones and Avise 1997b), males proved to have mated with one to three females each (Table 3, Figure 3). In addition, there was clear evidence for multiple mating by females. Quantitative estimates of the breeding population size and multiple maternity demonstrated that the rate of multiple mating by females of dusky pipefish is probably quite high (see Jones and Avise 1997b). Because both males and females often have multiple successful partners during a single male pregnancy, this species is genetically polygynandrous.

The Broad-Nosed Pipefish (*S. typhle*)

This western European species is sexually dimorphic, but less so than Gulf pipefish. Females are larger than males on average and have slightly deeper abdomens. They also possess a transient ornament that becomes visible during courtship and has been shown to be a target of sexual selection (Berglund et al. 1997). Nevertheless, the sexual dimorphism displayed by *S. typhle* is not nearly as dramatic as that in *S. scovelli*.

A population from Sweden's west coast was the focus of the genetic parentage assessment. Ninety percent of the males carried broods with two to six mothers each, indicating a much higher rate of multiple mating than in previously studied syngnathid species (Jones et al. 1999b). As in the Gulf and dusky pipefishes, multiple mating by females also was documented. These results are consistent with laboratory and field observations suggesting that both males and females frequently

have multiple mates (Berglund et al. 1986a; Vincent et al. 1995). Thus *S. typhle* is polygynandrous.

The Western Australian Seahorse (*Hippocampus subelongatus*)

Motivation for the study of seahorse parentage was twofold. First, seahorses tend to be the least sexually dimorphic of all syngnathids, so the mating system might reflect an apparently weak intensity of sexual selection (Vincent et al. 1992). Second, several seahorse species studied intently from a behavioral standpoint are socially monogamous, with long-term pair bonding (Masonjones and Lewis 1996; Vincent 1994b, 1995; Vincent et al. 1992; Vincent and Sadler 1995).

The species *H. subelongatus*, previously known as *H. angustus*, occurs along Australia's west coast (Lourie et al. 1999), and males and females do not differ in obvious secondary sexual characters (Table 3; unpublished data). Females and males do differ slightly in body proportions and in the frequency of body color morphs (Table 3; Kvarnemo C and Moore GI, unpublished data), but so far no evidence suggests that these differences are related to sexual selection. Jones et al. (1998) used microsatellite markers to provide the first evidence that seahorses are, indeed, genetically monogamous. A population near Perth, Australia, is the subject of ongoing study. In all cases examined ($n = 43$), a pregnant male carried eggs from only one female (Jones et al. 1998; Kvarnemo et al. 2000, unpublished data). Furthermore, in contrast to the previous studies of pipefish mating systems, no evidence was found for multiple mating by females. Thus molecular evidence supports the notion that *H. subelongatus* is strictly monogamous during a single reproductive interval (Figure 3, Table 3).

An additional question motivated by the unusual social system of seahorses was whether males mate with the same female repeatedly within a breeding season. Field and laboratory observations suggest that other species of seahorses do so (Vincent et al. 1992; Vincent 1995). Kvarnemo et al. (2000) addressed this by marking males in the field and sampling their broods repeatedly throughout the breeding season. Of 14 males successfully sampled more than once, the genetic data document that eight remained faithful to their original mates and six changed mates between broods. Males who switched mates moved much greater distances during the breeding season and displayed a somewhat lon-

ger interval (marginally significant) between the production of successive broods. Overall, results indicate that seahorses frequently maintain long-term pair bonds throughout the breeding season, and investigation into the costs associated with mate switching should be a fruitful area for future investigation.

Overview of Syngnathid Mating Systems

The parentage analyses described above can be considered in light of hypotheses relating the genetic mating system to the evolution of sexual dimorphism. The Gulf pipefish is polyandrous and also is the most sexually dimorphic species studied to date. The western Australian seahorse is monogamous with long-term pair bonds, a situation that should reflect weak sexual selection, and this species displays very little sexual dimorphism. The two other *Syngnathus* species are polygynandrous and they exhibit intermediate levels of sexual dimorphism. Thus available data indicate a positive correlation between the strength of sexual selection and the extent of polyandry in sex-role-reversed pipefishes. This comparative result is based on observations of only four species, however, so it must be considered provisional. Nevertheless, these genetic studies provide important baseline information for future comparative analyses of syngnathid mating systems.

Additional support for a connection between sexual selection and the mating system comes from observational studies on other syngnathid species. For example, the seahorses *H. zosterae*, *H. whitei*, and *H. fuscus* all appear to be monogamous and exhibit almost no sexual dimorphism (Masonjones and Lewis 1996; Vincent 1994b, 1995; Vincent et al. 1992; Vincent and Sadler 1995). Two pipefish species, *Hippichthys penicillus* and *Corythoichthys intestinalis*, appear to be monogamous as well (Gronell 1984; Watanabe et al. 1997) and exhibit little or no sexual dimorphism (Dawson 1985; Vincent et al. 1992). Within *Syngnathus*, two other pipefish species (*S. acus* and *S. rostellatus*) appear from field observations to be polygynandrous (Vincent et al. 1995). Neither species displays obvious secondary sexual traits, but *S. rostellatus* is sexually size dimorphic. Finally, the pipefish *N. ophidion* seems to be polyandrous and is extremely sexually dimorphic (Rosenqvist 1990), with females possessing secondary sexual characters (i.e., appearance-enlarging traits and anterior coloration in females) that are qualitative-

ly similar to those in *S. scovelli*. These observations should be interpreted with caution until they can be verified in nature using molecular markers, but current data appear to bolster the conclusion that more polyandrous species have experienced stronger sexual selection on females.

If the genetic mating system is indeed causally related to the evolution of sexual dimorphism, an important goal is to determine the ecological factors and evolutionary constraints that may shape the mating system. Although too little is known about syngnathid ecology to rigorously address causal hypotheses about mating system evolution in this group, some potentially important differences among species are listed in Table 3. For example, *S. scovelli* and *S. floridae* occur in relatively warm waters where breeding can occur year-round, whereas *S. typhle* occurs in northern latitudes where breeding is sharply seasonal, with individual pipefish experiencing many fewer reproductive episodes per lifetime. These conditions might promote a bet-hedging strategy in *S. typhle*, whereby males and females are inclined to seek multiple mates.

In addition, female pipefish in the genus *Syngnathus* mature eggs continuously and appear able to hold them until a mate becomes available. In contrast, a seahorse female prepares a batch of eggs all at once and, if not to be in vain, must deposit them in a male's pouch within a short time (Vincent 1994a). This feature of seahorse reproduction is certainly related to the evolution of monogamy, but whether it is a cause or consequence remains unknown. Additional factors possibly related to the evolution of mating systems in syngnathids include site fidelity (Table 3), male brood size (Table 3), variation in mate quality (Jones et al. 2000b), population density (Clutton-Brock et al. 1997), sex ratio (Emlen and Oring 1977), potential reproductive rates of the sexes (Clutton-Brock and Vincent 1991), predation risk (Fuller and Berglund 1996), resource abundance (Orians 1969), and phylogenetic constraints.

The Bateman Gradient and Sexual Selection

That a relationship between the genetic mating system and the evolution of sexual dimorphism is clear for the syngnathid species studied thus far may be due to the fact that the assayed species covered a

wide spectrum of mating systems, from polyandry to monogamy. Qualitative definitions of the mating system (Table 1) are useful verbal summaries, but they are inadequate for detailed quantitative comparisons. A major shortcoming is that polygynandry, probably the most common mating system in animal populations, is an overly broad category. Expectations for the strength of sexual selection under this system are not always clear, and the borders between polygynandry and other mating systems can be indistinct. Thus a major goal of research on syngnathid mating systems has been to test other methods of mating system quantification with respect to sexual selection.

Arnold and Duvall (1994) present a new theoretical framework that considers mating systems in the context of formal selection theory. This integration points to the relationship between mating success (the number of times an individual mates) and reproductive success (the number of offspring produced) as a central feature of the genetic mating system with respect to sexual selection. In his classic article, A. J. Bateman (1948) perceived the difference in this relationship in males compared to females as the cause of sexual selection. In species with typical sex roles, such as *Drosophila melanogaster*, males exhibit a strong positive relationship between mating success and reproductive success (Bateman 1948), whereas in females this relationship is weaker (Figure 4). Thus a male benefits greatly in terms of offspring number from having multiple mates, whereas a female does not, a situation that should promote stronger sexual selection in males than in females. The relationship between mating success and reproductive success can be quantified (and compared statistically) using a least-squares regression approach (Arnold and Duvall 1994). The resulting line is referred to as the sexual selection gradient (Arnold and Duvall 1994), or Bateman gradient (Andersson and Iwasa 1996).

One simple conceptualization of the sexual selection gradient's position in the sexual selection process is presented in Figure 5. The operational sex ratio, potential reproductive rates of the sexes, and relative parental investment, among other factors, no doubt interact in complex ways to affect the strength of sexual selection (Andersson 1994), and they typically do so by influencing patterns of mating success. In the context of sexual selection, variance in mating success can contribute to variance in fitness only through the sex-

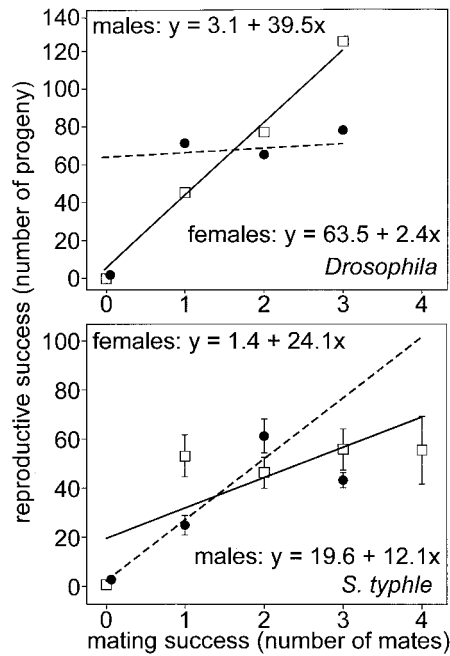


Figure 4. Sexual selection (Bateman) gradients for *Drosophila melanogaster* (top) and *S. typhle* (bottom) (after Jones et al. 2000a). The sexual selection gradient is given by a weighted least squares regression line relating reproductive success to mating success. Each symbol in the graph represents a mean (with one standard error for *S. typhle*). Males are shown as open squares, females as closed circles. In *Drosophila*, males and females exhibit sexual selection gradients that are steep and shallow, respectively, a pattern consistent with strong sexual selection on males. In pipefish, females have a significantly steeper sexual selection gradient than males ($P = .004$), a pattern consistent with a reversal in the intensity of sexual selection.

ual selection gradient (Arnold and Duvall 1994). Thus if the gradient is zero, variance in mating success has no effect on the strength of sexual selection and there can be no sexual selection due to differential mating success. However, if the gradient is steep, very strong sexual selection is possible. Under this view the sexual selection gradient concept does not diminish the importance of other methods related to the measurement of sexual selection, but rather should be seen as a quantitative framework that can be used to sort out the relative importance of the multifarious factors that contribute to sexual selection.

Pipefish provide a unique opportunity to test the validity of the sexual selection gradient concept. In sex-role-reversed species, a steep sexual selection gradient is expected in females and a shallow gradient in males. Jones et al. (2000a) tested this hypothesis in captive breeding populations of *S. typhle*. Microsatellite markers were used to describe parentage completely for small pipefish breeding groups, allowing the sexual selection gradients of

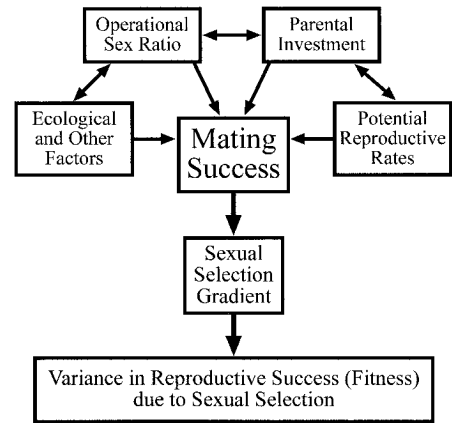


Figure 5. Path diagram showing the vital role of the sexual selection gradient in the sexual selection process. Traditional measures or correlates of sexual selection remain important, but the sexual selection gradient is the final path to fitness for all sexually selected traits (Arnold and Duvall 1994).

the sexes to be quantified. Results of the experiment were consistent with expectations based on the Bateman gradient model: The gradient of females was significantly steeper than that of males (Figure 4). In addition, the slopes of the lines responded as predicted to changes in the operational sex ratio. These results lend additional support to the Bateman gradient concept, suggesting that sexual selection gradients do provide a superior method for the quantification of mating systems with respect to the strength of sexual selection. Thus a goal in future studies of sexual selection in Syngnathidae, as well as in other taxa, should be to quantify Bateman gradients of natural populations in a comparative context.

Additional Insights from Studies of Syngnathid Mating Systems

Microsatellite-based studies of parentage in pipefishes and seahorses have also provided novel perspectives on the evolution of the markers themselves, and on the broader use of molecular parentage analyses in population studies. The following sections will highlight three such applications.

Clustered Microsatellite Mutations in *S. typhle*

In a large study of parentage involving 110 *S. typhle* families and 3195 progeny, Jones et al. (1999a) obtained direct estimates of mutation rates at two highly polymorphic microsatellites. Both loci contained tetranucleotide microsatellite repeats (motif GGAT or GGTT), and mutation rates were between 9.4×10^{-4} and 4.4×10^{-3} per

gamete. The pattern of mutations conformed well to a stepwise mutation model (Shriver et al. 1993), with all mutations resulting from the insertion or deletion of one or a few microsatellite motif units.

Another interesting result of the study was that mutations often occurred in clusters. Combining data from both loci, a total of 35 mutant progeny were assayed, but they apparently were produced by only 26 independent mutation events (Jones et al. 1999a). Thus multiple offspring in a single brood sometimes received the same mutant allele, a pattern indicative of multiple gametes carrying copies of one premeiotic mutation that arose in a parent's germline (Woodruff and Thompson 1992). The phenomenon of clustered mutations may have consequences for the reconstruction of parentage using microsatellite-based markers (Jones et al. 1999a), and also, under some conditions, for population genetics theory (Woodruff et al. 1996; see also Jones et al. 1999a).

Linked loci in the Study of Parentage

While documenting genetic monogamy in seahorses, Jones et al. (1998) encountered two loci that failed to segregate independently within families but displayed no linkage disequilibrium at the population level. This raised the question of whether tightly linked loci can be useful for parentage reconstruction. The authors found that linked loci can be useful if (1) the linkage phase of the parents can be ascertained with certainty, (2) the loci are situated close enough to one another that parental linkage relationships can be obtained reliably from progeny arrays, and (3) the loci are not in severe linkage disequilibrium at the population level. If these three criteria are met, then, due to the additional information gleaned from knowledge of the linkage phase of individuals, two linked loci can actually be more informative than two unlinked loci with similar levels of polymorphism (Jones et al. 1998).

Use of Parentage Assessment in Direct Population Size Estimation

Neutral molecular markers are frequently used to calculate indirect estimates of effective population size (Hartl and Clark 1989). In the context of microsatellite-based studies of parentage, a more direct measure of adult population size can be obtained using a "mark-recapture" framework (Jones and Avise 1997b). The procedure requires a collection from nature of

pregnant adult males, their intact broods, and adult females, and it takes advantage of the fact that microsatellite markers can match particular collected females to particular broods with great precision (see above). The method by which adult population size is estimated can be described by analogy to traditional mark-recapture procedures routinely employed by field ecologists (Pollock et al. 1990).

Under the Lincoln-Peterson method of a conventional mark-recapture experiment, an initial sample of n_1 animals is captured, marked, and released. A second sample of n_2 animals is later caught, and the number of marked animals (m_2) in the sample gives an indication of the population size. In the pipefish parentage study, the initial "marks" were provided by the inferred genotypes of females that contributed to the broods of pregnant males. Thus, in essence, a female marked herself by mating with one of our sampled males (Jones and Avise 1997b). The genetically assayed adult females were considered the second sample, and those females that matched particular broods were counted as "recaptures" of the marked individuals. Then an estimate of the population size is obtained by $\hat{n} = (n_1 + 1)(n_2 + 1)/(m_2 + 1) - 1$ (Pollock et al. 1990). Application of this methodology to the dusky pipefish population led to a surprisingly small population size estimate of only 85–192 adult females (Jones and Avise 1997b).

Summary and Future Directions

The study of pipefish and seahorse mating systems is in its early stages but has already provided some exciting results. A wide range of genetic mating systems have been uncovered in populations of syngnathid fishes—from long-term monogamy to polygynandry to polyandry. Only strict polygyny has not yet been found. These available studies support the notion that the genetic mating system is causally related to the intensity of sexual selection and the evolution of sexual dimorphism in sex-role-reversed taxa. In addition, studies of pipefish have provided the first characterization of Bateman gradients in a sex-role-reversed organism. The sexual selection gradient passed this important test, and it appears to be a promising new approach for the quantitative characterization of genetic mating systems. Studies of seahorses and pipefishes also have offered novel insight into the occurrence of clustered mutations, the utility of linked loci in parentage recon-

struction, and the use of parentage assessment for the estimation of adult population size.

Much research on syngnathids remains to be done. For example, all of the genetic studies conducted to date have dealt with mating patterns in only one population per species. Geographic variation in mating systems probably exists, the characterization of which should lead to progress in understanding the ecological causes and consequences of mating system evolution. In addition, all molecular studies of syngnathids have focused on species with completely enclosed brood pouches, yet patterns of mating and risks of cuckoldry may well differ in species where the pouch is absent or less enclosed. Comparative studies are also needed on species in other genera. Such studies will not only broaden the scope of analysis, but when interpreted in the context of syngnathid phylogeny should also help to identify possible phylogenetic constraints on mating system evolution.

Future studies of pipefishes and seahorses would also benefit from integrating genetic information with that from other areas relating to sexual selection and mating systems, including reproductive ecology, parent-offspring conflict, sexual conflict, and brood reduction. Overall these small fishes likely will continue to make disproportionately large contributions to the scientific understanding of sexual selection and reproductive evolution.

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Corresponding Editor: John C. Avise