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Microsatellite analysis of maternity and the mating system in the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal

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Abstract

Highly variable microsatellite loci were employed to study the mating system of the sexually dimorphic Gulf pipefish *Syngnathus scovelli*. In this species, like others in the family Syngnathidae, ‘pregnant’ males provide all parental care. Gulf pipefish were collected from one locale in the northern Gulf of Mexico, and internally carried broods of 40 pregnant males were analysed genetically. By comparing multilocus microsatellite fingerprints for the inferred mothers against expected genotypic distributions from the population sample, it was determined that: (i) only one male had received eggs from more than a single female; and (ii) on two separate occasions, two different males had received eggs from the same female. Given the high power to detect multiple matings by males, the first finding indicates that only rarely are individual males impregnated by multiple females during the course of a pregnancy. Conversely, given the lower power to detect multiple matings by females due to sampling constraints, the second finding suggests a high frequency of multiple successful matings by females. Thus, this population of Gulf pipefish displays a polyandrous genetic mating system. The relevance of these genetic findings is discussed with regard to the evolution of secondary sex traits in this species, and in other syngnathids.

Keywords: genetic markers, polyandry, parentage, sexual selection, sexual dimorphism

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Introduction

The family Syngnathidae is characterized by exclusive male parental care of offspring. A female pipefish or seahorse deposits eggs on the male’s ventral surface or in his brood pouch. He then fertilizes the eggs and carries them for several weeks until they are born as independent, free-swimming young. The female’s investment in offspring is complete after copulation, whereas the male provides the developing embryos with osmoregulation, nutrients and protection (Haresign & Shumway 1981; Berglund *et al.* 1986). To the caregiving male, this mode of fertilization and pregnancy provide assurance of paternity for his brood, probably an important element in the evolution of a high male-investment strategy in progeny rearing (Smith 1996). To the investigator interested in mating behaviours and sexual selection in natural syngnathid populations, this certainty of paternity for brooded embryos facilitates genetic appraisals of maternity, and hence of the mating system.

Extensive paternal care has the ultimate result that in some species of pipefish, sexual selection acts most strongly on females. Indeed, a stronger intensity of sexual selection operating on females than on males constitutes the definitional basis of sex-role reversal in recent sociobiological theory (Vincent *et al.* 1992). This reversal is thought to result from a female biased operational sex ratio (OSR), such that more females than males are available to mate at any given time (Emlen & Oring 1977). The net effect is that males become a limiting resource in reproduction and females are expected to compete for access to them (Clutton-Brock & Parker 1992). In pipefish, this competition is probably manifested either as male choice for more attractive females, or as dominance relationships among females with regard to mating (Rosenqvist 1990; Berglund 1991).

Whereas OSR appears to predict the sex on which sexual selection will act more strongly (Clutton-Brock &
there is a long-standing tradition in the study of sexual selection that the mating system is also related to the evolution of secondary sex characters. Darwin (1871) first noted that for species in which males compete for access to females, more polygynous species exhibit greater levels of sexual dimorphism than do less polygynous or monogamous species, presumably as a result of more intense sexual selection. Much subsequent work has been devoted to addressing this hypothesis in taxa with traditional sex-roles (Clutton-Brock et al. 1977, 1980; Payne 1984; Höglund 1989; Webster 1992; Winquist & Lemon 1994), but little attention has been directed toward the analogous hypothesis in sex-role reversed taxa (but see Jehl & Murray 1986; Andersson 1995).

The family Syngnathidae provides an excellent group to investigate the relationship between the mating system and sexual selection in sex-role reversed species. As a first step, we describe the genetic mating system in nature of the Gulf pipefish Syngnathus scovelli using molecular markers. The Gulf pipefish exhibits considerable sexual dimorphism relative to its congeners. Adult female Gulf pipefish are larger than males, have a deep V-shaped abdomen, and display silvery markings along the trunk (Brown 1972; personal observation). Most other species in this genus lack obvious secondary sex traits (Brown 1972; Dawson 1985). Although females of Syngnathus species often are larger than males, it is unclear that this evidences sexual selection (for example, it may simply be the consequence of natural selection for increased fecundity). The pronounced gender-specific markings on the Gulf pipefish, however, most likely are the result of sexual selection on females.

The discovery of highly polymorphic microsatellite markers (Tautz & Renz 1984; Tautz 1989) has opened possibilities for study of mating systems in natural populations in species where mating behaviours are difficult to observe directly (Brockmann et al. 1994; Craighead et al. 1995; Kellogg et al. 1995; Primmer et al. 1995; Colbourne et al. 1996). To this end, we have identified and employed microsatellite markers to study the genetic mating system of the Gulf pipefish. Microsatellite markers are ideal for such analyses in pipefish because they allow detection of multiple mating within a single brood, and because they can be assayed from small tissue samples such as single pipefish embryos. Empirical questions addressed in this study are: (i) how many Gulf pipefish females contribute eggs to a male’s brood?; (ii) do individual females mate with multiple males?; (iii) will the microsatellite PCR primers also prove suitable for studies of related syngnathid taxa?

**Materials and methods**

**Microsatellite characterization**

DNA was extracted from a single Gulf pipefish male using a standard phenol : chloroform extraction protocol. Three micrograms of genomic DNA were digested with NdeII and electrophoresed through a 2% agarose gel. The region of the gel containing 200–700-bp fragments was excised and the DNA purified using the Prep-A-Gene DNA Purification System (BioRad). The fragments were ligated into BamHI-digested, dephosphorylated pUC19. This ligation was heat-shock transformed into competent DH5α MCR™ E. coli (GIBCO BRL), which were subsequently plated on LB-ampicillin plates at a density of 100–150 colonies per plate.

The resulting library was screened with a synthetic GT₁₀ oligonucleotide that was end-labelled using γ³2P ATP. Bacteria were transferred to nitrocellulose filters and the DNA was crosslinked by heating in a vacuum at 80 °C for 1.5 h. Filters were prehybridized at 42 °C for 1.5 h in a solution containing 6 × SSC, 5 × Denhardt’s reagent and 0.1% SDS. Hybridization was carried out by adding 33.5 ng of the end-labelled GT₁₀ probe to the hybridization bag immediately after the 1.5-h prehybridization. Hybridization took place at 42 °C for 1.5 h. The filters were washed three times in 6 × SSC, 0.1% SDS for 15 min per wash at 42 °C and subsequently were placed on film overnight.

Plasmid DNA was purified from positive colonies using the Wizard Miniprep DNA Purification System (Promega). These potential positives were verified by performing a Southern analysis on the inserts of the plasmids.

<table>
<thead>
<tr>
<th>Locus</th>
<th>n</th>
<th>Number of alleles</th>
<th>Heterozygosity</th>
<th>Exclusion probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>micro11.1</td>
<td>42</td>
<td>22</td>
<td>0.929</td>
<td>0.922</td>
</tr>
<tr>
<td>micro22.3</td>
<td>42</td>
<td>19</td>
<td>0.952</td>
<td>0.916</td>
</tr>
<tr>
<td>micro25.10</td>
<td>78</td>
<td>28</td>
<td>0.885</td>
<td>0.892</td>
</tr>
<tr>
<td>micro25.22</td>
<td>81</td>
<td>29</td>
<td>0.877</td>
<td>0.947</td>
</tr>
</tbody>
</table>
Hybridization conditions were the same as above. Of ∼1200 total colonies screened, 56 potential positive clones were identified. Of these, 36 were verified as true positives by Southerns, five were not positive, and 15 were inconclusive (the miniprep or Southern failed).

Ten of the microsatellite clones were sequenced (fluorescent-dye method) by the Molecular Genetics Instrumentation Facility at the University of Georgia, and nine of these carried inserts which contained GT-rich microsatellites. Two of the sequenced clones proved identical. Primers were designed to amplify six of these microsatellite loci. One of the primer pairs failed to yield consistent amplification products and one has not yet been tested. The other four primer pairs consistently amplified fragments of the expected size and were polymorphic (Table 1).

The amplification of all microsatellite loci was carried out in 10 μL reaction volumes containing 1 × Promega Taq buffer, 1.5 mM MgCl₂, 0.15 μM each primer, 0.2 mM each dNTP, and 0.25 U Promega Taq polymerase. One primer was end-labelled with 1 μCi γ³²P ATP per five pmol of primer. The PCR amplification parameters consisted of an initial denaturing step of 3 min at 94 °C followed by 30 cycles of 92 °C for 1 min, an optimal annealing temperature (53–59 °C) for 1 min, and 72 °C for 1 min. The last cycle was followed by a final extension of 8 min at 72 °C. Microsatellites were resolved on standard 6% polyacrylamide denaturing sequencing gels. The primer pairs for two of the loci (micro25.10 and micro25.22) were designed to produce alleles that did not overlap in size. These loci could be coamplified in one PCR reaction and resolved simultaneously on a single sequencing gel. This was accomplished using the same PCR mixture as above, but by adding a second set of primers for the additional locus.

**Maternity assessment in the Gulf pipefish**

Pregnant Gulf pipefish males were collected on 1, 8 and 9 July 1994, from a single locale in the northern Gulf Coast of Florida (St Joseph State Park, Gulf Co.), using a 2.4-m seine with a 4-mm mesh. Numerous seine hauls taken through a 0.8-ha patch within a broader pasture of shallow seagrass meadows produced 64 pregnant males, eight nonpregnant adult males, and 49 adult females of the Gulf pipefish, which were then frozen in the field on dry ice. In the laboratory, embryos were dissected from pregnant males and rinsed in deionized water before being placed individually into 0.5-mL Eppendorf tubes. They were then placed in 100 μL of Gloor and Engels’ extraction buffer (10 mM Tris pH 8.0, 1 mM EDTA, 25 mM NaCl, 200 μg/mL proteinase K freshly diluted from a frozen 20 mg/mL stock) and incubated for 30 min at 37 °C followed by 95 °C for 2 min (Gloor & Engels 1992). This solution was then stored at −20 °C until needed. One microlitre of the embryo preparation was used for each PCR. A similar procedure was applied to adults. For males, a clip of the caudal fin was prepared in 50 μL of the buffer and a small tissue sample from the brood pouch was prepared in 100 μL, whereas for juveniles and females only the fin clip was used.

Genotypes for 20 pregnant males and for the embryos in their brood pouches were determined for two loci, micro25.10 and micro25.22. Sampling of the broods was designed to detect any incidence of multiple maternity, and involved a mean of 31 embryos typed per male (an average of 56.4% of each male’s brood). For males with less than 43 embryos, PCR was attempted on all individuals in the brood pouch. For males with 43 or more embryos, a sample evenly spaced along the brood pouch was employed. For example, from the male with the largest number of embryos (154) in our sample, every fourth embryo was assayed. The embryos could be sampled evenly because they are arranged in rows within the brood pouch and can be plucked with forceps after the brood pouch is opened.

To provide more power to detect multiple mating by females, broods from an additional 20 pregnant males were added to the analysis. For each of these males, an average of 10 embryos was typed. This reduced our ability to detect multiple mating by these males, but allowed us to infer the genotypes of the mothers of additional broods to determine whether multiple males sometimes had received eggs from the same female.

**Results**

**The microsatellite markers**

All four of the microsatellites for which a consistent amplification product was obtained were highly variable. Numbers of alleles observed per locus ranged from 19 to 29 (Fig. 1), and heterozygosities (both observed and expected) were near 90% (Table 1). These loci are ideal for studies of parentage, with expected exclusion probabilities (Chakraborty et al. 1988) on the order of 0.8–0.9 for a single locus (Table 1) and > 0.999 for all four loci combined. An example of the gel patterns is presented in Fig. 2.

The loci micro25.10 and micro25.22 were used for the majority of the analyses reported here. In analyses of the 20 males whose broods were sampled most extensively, neither locus deviated from the expectations of Mendelian segregation: In 75 tests, only four showed deviations at \( P \leq 0.05 \), an expected number of departures for this level of significance.

Null alleles have been noted in many reports which employ microsatellite polymorphisms (Callen et al. 1993; Koorey et al. 1993; Phillips et al. 1993; Pemberton et al.
1995), and our study also encountered this phenomenon. A null allele at micro25.22 appeared in four of the 40 males for which embryos were genotyped. The presence of a null allele was obvious because the male appeared to be homozygous for micro25.22 yet some of his offspring displayed only a single allele contributed by the mother, with no apparent contribution from the father. The presence of this low frequency null allele probably accounts for the observation of more homozygotes than expected at this locus (Table 1). No null alleles were observed for micro25.10. However, exceptionally large alleles were observed at both loci. These alleles often amplified less efficiently than smaller alleles such that they were not always visible on lighter autoradiograms and, thus, sometimes appeared as nulls. These faint alleles became visible upon longer exposure. All of the detected large alleles segregated in Mendelian fashion, indicating that they were not artefacts.

Contamination of embryos by paternal DNA was not observed in this study. For the few cases in which the embryo displayed the same genotype as the father, this was parsimoniously explained as resulting from the mother sharing a paternal allele. Invariably, the appropriate genotypic classes expected in progeny were observed.

**Mating behaviour of male Gulf pipefish**

The logic of deducing a mother’s genotype from the microsatellite data is exemplified in Table 2. Given the known father’s genotype at a locus, and the genotypes observed among his brooded progeny, the maternal genotype is evident by subtraction. By accumulating such information across loci, multilocus maternal assignments can be made.

The results of these maternity analyses were unambiguous. Among the 20 broods originally examined, no instances of multiple maternity were documented (Table 3). In other words, in no case did a male’s brood contain more than two nonpaternal alleles at a locus, nor did these inferred maternal alleles depart in frequency from the expectations of Mendelian segregation. The embryo sampling scheme described above, and the high variability of the markers, make it extremely unlikely that any incidence of multiple maternity would have remained undetected for any of these broods.

However, during the analysis of the additional 20 males for which fewer embryos were assayed per male, a single male (GM 8-2) was found to have mated with two females. This male’s multiple mating was first detected in a sample of eight embryos, which together displayed more than two maternal alleles. To examine this situation more fully, an additional 20 embryos from GM 8-2 were assayed for micro25.10 and micro25.22. Eighteen of these embryos also were typed at two additional loci, micro11.1 and micro22.3. In all cases, the data were consistent with this male having mated with two females. Thus, at three of the four loci the brood contained four maternally derived alleles (whereas at the fourth locus, three distinct maternal alleles were evident). Furthermore, the four-locus genotypes of the two mothers could be inferred from the
gametic phase disequilibrium in the progeny. Of the 28 embryos assayed, 12 were contributed by one of the mothers and 16 by the other.

**Mating behaviour of female Gulf pipefish**

For two of the males (GM 12-4 and GM 10-19) whose broods were analysed initially, the inferred genotype of the mother was identical at both micro25.10 and micro25.22 (Table 3), despite the fact that the expected frequency of this genotype in the population under random mating is only $6.1 \times 10^{-4}$. This finding suggested that these males may have mated with the same female. To investigate this possibility more fully, two additional microsatellite loci (micro11.1 and micro22.3) were scored for these two males and 10 embryos from each. The inferred genotypes of the mothers again matched, thus yielding a perfect four-locus identity overall (Table 4). The expected frequency of this four-locus genotype in the population is $3.2 \times 10^{-8}$ (Table 4). Thus, the most plausible explanation is that these two males had mated with the same female. Both males carried embryos of about the same stage of development, suggesting that the female mated with both individuals within a reasonably short span of time and had split a single batch of eggs. One male carried 81 embryos and the other carried 49.

In an attempt to document additional cases of multiple mating by females, another 20 pregnant males were examined with an average of 10 progeny assayed per brood (Table 5). This sampling scheme allowed us to infer two-locus genotypes for the mothers of the broods at micro25.10 and micro25.22. In some cases, only a partial two-locus genotype was obtained, but this was sufficient to establish the female either as unique to one brood or as
a possible mother of multiple broods. All broods except two proved to have unique mothers in this additional sample. For the two broods (GM 8-5 and GM 8-8) for which the inferred mothers had identical two-locus genotypes, additional loci revealed another perfect four-locus match (Table 4). In this case, the expected frequency of the four-locus genotype was $1.8 \times 10^{-11}$, thus providing strong evidence for multiple mating by females. The embryos of these two males were also at about the same developmental stage, indicating again that the female probably split a single batch of eggs between them. GM 8-5 and GM 8-8 carried 99 and 90 embryos, respectively.

<table>
<thead>
<tr>
<th>Male ID</th>
<th>Father’s genotype</th>
<th>Father’s maternal genotype</th>
<th>Inferred maternal genotype</th>
<th>exp. freq. maternal genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM 12-4</td>
<td>166/182</td>
<td>156/166</td>
<td>156/182</td>
<td>156/156</td>
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<tr>
<td>GM 12-8</td>
<td>176/182</td>
<td>176/190</td>
<td>176/194</td>
<td>190/194</td>
</tr>
<tr>
<td>GM 11-1</td>
<td>156/180</td>
<td>156/176</td>
<td>156/180</td>
<td>176/176</td>
</tr>
<tr>
<td>GM 11-2</td>
<td>156/180</td>
<td>156/170</td>
<td>156/180</td>
<td>170/180</td>
</tr>
<tr>
<td>GM 10-3</td>
<td>166/186</td>
<td>158/166</td>
<td>166/166</td>
<td>186/166</td>
</tr>
<tr>
<td>GM 10-5</td>
<td>182/192</td>
<td>176/186</td>
<td>180/192</td>
<td>200/192</td>
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<tr>
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<td>156/162</td>
<td>156/192</td>
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<td>156/168</td>
<td>156/196</td>
<td>156/204</td>
<td>196/204</td>
</tr>
<tr>
<td>GM 10-9</td>
<td>156/182</td>
<td>156/176</td>
<td>156/182</td>
<td>176/182</td>
</tr>
<tr>
<td>GM 10-6</td>
<td>156/174</td>
<td>156/160</td>
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<td>160/174</td>
</tr>
<tr>
<td>GM 10-10</td>
<td>192/194</td>
<td>182/192</td>
<td>192/194</td>
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<td>GM 10-17</td>
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<td>156/182</td>
<td>156/182</td>
</tr>
</tbody>
</table>

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Amplification of the microsatellite loci in other taxa

The capacity of all four PCR primer pairs to successfully amplify these microsatellite loci was also examined in several other gasterosteiform taxa: five congeners of Sygnathus scovelli (S. floridae, S. louisianae, S. leptorhynchus, S. californiensis and S. typhle), one species of pipefish (Micrognathus crinigerus) and two species of seahorses (Hippocampus erectus and H. zosterae) also in Syngnathidae, and the threespine stickleback (Gasterosteus aculeatus) representing Gasterosteidae. The evaluation of PCR conditions in these taxa was not extensive in that only the annealing temperature was optimized, so the possibility remains that with further effort better amplification results might be obtained. None the less, all four primer pairs yielded the expected PCR amplification products in all assayed Syngnathus species (example in Fig. 3). Outside this genus, amplification became more problematic. Some of the primers yielded products of the expected size in the pipefish and seahorses not in the genus Syngnathus, but in most cases the amount of product was reduced. None of the primers yielded expected amplification products in the stickleback (Fig. 3), suggesting that the primers may not be useful outside Syngnathidae.

Discussion

Pipefish mating systems

Despite the power to detect multiple maternity with the highly polymorphic microsatellite markers employed, in only one case in our sample of 40 paternal broods could a male be shown to have received eggs from more than one female. To bolster the conclusion of single mating by males, it is instructive to consider three ways in which a
male broods of loci (Table 1). In summary, only rarely (< 5%) do single
the rarity of observed and expected homozygotes at these
homozygous for different alleles. This too is unlikely given
sional male had mated with two females that were
often distribute their eggs among multiple males.

Second, perhaps an occasional male had mated with
two females that shared the same di-locus genotype,
resulting in an incorrect assessment of maternity. This is
unlikely because based on a model of random mating as
applied to the observed microsatellite allelic frequencies,
the expected population frequencies of the two-locus
genotypes assigned to the mothers were low (ranging
from 6 x 10^-4 to 5 x 10^-7; Table 3). Third, perhaps an occa-
sional male had mated with two females that were homozygous for different alleles. This too is unlikely given
the rarity of observed and expected homozygotes at these
loci (Table 1). In summary, only rarely (< 5%) do single-
male broods of S. scovelli at this north Florida locale have
multiple mothers. The factors that limit most males to a
single genetic mate remain unknown.

Conversely, the genetic data suggest that females
often distribute their eggs among multiple males.

Demonstrably, two pairs of assayed males carried broods
originating from the same mother. Given the severe sam-
ppling biases operating against the detection of multiple
matings by females in nature, such instances probably are
common. Collections were made within an extensive sea-
grass meadow such that no doubt only a minute fraction
of an enormous population was examined. Indeed, of 37
adult females collected from this locale and typed at
micro25.10 and micro25.22, all but two were excluded
(based on two-locus genotypes) as possible mothers of the
broods assayed, making it evident that we sampled only a
small fraction of the breeding adults at this locale. Thus,
females that appeared to have mated only once based
upon our sample of males may well have mated with
additional males that we failed to collect. In other words,
although we documented only two instances of multiple
matings by females, these genetic findings suggest that
females routinely mate with multiple males.

Given the current genetic observations on maternity
and paternity in S. scovelli, and in consideration of the
inherent asymmetry in the power to genetically document
multiple matings by males vs. females in large natural
populations of a species with male pregnancy, we con-
clude that the mating system of these Gulf pipefish can
best be described as tending toward genetic polyandry. In
the course of a single male pregnancy, most fathers have
only one successful mate, whereas females commonly dis-
tribute eggs to more than one male.

This study involves a single point sample from one
locale, and thus fails to address possible temporal or geo-
graphical variation in the Gulf pipefish mating system.
However, a comparison with Brown's (1972) study of a
Gulf pipefish population from Cedar Key, Florida, gives
no indication that our sample was atypical in certain
demographic parameters that might influence the mating
system. Numbers of embryos carried per male in our sam-
ple (mean = 66.3; max. = 154; n = 20) are similar to those
reported by Brown (mean = 55.6; max. = 175; n = 346).
Among adults, Brown found that the ratio of female to
male Gulf pipefish fluctuated throughout the year from
0.51 to more than 1.5; our observed ratio of 0.68 is within
this range. However, given that the OSR is considered a
key factor affecting the strength of sexual selection (Emlen
& Oring 1977; Vincent et al. 1994), the temporal variation
in sex ratio documented by Brown could be of interest for
future genetic investigations into possible variation in the
matting system of S. scovelli.

Among other Syngnathus species, detailed behavioural
information about the mating system is available only for
a European pipefish, S. typhle (Berglund & Rosenqvist
1993; Berglund 1994; Vincent et al. 1994; Rosenqvist &
Johansson 1995). In this species, it appears that during the
course of a male pregnancy, males mate with multiple
females and females mate with multiple males. This

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conclusion is based on direct observations of mating behaviour in the laboratory, and on the consideration that some field-caught males contain eggs of distinct colours believed to have originated from different females (Berglund et al. 1988). However, the frequency of multiple mating in natural populations of S. typhle has yet to be determined.

The genetic mating system of the Gulf pipefish is, perhaps, more similar to that of another pipefish species (Nerophis ophidion) in which a receptive male receives an entire brood from a single female, and females reportedly often mate with multiple males within the timeframe of a male pregnancy (Rosenqvist 1993). However, males of N. ophidion do not have a fully enclosed brood pouch as Gulf pipefish do; rather, eggs are glued to the male’s ventral surface. This is thought to have promoted an evolutionary tendency for male behavioural reluctance for matings with multiple females, because later matings could dislodge previously attached eggs (Rosenqvist 1993). N. ophidion females also differ from Gulf pipefish females in that they are thought to deposit a full load of eggs at each copulation, before preparing another batch for a different male (Berglund & Rosenqvist 1993). In contrast, our observations suggest that Gulf pipefish females are capable of splitting a single batch of eggs among more than one male.

The mating system of N. ophidion has been described as sequential polygyny (Vincent et al. 1992); during the timeframe of a single male pregnancy, males mate with one female only, whereas females mate sequentially with multiple males. This is similar to the definition of polyandry that we suggest also for the Gulf pipefish, and is in distinction to the polygynandrous system of S. typhle in which both males and females mate multiple times during the course of a male pregnancy. All three species can be considered polygamous, however, because at least one sex in each has multiple mates during a short and well-defined breeding interval.

Mating systems and sexual selection

Syngnathids have been proposed to fall into two separate categories with regard to sexual selection (Vincent et al. 1992): (i) monogamous pipefishes and seahorses which are not sex-role reversed (under the definition of sex-role reversal discussed in the introduction); and (ii) polygamous pipefishes which are. The former category includes the seahorses Hippocampus spp. and the pipefish genus Corythoichthys, both of which appear to consist of monogamous species in which competition for mates is greater among males than among females (Gronell 1984; Vincent 1994; Vincent & Sadler 1995). The latter category includes S. typhle, N. ophidion, and probably S. scovelli, in which the relative intensity of competition for mates by the two genders is thought to be the reverse. In these species, the stronger operation of sexual selection on females than on males can in some cases lead to the elaboration of female secondary sex traits. As already mentioned, S. scovelli females have a deep, enlarged abdomen and V-shaped silvery stripes along the trunk region. In N. ophidion, females have intense blue coloration on the trunk and appearance-enlarging skin folds (Rosenqvist 1993). In S. typhle, on the other hand, although females tend to be larger than males, only weak sexual dimorphism and little development of female secondary sex traits are displayed (Svensson 1988; Berglund et al. 1989). This is true also of many other species of Syngnathus (Brown 1972; Dawson 1985).

Why do some species of pipefish exhibit conspicuous secondary sex traits, whereas others do not? Perhaps there is a relationship in this family not only between the direction of sexual selection and the mating system (as suggested by Vincent et al. 1992), but also between the mating system and the strength of sexual selection. In species with traditional sex-roles, a simple prediction originally inspired by Darwin (1871) is that polygyny results in more intense sexual selection. Although this prediction has received general empirical support (Stamps 1983; Björklund 1990; Oakes 1992; Webster 1992; Winquist & Lemon 1994), the correlation between the extent of polygyny and the evolution of sexual dimorphism is far from perfect (Clutton-Brock et al. 1977; Leutenegger & Cheverud 1982; Payne 1984; Höglund 1989). It must be emphasized, however, that almost all studies conducted previously have examined social (behavioural) as opposed to genetic (realized) mating systems. A frequently observed discrepancy between the social and genetic mating systems (Birkhead & Möller 1992; Searcy & Yasukawa 1995) no doubt contributes to the difficulty of testing associations between the mating system and the evolution of sexual dimorphism (Möller & Birkhead 1994).

In any event, the analogous hypothesis for sex-role reversed species is that sexual dimorphism and expression of secondary sex traits in females may be more pronounced in species that are more strongly genetically polyandrous than in species that are less so (Jehl & Murray 1986). Thus, in the future the comparative mating systems of multiple syngnathid species might profitably be examined genetically. After grouping species into those that are genetically monogamous and not sex-role reversed, vs. those that are genetically polygamous and sex-role reversed, it may be informative to further subdivide the latter category based on the degree of polyandry vs. polygynandry. The hypothesis to be further evaluated is whether increased levels of polyandry, and hence in the presumed disparity in the intensity of sexual selection on females vs. males, are associated with increased gender dimorphism in secondary sexual characters. Although available information for S. typhle (a polygynandrous species

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lacking dimorphism) and for *N. ophidian* and *S. scovelli* (polymamous species with considerable dimorphism) appears to be consistent with (and indeed motivated) this hypothesis, further evaluation will require comparative study of genetic mating systems across additional syngnathid and other species with sex-role reversal.

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### References


The work described here is the first instalment of a dissertation project by Adam Jones that involves genetic examination of maternity and the mating system in species in which males (rather than females) provide parental care. Such species afford unusual perspectives on possible interrelationships between the mating system, sex-role reversal, intensities of sexual selection, and degrees of sexual dimorphism. This work is part of a broader research programme in John Avise’s laboratory on the use of molecular markers in ecology and evolution.