Geographical variation in the mating system of the dusky pipefish (*Syngnathus floridae*)

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**Abstract**

Differences among populations in the intensity of sexual selection resulting from distinct genetic mating systems can lead to divergent morphological evolution and speciation. However, little is known about how genetic mating systems vary between populations and what factors may contribute to this variation. In this study, we compare the genetic mating systems of two geographically distinct populations of the dusky pipefish (*Syngnathus floridae*), a species characterized by polygynandry and male pregnancy, from the Atlantic Coast of Virginia and the Gulf Coast of Florida. Our results revealed significant interpopulation variation in mating and reproductive success. Estimates of the opportunity for selection ($I$), the opportunity for sexual selection ($I_s$) and the Bateman gradient ($\beta$) were higher among males in the Florida population than in the Virginia population, suggesting that sexual selection on males is stronger in the Florida population. The Virginia population is larger and denser than the Florida population, suggesting that population demographics may be one of many causal factors shaping interpopulational mating patterns. This study also provides evidence that the adult sex ratio, operational sex ratio, population density and genetic mating system of *S. floridae* may be temporally stable over timescales of a month in the Florida population. Overall, our results show that this species is a good model for the study of mating system variation in nature and that Bateman’s principles may be a useful technique for the quantitative comparison of mating systems between populations.

**Keywords:** Bateman’s principles, geographical variation, mating system, pipefish, population density, temporal variation

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**Introduction**

Sexual selection can lead to rapid evolution of behavioural and morphological traits, and thus may be a salient force in species divergence (Kraaijeveld & Pomiankowski 2004). The recent flood of molecular appraisals of mating behaviour has established that the genetic mating system is closely related to the intensity of sexual selection in nature (Jones *et al.* 2001a; Shuster & Wade 2003). Consequently, it is now clear that knowledge of the mating system is fundamental to a complete understanding of sexual selection in any system. Data on mating systems from a wide variety of species indicate that mating behaviour can be evolutionarily labile with important evolutionary consequences (Kusmierski *et al.* 1997; Petrie & Kempenaers 1998; Griffith 2000; Avise *et al.* 2002). However, genetic characterizations of the mating system for most species involve only a single exemplar population, despite the fact that mating systems likely vary over space and time (Jones *et al.* 2001b). Such geographical or temporal variation in mating systems would provide excellent opportunities for comparative studies of mating system evolution. For example, understanding the causes of mating system variation among populations could resolve why some lineages experience intense sexual selection whereas others do not (Panhuis *et al.* 2001). Arguably, this question is one of the central issues in the study of sexual selection. Mating system divergence among populations also theoretically facilitates speciation (Kraaijeveld & Pomiankowski 2004). Lande (1982) showed that sexual selection has the potential to initiate sexual isolation and character divergence as distinct populations diverge with respect to secondary sexual traits and mating preferences. Such divergence is most effective when populations evolve dissimilar mating systems. In addition, Zeh & Zeh (2000) have speculated...
that mating system divergence could facilitate speciation through parent-offspring conflict. Despite the apparent potential of studies of geographically or temporally varying mating systems, such studies are thus far rare.

Several factors have slowed progress in the comparative study of mating systems within species. An understanding of evolutionary processes such as sexual selection requires the knowledge of biological parentage at a level of detail that in most species can be achieved only through the application of molecular methods (Avise 2004). Such studies are expensive and time-consuming. A comprehensive study of the mating system in a single population may involve hundreds or thousands of genotypes (Avise et al. 2002), so scaling up to multiple populations can be a major logistical problem. Mating system studies also require samples of breeding adults and their offspring, which can be difficult to obtain from multiple populations across the range of a species, especially for species whose abundance varies temporally and geographically. As a consequence of these limitations, studies of sexual selection often are forced to assume that mating systems are fixed or nearly fixed over time and space within a species. For many species, this assumption may be false since populations that inhabit distinct environments may experience local environmental selection pressures, such as predation, that can influence the genetic mating system (Kelly et al. 1999; Bronikowski et al. 2002). In addition to geographical variation in mating systems, populations can be temporally dynamic with respect to population density, the availability of mates, and the breeding condition of individuals, all of which may affect the mating system (Emlen & Oring 1977; Shuster & Wade 2003; Soucy & Travis 2003). Thus, to gain a more complete understanding of the ecological factors that influence the evolution of genetic mating systems, a deeper appreciation of within-species mating system dynamics is necessary.

The few studies that have addressed geographical variation in mating systems have produced mixed results, ranging from significant intraspecific variation among populations of fishes (Trexler et al. 1997; Kelly et al. 1999; Soucy & Travis 2003), birds (Griffith et al. 1999; Griffith 2000; Durrant & Hughes 2005) and mammals (Taylor et al. 2000; Clinchy et al. 2004) to essentially no variation between geographically distinct populations (Zane et al. 1999; Jones et al. 2001b; Goodisman et al. 2002). A handful of comparative studies have attempted to correlate variation in mating patterns with environmental factors. While these studies have also led to mixed results (Weatherhead & Boag 1997; Griffith et al. 1999), some biologically important patterns have emerged. First, in birds, extra-pair fertilizations are more frequent in mainland populations than in island populations (Griffith et al. 1999, 2002; Griffith 2000). Second, in Trinidadian guppies, the frequency of multiple insemination is related to the intensity of predation (Kelly et al. 1999; Bronikowski et al. 2002). Finally, in a study of three populations of least killifish, rates of multiple paternity were higher in populations with greater population densities (Soucy & Travis 2003). These studies provide an interesting preliminary glimpse into environmental factors that likely shape mating systems, and hence sexual selection, in nature, but are of insufficient number and taxonomic breadth to provide any clear generalities. Additional studies of geographical variation in mating systems are warranted.

In this study, we characterize the genetic mating system of two geographically distinct populations of the dusky pipefish, Syngnathus floridae. The reproduction of this species is characterized by male pregnancy in which females transfer eggs into a specialized brood pouch on the male’s trunk during copulation (Dawson 1982; Jones & Avise 2003). Males then provide parental care during development until the young are released as independent juveniles (Dawson 1982). This species is likely sex-role reversed with respect to the intensity of sexual selection, such that sexual selection acts more strongly on females than on males (Jones & Avise 1997b). The high paternal investment in this and other syngnathid species can depress the potential reproductive rates of males to such an extent that the direction of sexual selection becomes reversed relative to that of most taxa (Berglund et al. 1989; Jones et al. 2005). Characterization of the mating system is facilitated in this species because maternal genotypes can be reconstructed from genetic analysis of pipefish embryos contained within the male brood pouch through the use of highly polymorphic molecular markers (Jones & Avise 1997b). The dusky pipefish is ideally suited for this type of inquiry because it has a wide geographical distribution, and large numbers of pipefish can be harvested from relatively small areas during the breeding season, yielding high numbers of pregnant males and their potential mates.

The study of geographical variation in the mating system of the dusky pipefish provides a good opportunity to take advantage of quantitative measures of the potential for sexual selection based on Bateman’s principles. Even though these techniques have been advocated as methods for the quantification and comparison of mating systems (e.g. Arnold & Duvall 1994; Arnold 1994; Jones et al. 2001a), they have not yet been employed to compare mating systems among populations of the same species. One major advantage to the application of Bateman’s principles for the characterization of mating systems in an interpopulation framework is that it allows strict quantitative comparison of the potential for sexual selection between populations and between sexes.

The present study has several goals. First, we use Bateman’s principles to test for significant geographical variation in the mating systems of two geographically isolated populations of dusky pipefish. Second, we examine temporal variation in the mating system by comparing
two collections made in 2003 within one Florida population. Third, we consider the results in light of differences in population density and other environmental factors that may influence the genetic mating system.

Materials and methods

Study species

The dusky pipefish, Syngnathus floridai, is a geographically widespread species, occurring in the western Atlantic Ocean from the Chesapeake Bay and Bermuda to the southern Florida coast and the Florida Keys as well as in the Gulf of Mexico from the west coast of Florida to Panama (Dawson 1982). This species inhabits shallow seagrass beds in coastal waters, and its diet comprises primarily zooplankton such as copepods, amphipods, isopods and mysids (Brown 1972; Mercer 1973). During mating, females deposit eggs in a highly specialized brood pouch on the male’s ventral surface. The male then fertilizes the eggs and carries the embryos until they hatch approximately 10 days later (Dawson 1982). In the Chesapeake Bay, near the northern limit of its range, S. floridai migrates inshore during the spring and breeds from April to October with peaks in population density and male pregnancy in July and August (Mercer 1973). In the northern Gulf of Mexico, populations of S. floridai migrate from April through November with a peak in population density and male pregnancy in August and September (Brown 1972).

Collection of specimens

We collected 150 adult male and 91 adult female S. floridai on 5 and 6 August 2003 from Mobjack Bay (37.18423°N, 76.24752°W), near the mouth of the York River, Virginia (Table 1). Two samples of S. floridai were collected from Saint Joseph Bay, Florida (29.47765°N, 85.18237°W) on 18–20 July 2003 (19 males, 34 females) and 24–25 August 2003 (17 males, 30 females) near the collection site reported in Jones & Avise (1997b). Individuals were captured at each location by seine net (2 mm mesh) from measured plots marked with stakes inside a shallow (depth less than 1 m), continuous seagrass meadow. Our goal was to capture as many pregnant males as possible along with their potential mates within the designated area. Each plot was seined completely during low tide a minimum of three full sweeps or until a full sweep of the area captured less than 5% of the original sweep. We measured standard lengths (SL, tip of snout to base of caudal fin) of all individuals. All fishes were sacrificed in the field by severing their spinal column above the operculum and preserved in 95% ethyl alcohol.

Males were considered sexually mature if they possessed a mature brood pouch, whereas females were considered sexually mature if they possessed ripe ova. All females of less than 120 mm standard length were dissected to assess the presence of ripe ova, whereas females longer than 120 mm were assumed to be sexually mature (Brown 1972). This assumption was confirmed by random dissections on 15 females > 120 mm from each population, all of which contained ripe ova. Based on these criteria, all individuals collected from these sites were sexually mature adults and were included in all analyses. The adult sex ratio (ASR) is given as the number of adult males divided by the total number of adults collected, whereas the operational sex ratio (OSR) is the ratio of receptive adult males to the total number of receptive adults. In the case of sex-role-reversed pipefish, such as S. floridai, the OSR is the number of nonpregnant males divided by the sum of nonpregnant males and adult females (Kvarnemo & Ahnesjö 1996).

Molecular parentage analysis

Parentage analysis was conducted on the broods of 30 pregnant males from the Virginia population and 11 pregnant males from each of the Florida collections. Brood pouches were dissected following the protocol of Jones & Avise (1997b). Briefly, each brood pouch was divided into

### Table 1

Comparison of sample sizes of adults (n), males (m), nonpregnant males (m′) and females (f), adult sex ratios (ASR; ratio of males to total adults), operational sex ratios (OSR; ratio of nonpregnant males to females + nonpregnant males), population density (ind./m²) and mean adult female population size (estimated using the modified Lincoln–Peterson method with 95% confidence intervals) of Syngnathus floridai from different sample sites in Virginia (VA) and Florida (FL).

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Sample size</th>
<th>ASR</th>
<th>OSR</th>
<th>Population density (ind./m²)</th>
<th>Adult female population size (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n  m  m′ f</td>
<td>m/n</td>
<td>m′/(m′ + f)</td>
<td>Total  m  f</td>
<td></td>
</tr>
<tr>
<td>Mobjack Bay, VA</td>
<td>8/03</td>
<td>241 150 3 91</td>
<td>0.62</td>
<td>0.03</td>
<td>0.18 0.11 0.07</td>
<td>635 (323–947)</td>
</tr>
<tr>
<td>St Joseph Bay, FL</td>
<td>7/03</td>
<td>53 19 6 34</td>
<td>0.36</td>
<td>0.15</td>
<td>0.06 0.02 0.04</td>
<td>122 (52–191)</td>
</tr>
<tr>
<td></td>
<td>8/03</td>
<td>47 17 6 30</td>
<td>0.36</td>
<td>0.17</td>
<td>0.03 0.01 0.02</td>
<td>162 (42–282)</td>
</tr>
<tr>
<td></td>
<td>7/94*</td>
<td>93 50 7 43</td>
<td>0.54</td>
<td>0.14</td>
<td>— — —</td>
<td>138 (85–192)</td>
</tr>
</tbody>
</table>

*Data for the June 1994 St Joseph Bay collection from Jones & Avise (1997b).
14 roughly equal sections (once lengthwise and six times laterally) and marked with an alcohol-resistant pen. Three embryos were selected at random from each section, extracted with flame-sterilized forceps and placed into a 96-well plate for genotyping. Using this sampling scheme, we genotyped a total of 42 offspring from each male’s brood pouch, and counted the total number of embryos in each section of the pouch.

We used three polymorphic microsatellite loci to characterize the mating system of *S. floridae* (*Micro11.1, Micro22.3* and *Micro25.22*) and four polymorphic microsatellite loci to genotype all sexually mature adults in each population (*Micro11.1, Micro22.3, Micro25.10 and Micro25.22, Table 2). Microsatellite markers employed in this study were originally developed for the Gulf pipefish, *Syngnathus scovelli* (Jones & Avise 1997a; Jones et al. 1999). DNA was extracted using a standard Proteinase K and 5% Chelex digestion in 96-well plates (Miller & Kapuscinski 1996), and microsatellite markers were amplified using polymerase chain reaction (PCR) conditions modified from Jones & Avise (1997a). We used annealing temperatures of 54 °C for *Micro11.1* and *Micro22.3*, and 56 °C for *Micro25.10 and Micro25.22*. All thermal cycling ran for 36 cycles, except that for *Micro11.1*, which ran for 40 cycles to yield sufficient PCR product for analysis. Primers were labelled with a fluorescent dye and sized on an ABI 3730 Genetic Analyser (Applied Biosystems). All microsatellite fragment analyses were accomplished using GENOTyper or GENEMAPPER software (Applied Biosystems).

Maternal genotypes were reconstructed from progeny arrays using GERUD2.0 (Jones 2001, 2005), and the resulting inferred maternal genotypes were compared with each other and with the genotypes of collected adult females in the population using MICRO SATellite toolkit 3.1 for Microsoft Excel (Park 2001). Because eggs are spatially segregated by maternity in male brood pouches (Jones & Avise 1997b), we were able to assign embryos to mothers with great accuracy based on data from the genotyped offspring in each of the 14 demarcated sections. If only one maternal genotype was found in all three embryos for a particular section, all embryos counted in that section were ascribed to that inferred female. When a section transitioned from one mother to the next, we assigned one-third and two-thirds of the total embryos to each of the two mothers, depending upon the proportion of genotyped embryos genetically assigned to each mother.

Female reproductive contribution was calculated by tallying all embryos assigned to each mother for all sections. Eggs that had no visible signs of embryonic development were considered undeveloped eggs. These eggs were likely the result of unsuccessful fertilization or incomplete development. Because undeveloped eggs contribute a small proportion to the numbers of eggs received by males (2.4% ± 1.5 SE for the Virginia population and 8.4% ± 1.7 SE for July and August Florida populations) and because it is unlikely that these eggs would result in viable offspring, undeveloped eggs were not included in the female reproductive contribution or estimates of male reproductive success.

All microsatellite data were analysed with GENEPOP version 3.4 (Raymond & Rousset 1995) to test for Hardy–Weinberg equilibrium (Fisher’s exact test) and for genotypic disequilibrium for pairs of loci within the population (Fisher’s exact test). Wright’s F-statistic ($F_{ST}$) was also calculated using GENEPOP version 3.4 (Table 2). The cumulative probability of identity ($P_{ID}$) was estimated from microsatellite data for adult females and reconstructed female genotypes from the two different populations using LOCUSEATER version 2.4 (Hoyle et al. 2005). A modified Lincoln–Peterson method of capture-recapture was used to estimate female local population size in the Virginia and Florida populations based on number of reconstructed female genotypes (Jones & Avise 1997b).

### Table 2 Microsatellite data comparing the number of alleles (A), sample sizes (N), observed and expected heterozygosities ($H_O$ and $H_E$, respectively) and $F_{ST}$ estimates between Virginia (VA) and pooled Florida (FL) adult *Syngnathus floridae* collections

<table>
<thead>
<tr>
<th>Locus</th>
<th>Mobjack Bay, VA</th>
<th>St Joseph Bay, FL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>N</td>
</tr>
<tr>
<td><em>Micro11.1</em></td>
<td>43</td>
<td>228</td>
</tr>
<tr>
<td><em>Micro22.3</em></td>
<td>21</td>
<td>191</td>
</tr>
<tr>
<td><em>Micro25.10</em></td>
<td>18</td>
<td>185</td>
</tr>
<tr>
<td><em>Micro25.22</em></td>
<td>20</td>
<td>228</td>
</tr>
</tbody>
</table>

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Quantification of the genetic mating system by Bateman’s principles

The genetic mating system for males from each geographical location was quantified using mean mating success ($\bar{X}_{ms}$), mean reproductive success ($\bar{X}_{s}$) and Bateman’s three principles: the opportunity for selection ($\bar{F}$), the opportunity for sexual selection ($\bar{I}$), and the sexual selection gradient or Bateman gradient ($\bar{b}_s$). Mean mating success is the average
number of mates per male for each population. Similarly, \( \bar{X}_n \) is the mean number of offspring per male for each population. The opportunity for selection is the standardized variance in reproductive success \( I = \frac{\sigma_n^2}{\bar{X}_n^2} \), and the opportunity for sexual selection is the standard variance in mating success \( I_s = \frac{\sigma_{ms}^2}{\bar{X}_{ms}^2} \) (Arnold & Duvall 1994; Arnold 1994). The Bateman gradient is the slope of the weighted least-squares regression of relative reproductive success (number of offspring divided by the mean) on mating success (Arnold 1994; Jones et al. 2002). All measures of the genetic mating system include an estimate of nonbreeding males (Wade 1979; Arnold 1994; Shuster & Wade 2003). The number of nonbreeding males was calculated based on the frequency of nonpregnant males encountered at each location and during each sampling period.

**Statistical analysis**

Statistical analyses were performed with JMP IN version 5.1 statistical software package (SAS Institute). All statistics were analysed first for normality and equal variances. If these assumptions were not met, data were transformed or if no transformation satisfied a priori assumptions, appropriate nonparametric tests were applied. Statistical tests as well as any transformations are indicated throughout the text. All means are reported ± one standard error of the mean (SE).

**Results**

**Microsatellite markers**

Four microsatellite loci revealed high levels of polymorphism and heterozygosity among adult *Syngnathus floridae* in both populations (Table 2). The Virginia population displayed between 18 and 43 alleles per locus with expected heterozygosities ranging from 0.80 to 0.87 (Table 2). The Florida sample displayed between 21 and 47 alleles per locus with expected heterozygosities ranging from 0.89 to 0.96 (Table 2). Fisher’s exact tests indicated no significant departures (\( \alpha = 0.05 \)) from Hardy–Weinberg equilibrium or linkage equilibrium. A low-frequency (0.002) null allele was detected at Micro11.1 in the maternal parent of the brood of a single male from the Virginia population. The allele was discovered after GERUD2.0 (Jones 2001, 2005) indicated the presence of two maternal genotypes in the male’s offspring that were inconsistent with the expected spatial clustering of offspring by maternity. The null allele manifested itself clearly as sets of embryos homozygous for each paternal allele with an absence of embryos possessing the expected heterozygous genotype comprising both paternal alleles. A second null allele at Micro11.1 was discovered in the maternal contribution to the broods of two Florida males that apparently had mated with the same female, as evidenced by identical reconstructed maternal genotypes. These null alleles were infrequent enough in both populations that their presence did not cause a heterozygosity deficit (Fisher’s exact test, \( P < 0.05 \)), and they did not compromise the interpretation of the parentage analysis. Moderate population differentiation was evident from the \( F_{ST} \) values estimated for these four microsatellite markers (Table 2). Single-locus \( F_{ST} \) estimates ranged from 0.007 to 0.101 with a global \( F_{ST} \) value of 0.051.

**Comparison between Virginia and Florida populations**

The Florida and Virginia sites differed in both ASR and OSR estimates. The ASR of the Virginia population caught in August 2003 was heavily male biased with a significant departure from an equal sex ratio (\( \chi^2 = 14.4, \text{d.f.} = 1, P < 0.0001 \), Table 1). The ASRs of the Florida collections were similar to one another and showed a significant female bias in the July (\( \chi^2 = 9.3, \text{d.f.} = 1, P = 0.002 \)) but not in the August sample (\( \chi^2 = 3.6, \text{d.f.} = 1, P = 0.058 \); Table 1). In contrast, OSRs were heavily female biased in all of the populations due to the rarity of nonpregnant males (Table 1). The two Florida collections showed similar OSRs, both of which were less female biased than the OSR in the Virginia population, suggesting that competition for males may be more intense in the latter population.

Males and females were significantly larger in body size (as measured by SL) in both Florida collections than in the Virginia population as shown by a two-way analysis of variance (ANOVA) and a Tukey–Kramer post-hoc analysis (male ANOVA: \( F_{2,184} = 43.6, P < 0.0001 \), female ANOVA: \( F_{2,153} = 30.8, P < 0.0001 \)). Some evidence of sexual dimorphism is present in the Virginia collection as females were slightly, but significantly, larger than males (mean SL = 128 ± 1 mm, female SL = 133 ± 2 mm, ANOVA: \( F_{1,238} = 5.94, P = 0.016 \). No evidence of sexual dimorphism was present in the Florida samples as ANOVA detected no significant difference in SL between sexes (\( F_{1,96} = 0.0050, P = 0.94 \)). However, a significant difference in body size between the two Florida collections was observed (ANOVA: \( F_{1,96} = 7.37, P = 0.008 \)). Adult *S. floridae* were, on average, 8 mm larger in the August population (July SL = 148 ± 2 mm; August SL = 156 ± 2 mm). No significant interaction between time and sex was observed between Florida populations (ANOVA: \( F_{1,96} = 0.0095, P = 0.92 \)).

**Male mating behaviour**

An evaluation of the July and August Florida collections revealed similar mating system estimates. The July population had six unmated males, one singly mated male, seven males that mated with two females, and three males that mated with three females (\( \bar{X}_{ms} = 1.5 ± 0.3 \)). The August population had six unmated males, five singly mated males, four males that mated with two females, one triply...
Table 3  Comparison of the opportunity for selection, \( l \), the opportunity for sexual selection, \( I_s \), and the Bateman gradient (\( \beta_s \pm SE \)) between Florida (FL) and Virginia (VA) populations of male Syngnathus floridensis. Average reproductive (\( \bar{x}_{rs} \)) and mating (\( \bar{x}_{ms} \)) successes per male and their respective variances (\( \sigma^2 \)), including unmated males, are also listed.

<table>
<thead>
<tr>
<th>Site</th>
<th>Date</th>
<th>Reproductive success</th>
<th>Mating success</th>
<th>Bateman gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \bar{x}_{rs} )</td>
<td>( \sigma^2_{rs} )</td>
<td>( l )</td>
</tr>
<tr>
<td>Mobjacks Bay</td>
<td>8/03</td>
<td>251.3</td>
<td>9165.8</td>
<td>0.15</td>
</tr>
<tr>
<td>St Joseph Bay</td>
<td>7–8/03</td>
<td>180.2</td>
<td>23911.8</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>7/94*</td>
<td>440.4</td>
<td>60837.4</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Data for the June 1994 St Joseph Bay collection from Jones & Avise (1997b).

Males in Virginia had higher rates of mating and higher numbers of viable embryos per male than the Florida population. Among the 30 males analysed, only two males had broods consisting entirely of full siblings. Of the remaining 28 males, 11 had two matings, 14 had three matings, three had four matings and three were unmated (\( \bar{x}_{ms} = 2.5 \pm 0.2 \), Table 3). A chi-square test showed a significant difference in the distributions of mating success between the Florida and Virginia populations (\( \chi^2 = 13.1; \text{d.f.} = 4; P = 0.011 \); Fig. 1). Virginia males had, on average, a mean reproductive success of 251.3 ± 95.2, a value significantly higher than the pooled Florida estimate of 180.2 ± 26.91 (ANOVA: \( F_{1,62} = 4.82, P = 0.032 \)). The lower mean estimate of reproductive success for Florida males is driven primarily by the relatively high frequency of unmated males in the Florida population, despite similar values for the number of viable embryos per mated male in both Florida and Virginia collections (FL = 270.2 ± 20.0, VA = 259.7 ± 17.1; ANOVA: \( F_{1,62} = 0.16, P = 0.69 \)).

An analysis of covariance (ancova) revealed mean mating success was positively correlated with SL for males in both populations (\( F_{1,49} = 26.90, P < 0.0001 \); Fig. 2). Similarly, the relationship between number of embryos and SL was significantly positive in both populations (ANOVA: \( F_{1,49} = 7.14, P = 0.01 \)), and mated Virginia males were significantly smaller than their Florida counterparts (ANOVA: \( F_{1,49} = 23.81, P < 0.0001 \); Fig. 3).

Estimates of both \( l \) and \( I_s \) were significantly higher in the Florida than in the Virginia population (Levene’s homogeneity of variances test: \( F_{1,64} = 33.04, P < 0.0001 \); \( F_{1,64} = 25.15, P < 0.0001 \), respectively; Table 3). An analysis of covariance revealed that Florida males have a significantly steeper estimate of \( \beta_s \) than Virginia males (\( F_{1,64} = 9.32, P = 0.003 \), Table 3, Fig. 4). Both the Virginia and Florida collections exhibited a significant positive relationship between relative fitness and mating success, resulting in slopes of Bateman gradients significantly greater than zero (VA \( P = 0.0007 \); FL \( P < 0.0001 \), Fig. 4).

**Female mating behaviour**

The high variability of the microsatellite markers allowed reconstruction of female genotypes, which we then compared to the genotypes of individual females caught in the field. We reconstructed 77 female genotypes from the Virginia population, 24 from the July Florida collection and 20 from the August Florida collection. The average probability of identity for collected adult females (FL = 8.6
× 10⁻⁷, VA = 8.1 × 10⁻⁹) and reconstructed female genotypes (FL = 3.6 × 10⁻⁷, VA = 3.5 × 10⁻⁹) were low, suggesting that a match between a reconstructed genotype and a female would only occur if we had collected the true mother of a progeny array. Ten adult females in the Virginia population shared identical three-locus genotypes with particular inferred mates of sampled males. Although not present in collected females, two identical reconstructed female genotypes were detected in more than one male’s brood from the Virginia population, indicating that females have the potential to mate with more than one male. The July Florida population yielded five reconstructed maternal genotypes that matched females caught in the field. Two of these reconstructed genotypes were each recovered in the embryos of two distinct males (i.e. each of two females had deposited eggs in two of our sampled males). In addition to these two collected females that mated with at least two males, two additional reconstructed female genotypes were each found in two separate progeny arrays. Thus, two females that we did not collect also mated with at least two males each. Similarly, three females collected from Florida in August possessed genotypes that matched reconstructed maternal genotypes. Hence, both Virginia and Florida populations are polygynandrous, an observation consistent with the data for dusky pipefish reported by Jones & Avise (1997b). Although the July and August collections were taken in the same seagrass meadow, no reconstructed female genotypes matched field-caught females across the two separate collections.

Females from the Virginia and Florida populations differed in the number of eggs transferred per copulation. Females from the Florida population produced significantly more embryos per successful mating event than did those from the Virginia population (FL = 135.1 ± 10.1; VA = 99.9 ± 7.6; Mann–Whitney U-test: χ²₁,₉₀ = 4.3, P = 0.038). Hence, females exhibit a lower rate of multiple mating in Florida but transfer a larger number of eggs per mating event than do females in Virginia. The fact that every offspring has exactly one mother and one father, coupled with our
estimate of male mating success, allows calculation of the mean mating success of females for a given sex ratio. The mean mating success for females (including females with zero mating success) is the product of the ratio of males to females and the mean mating success of all males. By this reasoning, the mean mating success, including non-mating females with zero mating success, for females in Virginia must be about 4.2 on average \( (2.52 \text{ mates per male} \times 150 \text{ males})/91 \text{ females} \), whereas the mean mating success for females in Florida is only about 0.75 mating event per female \( (1.33 \text{ mates per male} \times 36 \text{ males})/64 \text{ females} \), a dramatic difference between populations.

### Population size and density

Mean population size for the Virginia site was estimated to have 653 adult females using the modified Peterson–Lincoln capture–mark–recapture method (Jones & Avise 1997b; Table 1). This result contrasts sharply with the estimates of 122 and 162 adult females in the July and August Florida collections, respectively (Table 1). Given the observed adult sex ratios, these values translate into local breeding population sizes of 1682 adults for the Virginia population and 190 and 254 for the July and August Florida collections, respectively.

Direct measurements of population density based on the numbers of individuals collected given the area seined showed that the Virginia population was between three to six times as dense as the Florida populations (Table 1). This difference in density is similar to the difference in population size estimates based on the modified capture–mark–recapture method, which showed a 6- to 9-fold higher number of breeding adults in Virginia. Overall, these results show clearly that dusky pipefish in the Virginia population occur at higher densities and exhibit larger local breeding populations than those in our Florida population (Table 1).

### Temporal variation in the genetic mating system

It is clear from other studies of syngnathid fishes that both the adult sex ratio and operational sex ratio vary throughout the breeding season, providing the potential for the intensity of sexual selection to vary temporally (Vincent et al. 1995). In earlier studies of Atlantic and Gulf Coast populations conducted throughout the year, females on average tended to be more abundant than males (Brown 1972; Mercer 1973). However, the adult sex ratios tend to show a higher proportion of males during the summer (June to August) than during the fall and winter months (September to April), presumably because males depart the seagrass beds for deeper waters earlier than females (Brown 1972; Mercer 1973). An alternative hypothesis is that males may suffer higher mortality than females, as has been observed in other species with high paternal investment in offspring (Forsgren et al. 2004).

While previous studies (Brown 1972; Mercer 1973; Vincent et al. 1995) of syngnathids have shown that adult and operational sex ratios can vary throughout the year, we did not find temporal variation in several population parameters between the two sampling periods of the Florida collections. The collections made in the same seagrass bed during July and August of 2003 showed similar ASRs and OSRs, as well as similar population densities.
and population sizes (Table 1). Genetic mating system parameters such as $X_{s0}$, $X_{s1}$, $I_s$, and $B_{ss}$ also did not differ significantly between the two time periods. These data suggest that population demographics and the genetic mating system are locally stable over short periods of time.

Between-year temporal variation also has the potential to contribute to meaningful variation in the genetic mating system. However, the genetic mating system also shows a pattern of long-term stability in *Syngnathus floridae*. An earlier study of the Saint Joseph Bay site by Jones & Avise (1997b) in July of 1994 revealed a similar OSP and estimated population size of females despite dissimilar ASRs (Table 1). The Bateman gradient, $\beta_{ss}$, and the number of mates per male also show surprisingly similar estimates between years (Table 3) despite differences in body size (SL), mean number of eggs transferred per copulation by females and mean number of eggs per male. In the Florida 1994 collection, adults were significantly larger, females transferred significantly more eggs per copulation, and males had more viable embryos than in the 2003 collections (Jones & Avise 1997b). The differences in number of eggs transferred per copulation and male reproductive success are a direct result of the larger body size of the 1994 collections and may represent variable growth rates among years. In addition, the differences in body size may also be related to the differences in $I$ and $L$ between the 1994 and 2003 samples. Thus, even though we see some interesting temporal changes in life history and mating parameters across a 9-year period in the Florida population, among-population differences appear to be much larger than the temporal differences. Nevertheless, this study suggests that the examination of temporal variation in pipefish mating systems could be a fruitful area for future research.

**The effect of population density on the genetic mating system**

Higher population densities may facilitate greater rates of multiple mating by both sexes as a consequence of a higher rate of mate encounter (Kokko & Johnstone 2002; Kokko & Rankin 2006). In the present study, the Virginia site exhibited a threefold to sixfold higher population density than the Florida population. Our results are consistent with the prediction that higher population densities result in higher rates of multiple mating since the Virginia population had higher mating and reproductive success. We also observed evidence for significantly stronger sexual selection on males in the less dense Florida population. Lower population density may make it difficult for small, unattractive males to find suitable mates, because larger females probably prefer to mate with larger males, as has been observed in *Syngnathus typhle* (Berglund *et al.* 1988).

While our study represents a comparison of only two populations and hence cannot resolve whether population density affects the genetic mating system of *S. floridae* by itself, it is instructive to consider our results in light of other comparative studies of mating systems as a function of population density. For example, Soucy & Travis (2003) found that rates of multiple paternity were higher in populations with greater densities of adult individuals in the least killifish, *Heterandria formosa*. Several studies of other taxa also have shown a positive relationship between rates of multiple mating and population density. For example, in some species of birds, increasing breeding density appears to be associated with a higher rate of extra-pair paternity at the within-species level (Møller 1991; Reyer *et al.* 1997; Westneat & Sherman 1997; Sellgren & Burke 2001; Charmantier & Perret 2004; Mouget 2004). However, other studies in a variety of taxa have failed to find a positive relationship between population density and multiple paternity. In several species of birds, for example, there appears to be no relationship between extra-pair fertilization frequency and nesting density (Dunn *et al.* 1994; Veiga & Boto 2000; Conrad *et al.* 2001; Ratti *et al.* 2001). Similarly, population density was not positively related to the frequency of concurrent multiple paternity in *Drosophila melanogaster* (Ochando *et al.* 1996). Very few studies thus far have focused on nonavian taxa, so additional research on the relationship between population density and mating patterns in a wider variety of taxa is clearly warranted.

**Other environmental factors that may affect the genetic mating system**

In addition to population density, a wide range of other parameters could conceivably contribute to the observed variation in mating patterns between our populations. Differences in mating preferences among males and females with respect to body size (Berglund *et al.* 1988), parasite load (Rosenqvist & Johansson 1995), or ornaments (Berglund & Rosenqvist 2001) may contribute to variation in local genetic mating systems. A large number of biotic and abiotic environmental factors, such as food availability (Kvarnemo 1997) and predation (Kelly *et al.* 1999; Bronikowski *et al.* 2002), may also shape genetic mating systems. Probably the most important abiotic environmental parameter for pipefish reproduction is water temperature. Previous investigations of *S. typhle* show that as little as a 4 °C difference in temperature regimes has a large effect on the potential reproductive rates of males and females (Ahnesjö 1995; Kvarnemo & Ahnesjö 1996). Our Virginia population occupied a site with lower mean water temperatures than those experienced by the Florida population. The yearly temperature for the Chesapeake Bay near the York River, Virginia averages 15 °C (data available from the Chesapeake Bay Observing System, www.cbos.org), whereas the yearly surface water temperature of Panama City Florida (~50 km from Saint Joe Bay) averages 20 °C.
Conclusions

Understanding the biotic and abiotic factors affecting genetic mating systems is a central goal of much research in evolutionary biology and behavioural ecology. Our study contributes to this goal by providing empirical evidence that critical mating system parameters, such as $\tilde{X}_{\text{mat}}, \tilde{X}_{\text{rep}}, I, I_d,$ and $\beta_{\text{mat}}$ can vary among populations within a species. Such variation may underlie divergent evolutionary trajectories for population-specific morphology, and thus play a significant role in the process of speciation. Much more work on geographical variation in genetic mating systems of this and other species will be required for a complete understanding of the ecological factors contributing to mating system evolution.

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