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Microsatellite evidence for monogamy and sex-biased recombination in the Western Australian seahorse *Hippocampus angustus*

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Abstract

Four polymorphic microsatellite loci were used to assess biological parentage of 453 offspring from 15 pregnant males from a natural population of the Western Australian seahorse *Hippocampus angustus*. Microsatellite genotypes in the progeny arrays were consistent with a monogamous mating system in which both females and males had a single mate during a male brooding period. Multilocus genotypes implicated four females in the adult population sample as contributors of eggs to the broods of collected males, but there was no evidence for multiple mating by females. Based on genotypic data from the progeny arrays, two loci were linked tightly and the recombination rate appeared to be ≈ 10-fold higher in females than in males. The utility of linked loci for parentage analyses is discussed.

Keywords: linkage disequilibrium, mating system, parentage, pipefish, sex-role reversal, sexual selection

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Introduction

Similar to other fishes in the family Syngnathidae, seahorse males provide all postzygotic care. A female transfers eggs to a male's ventral pouch where he fertilizes them and provides protection, nutrients and osmoregulation to the developing brood until parturition (Haresign & Shumway 1981; Berglund *et al*. 1986). Unlike many syngnathids, seahorses are socially monogamous, exhibiting a long-term pair bond between mated individuals (Vincent 1994a; Vincent & Sadler 1995; Masonjones & Lewis 1996). This interaction manifests itself as predictable, ritualistic daily greetings that can be observed consistently among the same pairs of individuals throughout a breeding season (Vincent & Sadler 1995).

A recent plethora of molecular studies documenting extra-pair paternity in avian species underscores the need for care in relating a social mating system to a realized genetic mating system (reviews in Birkhead & Møller (1992), Avise (1994) and Westneat & Webster (1994)). Observationally, the social systems of studied seahorse species resemble those of some socially monogamous birds, in which situations between two extremes are possible: (i) social monogamy reflects an underlying genetic mating system that truly is monogamous (Decker *et al*. 1993; Dickinson *et al*. 1995; Mauck *et al*. 1995); or (ii) social monogamy conceals a polygamous genetic mating system replete with mating infidelities by one or both sexes (e.g. Lifjeld *et al*. 1993; Blakey 1994; Yezerinac *et al*. 1995; Burley *et al*. 1996).

Studies of biological parentage in seahorses assume greater significance when we consider that other syngnathids for which the genetic mating system has been investigated are either polyandrous (as in the Gulf pipefish *Syngnathus scovelli* (Jones & Avise 1997a)) or polygynandrous (dusky pipefish *S. floridae* (Jones & Avise 1997b)). Laboratory and field observations suggest that polygamy also characterizes some other pipefish species (*S. typhle* (Berglund *et al*. 1988), *Nerophis ophidion* (Rosenqvist 1993)). In seahorses, although males and females of a mated pair greet each other daily, much of their time is spent apart (Vincent & Sadler 1995), such that possible opportunities for extra-pair matings do exist.

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Seahorses have been discussed as classic examples of sex-role reversal in the sense that a high investment in offspring by fathers was thought to promote stronger sexual selection on females than on males (Trivers 1972; Williams 1975). In other words, males (being a limiting resource in reproduction) presumably evolve to be choosy and females compete for access to mates. Ironically, the only syngnathid species studied thus far that appears to lack sex-role reversal (under the definition given above based on asymmetric intensity of sexual selection) is a seahorse (Vincent 1994a). In general, mating systems may be important determinants of the strength and direction of sexual selection in syngnathids (Vincent *et al*. 1992). Thus, a comparison of the genetic mating systems of seahorses with those reported previously for sex-role-reversed pipefish species may provide insights into the evolution of mating systems and sexual selection in the Syngnathidae.

Microsatellite loci offer great power for assessing biological parentage, and they have been employed successfully to study mating behaviour in natural populations of several fish species (Kellogg *et al*. 1995; Colbourne *et al*. 1996; Parker & Kornfield 1996; Jones & Avise 1997a,b; Jones *et al*. 1998). The primary goal of the current study was to assess the genetic mating system of the Western Australian seahorse (*Hippocampus angustus*) using microsatellite markers. Two of the cloned microsatellite loci proved to be linked, so a secondary goal was to assess the recombination rate between these loci and to consider the consequences of linkage for parentage analysis.

Materials and methods

Collections

Specimens were collected by SCUBA in coastal waters 5–18 m deep, immediately south of Perth, Australia on 14, 20 and 28 January 1997. Pregnant males were returned live to the laboratory and held in tanks until birth of their progeny. Fifty progeny sampled at random from each brood were frozen for microsatellite analysis, as were the known fathers and the adult females also collected. Other individuals were returned live to the collecting locale.

Microsatellite loci

Attempts to obtain polymorphic products in *Hippocampus angustus* using available polymerase chain reaction (PCR) primers for pipefish microsatellites (Jones & Avise 1997a) failed. Thus, novel microsatellite loci were cloned from a single *H. angustus* individual from which genomic DNA was extracted using a standard proteinase K, phenol–chloroform procedure. The DNA was digested with *Nde*II and the 300–700 bp fragments were ligated into *Bam*HI digested, dephosphorylated pBluescript phagemid (Strategene, La Jolla, CA, USA). The resulting product was transformed into competent XL1-Blue *Escherichia coli* (Stratagene). This partial genomic library was screened with two distinct cocktails of radiolabelled oligonucleotides: $(GT)_{10}$, $(GGAT)_4$, $(GACA)_4$, and $(TAG)_6$, followed by $(GATA)_4$, $(GA)_{10}$, $(TCC)_5$, and $(TTAGGG)_3$. Of ≈ 1000 colonies screened, 20 clones hybridized to one or more of the oligonucleotides, and their inserts were sequenced using the *fmol* DNA sequencing system (Promega, Madison, WI, USA). Four pairs of microsatellite-flanking primers were designed.

Tissue samples from adults and fry were prepared for PCR using the Gloor & Engels (1992) technique as described in Jones & Avise (1997a). Before PCR, one primer was end-labelled with 1 μCi [γ^{32}P]-ATP per 5 pmol of primer. The 10 μL reaction mixture consisted of 1 × Promega *Taq* buffer, 1.25–2.0 mM $MgCl_2$, 0.15 μM of each primer, 0.1 mM of each dNTP, and 0.5 units Promega *Taq* polymerase. The thermal cycling, preceded by 2 min at 95 °C and followed by 4 min at 72 °C, consisted of 30 cycles of 95 °C for 1 min, an optimal annealing temperature for 1 min, and 72 °C for 1 min. The loci *Han*03 and *Han*05 were amplified with 1.25 mM $MgCl_2$ and an annealing temperature of 54 °C, whereas *Han*06 and *Han*15 were amplified with an annealing temperature of 50 °C, and $MgCl_2$ concentrations of 2.0 mM and 1.5 mM, respectively.

Fifteen broods (453 total fry) were genotyped successfully for *Han*03 and *Han*05, together with the fathers of 13 of the broods and 26 adult females. Two fathers did not yield useable DNA despite repeated attempts (in each case the specimen had been left at room temperature for an extended time before DNA was extracted). Additional loci were used to establish four-locus genotypes for the inferred mothers of broods of special interest (see below), and the microsatellite locus *Han*06 was assayed in a large number of progeny to investigate its linkage with *Han*03.

Results

Hardy–Weinberg and linkage

All four microsatellite loci were polymorphic, displaying from nine to more than 22 alleles per locus (Fig. 1, Table 1). Observed heterozygosities ranged from 0.727 to 0.949 across loci (Table 1). In the adult sample, no significant deviations from Hardy–Weinberg proportions were detected (exact test in GENEPOP version 3.1 (Raymond & Rousset 1995)), and no significant genotypic disequilibria between pairs of loci were observed. However, tests for independent assortment within progeny arrays revealed strong linkage disequilibria for *Han*03 and *Han*06 at the within-family level (contingency χ^2, $P < 0.001$). All other pairs of loci appeared to assort in independent Mendelian fashion within families and, thus, provided no indication

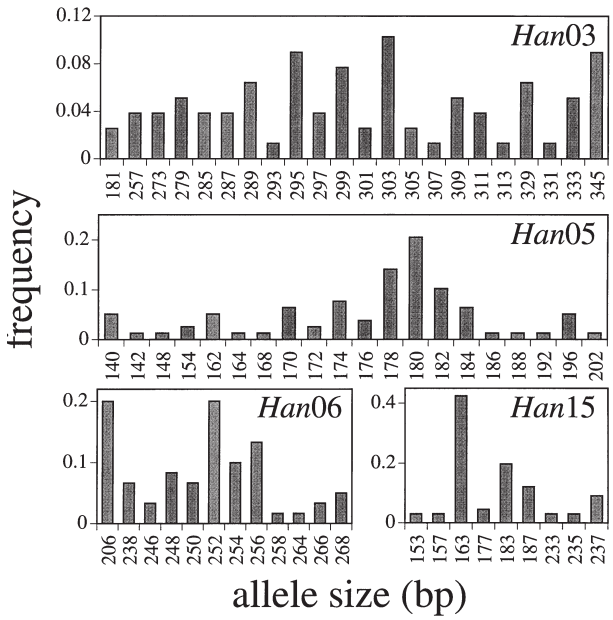

Fig. 1 Allele frequency histograms for four microsatellite loci in *Hippocampus angustus*. Allelic designations represent sizes in base pairs (bp) of the amplified product. For the locus *Han*03, several alleles greater than 340 bp in length were grouped together in the 345 bp class.

of linkage. The inheritance pattern of alleles within families and the lack of deviations from Hardy–Weinberg equilibrium indicated that null alleles were not present at detectable frequencies for any locus. Exclusion probabilities (Chakraborty *et al*. 1988) are shown in Table 1.

Mating system assessment

The 15 males assayed in this study gave birth to a mean of 458.4 offspring, of which ≈ 30 were assayed per brood (Table 2). For 13 of the broods, the father's genotype was available and the mother's allelic contribution to each embryo could be determined unambiguously by subtraction, except in the rare cases in which an embryo's genotype was the same as the father's. For a more complete description of the methodological details, see Jones & Avise (1997a). In no case did a brood display more than two maternal alleles per locus, suggesting that each brood had a single mother. The two broods for which the paternal genotypes were not available also contained progeny genotypes consistent with a single pair of parents (Table 3). Thus, no evidence was uncovered that any pregnant male had received eggs from more than one female. Also, multiple mating by females was not in evidence in our sample, as each mother's inferred genotype proved unique to a single brood (Table 2).

By comparing the genotypes of the inferred mothers of assayed broods with the two-locus genotypes of field-collected adult females, four matches were identified (i.e. in each case a female in the field sample displayed the same genotype as an inferred mother; Table 2). These matches were investigated further using *Han*06 and *Han*15, and in each case a four-locus match resulted. The expected frequencies of these four-locus genotypes range from 1.2×10^{-8} to 7.6×10^{-9} if all four loci are considered to be in linkage equilibrium, or from 1.6×10^{-5} to 7.0×10^{-7} if *Han*06 is (conservatively) disregarded by virtue of its linkage to *Han*03. In either case, the results suggest that the four collected females are the true mothers of the four broods.

Two broods contained progeny for which the brooding male appeared to be excluded as the true father at one or more loci. In the first instance, a single embryo in the brood of male SM69 had the genotype 178/188 at *Han*05, whereas the father had the genotype 180/184. This embryo was typed for the other three loci, none of which confirmed the exclusion. As allele 178 appears to be derived from the

Table 1 Summary information for each assayed microsatellite locus of *Hippocampus angustus*. Shown are polymerase chain reaction (PCR) primer sequences, the microsatellite motif in the cloned sequence, the number of alleles observed in the *n* adult seahorses, observed and expected heterozygosities, and the average exclusion probability (Chakraborty *et al*. 1988)

Locus	Primer sequences (5'–3')	Cloned repeat	No. of alleles	*n*	Heterozygosity		Exclusion probability
					observed	expected	
*Han*03	AAATCTGTTACGAAATCTTATGAA GTTAGGGGCTGACATTTAATC	$[GT]_{43}$	22	39	0.949	0.952	0.878
*Han*05	TGGAATACAGATGACAAACAAGA CCCAAAGTACACTCAATCACAG	$[GT]_{43}$	20	39	0.949	0.913	0.805
*Han*06	TTGGTTTCGCACTGACAT ATAGTTGGGAGTATTGTTACATTAT	$[GT]_{18}$	12	36	0.917	0.877	0.731
*Han*15	GATCAAAACATTCTACTCAGTATT TGATATTGGCTTTCACTGC	$[GT]_{9}$	9	33	0.727	0.764	0.560

Table 2 Two-locus genotypes of the 13 males for which complete genotypic information was available. Also shown are the deduced genotypes of each male's mate. Columns 2–3 indicate the total number of progeny carried by the male and the number of these that were assayed for both *Han*03 and *Han*05. Also shown is the expected probability of identity (assuming random mating) for the inferred maternal genotype. Mothers present in the adult population sample have an alpha-numeric I.D. shown in the last column (n.p. indicates an inferred mother who was not present in the collection)

			Father's genotype		Mother's genotype			
Male I.D.	No. of fry	No. assayed	*Han*03	*Han*05	*Han*03	*Han*05	Prob. (iden.)	Female I.D.
SM06	118	30	181/289	142/180	285/295	170/174	6.8×10^{-5}	n.p.
SM15	309	30	273/303	178/180	295/295	172/184	2.6×10^{-5}	n.p.
SM19	374	31	333/345	176/196	295/301	188/196	6.1×10^{-6}	n.p.
SM29	639	30	287/299	182/182	299/303	182/188	4.1×10^{-5}	SF47
SM31	—	30*	257/305	178/180	295/299	148/186	4.5×10^{-6}	n.p.
SM43	208	30	181/287	182/202	311/345	140/154	1.8×10^{-5}	SF72
SM50	720	30	273/279	140/180	285/303	180/180	3.3×10^{-4}	n.p.
SM64	—	16	295/305	174/180	303/345	180/184	4.8×10^{-4}	n.p.
SM66	463	30	311/333	170/180	257/287	182/204	7.8×10^{-6}	n.p.
SM68	582	30	329/345	178/182	273/301	140/174	1.6×10^{-5}	n.p.
SM69	280	30†	295/309	180/184	279/329	170/178	1.2×10^{-4}	SF07
SM70	582	30	285/287	172/174	285/345	180/190	3.6×10^{-5}	n.p.
SM80	589	30	295/309	140/180	285/329	174/180	1.6×10^{-4}	SF57

*An additional 15 assayed embryos originally attributed to SM31 (not shown) appeared from molecular data to have been involved in a sample mix-up (see text).
†One of the 30 typed embryos of SM69 had the genotype 178/188 at *Han*05. This apparent exclusion most probably resulted from a mutation.

mother (Table 2), the most likely explanation is that a germline mutation, consisting of an insertion of either 4 or 8 bp, occurred at the *Han*05 locus.

The second unusual pattern occurred in the brood of SM31. Among 45 offspring assayed for *Han*03 and *Han*05, 15 were excluded as SM31's progeny at one or both loci. These 15 fry had genotypes consistent with a single mother and father for the entire group (i.e. no more than four alleles per locus), but they clearly were not the progeny of either SM31 or the mother of the other 30 embryos (which SM31 did sire). Furthermore, the genotypes of these 15 offspring indicated that they could not have been produced by any of the other males assayed in the study. Our preferred post hoc explanation is that a male who appeared not to be pregnant gave birth to these fry (without our notice), that the fry became mixed with SM31's true progeny, and that the additional male was returned to the collecting site without being assayed. We cannot, however, rule out other possibilities. In any case, all of the progeny assayed from SM31 are consistent with a monogamous mating system.

Sex-biased recombination

Although *Han*03 and *Han*06 displayed no detectable genotypic disequilibrium at the population level, within-

Male I.D.	Embryo genotypes								Parental genotypes
	Class 1	*n*	Class 2	*n*	Class 3	*n*	Class 4	*n*	
SM27	140/170	10	140/182	8	154/170	9	154/182	6	140/154 ×170/182
SM36	154/172	8	154/180	9	170/172	9	170/180	6	154/170 ×172/180

Table 3 Progeny array data from the locus *Han*05 for the two families (SM27, SM36) for which paternal genotypes were not determined (due to sample degradation). Shown are the four classes of embryo genotypes observed in each progeny array, the number of assayed embryos that fell into each class (*n*), and the most plausible parental genotypes that could have led to such a progeny array. These offspring genotypes are consistent with monogamous pairings. A similar result was obtained by assaying the same embryos for locus *Han*03

family tests indicated a strong nonrandom association of alleles at the two loci. To document this more fully, 10 broods in which one or both parents were heterozygous at both loci were assayed, with the results shown in Table 4. Because each brood had a single parent pair, recombination events between the two loci in the male parent will result in progeny that differ in linkage phase from the father. Similarly, recombination in the female parent results in progeny that differ in linkage phase from the mother.

Among a total of 418 assayed opportunities for recombination, only 21 recombinant chromosomes were observed, 19 of which were deduced to have occurred in the production of female gametes. This represents a significant departure from the null expectation of equal recombination in each gender ($\chi^2 = 13.76$, d.f. = 1, $P < 0.01$).

Discussion

The genetic mating system

The microsatellite loci employed were sufficiently variable to resolve the genetic mating system that produced the assayed broods of *Hippocampus angustus*. The molecular data indicate that this population is genetically monogamous.

In principle, our capacity to detect multiple matings could have been limited if: (i) a male mated with two females of identical genotype; (ii) a male mated with two homozygous females; or (iii) limited sampling of broods resulted in a failure to detect some genotypes (see Jones & Avise (1997a) for a detailed discussion). However, given the low expected frequencies of two-locus genotypes among the inferred *H. angustus* mothers (Table 2) and the scarcity of homozygotes (Table 1), the first two possibilities are unlikely. Nonexhaustive sampling is the most likely source of error, but, in a multiply mothered brood, a random sample of 30 fry virtually ensures sampling of both mothers' progeny if maternity is equally shared ($P > 0.999$). The same sample gives a > 0.95 binomial probability of including at least one fry from a second female who mothered only 10% of the progeny in a brood.

To investigate the consequences of combining these different sources of error, we ran computer simulations involving two-mother broods. For each simulated brood, two-locus genotypes for the two hypothetical mothers were assigned randomly, based on population allele frequencies for *Han*03 and *Han*05. Each brood contained 458 embryos (the mean in our *H. angustus* sample) and was constructed by assigning a predetermined proportion of offspring to the first mother and the rest to the second mother. Based on its predetermined maternity and the genotype of its mother, each embryo was randomly assigned one maternal allele for each of the two loci. Finally, a random sample of 15, 30 or 60 offspring genotypes was drawn from each 458-embryo brood without replacement. If three or more maternal alleles were present in the sample at either locus, multiple mating was deemed to have been documented. Otherwise, multiple mating remained undetected. For each combination of sample size (15, 30 or 60) and each proportion of embryos from the first mother (0.50, 0.60, 0.70, 0.80, 0.85, 0.90, 0.95, 0.97, 0.98, or 0.99), 5000 complete simulations were run.

The results of the simulations are shown in Fig. 2. With maternity equally shared (229 fry from each mother), the proportion of samples for which multiple maternity was detected was 0.999, whether the sample of progeny was 15, 30 or 60. As maternal contributions became more skewed, the ability to detect multiple matings understandably dropped. For example, when one female mothered 90% of the progeny and 30 progeny were sampled per brood, the proportion of samples in which multiple maternity was detected was 0.949; and, with 98% of the fry attributable to one mother, multiple maternity was detected with a probability of 0.437 using a sample of 30

	Recombination in males		Recombination in females	
Male I.D.	No. of tests	No. of recombinants	No. of tests	No. of recombinants
SM06	30	0	0	0
SM19	0	0	30	4
SM29	0	0	8	2
SM43	28	0	28	0
SM50	16	1	0	0
SM66	25	0	25	0
SM68	29	1	29	2
SM69	29	0	29	5
SM70	28	0	28	2
SM80	28	0	28	4
Total	213	2	205	19

Table 4 Observed recombination events between *Han*03 and *Han*06 within the assayed families. The number of tests is the number of progeny for which the father, mother, or both were heterozygous at both assayed loci. The number of recombinants indicates the number of fry whose linkage phase differed from either the father (sperm) or mother (eggs). Significantly more recombination events occurred in females than in males ($\chi^2 = 13.76$, d.f. = 1, $P < 0.01$)

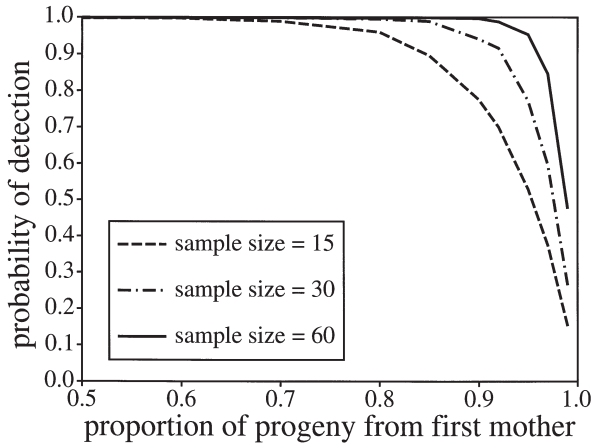

Fig. 2 Our ability to detect multiple maternity in an average male's brood was investigated using computer simulations (see text). Each simulated brood of 458 fry (the mean brood size in our sample) was created from two mothers with randomly assigned genotypes for *Han*03 and *Han*05. A sample of 15, 30 or 60 fry was drawn at random without replacement from each brood. If three or more alleles were present at either locus in the sample, multiple mating in the brood was considered to have been detected. The probabilities are based on 5000 replicates for each proportion/sample size combination.

progeny. Most of the failures to detect multiple mating were, as expected, due to nonexhaustive sampling of broods. From these simulations and our genetic results, we conclude that multiple maternity within a brood rarely (if ever) occurred in our sample of *H. angustus*.

Inferring monogamy for females is more problematic because the inferred mothers in our sample, although they mated with only a single collected male, may have mated with other males that were not sampled. The probability of this occurrence depends upon the size of the breeding population in comparison with the sample size. Although microsatellite-based studies of pipefish mating systems have found evidence for multiple mating by females (Jones & Avise 1997a,b), an absence of evidence should be interpreted with caution, given the low power to detect this phenomenon from field samples. In addition, the available genetic data cannot address whether males mate with the same partners sequentially over one or more breeding seasons, or whether there exists temporal or interpopulation variation in the genetic mating system.

The results of the genetic analysis of *H. angustus* are in agreement with behavioural studies of other seahorse species. Laboratory observations have documented monogamous mating systems for *H. fuscus* (Vincent 1994a) and *H. zosterae* (Masonjones & Lewis 1996), and extensive field data suggest that *H. whitei* is also monogamous (Vincent & Sadler 1995). These uniformly monogamous outcomes in seahorses are in contrast to the variously polygamous mating systems of pipefishes in the genus *Syngnathus*, where populations of *S. scovelli* and *S. floridae* were documented by microsatellites to be polyandrous and polygynandrous, respectively (Jones & Avise 1997a,b).

Behavioural studies on sexual selection in the Syngnathidae show that the polygamous pipefish species thus far studied are sex-role reversed (*sensu* Vincent *et al*. 1992), with sexual selection operating more strongly on females than on males (Rosenqvist 1990; Berglund 1991; Berglund & Rosenqvist 1993; Berglund *et al*. 1997). Furthermore, from the limited number of comparisons available, sexual selection appears to operate more strongly on populations of species that are more highly polyandrous (Berglund *et al*. 1986; Jones & Avise 1997a,b). By contrast, monogamous seahorse species seem not to be sex-role reversed, experiencing somewhat stronger sexual selection on males than on females (Vincent *et al*. 1992; Vincent 1994a; Masonjones & Lewis 1996). *H. angustus* conforms to this pattern, as behavioural observations suggest that males are more competitive than females for mates, even though the relative sexual monomorphism suggests that sexual selection is weak (G. I. Moore, unpublished data).

Recent theory suggests that the direction of sexual selection may be determined primarily by the relative rate at which males and females can engage in reproduction; sexual selection should operate more strongly on the sex that can reproduce more rapidly (Clutton-Brock & Parker 1992; Parker & Simmons 1996). For some pipefishes, females are able to produce more eggs than males can brood (Berglund *et al*. 1989; Ahnesjö 1995). This disparity may also exist for seahorses (Vincent 1994b), yet the operation of sexual selection differs between these taxa. As suggested by Vincent (1994b), the answer may lie partly in differences in the physiology of egg production. A female pipefish can mature eggs continuously, whereas a female seahorse matures an entire clutch before it is delivered to the male's pouch (Wallace & Selman 1981; Vincent 1994b). Hydration of the eggs during clutch maturation in *H. fuscus* requires 3 days, and the eggs are dumped within 24 h if a male is unavailable to receive them (Vincent 1994b). Thus, females should not begin hydrating a clutch unless they are certain that a receptive male will be available to receive it. The male reproductive rate may be maximized by remaining with a female, as switching to a new female will require a further 3-day premating association. Potential reproductive rates of male and female seahorses may, therefore, be linked by the necessity to synchronize clutch hydration with male availability. Such reproductive synchronization may favour the evolution of a monogamous mating system and would constrain sexual selection intensity on females (Price *et al*. 1987).

Interestingly, there are pipefish species that exhibit behavioural monogamy (reviewed in Vincent *et al*.

(1992)) and it would be informative to know if they also exhibit genetic monogamy. If so, an additional question would be whether they have clutch, as opposed to continuous, egg maturation. With more than 30 species in the genus *Hippocampus*, the possibility remains that some seahorse species depart from strict monogamy, and these species would be of special interest for interpreting mating system evolution and sex-role reversal in the family Syngnathidae. Genetic monogamy, polyandry, and polygynandry have now been documented in various syngnathid taxa. The only mating pattern not yet observed is strict polygyny, in which a male mates with multiple females but each female deposits eggs in the brood pouch of only one male.

Linked loci in parentage studies

Two microsatellite loci cloned from *H. angustus* exhibited strong linkage disequilibrium within families. This observation raises the issue of the utility of linked loci for genetic analyses of parentage. Physically linked loci need not be in complete gametic-phase disequilibrium within a population and, thus, may still provide exclusionary power (Chakraborty & Hedrick 1983). For *Han*03 and *Han*06, the overall recombination rate (r) is 0.05. Because any initial disequilibrium decays by a factor of $1 - r$ per generation in a random mating population (Hedrick 1985; Hartl & Clark 1989), within ≈ 50–100 generations such loci should approach gametic-phase equilibrium at the population level. Tests for genotypic disequilibria within the adult population sample of *H. angustus* were nonsignificant, but with 22 and 12 alleles at the two loci, our ability to detect such associations is extremely limited, and a sample much larger than 36 adults would probably be necessary (Thompson *et al*. 1988).

For studies in which many progeny are assayed per parent, the linkage phase of each parental chromosome can be determined and the two linked loci can be viewed as one super-locus. Under linkage equilibrium, the expected number of alleles at this super-locus is equal to the product of the number of alleles at each individual locus, and the expected frequency of each super-allele is the product of the frequencies of the two alleles at the separate loci. Combining *Han*03 and *Han*06 in this way results in a total of 264 expected alleles at the super-locus, each in extremely low frequency.

An interesting outcome of this exercise is that by combining two loci in this manner, an increase in the expected exclusion probability can arise for the super-locus as compared with two comparable unlinked loci. As unlinked loci, the combined exclusion probability for *Han*03 and *Han*06 is 0.967. However, as a super-locus with 264 alleles, the exclusionary probability is 0.984. In effect, this increase in exclusionary power results from the added information (beyond the two-locus genotype *per se*) provided by knowledge of the linkage phase for the two loci in each individual.

This increase in exclusionary power is a general phenomenon, and the relative benefit is greater with less informative loci (Fig. 3). For example, for two independent, hypothetical loci with four equally frequent alleles, the combined exclusion probability is only 0.754. However, with these two loci physically linked, but in complete linkage equilibrium (a 16 allele super-locus), the exclusion probability increases to 0.872. These results are in agreement with previous treatments of the same phenomenon for di-allelic loci (Chakraborty & Hedrick 1983; Smouse & Chakraborty 1986). In practice, it may seldom be feasible to use linked loci in this way, due to difficulties in detecting linkage disequilibrium, estimating allele frequencies, and determining the linkage phase of individuals in finite population samples, but this exercise demonstrates that the potential utility of linked loci in parentage assessments should not necessarily be ignored.

Sex-biased recombination

An unanticipated finding is that recombination rates between *Han*03 and *Han*06 in *H. angustus* are ≈ 10-fold higher in female gametes than in male gametes (Table 4). Moderate to extreme differences in the recombination rate between the sexes have been observed in many taxa. For example, the autosomal genetic map in humans is ≈ 90% longer in females than in males (Donis-Keller *et al*. 1987). In the fruit fly *Drosophila melanogaster*, males show no

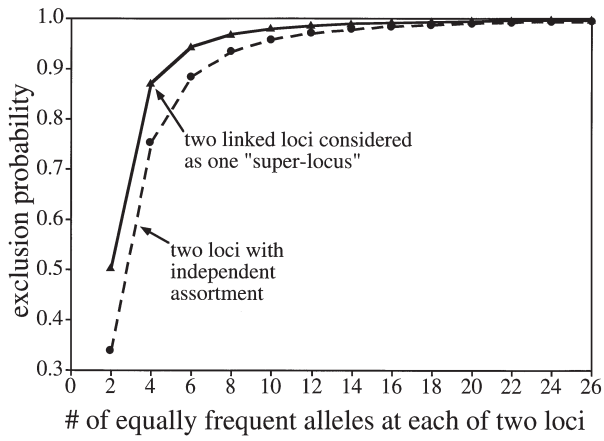

Fig. 3 Comparison of the exclusion probabilities for (i) two loci in linkage equilibrium which behave as if unlinked and (ii) two physically linked loci in linkage equilibrium for which the linkage phase can be discerned with certainty. For each comparison, the two loci are assumed to have the same number (2–26) of equally frequent alleles. The exclusion probabilities are calculated for the two independent loci as in Chakraborty *et al*. (1988) and for the two linked loci by combining them into a single super-locus in which each allele is a composite haplotype (with one allele from each locus).

recombination (Morgan 1914); in the moth *Bombyx mori*, females lack recombination (Tazima 1964); and in several species of marsupials, recombination is reduced fivefold or more in females (Bennett *et al*. 1986; van Oorschot *et al*. 1992). Other studies have documented that recombination rates and gender biases can vary strikingly among different segments of a genome (Davisson & Roderick 1981; Pàldi *et al*. 1995). Whether female-biased recombination in *H. angustus* is a genome-wide phenomenon remains to be determined.

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This work represents a continuing effort by Adam G. Jones to apply microsatellite markers to the study of mating systems and sexual selection in natural fish populations. Charlotta Kvarnemo and Leigh W. Simmons focus on the influence of operational sex ratios on the control of sexual selection and on sperm competition. This study is part of a Master of Science project on the Western Australian seahorse by Glenn I. Moore. The molecular assays were carried out in the laboratory of John C. Avise, whose research interests are in the application of molecular markers to questions in organismal behaviour and natural history.
