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Surprising similarity of sneaking rates and genetic mating patterns in two populations of sand goby experiencing disparate sexual selection regimes

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Abstract

Molecular markers have proved extremely useful in resolving mating patterns within individual populations of a number of species, but little is known about how genetic mating systems might vary geographically within a species. Here we use microsatellite markers to compare patterns of sneaked fertilization and mating success in two populations of sand goby (*Pomatoschistus minutus*) that differ dramatically with respect to nest-site density and the documented nature and intensity of sexual selection. At the Tvärminne site in the Baltic Sea, the microsatellite genotypes of 17 nest-tending males and mean samples of more than 50 progeny per nest indicated that approximately 35% of the nests contained eggs that had been fertilized by sneaker males. Successful nest holders mated with an average of 3.0 females, and the distribution of mate numbers for these males did not differ significantly from the Poisson expectation. These genetically deduced mating-system parameters in the Tvärminne population are remarkably similar to those in sand gobies at a distant site adjoining the North Sea. Thus, pronounced differences in the ecological setting and sexual selection regimes in these two populations have not translated into evident differences in cuckoldry rates or other monitored patterns of male mating success. In this case, the ecological setting appears not to be predictive of alternative male mating strategies, a finding of relevance to sexual selection theory.

Keywords: cuckoldry, mating strategies, microsatellites, *Pomatoschistus minutus*, sexual selection

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Introduction

Lineages in which alternative mating strategies have evolved provide excellent opportunities to investigate behavioural and morphological evolution. In such populations, males often exhibit dramatic phenotypic variation (Gross 1984; Sinervo & Lively 1996; Shuster & Sassaman 1997), which can be maintained by a variety of factors involving both genetic polymorphisms and phenotypic plasticity. Alternative mating strategies in animal populations can take several forms (Thornhill & Alcock 1983; Birkhead &

Møller 1992; Taborsky 1994), perhaps the most common of which occurs as a dichotomy involving nest-holding males or suitors who attract females using dominance or courtship, and sneaker males who 'parasitize' the reproductive efforts of suitor males by fertilizing eggs through stealth or force (Taborsky 1994; Gross 1996; Henson & Warner 1997). This phenomenon certainly can affect the evolution of the male phenotype in interesting ways, but it also may be important because of its potential influence on the dynamics of mating conflicts within and between the sexes (Alonzo & Warner 1999a).

Despite extensive empirical and theoretical efforts (Davies 1991; Birkhead & Møller 1992; Gross 1996), there remain substantial gaps in our understanding of the factors that contribute to the evolution and maintenance of

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alternative mating strategies. Until recently, one formidable barrier to the empirical study of differing mating tactics was the difficulty of assessing male fertilization success. This situation changed with the advent of DNA fingerprinting and microsatellite-based techniques which can document parentage and, hence, provide opportunities to reconcile observed social interactions with eventual reproductive success (Hughes 1998). These molecular techniques have been employed to study extra-pair fertilizations in birds (Birkhead & Møller 1992; Avise 1994; Westneat & Webster 1994), parentage in mammals (Coltman *et al.* 1999; Gagneux *et al.* 1999; Wilmer *et al.* 1999), and (to a much lesser extent) mating systems in fishes (Rico *et al.* 1992; Jones & Avise 1997a,b; DeWoody *et al.* 1998, 2000; Jones *et al.* 1998), amphibians (D'Orgeix & Turner 1995), reptiles (Bull *et al.* 1998; McCracken *et al.* 1999; Pearse *et al.* submitted) and other animals (Evans 1998; Fjerdingstad *et al.* 1998; Harshman & Clark 1998; Urbani *et al.* 1998). Such techniques promise continued progress in the study of mating-system evolution in natural populations.

Alternative reproductive strategies can evolve as the result of two different types of selective pressures. First, in the face of strong sexual selection, males may evolve alternative strategies that provide various methods by which they can compete for access to the gametes of females (Taborsky 1994; Gross 1996). In this case, the alternative male strategies evolve as a result of disruptive sexual selection on the male phenotype. Second, in species with parental care, alternative reproductive strategies may evolve as a way for individuals to avoid parental responsibilities and thereby increase their reproductive potential beyond the constraints imposed by the limited number of offspring for which they can care. This selective pressure can result in the evolution of alternative reproductive strategies in the absence of strong sexual selection. Intraspecific brood parasitism by female birds, for example, provides one such example (McRae 1997; Alves & Bryant 1998; Zink 2000). In many fish species, males care for offspring and nevertheless experience strong sexual selection. In such species, both types of selective pressures probably contribute to the evolution of alternative mating strategies. Thus, a change in the strength of sexual selection typically should be expected to affect the intensity of disruptive selection for male reproductive tactics.

One way to investigate this expectation is to compare mating patterns in distinct populations that differ with respect to relevant ecological factors or phenotypic features (Kelley *et al.* 1999). Most genetic studies have been restricted to a single population, so little is known about geographical variation in animal genetic mating systems and its possible association with reproductive ecology. In this study, we used microsatellite markers to compare the genetic mating systems of two populations of the sand

goby (*Pomatoschistus minutus*) that differ substantially in terms of sexual selection regimes due to a difference in nest-site availability (Forsgren *et al.* 1996). Our expectations were that mating patterns and rates of fertilization by sneaker males would differ in these populations, because both the genetic mating system and the evolution of mating tactics should be tightly linked to the intensity of sexual selection (Andersson 1994; Arnold & Duvall 1994; Sinervo & Lively 1996).

Biological background

The sand goby is a small marine fish common in shallow waters along European coasts (Miller 1986). Males provide all the parental care in nests constructed under hard substrates such as mussel shells or stones. The spawning process (Forsgren 1997) begins when a female briefly inspects and then enters a nest. She attaches eggs in a single layer to the nest's ceiling where they are fertilized, typically by the nest-holding male who will fan and protect the developing embryos for 1–3 weeks until they hatch (Lindström & Hellström 1993). Sneaking behaviour has been observed in some sand goby populations (Forsgren 1997), wherein a sneaker male enters the nest immediately after the female. Typically, the nest holder quickly evicts the intruder, but the sneaker might achieve an unknown percentage of fertilizations. As is also true for the common goby, *Pomatoschistus microps* (Magnhagen 1994, 1998), a distinct sneaker morph apparently does not exist in the sand goby. Rather, sneaking is thought to be a facultative strategy adopted by young males or by unsuccessful nest holders (Magnhagen 1994, 1998).

Our two study populations (Fig. 1) differ in several important ways (Table 1), not the least of which is a nearly 50-fold difference in the density of potential nest sites. To construct a nest, a male goby requires a suitable substrate. At the Tvärminne location, nest sites are relatively rare (0.2/m²) and 41% are occupied. By contrast, nest sites at Klubban are abundant (9.6/m²), but only 0.2% are occupied during the breeding season. These discrepancies have important consequences for the relative strengths of sexual selection. Tvärminne males compete intensely for access to good nest sites (Lindström 1988), whereas Klubban males have the option to build nests without competition from other males (Forsgren *et al.* 1996). Consequently, intrasexual selection is thought to be strong at Tvärminne but weaker at Klubban, an idea supported by the observation that nest sites artificially introduced are occupied by males at a much higher rate in the Tvärminne population (Table 1). As expected, Tvärminne fish are sexually dimorphic whereas Klubban fish are not, and at Tvärminne (but not at Klubban) nest-holding males are significantly larger than non-nesting males, a sign of intrasexual selection (Table 1). On the other hand,

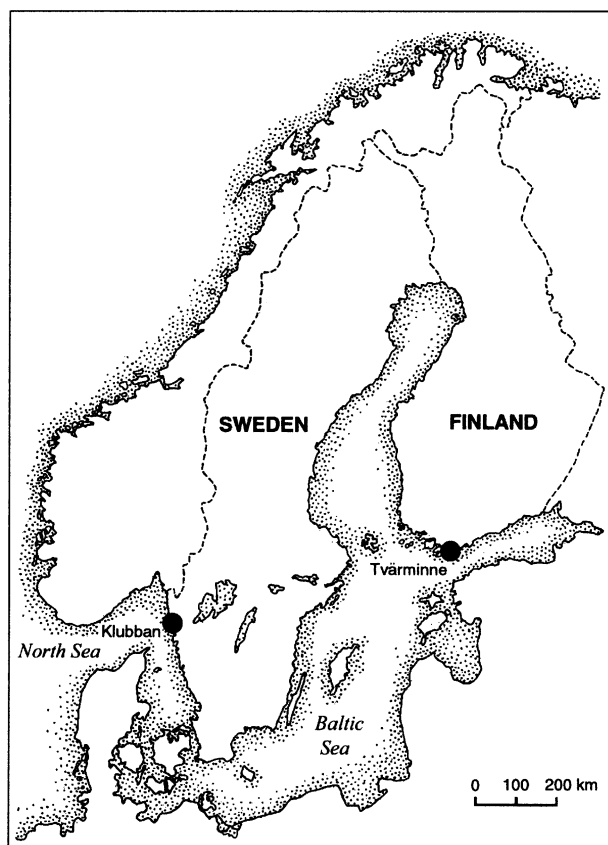


Fig. 1 Partial map of northern Europe showing the two study sites [reproduced from Forsgren *et al.* (1996)].

Table 1 Comparison of important ecological and phenotypic differences between Klubban and Tvärminne sand goby populations. Data are from Forsgren *et al.* (1996)

Ecological feature	Population	
	Klubban	Tvärminne
Nest-site abundance	9.6 sites/m ²	0.2 sites/m ²
Percentage of nest sites occupied	0.2%	41%
Artificial nest-site colonization rate	17/96 h	≥30/54 h
Males larger than females?	No ($P = 0.33$)	Yes ($P < 0.05$)
Nest holders larger than non-nesting males?	No ($P = 0.29$)	Yes ($P < 0.001$)
Percentage of nest-holding males with eggs	58%	96%

intersexual selection in the form of female choice is probably weaker at the Tvärminne locale, as evidenced by the fact that almost all nest-holding males there tend eggs whereas only 58% do so at Klubban (Table 1).

Genetic parentage analyses might document additional differences between the two populations potentially

related to these ecological and phenotypic distinctions. For example, at the outset, we predicted that the stronger competition for nest sites and, hence, larger proportion of males who cannot adopt the parental strategy at Tvärminne might result in a far higher incidence of cuckoldry than at Klubban. We also expected the average mating success per nest-holding male to be higher in the Tvärminne population because there are fewer nests per female at this locale.

Materials and methods

Collection of field samples

The Klubban site [previously studied by Jones *et al.* (submitted)] is adjacent to the Klubban Biological Station on Sweden's west coast; the Tvärminne population is near the Tvärminne Zoological Station on Finland's south coast (Fig. 1). At the latter site, field work was carried out in the summer of 1997, during the peak of the sand goby breeding season. On 21 and 22 June, artificial nest sites (7.5 × 7.5 cm square tiles) were placed on the sandy bottom in shallow water (<1 m) to allow colonization by males. Neighbouring tiles were separated by at least 1 m. The nests were checked after 10 days, and those containing sufficiently developed embryos were collected together with the guardian male. The embryos and males were frozen and stored at -80 °C for molecular analysis. The testes and guts were separated from the body of each male. These parts were dried at 70 °C for 48 h and weighed.

Microsatellite-based parentage assessment

For the current analysis, we used three microsatellite loci previously described for the sand goby (Jones *et al.* submitted). Polymerase chain reaction (PCR) conditions and DNA extraction protocols also followed the previous report. One primer from each locus was dye labelled and the PCR products were resolved using an ABI 377 automated sequencer. The relative sizes of the alleles were verified by running individuals from distinct nests side by side. Population-level allele frequencies were estimated by tallying the observed genotypes of adult males, together with the genotypes of adult females as deduced from the maternity analyses based on a minimum of 10 full-sib embryos per clutch.

For the Tvärminne population, 17 nests with resident males were examined genetically. All males and samples of 47–59 embryos per nest were assayed for two loci (*Pmin05* and *Pmin01*); a third locus (*Pmin10*) was used only when needed to resolve ambiguities. Each sneaked progeny was unambiguously detected when the male tending its nest was genetically excluded as the embryo's biological sire. After removing sneaked progeny from

consideration, the maternally derived alleles in a nest were deduced by subtracting the paternal allele from each embryo's diploid genotype, and the maternal genotypes were reconstructed by examination of the associations of maternal alleles across loci as described in Jones & Avise (1997b). For each progeny array, we conservatively invoked the minimum number of sneakers and mothers necessary to explain the data. In two cases (males TV07 and TV17), one embryo had an allele at a single locus that would require the addition of one more mother for the nest. In these cases, rather than invoke the existence of an additional mate for the resident male, we provisionally interpreted the embryo as possessing a *de novo* mutant allele.

Exclusion probabilities, which provide information about the power of molecular markers to resolve parentage, were calculated in two ways. First, for comparison with other marker systems, we present the probability of paternity exclusion given a known parent-offspring pair (Chakraborty *et al.* 1988). Second, for each paternal genotype in our sample, we calculated the probability of exclusion when neither parent is known with certainty (Dodds *et al.* 1996). This latter probability corresponds to the expected proportion of unrelated offspring in the population that would be genetically excluded as progeny of a given male (or, in other words, the probability of correctly identifying a sneaked embryo, if sampled, from a male's nest).

Results

The microsatellite markers

The microsatellite loci were extremely polymorphic in the Klubban population from which they were originally identified (Jones *et al.* submitted), and they proved to be hypervariable in the Tvärminne population as well, with 15–47 alleles per locus (Table 2). The genetic heterozygosities and exclusion probabilities are very high in both sand

goby populations (Table 2), making these loci extremely useful for parentage analysis. Indeed, the combined expected exclusion probability (Chakraborty *et al.* 1988) for all three loci in the Tvärminne population was 0.9992. The results from this study support the notion that *Pmin01* and *Pmin10* are physically linked (Jones *et al.* submitted), as they did not segregate independently in progeny arrays. We did not use *Pmin10* extensively, however, so this linkage did not affect the results. We used the exact test in GENEPOP (Raymond & Rousset 1995) to test for departures from Hardy-Weinberg equilibrium and found none. The two-locus exclusion probabilities (for *Pmin05* and *Pmin01*) calculated for each male (for the case in which neither parent of the embryo is known) ranged from 0.956 to 0.998 (mean = 0.986), indicating an extremely high power to detect sneaked eggs.

Evidence for sneaking and multiple mating at Tvärminne

In 10 of the 17 assayed nests, the resident male appeared to be the true sire of all progeny. However, six nests clearly contained a subset of embryos that resulted from fertilizations by sneaker males (Table 3), and in one additional nest the resident male (TV16) was excluded as the sire for all 47 embryos assayed. The pattern in TV16's nest could have resulted from an extremely successful sneaking event, but it also may be the signature of a nest takeover or some more complicated scenario. Thus, this male was excluded from further analysis. In all other sneaked nests, the resident male clearly fathered some (and usually most) of the embryos. In each of the following features, males whose nests contained sneaked eggs did not differ significantly from males whose nests had not been sneaked: number of eggs assayed ($t = 0.52$, d.f. = 14, $P = 0.61$), number of mates ($t = 1.47$, d.f. = 14, $P = 0.16$), dried bodyweight ($t = 1.06$, d.f. = 14, $P = 0.31$), testes weight ($t = 0.40$, d.f. = 14, $P = 0.70$), and gut weight ($t = 0.41$, d.f. = 14, $P = 0.69$). The multilocus genotypes of sneakers were reconstructed when possible, and in no case was a

Table 2 Comparison of microsatellite variability data for sand gobies from the Klubban and Tvärminne populations. Shown are the number of alleles per locus in a sample of n adults, the observed and expected heterozygosities, and the exclusion probabilities (given a known parent-offspring pair; Chakraborty *et al.* 1988) associated with each locus. The primer sequences and Klubban data can be found in Jones *et al.* (submitted)

Locus	Population	Number of alleles	n	Heterozygosity		Exclusion probability
				Observed	Expected	
<i>Pmin05</i>	Klubban	56	62	0.968	0.979	0.942
	Tvärminne	46	43	1.000	0.977	0.930
<i>Pmin01</i>	Klubban	63	62	0.968	0.984	0.952
	Tvärminne	47	43	0.977	0.981	0.938
<i>Pmin10</i>	Klubban	17	16	0.938	0.956	0.850
	Tvärminne	15	14	1.000	0.939	0.811

Table 3 Results of the microsatellite parentage assessment for males from the Tvärminne population of sand gobies. Shown are the dried body mass of each male, the number of embryos assayed genetically from his nest, and the number of females with whom he sired offspring. Also shown are the number of sneakers that successfully fathered eggs in each male's nest and the total number of eggs fertilized by these sneakers. Male TV16 was not the genetic father of any embryo in his nest, so his case was scored as a nest takeover and was excluded from further analysis

Male	Dried body weight (mg)	No. of embryos assayed	No. of females mated	No. of sneaker males	No. of eggs sneaked
TV01	130	52	3	0	0
TV02	108	52	4	0	0
TV03	152	58	2	0	0
TV05	149	57	4	1	21
TV06	120	50	3	2	7
TV07	105	56	2	0	0
TV08	190	50	5	2	2
TV09	147	51	3	0	0
TV10	146	52	3	0	0
TV11	159	55	1	1	2
TV12	190	58	3	0	0
TV13	168	56	3	0	0
TV14	155	59	3	6	45
TV15	164	55	2	0	0
TV16	140	47	0	n/a	n/a
TV17	146	47	2	0	0
TV18	186	57	5	2	2

sneaker's genotype identical to that of a male from our sample of adults. Thus, we were not able to implicate particular collected males as sneakers.

The rate of multiple mating by the Tvärminne males was high: 15 of the 16 males (94%) each had fertilized eggs from more than one female who laid in his nest, and the deduced number of such mates ranged from two to five (Table 3). We did not find a significant relationship between mating success and male bodyweight in this population (Fig. 2).

Comparison of Tvärminne and Klubban

The results from our microsatellite assessment of parentage in the Tvärminne sand gobies were remarkably similar to those from the Klubban population. The distributions of mating success in both populations were similar (Fig. 3), and neither differed significantly from the relevant Poisson distribution (Tvärminne: $\chi^2 = 5.29$, d.f. = 4, $P > 0.10$; Klubban: $\chi^2 = 7.42$, d.f. = 5, $P > 0.10$). These comparisons did not include nest-holding males without eggs in their nests, non-nesting males that failed to mate, or successful sneakers, because we did not have access to data for such males. In neither population was there a

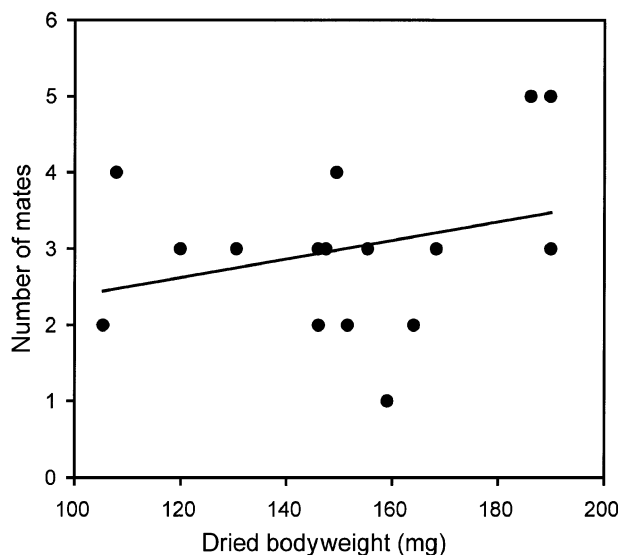


Fig. 2 Plot of mating success (number of mates) as a function of the size of nest-holding males. The regression is not significantly positive ($n = 16$, $r = 0.29$, $P = 0.28$).

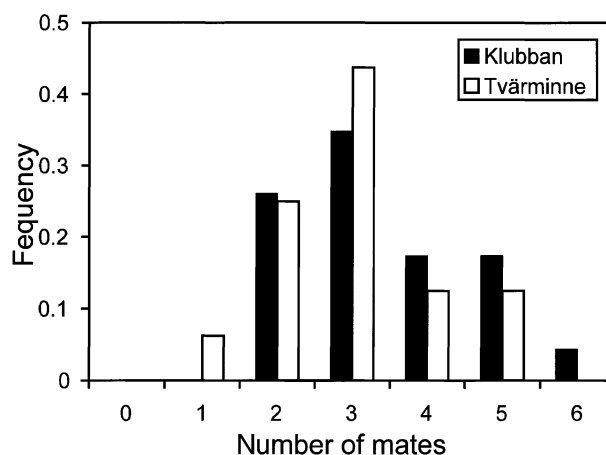


Fig. 3 Comparison of mating success histograms for nest-holding males from the Klubban and Tvärminne populations. These data are derived only from males with eggs in their nests, so no males with zero mates are represented in this figure. The Klubban data are from Jones *et al.* (submitted).

significant relationship between male body size and mating success (Fig. 2; Jones *et al.* submitted). Furthermore, the populations did not differ significantly in the number of mates per nest ($t = 1.0$, d.f. = 37, $P = 0.31$), the number of sneakers per nest ($t = 0.8$, d.f. = 37, $P = 0.41$), or the number of eggs sneaked in those nests containing sneaked eggs ($t = 0.7$, d.f. = 16, $P = 0.47$). Figure 4 permits a direct inspection of the proportion of sneaked eggs in each nest, and it also underscores the overall similarity of mating patterns in sand gobies from Klubban and Tvärminne.

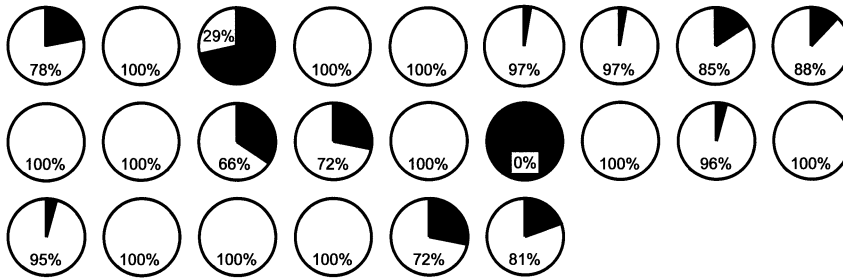
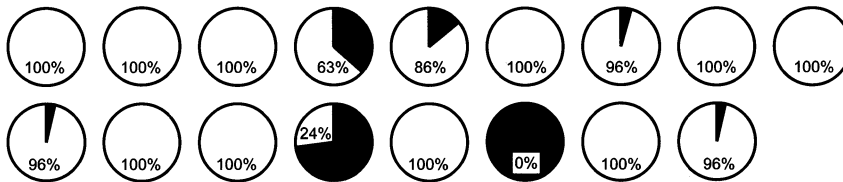
Klubban sand gobies:**Tvärminne sand gobies:**

Fig. 4 Pie diagrams showing (dark areas) the relative proportion of eggs fertilized by sneakers in each of 41 sand goby nests from Klubban and Tvärminne. The numerals indicate the percentage of assayed progeny that were sired by each nest-tending male. The nests are shown (from left to right) in the same order in which they appear in Table 2 of Jones *et al.* (submitted) for Klubban, and in Table 3 of this paper for Tvärminne.

Discussion

The microsatellite assays demonstrate unambiguously that successful sneaking is common in sand goby populations. For example, of the 17 nests assayed at Tvärminne, seven contained embryos that were not sired by the guardian male, and successful intrusion by sneaker males is the only feasible explanation for the paternity patterns in six of those nests. In addition, multiple mating was the norm (Table 3). These genetic results confirm and extend previous behavioural observations suggesting that sneaked fertilizations might occur in this species (Forsgren 1997) and that multiple mating by males may be common (Lindström 1992).

These conclusions are particularly robust because they invoke the minimum numbers of mates and sneakers necessary to explain the genetic data, and because all of the paternity exclusions and maternal genotypic assignments treated as confirmed were those that could not be explained by null alleles or *de novo* mutations. Furthermore, given the high levels of microsatellite polymorphism in this species, our current estimates of male sneaking rates and mating success must closely approximate their true values in these populations [see Jones *et al.* (1998) for detailed discussions of such issues].

Sneaking rates in the Klubban and Tvärminne populations

One surprising result from this study was the close similarity in mating patterns between two sand goby populations that differ significantly in availability of nest sites, a disparity that translates into dissimilar sexual selection regimes (Forsgren *et al.* 1996). From the genetic evidence, these populations failed to differ significantly

with respect to the proportions of nests containing sneaked eggs, numbers of sneakers detected per nest, or numbers of eggs sneaked per nest. In addition, we found a single nest in each population in which the resident male failed to sire any of the embryos, a pattern that may reflect a nest takeover. Thus, our genetic results indicate that pronounced ecological differences between these two sand goby populations have not appreciably impacted on the expression of alternative mating strategies.

Our a priori expectation was that sneaking rates would be higher at Tvärminne because nest sites are rare, males compete intensely for access to them (Lindström 1988), and males with nests frequently achieve high reproductive success (Forsgren *et al.* 1996). The only reproductive option for many males at Tvärminne is to sneak fertilizations from nest holders. Thus, if sneaking evolves as a result of disruptive sexual selection on the male phenotype, then at Tvärminne, more so than at Klubban, there should be a strong reproductive premium on any capacity for males to adopt a successful sneaker strategy. Yet, the genetic data give no indication that sneaking behaviour is more prevalent at Tvärminne.

Sand gobies are like many other species in that alternative male mating tactics are probably facultative, meaning that an individual can switch between nest-holding and sneaking behaviours during its lifetime (Magnhagen 1994, 1998). For such a system, existing mathematical theory does not offer a framework that can be used to predict how alternative mating strategies should change in response to ecological factors such as nest-site abundance. Rather, most of the existing theory has focused on the less common (but more tractable) case in which alternative male phenotypes are genetically hard-wired and their population frequencies can be modelled as evolutionarily stable strategies (Dominey 1984; Gross 1984; but see also

Gross & Repka (1998)). Additional genetic studies of the current sort, which compare mating-system properties in populations that differ in important ecological attributes, should provide both a stronger incentive and an empirical foundation for the development of useful theory in this area.

Several factors may contribute to the similarities of sneaking behaviour in our two study populations. First, the fact that nest holders at Tvärminne are larger on average than non-nester adult males (Table 1) may mean that they can better defend against sneakers, thereby lowering cuckoldry rates below what might be anticipated from the limited availability of nest sites alone. Larger males did suffer less sneaking at Klubban (Jones *et al.* submitted), but we found no significant relationship between a nest tender's size and his probability of being cuckolded at Tvärminne. Second, the expectation of a higher sneaking rate at Tvärminne presumes that there are more sneaker opportunities per nest at this locale, but this may not be the case if, for example, nest-holders themselves frequently attempt parasitic spawning. Then, the number of potential cuckoldry events in the Klubban population may be higher than it would appear at face value. A related possibility is that the density and dispersion of occupied nests in the two populations differ in important ways that affect rates of cuckoldry (Westneat & Sherman 1997). If these factors operate independently from sexual selection to affect sneaking rates, then they may mask any differences in alternative mating tactics due to nest-site availability. In addition, sneaking rates could conceivably vary temporally in these populations. Breeding takes place later at Tvärminne than at Klubban, and our Tvärminne samples were collected closer to the end of the sand goby breeding season than our Klubban samples. These topics have not been examined in detail in sand gobies. Other ecological and behavioural factors that might influence the success rates of alternative mating strategies include predation pressures (Kelley *et al.* 1999), female responses to the presence of sneakers (Alonzo & Warner 1999b), and intersexual conflict (Alonzo & Warner 1999a).

Mating systems and sexual selection

Another major surprise was the striking similarity in patterns of nest-holding male mating success in the two sand goby populations. At both geographical sites, males mated with a mean of approximately three females, and in neither population did the distribution of mating success for males with eggs in their nests differ significantly from Poisson expectations.

There can be no doubt that the sexual selection regimes in these populations are quite distinct (Forsgren *et al.* 1996). At Klubban, all males have the ecological option

of building nests, so sexual selection is primarily intersexual, resulting from variation among males in the ability to attract females to a nest (Forsgren *et al.* 1996). By contrast, sexual selection at Tvärminne is primarily intra-sexual as males compete for nest sites (Forsgren *et al.* 1996). Nonetheless, these differences in sexual selection mode have not translated into any significant differences between the two populations in the mating-system parameters examined in the current genetic analysis. Without more information about the mating and reproductive success of individual sneaker males, and precisely who is doing the sneaking, we cannot ascertain how sneaked fertilizations influence the dynamics of sexual selection in these populations. If nest-holders routinely sneak, then these males may circumvent their intrinsic brooding limitations by leaving the care of some of their genetic progeny to other males. However, if non-nesters are doing the sneaking, then they may decrease the monopolization of females by the nest holders. In either case, sneaking should influence the strength of sexual selection, but the sign and magnitude of the effect will depend upon the details of sneaker reproduction.

Geographical variation in mating systems and alternative mating strategies

The current study is among the few now available on genetic mating systems in geographically distinct populations. Much more work is clearly needed in this area. This sentiment is underscored by the current results and colleagues who have failed to find clear relationships between ecological variables and mating patterns among conspecific populations. For example, in red-winged blackbirds (*Agelaius phoeniceus*; Weatherhead & Boag 1997; Westneat & Gray 1998) and house sparrows (*Passer domesticus*; Griffith *et al.* 1999), particular populations differed dramatically in some aspects of the mating system, yet comparisons across geographical sites failed to identify definitively the ecological factors that might be responsible. Such investigations are labour-intensive, and a shortcoming of these studies (and ours) is that only a few populations were examined. For example, a microsatellite-based study of multiple paternity in 10 populations of the guppy (*Poecilia reticulata*) did yield a clear positive association between predation intensity and rates of multiple insemination (Kelley *et al.* 1999). Thus, a major goal of future studies of genetic mating systems should be to increase the number of populations under consideration. Continued comparative study of mating systems and ecological factors in geographically distinct populations no doubt will provide additional insights into important evolutionary processes, such as mating-system evolution, sexual selection, and the evolution of alternative mating strategies.

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This research resulted from a lively collaboration between American and Scandinavian scientists. Adam Jones, DeEtte Walker, and John Avise are interested in the use of molecular genetic markers to enhance our understanding of evolution and natural history in wild populations. Charlotta Kvarnemo and Kai Lindström are field biologists immersed primarily in the reproductive ecology of fishes and the evolution of paternal care.
