Title

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Journal

Annual Review of Genetics, 36(1)

ISSN

0066-4197

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Publication Date

2002-12-01

DOI

10.1146/annurev.genet.36.030602.090831

Annu. Rev. Genet. 2002. 36:19–45
doi: 10.1146/annurev.genet.36.030602.090831

GENETIC MATING SYSTEMS AND REPRODUCTIVE NATURAL HISTORIES OF FISHES: Lessons for Ecology and Evolution

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Key Words molecular markers, parentage analysis, cuckoldry, sexual selection, alternative reproductive tactics

■ **Abstract** Fish species have diverse breeding behaviors that make them valuable for testing theories on genetic mating systems and reproductive tactics. Here we review genetic appraisals of paternity and maternity in wild fish populations. Behavioral phenomena quantified by genetic markers in various species include patterns of multiple mating by both sexes; frequent cuckoldry by males and rare cuckoldry by females in nest-tending species; additional routes to surrogate parentage via nest piracy and egg-thievery; egg mimicry by nest-tending males; brood parasitism by helper males in cooperative breeders; clutch mixing in oral brooders; kinship in schooling fry of broadcast spawners; sperm storage by dams in female-pregnant species; and sex-role reversal, polyandry, and strong sexual selection on females in some male-pregnant species. Additional phenomena addressed by genetic parentage analyses in fishes include clustered mutations, filial cannibalism, and local population size. All results are discussed in the context of relevant behavioral and evolutionary theory.

CONTENTS

INTRODUCTION

Molecular markers can unveil and quantify the incidence of organismal reproductive behaviors that otherwise may remain hidden from field naturalists (6, 9, 52). Here we highlight genetic findings on fish mating systems and alternative reproductive tactics in nature as gleaned from DNA-level analyses of maternity and paternity, and interpret the results in conjunction with behavioral observations. Genetic parentage analyses of fish clutches have unearthed several fascinating details of reproductive natural history, and have also yielded fresh insights into broader theories on animal mating systems and sexual selection. This review focuses on conspecific populations of sexually reproducing fishes in the wild; special cases of parentage assessment in interspecific hybrids and in unisexual fishes have been reviewed elsewhere (7, 10).

The Natural History of Fish Reproduction

Fish have remarkably diverse reproductive behaviors (21, 63, 130, 131, 136). A rich natural-history literature documents mating systems ranging from pelagic group spawning to cooperative breeding to social monogamy. Subsequent to spawning, adult care of fertilized eggs and larvae may be nonexistent, confined to one gender, biparental, or communal. When parental care is offered, it may take such varied forms as oral or gill brooding, use of natural or constructed nests, internal gestation by a pregnant mother or by a pregnant father, or open-water guarding of fry.

For reasons that will become apparent, most genetic studies of fish mating behaviors have been conducted on species displaying parental care of offspring (8). In the bony fishes (Osteichthyes), approximately 89 of the 422 taxonomic families (21%) contain at least some species in which adults provide direct postzygotic services, and in nearly 70% of those families, the primary or exclusive custodian is the male (18, 19). Parental care by males alone is otherwise extremely rare in vertebrates other than anuran amphibians (26, 149). Thus, the evolutionary elaboration of paternal devotion makes fishes particularly favorable for testing traditional parental-investment and sexual-selection theories originally motivated by research on mammals and birds, where females typically are the primary caregivers.

Also intriguing for genetic analysis are alternative reproductive tactics (ARTs) *within* a species (61), or sometimes even within an individual during its lifetime. An example of the latter occurs in sequential hermaphroditic species in which an individual fish may switch its gender (and associated mating behavior) from female to male or vice versa (148). Both sex-changing and nonchanging fish are present in some populations (146).

Most fish species are gonochoristic (separate sexes), and here too, within a gender, ARTs may be prevalent. In theory, a male fish may maximize the number of eggs he fertilizes by being quicker than rivals in "scramble competition," monopolizing mates or resources such as nests or territories, exploiting the resources of other males via reproductive parasitism, or cooperating or trading with resource holders via mutualism or reciprocity (132). Two or more such tactics are often observed in a population. For example, four types of males co-occur in the ocellated wrasse (*Symphodus ocellatus*): large "bourgeois" males that build nests and tend progeny; small males that parasitize (or cuckold) a bourgeois spawner by sneaking into a nest and "stealing" some of the fertilization events; medium-sized males that defend another male's nest from sneakers, but also court females and occasionally spawn; and extra-large males (pirates) that temporarily usurp the nest of another male (133).

In another example, male salmon spawn either as full-sized anadromous adults after returning from the sea, or as dwarf precocious parr that have remained in fresh water. In marker-based parentage analyses of Atlantic salmon (*Salmo salar*), parr have been shown to fertilize widely varying proportions (5–90%) of the total eggs in various populations (55, 56, 68, 83, 104, 135). They also produce physiologically superior spermatozoa, a feature that partially compensates for their behavioral subordinance to dominant anadromous males (143).

The ARTs of anadromous salmon males and resident parr appear tied to an individual's environmental exposure, but ARTs in some fish species might be genetically hard-wired. Rearing fry under controlled conditions can help in evaluating developmental plasticity, as has been demonstrated with respect to alternative trophic morphs in several fish species (102, 124, 140). Regardless of their mechanistic basis, ARTs are common in fish, their occurrence facilitated by the prevalence in this group of external fertilization, a high incidence of paternal investment, and extensive intrasexual size variation attendant with indeterminate growth (132).

On the female side of the ledger, strategies of mate choice and parental investment can also vary (65). For example, in the peacock wrasse (*Symphodus tinca*), a female normally spawns with territorial males who care for her eggs on the nest, but she also may spawn off-territory with males who provide no parental care (147). Under some ecological circumstances, the cost of nest-searching by a female may outweigh the lower survival of her untended offspring from these latter matings (141). Other ARTs known in female fish include additional variations on patterns of mate choice, parental care, resistance to coercion, and mating mode (1, 65).

Although such ARTs are well described in the fish behavioral literature, their fitness consequences cannot be fully assessed from field observations alone.

Genetic markers can shed new light on the realized success of ARTs in nature by disclosing actual biological parentage.

Historical Precedents for Genetic Reassessments

Three broad developments in the 1980s set a stage for refined genetic appraisals of fish reproductive activities. First, highly variable satellite DNA regions (70) were found to be common features of eukaryotic genomes (57), and their utility in parentage assessment was quickly appreciated (24).

Second, revolutionary insights came from analogous genetic studies in other taxonomic groups, notably insects and birds (16). For example, ornithologists supposed that most songbirds were genetically monogamous within a breeding season (92), but the new genetic data often excluded a nest-tending adult as the sire or dam of some nestlings. Such findings led to the realization that extra-pair copulations and other clandestine reproductive activities, including conspecific nest parasitism, are routine phenomena in many avian species (17).

A third development was the realization that patterns of genetic parenthood are important for theoretical models of behavioral evolution (2, 4, 64, 139). For example, surreptitious cuckoldry can yield a "genetic mating system" that departs from the apparent "social mating system" of a population, and this can impact the intensity of sexual selection and the evolution of secondary sexual traits. Furthermore, genetic analyses of birds, insects, and other taxa have shown that realized parentage can reflect postcopulatory processes too, including sperm competition and female sperm choice (47, 129).

BACKGROUND

Microsatellite Markers

Microsatellites are well-suited for genetic parentage analyses because the DNA repeat units are small (2–5 bp each) such that alleles separated through suitable gels usually can be identified cleanly; the assays are applied locus-by-locus so the data can be interpreted in simple Mendelian terms; the polymerase chain reaction (PCR) is employed, so data can be recovered from even small amounts of tissue such as a single fish embryo; and allelic variation in most fish populations is extremely high (35).

The Logic of Molecular Parentage Analysis

A variety of statistical approaches for parentage analysis have been developed for particular biological settings (e.g., 37, 53, 69, 106). However, the basic logic of parentage analysis is generalizable. One common situation in fishes is when the male parent of a brood is known or suspected from genetic or behavioral evidence, and maternity is in question. For each offspring at each locus, the maternal allele can be deduced by subtraction (except when the sire and offspring are identically

heterozygous). Then, any female whose genotype is inconsistent with these maternally deduced alleles at multiple loci is excluded as the dam.

An average exclusion probability refers to the mean probability of excluding an unrelated adult as a parent of a randomly chosen juvenile. In nearly all cases considered in this review, genetic markers were sufficiently variable that mean multilocus exclusion probabilities (126) were well above 0.95. Such exclusionary power may earmark the true dam, but this also depends on the number of candidate females in the population, how thoroughly they have been sampled, and their genetic relationships.

Similar logic applies to paternity exclusions when particular offspring display alleles incompatible with those of their male custodian. Cuckoldry (stolen fertilizations by other males), nest piracy, and egg thievery are among the behavioral possibilities that can lead to male foster parentage, and these can often be distinguished by considering details of the particular natural-history setting. Finally, when neither fish parent is available for genetic examination (as is normally true in species lacking parental care), the statistical exclusionary power and, hence, the capacity to draw biologically informative conclusions, usually is reduced considerably.

In most of the genetic appraisals of parentage and reproductive behaviors in fishes published to date, discrete cohorts of embryos within a nest (or inside a gestating parent) were genotyped in conjunction with a custodial adult and other individuals sampled nearby. By straightforward chains of reasoning, these multilocus genotypic data permit powerful deductions about the genetic parentage of particular juveniles, and such information accumulated across hundreds or thousands of fish from multiple nests can reveal the relative success of ARTs as well as the genetic mating system of a natural population.

REPRODUCTIVE PHENOMENA IN VARIOUS FISH GROUPS

What follows are brief synopses of various natural-history phenomena illuminated by genetic parentage analyses in fishes, particularly species with parental care of offspring (Table 1).

North American Sunfishes

In nearly all 30 species of North American sunfish (Centrarchidae), males guard eggs and embryos in shallow depression nests swept in the soft substrate of a lake or stream (20). One species—the bluegill, *Lepomis macrochirus*—has been a model system for the study of ARTs (59–61, 107).

CUCKOLDRY BY MALES Various routes to paternity are available to bluegills at the study sites in eastern Canada (45, 62). “Parental” or bourgeois males, which mature at seven years of age, construct nests in colonies, attract females, spawn with them on the nest, and vigorously defend the nest and embryos against intruders.

TABLE 1 Summary of salient findings in microsatellite-based studies of genetic parentage in various natural fish populations[a]

Mode of brood care by adult custodian	Species	# Young assayed	# Nests or broods assayed	# Sneaked nests or broods	# Nest takeovers or egg-theft events	% Offspring not parented by custodian	Average minimum (& estimated) # mates[b] per nest or brood	References
Nest-tending by males	*Lepomis auritus* (redbreast sunfish)	996	25	11	2	12	3.6	41
Nest-tending by males	*Lepomis punctatus* (spotted sunfish)	1434	30	13	1	1	4.4 (7.2)	38
Nest-tending by males	*Lepomis macrochirus* (bluegill sunfish)	1677	38	35	0	21	Not estimated	106
Nest-tending by males	*Lepomis marginatus* (dollar sunfish)	1015	23	2	1	4	2.5 (3.7)	Mackiewicz et al., submitted
Nest-tending by males and females	*Micropterus salmoides* (largemouth bass)	1088	26	1	1	7	1.2	42
Nest-tending by males	*Etheostoma olmstedi* (tessellated darter)	610	16	2	3	14	3.2 (9.2)	39
Nest-tending by males	*Spinachia spinachia* (15-spined stickleback)	1307	28	5	4	19	2.6	75
Nest-tending by males	*Pomatoschistus minutus* (sand goby)	981	24	12	1	15	3.4	81, 82[c]
Nest-tending by males	*Etheostoma virgatum* (striped darter)	987	19	0	4	17	4.7 (6.1)	118
Nest-tending by males	*Cottus bairdi* (mottled sculpin)	1259	23	0	1	2	2.8	53a
See footnote d	*Salmo salar* (Atlantic salmon)	250	10	10	0	NA	5.1	97, 103
See footnote d	*Salmo trutta* (brown trout)	85	33	4	0	NA	2.2	56

Internal gestation by females	*Gambusia holbrooki* (mosquitofish)	823	50	NA	NA	NA	2.0	155
Internal gestation by females	*Poecilia reticulata* (guppy)	1812	253	NA	NA	NA	1.5	87
Internal gestation by males	*Syngnathus scovelli* (gulf pipefish)	838	40	0	NA	0	1.0	71, 80
Internal gestation by males	*Syngnathus floridae* (dusky pipefish)	924	22	0	NA	0	1.9	72
Internal gestation by males	*Syngnathus typhle* (Swedish pipefish)	1344	30	0	NA	0	3.1	77
External gestation on male's body	*Nerophris ophidion* (straight-nosed pipefish)	361	15	0	NA	0	1.0	101
Internal gestation by males	*Hippocampus angustus* (W. Australian seahorse)	453	15	0	NA	0	1.0	74
Mouthbrooding by females	*Pseudotropheus zebra* (a Lake Malawi cichlid)	99	7	0	0	0	3.8	110
Mouthbrooding by females	*Protomelas spilopterus* (a Lake Malawi cichid)	200	6	4[e]	4[e]	29	1.7	86
Mouthbrooding by females	Seven other species of Lake Malawi cichlids	203	16	0	0	0	2.1	85

[a]Modified and expanded from (36).

[b]Usually, the mean minimum number of female mates of the male parent (but sometimes male mates of the female parent, as in the cases of *Salmo* and *Gambusia*) per nest or per brood as deduced directly from counts of relevant gametotypes in the progeny array; estimated numbers (where presented) were statistically corrected by the original authors according to procedures in DeWoody et al. (37, 43).

[c]Data from the Klubban population only (see text for additional information).

[d]Male *Salmo* do not "tend" nests, so the numbers refer merely to the incidences of multiple parentage.

[e]The genetic data revealed that each of 4 females was mouthbrooding some fry that were not her own biological progeny. Because the field behavior(s) underlying such brood-mixing remain unknown, it is unclear whether these fry should be considered "sneaked" or "stolen".

Precocious cuckolder males, by contrast, attempt to steal fertilizations from nest-holders. These can often be 2- to 3-year-old "sneakers" that dart into a nest and release sperm as the bourgeois male spawns with a female, or older "satellites" that mimic females in color and behavior but release sperm as the primary couple spawns. Cuckolders leave the nest after spawning and show no parental care. They represent an "alternative life history" to that of bourgeois males (62).

What fraction of the reproductive output is attributable to bourgeois versus cuckolder males? Molecular markers provided the answer (29, 106, 116). In the largest genetic study of bluegills, involving 38 nests in one colony, the percentage of offspring per nest sired by the resident male ranged from 26–100 (mean 79%; Figure 1). Cuckoldry by neighboring bourgeois males was rare, so about 20% of the embryos were the result of fertilization thievery by satellites or sneakers (106). The levels of cuckoldry per nest also were evaluated in conjunction with behavioral observations (107). The comparisons showed that as bourgeois males detect paternity lost to cuckolders (by assessing intrusion rates of sneakers and

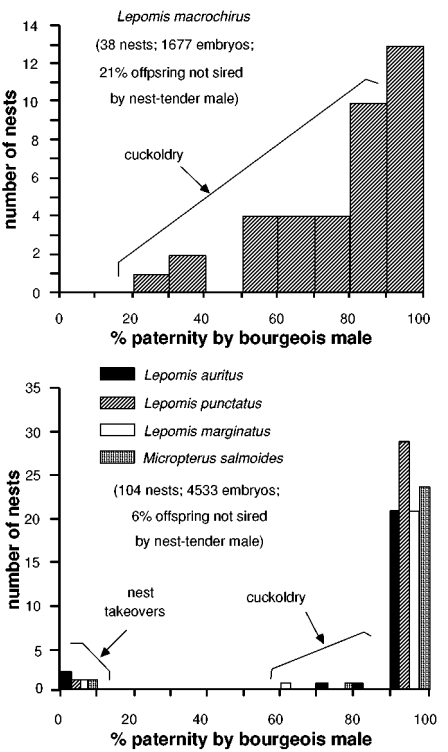

Figure 1 Paternity found for bourgeois male sunfish. Shown are the percentages of progeny per nest sired by the respective attendant males for (*above*), 38 nests of the bluegill, *Lepomis macrochirus*; and (*below*), a total of 104 nests in four other centrarchid species (see Table 1 for literature citations).

perhaps by using olfactory cues on offspring relatedness), they adaptively lower their level of parental care.

The overall rate of cuckoldry in this population closely matched the observed proportion (20%) of males at age 2 destined to become cuckolders (62). This raises the possibility that the genetic fitness of an individual may be similar for bourgeois males and cuckolders, a finding consistent with the notion that these two ARTs might be near an equilibrium frequency in an evolutionarily stable system (100) perhaps maintained by frequency-dependent selection (60–62). This conclusion remains tentative, however, because snapshot appraisals of genetic parentage do not yield estimates of lifetime fitness. Furthermore, although not necessarily crucial to the question of evolutionary stability (32, 54), it remains uncertain whether the ARTs in bluegill reflect a genetic polymorphism or a conditional ontogenetic switch regulated by the social or environmental experiences of a male during its development.

Microsatellite assessments of paternity rates for bourgeois males have recently been gathered for four other centrarchid species (Figure 1). Most or all embryos in a majority of nests proved to have been sired by the nest attendant. If we temporarily disregard the nests in which the custodial male fathered none of the young (see below), then mean cuckoldry rates were about 2%, or roughly an order-of-magnitude lower than in *L. macrochirus*. Two factors probably contribute to this difference. First, at the sites studied, the other centrarchid species were either solitary nesters or less colonial than the bluegills assayed, and, all else being equal, lower nesting densities probably reduce the opportunities for cuckoldry (36, 60, 116). Second, specialized cuckolder morphs are not known in *L. auritus*, *L. marginatus*, or *M. salmoides*, and although such morphs have been reported in *L. punctatus* (38), they were rare at the study site.

NEST TAKEOVERS Among the total of 142 centrarchid nests genetically surveyed, the custodial male in six instances (4.2%) had sired none of the young. One such instance involved a nest tended by a sterile F_1 hybrid between *L. macrochirus* and *L. gibbosus*, so the author concluded that this hybrid male had been 100% cuckolded (106). However, most of the other cases (all intraspecific) probably reflected nest-takeover events, and these nest piracies account for the majority of the documented foster parentage. Perhaps nest takeovers are opportunistic responses to limited nest-site availability, or, perhaps the captured "nest-holder" at the time of sampling was merely a temporary visitor (e.g., was there to cannibalize embryos).

CUCKOLDRY BY FEMALES Largemouth bass (*Micropterus salmoides*) are unusual among fishes for tendencies toward biparental care of young and for staying with schooling fry for up to a month post-hatching. Most of the 26 offspring cohorts genetically assayed in *M. salmoides* proved to be composed of full-sibs (consistent with the social monogamy suspected for this species), but four cases were documented in which the custodial female was the dam of most but not all of the juveniles that she and her mate tended (42). Most likely, a second female in each case had laid some eggs in another's nest and then left her offspring

in the care of their father and stepmother. "Cuckoldry" is normally meant to imply a reproductive behavior by which a breeding individual surreptitiously usurps parental services of another adult of the same gender, so this can qualify as a case of "female cuckoldry" analogous to male cuckoldry discussed above.

MULTIPLE MATING BY BOURGEOIS MALES In *M. salmoides*, the genetic parentage data show that successful spawning was usually by one female (and one male) per nest (42). However, in three other sunfish species similarly assayed—*L. auritus*, *L. marginatus*, and *L. punctatus*—the genetic data showed that multiple females typically had spawned with each bourgeois male (Table 1). In other words, such nests contained mixtures of full-sib and paternal half-sib embryos. In the spotted sunfish, for example, the mean number of mothers per nest was at least 4.4, and statistical adjustments suggest that the true number occasionally may have been 10 or more (38).

Other Nest-Tending Species

In several other nest-tending fish species from both the freshwater and marine realms, parentage analyses by genetic markers have likewise been used to estimate numbers of dams per nest, rates of non-paternity for bourgeois males, and cases of nest piracy (Table 1). Additional reproductive phenomena have been uncovered as well, as described next.

EGG THIEVERY Another behavioral route to non-paternity for custodial males is egg-stealing, a nest-raiding phenomenon occasionally observed, for example, in sticklebacks, Family Gasterosteidae (94, 154). A bourgeois male uses kidney-secreted glue to construct a nest in vegetation, into which females lay eggs (14). Then the resident male (and, sometimes, sneakers) swim through the nest, releasing sperm. Occasionally, a bourgeois male is also seen transporting to his own nest a discrete cluster of eggs (clutch) that he stole from a neighbor.

Are these stolen eggs viable, and had they been fertilized by the neighbor? Applying microsatellite markers to the problem, Jones et al. (75) documented probable cases of egg piracy in about 17% of the nests in a population of fifteenspine sticklebacks (*Spinachia spinachia*). Each such instance was adduced when one of two or more clutches of viable embryos in a nest (rather than a few scattered progeny) had been sired by a male other than the bourgeois attendant. Using a slightly different DNA fingerprinting approach, Rico et al. (121) similarly found that about 18% of nests in the threespine stickleback, *Gasterosteus aculeatus*, contained some stolen eggs.

Why would nesting males often pilfer fertilized clutches? Several hypotheses have been advanced for how natural selection on males might have promoted the evolution of egg-stealing tendencies: (*a*) the pump-priming effect—females in several species, including sticklebacks, are known to spawn preferentially in nests that already contain eggs (89, 122, 123); (*b*) the predator-dilution effect—extra eggs in a nest might ameliorate predation on the guardian's own embryos (150);

(*c*) kin selection—if the larcenist and his victim are close genetic relatives but the thief has much higher prospects for successfully rearing offspring, both individuals might benefit from the theft in terms of inclusive fitness; or (*d*) the larder-stocking effect—males may steal eggs only to eat them later.

EGG MIMICRY In *Etheostoma* darters of the eastern United States, males of several species appear to have evolved bodily structures (typically on the tips of fins) that closely resemble eggs of these species and have been interpreted as "egg mimics." In a population of one of these species, *E. virgatum*, the egg mimics are displayed as pigment spots on the pectoral fins. In a genetic maternity analysis of fertilized eggs in the nests of 10 males, Porter et al. (118) found a significant correlation between the number of egg mimic spots on nesting males and their respective numbers of genetically deduced mates. Results are consistent with the hypothesis that egg mimicry by bourgeois males helps to attract gravid females to a nest.

BROOD PARASITISM BY HELPER MALES Cooperative breeding is fairly common in avian and mammalian species (23), but is known in only eight species of fish (130). In a nest-guarding cichlid fish from Lake Tanganyika, *Neolamprologus pulcher*, a pair of breeders often shares brood-care duties with individuals from previous clutches (134). In general, nest helpers might gain personal benefits such as food, protection, parental experience, or inheritance of a territory or mate (e.g., 23, 49), and/or they might gain in terms of inclusive fitness by rearing kin (64). Might they also profit in the immediate currency of personal fitness by siring some of the offspring within the brood? Yes. Using multilocus DNA fingerprinting assays, Dierkes et al. (44) showed that about 10% of the progeny in seven assayed families of *N. pulcher* were fathered by helpers.

REPRODUCTIVE VARIANCE AND THE OPPORTUNITY FOR SEXUAL SELECTION One common notion, supported by many studies of avian species, is that extra-pair fertilizations enhance the opportunity for sexual selection by increasing the variance in male reproductive success. By stealing fertilizations from neighbors, some males become bigger winners (and others bigger losers) in the reproductive sweepstakes. Although this view may generally be true in socially monogamous species such as many birds, it may not hold in all situations. Namely, whenever the variance in reproductive success among males is larger in the absence of cuckoldry than in its presence, the opportunity for sexual selection actually may decrease with increased levels of fertilization thievery (82).

Such may well be the case in the sand goby, *Pomatoschistus minutus*, a small European marine species in which males build and defend nests under mussel shells. Nest sites can be at a premium, and males often mate with multiple females. Thus, in total reproductive output, successful bourgeois males might be expected to greatly surpass other males in the population, especially those unable to secure nesting sites. However, as demonstrated genetically by Jones et al. (81, 82), fertilization thievery via sneaking is also extremely common in this species, occurring

in about 50% of all nests. By interpreting these empirical findings in the context of models relating the intensity of sexual selection to variances in male reproductive success, the authors conclude that for this species, cuckoldry by sneaker males probably substantially reduces the opportunity for sexual selection.

Oral Brooders

Adults in many fish species protect their offspring by carrying eggs and hatchlings in the mouth or gill cavity. Oral incubation is particularly prevalent, and has evolved many times independently, in fishes of the Family Cichlidae (58, 84, 88). Microsatellite-based paternity analyses (Table 1) in several cichlid species have documented multiple paternity of broods, with up to six males fertilizing a single clutch (85, 110).

INTRASPECIFIC BROOD MIXING Another otherwise cryptic phenomenon in mouthbrooders, documented by microsatellite markers, is the shuffling of conspecific broods. In four of six orally brooded cohorts of fry examined in a Lake Malawi cichlid, *Protomelas spilopterus*, the proportions of juveniles not dammed by the female who held them ranged from 6% to 65% (86). Several possible explanations for the origin and significance of brood mixing remain highly speculative (86), but based on genetic as well as other evidence (84, 120), this foster behavior in cichlids is remarkably common.

Female-Pregnant Species

In several fish groups, including the Poeciliidae (a large New World Family of live-bearers) and the Embiotocidae (the only Family of marine teleosts that is exclusively viviparous), a female is impregnated by one or more males and carries the resulting embryos internally, giving birth weeks or months later. Internal gestation guarantees that a pregnant female is the biological mother of her brood.

MULTIPLE MATING BY FEMALES In most nest-tending fishes, there is an inherent gender asymmetry in the genetic power to detect multiple mating. Each clutch typically is associated with a male guardian, so any multiple in situ mating by that male will be apparent in suitable molecular assays of progeny in his focal nest. However, multiple mating by a female can only be revealed if separate nests containing her progeny were included in the field collection, and this may seldom be the case when populations are large or sparsely sampled. Thus, if only for this bias, multiple mating by females has rarely been genetically verified in nest-tending fishes (42, 75). However, this detection bias is reversed in female-pregnant fishes, where multiple mating (if present) by females is normally far easier to document genetically than is multiple mating by males.

In the sailfin molly, *Poecilia latipinna*, allozyme-based paternity analyses revealed that at least 52% of assayed broods were composed of embryos sired by two or more males (137), and that larger females were more likely to have had

multiple mates (138). Constantz (31) summarized other allozyme-based estimates of multiple insemination rates in poeciliids. For example, at least 56% of pregnant mosquitofish (*Gambusia affinis*) carried broods of mixed paternity (25). However, marker variability can affect such estimates, as suggested by a later microsatellite analysis of mosquitofish in which multiple paternity was documented in nearly 100% of the surveyed broods (155).

SPERM STORAGE BY FEMALES Ovarian tissues in poeciliids can store functional sperm for at least 1–2 weeks post-copulation, but storage of viable sperm by female surfperches (Embiotocidae) routinely occurs across several months (145). Using allozyme assays, Darling et al. (33) showed that most broods in the embiotocid shiner perch, *Cymatogaster aggregata*, are sired by multiple males, despite the fact that the matings preceded fertilization by 25 weeks or more (thus evidencing long-term sperm storage by females). This may be the longest known duration in fishes for potential sperm competition and postcopulatory female choice. However, even this pales in comparison to the multiyear utilization of female-stored sperm that has been genetically documented in some turtles (114).

BIOLOGICAL BENEFITS OF FEMALE PROMISCUITY In female-pregnant fish, promiscuous mating tendencies by the males are evident in their vigorous sexual behavior (30) and are easy to understand, but why would females also mate promiscuously? Multiple mating may expose a female to higher risks from sexually transmitted diseases, predation, copulation brutality, or other time or energy expenses associated with the mating process, and these costs might seem to outweigh any benefits in genetic fitness. However, a female also might gain any of several fitness advantages by mating with multiple males, including fertilization insurance against male sterility, access to more or better quality territories, success in "prospecting" better genes for her progeny, production of broods with more diverse and potentially adaptive genotypic arrays, and avoidance of inbreeding depression if some of her matings might be with close kin.

Female guppies, *Poecilia reticulata*, often solicit matings from multiple males (67), and many broods have multiple sires, especially in high-predation regimes (87). In microsatellite-based paternity analyses, Evans & Magurran (50) discovered that females who had mated with multiple males had shorter gestation times and produced larger broods containing progeny with better-developed schooling behaviors and predator avoidance. These findings provide some of the first experimental evidence in fishes that promiscuity can be genetically rewarding for females as well as males.

Male-Pregnant Species

Two motivations have guided most genetic parentage analyses in fish: intellectual curiosity about a species' natural history and a desire to test broader mating system theories. Nowhere has the latter objective been more evident than in recent molecular appraisals of the Syngnathidae (73). A universal feature in the more than

200 living species of pipefishes and 30 species of seahorses is male pregnancy. One or more females lay eggs into a male's brood pouch or ventral surface, where they are fertilized by the assured sire and then housed as developing embryos until parturition weeks later. Such high paternal investment in offspring, and a freedom from parental responsibility for females, contrast diametrically with the situation in most mammals and many birds, making the syngnathids ideal subjects for testing, from a mirror-image perspective, traditional notions about gender roles in the context of mating system theories (3, 28, 139, 151).

MATING SYSTEMS, SEXUAL SELECTION, AND SEXUAL DIMORPHISM In the behavioral literature on syngnathid fishes, "sex-role reversal" is usually defined not as male pregnancy per se, but rather as any situation in which females compete more intensely for access to mates than do males (15, 142). By this definition, some syngnathids are sex-role-reversed and some are not (142). In other words, in some but not all syngnathid species, females potentially produce more eggs during a breeding season than the available brood pouches of males can accommodate, such that males are the limiting resource in reproduction. This situation differs from that in most nest-tending teleosts, where rates of egg care by guardian males usually exceed rates of egg production by females (28).

The reason for defining sex-role reversal in this fashion (whether stemming from male pregnancy, or from any other impacts on the relative reproductive rates of the sexes) is that the phenomenon then ties rather directly to broader theories on mating systems and sexual selection (142). Namely, because sex-role reversal produces a female-biased "operational sex ratio," it presumably is associated with higher intensities of sexual selection on females, a greater potential for the elaboration of secondary sexual traits in that gender, and mating systems tending toward polyandry (Figure 2). All of these predictions fall on the opposite end of a mating-system spectrum from the polygynous behaviors that characterize, for example, many mammal and bird species with traditional gender roles. In these other organisms, males often have the potentially larger variances in fitness, compete actively for females (the limiting resource in reproduction), experience more intense sexual selection, and often display sexually selected behavioral or morphological traits (Figure 2).

In some syngnathid species, sex-role reversal has been evaluated experimentally, for example, as the potential reproductive rates of males versus females (99, 142). In other syngnathid species, the evidence for or against the phenomenon is indirect, involving, for example, the observed degree of dimorphism in secondary sexual characters. In syngnathids, when one gender is more brightly colored or otherwise sexually adorned, it is, indeed, normally the female (34). Given that syngnathid species appear to vary considerably along the sexual-selection continuum, conventional theory suggests that their mating systems may also vary accordingly (Figure 2).

In initial tests of this hypothesis, microsatellite-based appraisals of the genetic mating system have recently been conducted in each of five syngnathid species

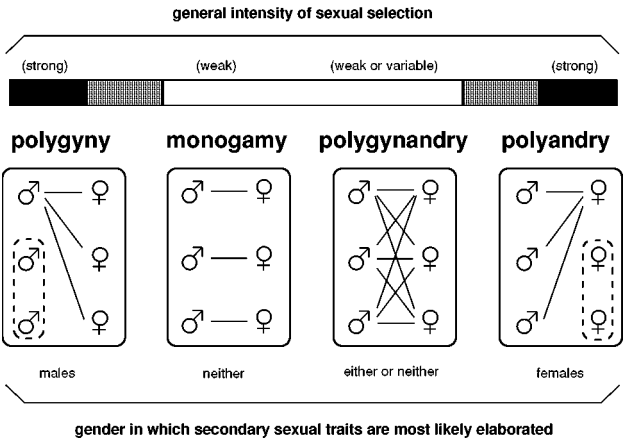

Figure 2 Pictorial definitions of four genetic mating systems possible in fishes. Lines connecting males and females indicate spawning partners that produced offspring. Also shown are the theoretical gradients in sexual-selection intensities and the degrees of gender dimorphism in secondary sexual traits often associated with these mating systems.

(Table 1) that display differing degrees of sexual dimorphism. The results proved to be in general agreement with broader mating system theory in at least two regards (Figure 3). First, the genetic mating systems fell along the monogamy-polyandry end of the mating system continuum, rather than in the monogamy-polygyny range of the spectrum as is normally true in most mammals and birds. Second, the strongly sexually dimorphic pipefish species (*Syngnathus scovelli* and *Nerophis ophidion*) proved to be genetically polyandrous (71, 80, 101), whereas a seahorse species (*Hippocampus subelongatus*, formerly *H. angustus*) in which males and females show no elaboration of secondary sexual traits was genetically monogamous within a breeding episode (74, 91). Furthermore, two pipefish species (*S. typhle* and *S. floridae*) that are intermediate in level of sexual dimorphism displayed a polygynandrous genetic mating system in which many females and males probably had multiple mating partners during the course of a male pregnancy (72, 77, 79).

These initial genetic findings for syngnathids conform to the general expectations of sexual-selection theory and mating-system evolution as applied to taxonomic groups containing role-reversed species. Caution is warranted, however, because many proximate ecological factors (as well as phylogenetic constraints) may also influence mating systems (73). In only a few fish species have molecular parentage analyses been applied to two or more populations, and pronounced geographic variation in the genetic mating system sometimes has (87, 138) but at other times has not (81) been present.

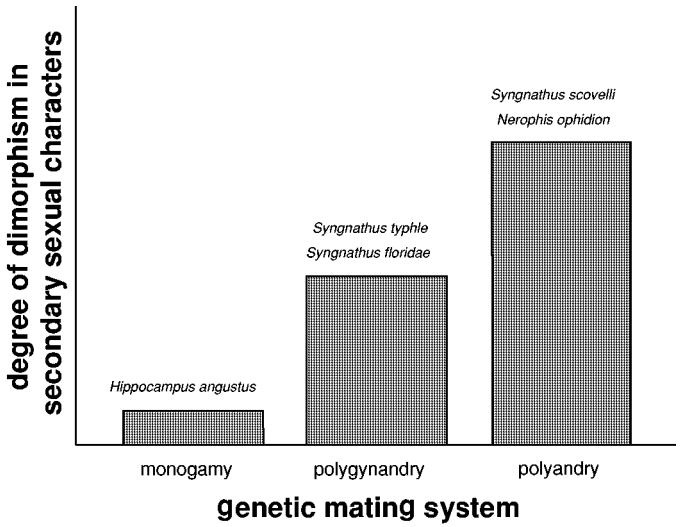

Figure 3 Observed relationships between the degree of sexual dimorphism and the genetic mating system (as deduced from molecular parentage analyses) in each of four pipefish (*Syngnathus* and *Nerophis*) and one seahorse (*Hippocampus*) species (see text).

SEX-ROLE REVERSAL AND BATEMAN'S GRADIENTS In the literature on animal mating systems, the relative intensities of sexual selection on the two genders have been variously attributed to differences in parental investment (111, 139), operational sex ratio (90), relative variances in reproductive success (112, 144), and potential reproductive rates of the sexes (27, 28), among others. Although such factors certainly impact the nature of sexual selection on males and females, Bateman (12) argued more than 50 years ago that they all do so via one common denominator or first-order factor: the average relationship between the number of mates an individual obtains (its mating success) and the number of offspring it produces (its reproductive success or genetic fitness).

Working with experimental populations of *Drosophila*, Bateman noticed that males' mean genetic fitness tended to increase rapidly with mating success (yielding a steep, linear selection gradient), whereas females gained little in offspring counts by mating with multiple males (a shallow or flat selection gradient). Bateman saw this disparity as the true cause of differential sexual selection; multiple mating afforded to males a higher fitness payoff than it did to females. More recently, "Bateman's gradients" have been touted by some authors as quantitative keys to comparing the intensities of sexual selection across species as well (3, 5).

In sex-role-reversed taxa, steeper slopes in Bateman's gradients are predicted for females than for males (the reverse of the usual situation in *Drosophila*, and in many mammals, birds, and other taxonomic groups). Using microsatellite-based

paternity analyses to assay the reproductive success of genetically marked individuals, Jones et al. (76) critically tested this expectation using aquarium populations of a role-reversed pipefish species, *S. typhle*. Consistent with theory, the sexual selection gradients proved to be significantly steeper for females than for males. Results supported the Bateman-gradient approach for characterizing the strength and direction of sexual selection, and its underlying notion that the relationship of mating success to fitness is a cardinal feature in the process of sexual selection.

EXTREME POLYANDRY AND SEX-ROLE REVERSAL A focused genetic study of the gulf pipefish, *S. scovelli*, uncovered the most compelling data yet available for any vertebrate species that sexual selection in nature can act more strongly on females than on males (80). One small population from a well-demarcated patch of seagrass was sampled exhaustively, thus enabling more complete evaluations than are normally possible on rates of genetic parentage by individuals of both genders. From genetic maternity and paternity analyses of the 21 broods, each pregnant male had mated with only one female, but on average a female had mated with 2.2 males. Furthermore, the standardized variance in female mating success (the variance in the number of embryos dammed by females, divided by the square of the mean—a gauge of the opportunity for sexual selection), proved to be at least seven times greater than the standardized variance in the mating success of males (including those not pregnant). This may represent the highest female-biased asymmetry of reproductive roles yet documented in nature for any vertebrate species, including several of the well-known shorebirds with sex-role reversal such as the phalaropes and jacanas [see citations in (80)].

PHYLOGENETIC CHARACTER MAPPING A popular approach in recent years is to trace the evolutionary origin and modification of particular morphological or behavioral features through species' phylogenies estimated independently from molecular or other evidence (98). For example, a cladogram for the Syngnathidae, based on mtDNA sequences, was recently generated and used as a phylogenetic backdrop for interpreting the diversification of varied brood pouch morphologies within the Family (152).

This same phylogeny also provided a foundation for interpreting genetic paternity data in the context of the evolutionary rationale for brood pouch elaboration (101). *Nerophis ophidion* is unusual among syngnathid species in that adult males fertilize eggs externally and carry the resulting embryos on the outside of their bodies, rather than in an enclosed brood pouch. This arrangement opens a possibility for fertilization thievery by other males. Nonetheless, paternity analyses based on microsatellites showed that cuckoldry in this species is rare or nonexistent (101). The basal lineage leading to *N. ophidion* branched off early in the syngnathid family tree. Thus, the genetic paternity data suggest that the evolutionary elaboration of enclosed brood pouches in other species of pipefishes and seahorses probably was not in response to strong selection pressures on pregnant males to circumvent cuckoldry, but rather as a means to enhance offspring care and protection (101).

Broadcast Spawners

The majority of fish species provide no parental care to their offspring (18, 19). Typically, the dispersed fry from a spawning event do not remain associated with particular candidate sires or dams, so parentage analyses are far more challenging and problematic. Nonetheless, such focused genetic appraisals have proved fruitful under some circumstances.

Avise & Shapiro (11) used allozyme markers to test the hypothesis (127) that schooling juveniles of an open-water-spawning coral reef fish, *Anthias squamipinnis*, had remained together throughout the pelagic dispersal stage and settled onto a reef as full-sib cohorts. The genetic data proved that juveniles in each school were not close relatives, but instead were a random draw from the local gene pool. In a study of a European minnow, *Phoxinus phoxinus*, Naish et al. (105) likewise showed that discrete schools consisted of unrelated individuals. Conversely, microsatellite markers revealed that discrete fish shoals in the tilapia, *Sarotherodon melanotheron*, often consisted of closely related kin (119).

In experimental populations of free-spawning cod, *Gadus morhua*, microsatellite assessments revealed that although larger males typically achieved a higher reproductive output, multiple males contributed sperm to most of the monitored spawnings (13). Such genetic studies can be used to quantify the variance in reproductive success across males. In cod, these variances were large, and the authors suggest that highly skewed paternity is an important factor contributing to the low ratios of effective population size to census population size often reported in abundant marine organisms.

OTHER PHENOMENA ELUCIDATED

Apart from revealing mating behaviors and reproductive phenomena per se, microsatellite analyses of fish parentage have also found innovative service in some unexpected applications. Three such examples follow.

Clustered Mutations

Population genetic theory traditionally treats each de novo mutation event as producing a variant allele that enters the population as a singleton. However, any mutation arising in a premeiotic germ cell lineage is likely to be copied and distributed to multiple gametes, and, hence, to two or more of that parent's progeny. Such "clustered mutations" have been theoretically neglected and empirically overlooked except in a few organisms such as *Drosophila* (153).

Fish species with large clutches and offspring care by a known parent provide favorable settings to search for clustered mutations. In one microsatellite study of 3195 brooded embryos screened in 110 families of the pipefish species *S. typhle*, a total of 35 de novo mutations were detected (78). These conformed well to a stepwise mutation model, had arisen in both paternal and maternal lines, and yielded standard mutation-rate estimates for microsatellite loci in vertebrates

(about 10^{-3} per gamete per generation). Of greater interest, however, was the fact that several of the mutations were clustered—present in multiple progeny within a brood. These findings in a fish extend the documentation of clustered mutations to another major taxonomic group.

Filial Cannibalism

Cannibalism is a widespread phenomenon in fishes: Partially digested remains of conspecific juveniles are often found inside the stomachs of adults (46, 48). Especially intriguing is filial cannibalism, wherein an adult purportedly has eaten some of his own biological offspring (66, 95, 108, 109). This counterintuitive behavior might be rationalized as an adaptive response for removing fungal-invested embryos from a clutch, thereby improving overall offspring survival in the nest (31), or for otherwise enhancing a guardian's genetic fitness if his longer-term net gain in reproductive output (e.g., by avoiding starvation, or circumventing nest desertion) outweighs the immediate fitness cost of reducing offspring numbers via filial cannibalism (66, 123, 125, 128).

Despite extensive theory and field observations on suspected filial cannibalism, the phenomenon itself, by hard criteria, was not definitively confirmed in nature until molecular paternity analyses were applied. Using PCR-based assays, DeWoody et al. (40) genetically proved that the partially digested remains of several dozen embryos recovered from the stomachs of nest-tending tessellated darters (*Etheostoma olmstedi*) and sunfish (*Lepomis punctatus* and *L. auritus*) were indeed the biological progeny of their respective fathers. This outcome was not a foregone conclusion. For reasons described above, fish nests often contain some embryos not sired by the guardian male, and many cold-blooded organisms, including some fish (22), possess refined kin-recognition capabilities (e.g., 51, 93) that might be suspected to play a role in lessening cannibalism rates on close kin (96, 115).

Local Population Sizes

A widespread approach in wildlife biology is to use physical traps in mark-recapture protocols to estimate the contemporary size (n) of a local population. For example, under the commonly employed Lincoln-Peterson statistic to analyze such data, $n = (n_1 + 1)\,(n_2 + 1)/(m_2 + 1) - 1$, where n_1 is the number of animals captured and physically marked in an initial sample, n_2 is the number of animals caught later, and m_2 is the number of recaptured (marked) animals in the second sample (117).

In a modification of this approach, Jones & Avise (72) used the data from genetic parentage analysis as a novel part of the mark-recapture protocol. In a population of the pipefish *S. floridae*, the initial marks were provided by the deduced genotypes of females (n_1) who had contributed to the broods of pregnant males, the genotypes of assayed adult females were considered the second sample (n_2), and females that matched the maternal genotypes in particular broods were the "recaptures" (m_2).

These observed counts produced an estimate of about 85–192 adult females in the local population.

As discussed by Pearse et al. (113), several modifications of this genetic approach also can be envisioned. For example, in a population that is monitored over multiple breeding seasons, both the marks and recaptures could come from the genetically deduced maternal (or paternal) genotypes in successive clutches of embryos. This method has the distinct advantage for some species in that there is never a need to physically trap the alternate sex because genes provide the marks, and breeding individuals of one sex in effect provide both the captures and the recaptures of the opposite gender (via mating). Also, the resulting estimates of *n* for a given population refer explicitly to number of successful breeders, a parameter that is of special interest in many circumstances.

CONCLUSIONS

Fish parentage analyses based on microsatellites or other molecular markers have unveiled facets of reproductive natural history and mating systems that would be difficult if not impossible to detect by other means. Furthermore, as we hope to have demonstrated, many of these findings have important ramifications for broader scientific thought about the ecology and evolution of animal reproductive strategies.

ACKNOWLEDGMENTS

Recent work in the Avise laboratory has been supported by funds from the University of Georgia and by a Fellowship in Marine Conservation from the Pew Foundation.

The *Annual Review of Genetics* is online at http://genet.annualreviews.org

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