

Genetic Perspectives on the Natural History of Fish Mating Systems

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Molecular analyses of bird and mammal populations have shown that social mating systems must be distinguished from genetic mating systems. This distinction is important in fishes also, where the potential for extrapair spawning and intraspecific brood parasitism is especially great. We review studies on fishes that have used molecular markers to document biological parentage and genetic mating systems in nature, particularly in species with extended parental care of offspring. On average, nest-guarding adults parented about 70–95% of their custodial offspring, and approximately one-third of the nests were cuckolded to some extent. Furthermore, nearly 10% of the assayed nests contained offspring tended by foster fathers either because of nest takeovers or egg thievery. On average, fish that provide parental care on nests spawned with more mates than did fish with internal fertilization and pregnancy. Overall, genetic markers have both confirmed and quantified the incidence of several reproductive and other social behaviors of fishes, and have thereby enhanced our knowledge of piscine natural history.

The diversity of reproductive modes in fishes is astounding (Breder and Rosen 1966; Dawley and Bogart 1989; Helfman et al. 1997; Taborsky 1994, 1998; Turner et al. 1992). Various species may be unisexual, hermaphroditic, or have separate sexes. Mating systems can range from self-fertilization to monogamy to obligate trio spawning to group spawning. Spawners which broadcast gametes into the open water usually provide no parental care, whereas, at the other end of the continuum, many fish species brood and nurture embryos internally. Molecular-genetic markers have clarified evolutionary aspects of reproductive patterns in several fish groups (e.g., Avise et al. 1992; Kocher and Stepien 1997; Wilson et al. 2001), and they have the potential to address issues in behavioral ecology as well.

For example, females in the family Poeciliidae carry embryos internally and give birth to live young. They often mate with multiple males, and genetic evaluations have confirmed that most broods are indeed multiply sired. Many other fish species build nests and fertilize large clutches of eggs externally. Despite an opportunity for “fertilization thievery,” molecular analyses have shown that a nest-attendant male often (but not invariably) has sired a high proportion of the offspring he tends.

Here we summarize the available literature on genetic parentage in fishes, primarily in species with extended parental care of offspring. We focus mostly on microsatellite data because their variability offers high resolution of maternity and paternity (DeWoody and Avise 2000; O'Reilly et al. 1998). We begin with a brief overview of statistical considerations on genetic parentage analyses and then discuss how genetic markers have influenced thought on the reproductive behaviors of broadcast spawners, nest builders, and brooders.

Statistical Background

A fish clutch often contains hundreds or thousands of offspring. In a typical parentage study, genotypes are examined at several hypervariable loci in a sample of juveniles and one or both of their candidate parents. Based on these genetic data, statistical methods are used to eliminate most of the candidate progenitors (DeWoody et al. 2001; Dodds et al. 1996; Jamieson and Taylor 1997). The analyses are most straightforward when one parent is known from independent evidence (e.g., a pregnant adult carrying embryos), because alleles contributed by the other parent are evident by subtraction.

Assuming that *de novo* mutation has

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been eliminated as a confounding factor, a parental “exclusion” is accomplished when these deduced alleles in a focal offspring are inconsistent with the candidate parent’s genotype. The exclusion probability (P_E) is the expected proportion of adults who can be disclaimed as parents of a randomly chosen juvenile. Its value depends on the level of assayed genetic diversity in the local population, which often has proved to be greater in marine than in freshwater fish species (DeWoody and Avise 2000; Ward et al. 1994).

Practical limitations of genetic profiling commonly lead to cases where two or more candidate adults remain nonexcluded as parents of the focal juvenile. Then, likelihood-based methods can be used for provisional parentage assignment (Bernatchez and Duchesne 2000; Marshall et al. 1998; Meagher and Thompson 1986). However, robust assignments require that all candidate parents be genotyped, and such exhaustive sampling is usually impractical. Thus, in fish species with extended adult care of broods or clutches, most studies of cuckoldry and other parentage topics have relied on parental exclusions to make behavioral inferences.

In genetic assays of large clutches, the microspatial distribution of offspring from different parents has relevance to how embryos from a nest or brood should be sampled to reveal all parents. In some species (see below), males brood their offspring in internal pouches (Jones and Avise 2001), and genetic parentage analyses have revealed that embryos from different mothers are spatially layered within the pouch. However, detailed mapping of embryos suggests that egg deposition by different females is spatially random in the nests of some *Lepomis* sunfish (DeWoody et al. 1998).

When a male tends a nest containing embryos that might have come from more than one mother, a logical question is how many of these embryos must be sampled to adequately document maternal numbers. In the simplest case, assume that the concern is merely to detect a contribution from the least represented mother who laid some proportion p of eggs in the nest. From binomial probability theory (Ross 1997), a random sample of size n will include eggs from that female with 95% certainty if

$$n \geq \ln(0.05)/\ln(1 - p).$$

Thus if two mothers contributed equitably to a nest ($p = 0.5$), a sample of about five embryos would include at least one from

each mother 95% of the time; if $p = 0.1$, n increases to about 28. These calculations assume that eggs from each mother can be unambiguously differentiated from one another. In practice, such delineations often rely on molecular markers. Despite the fact that fish microsatellites are often hypervariable (DeWoody and Avise 2000), allele sharing between parents complicates such genetic demarcations. Thus different techniques have been developed which account for marker polymorphism, reproductive skew, and other variables.

Analytical rarefaction curves can be used to determine the number of maternal gametotypes within a nest as an increasing function of the number of embryos sampled, but these curves are nest specific (DeWoody et al. 2000e). Alternatively, computer simulations have been developed to estimate sample sizes of offspring required per nest (DeWoody et al. 2000a; Fiumera et al. 2001). These programs involve resampling simulated genetic datasets across many nests, and they provide distributions from which mean sample sizes may be determined. Given a suite of markers with a P_E of roughly 0.9, a sample size of about 30–80 embryos per nest is usually sufficient to genetically detect all parents who contributed to a nest.

Species Without Parental Care

Additional challenges arise in genetic parentage studies of broadcast spawners or other species that lack parental care. One is the logistic difficulty of sampling relevant juvenile groups. A second complication for such species is that multilocus parental genotypes usually cannot be reconstructed from full-sib offspring data (DeWoody et al. 2000d).

Nevertheless, biologically meaningful questions sometimes can be addressed for offspring arrays even when neither candidate parent is known. An example is provided by an allozyme study of *Anthias squamipinnis*, a coral reef fish in which spawning pairs produce fertilized eggs that immediately begin a pelagic existence. Several weeks later, juveniles “settle” on the reef in discrete aggregations. Avise and Shapiro (1986) tested whether each juvenile school consisted primarily of full sibs that had stayed together through the pelagic phase (as hypothesized by Shapiro 1983). From the genetic analyses, juvenile aggregations proved to be composed of unrelated individuals representing a random draw from the local gene pool.

Few other studies have examined genetic relatedness in discrete groups of fish where neither suspected parent is available for analysis. However, Naish et al. (1993) showed that schools of a European minnow (*Phoxinus phoxinus*) also consist of unrelated individuals.

Nest Tenders

In species where one or more adults tend a nest, and hence are candidate parents, genetic analyses can do more than determine whether juveniles are related. Microsatellite analyses of several nest-tending species have documented multiple paternity and maternity, nest-takeover events, and egg thievery (Table 1). A fish nest may contain offspring from multiple adults of either or both gender, so questions of fatherhood and motherhood are treated separately here.

Paternity

In fishes with uniparental care, a male (rather than female) typically guards the nest (Blumer 1979). What fraction of embryos is sired by the custodian? In genetic studies of several nest-tending species, on average about 70–95% of the embryos in a nest have proved to be sired by the current attendant (Table 1, Figure 1). In other words, an attendant was genetically excluded as sire for about 5–30% of the embryos in his nest. When embryos in a tended nest are genetically inconsistent with their guardian, possible explanations include cuckoldry, nest takeovers, and egg thievery.

Cuckoldry. Stolen fertilization events are usually inferred when genetic data exclude the nest attendant as the sire of some (but not all) of the embryos in the nest. The offspring presumably resulting from cuckoldry may have been fathered by other nest-holding males (DeWoody et al. 1998) or by reproductive parasites that never build nests (Philipp and Gross 1994). Genetic studies of nesting male fish and their custodial young (Table 1) have revealed that multiple paternity may be virtually nonexistent (DeWoody et al. 2000d), moderate in frequency (DeWoody et al. 1998, 2000b,c; Jones et al. 2001), or common (Martinez et al. 2000; Philipp and Gross 1994).

However, even where the mean proportion of embryos sired by nest-holding males is high, a large fraction of nests may display multiple paternity. For example, guardian males sire more than 90% of the surveyed offspring in the redbreast sun-

Table 1. Summary of microsatellite-assessed parentage in natural populations of nesting fishes

Species	No. of young assayed	No. of nests assayed	No. of sneaked nests	No. of nest takeovers or egg-theft events	Offspring not sired by nest-tending male (%)	Minimum number of mothers (SD) per nest ^a	Estimated number of mothers (SD) per nest ^a	Reference
<i>Lepomis auritus</i> (redbreast sunfish)	996	25	11	2	11.7	3.6 (1.4)	—	DeWoody et al. 1998
<i>Lepomis punctatus</i> (spotted sunfish)	1434	30	13	1	4.5	4.4 (1.4)	7.2 (3.5)	DeWoody et al. 2000b
<i>Lepomis macrochirus</i> (bluegill sunfish)	10	1	1	0	30.0	2.0	—	Colbourne et al. 1996
<i>Micropterus salmoides</i> (largemouth bass)	1088	26	1	1	7.3	1.2 (0.5)	—	DeWoody et al. 2000d
<i>Etheostoma olmstedii</i> (tessellated darter)	610	16	2	3	14.3	3.2 (0.9)	9.2 (3.4)	DeWoody et al. 2000d
<i>Spinachia spinachia</i> (fifteenspine stickleback)	1307	28	5	4	19.0	2.6 (1.7)	—	Jones et al. 1998b
<i>Pomatoschistus minutus</i> (sand goby)	981	24	12	1	15.0	3.4 (1.2)	—	Jones et al. 2001 ^c
<i>Gasterosteus aculeatus</i> (threespine stickleback)	170	17	2	3	13.5	?	—	Rico et al. 1992
<i>Salmo salar</i> (Atlantic salmon)	50	1	1 ^d	0	NA	6.0 (0.0) ^a	—	Moran and Garcia-Vazquez 1998
<i>Salmo salar</i> (Atlantic salmon)	200	9	9 ^d	0	NA	5.0 (2.1) ^a	—	Martinez et al. 2000
Total or mean	6846	177	57	15	14.4	3.49	8.2	

^a Mean minimum number of females (or, in the case of Atlantic salmon, males) contributing to a nest as deduced directly from gametotypic counts.

^b Mean number of females (or, in the case of Atlantic salmon, males) contributing to a nest as estimated by statistical procedures in DeWoody et al. 2000a,e.

^c Data included here are only from the Klubbán population.

^d Male salmon do not “tend” nests, so the numbers refer merely to the incidences of multiple parentage.

fish (*Lepomis auritus*) and spotted sunfish (*L. punctatus*), yet about 44% of the nests in both species showed evidence of reproductive parasitism (DeWoody et al. 1998, 2000c). Across all species genetically surveyed to date, roughly one-third of the guardian males (57 of 177 nests) were cuckolded to some extent (Table 1).

Cuckoldry rates appear to be related to the density of both parental males and reproductive parasites (DeWoody et al. 2000c; Gross 1991; Philipp and Gross 1994). The availability and density of nest sites are additional environmental factors identified as potential influences on cuckoldry rates in various surveyed species

(DeWoody et al. 2000b; Fletcher D, et al., unpublished data; Martinez et al. 2000).

In some cases, multiple sires are nearly always deduced for the offspring in a fish nest. An example is provided by Atlantic salmon, where several males often spawn simultaneously with a single female (Martinez et al. 2000; Moran and Garcia-Vazquez 1998; Moran et al. 1996). In such cases it is sometimes possible via molecular markers to estimate the males' relative contributions (paternal skew) to each nest (Figure 2).

Nest takeovers and egg thievery. Occasionally a nest-tending male displays a multilocus genotype that is incompatible

with all of the embryos in his nest. Although other explanations are possible (e.g., extensive cuckoldry of an attendant who is sterile), such cases probably often reflect “nest takeovers” resulting from the death, abandonment, or expulsion of the resident male. Nest takeovers may also be inferred if young embryos from a nest are consistent with paternity by the guardian male whereas older embryos are not. Among sunfishes, gobies, and darters, 8 of 122 nests surveyed (~7%) were probable takeovers (DeWoody et al. 1998, 2000b–d; Jones et al. 2001; Table 1). It is not certain that selective factors must be invoked to explain the evolution of such male behaviors, but some authors have argued that “stepfathers” might provide alloparental care in hopes of attracting females that are more willing to spawn in nests that already contain eggs (Constantz 1985; Sargent 1989).

Genotypic incompatibility between an embryo and its guardian may also result from “egg thievery.” In some species, eggs clump together, and genetic data have helped to confirm observations from the field that males sometimes steal a discrete clump of fertilized eggs from another nest (Rico et al. 1992). In the fifteenspine stickleback (*Spinachia spinachia*), 4 of 24 nests (~17%) appeared by genetic evidence to contain embryo clumps that the guardian male probably stole (Jones et al. 1998b). Again, a possible adaptive explanation is

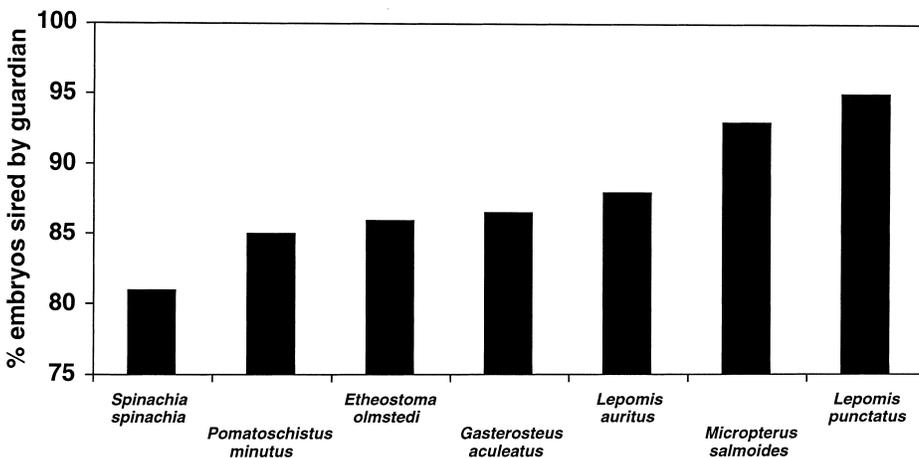


Figure 1. The mean proportion of embryos sired by the caregiving adult male in nests of each of seven fish species (for citations, see Table 1).

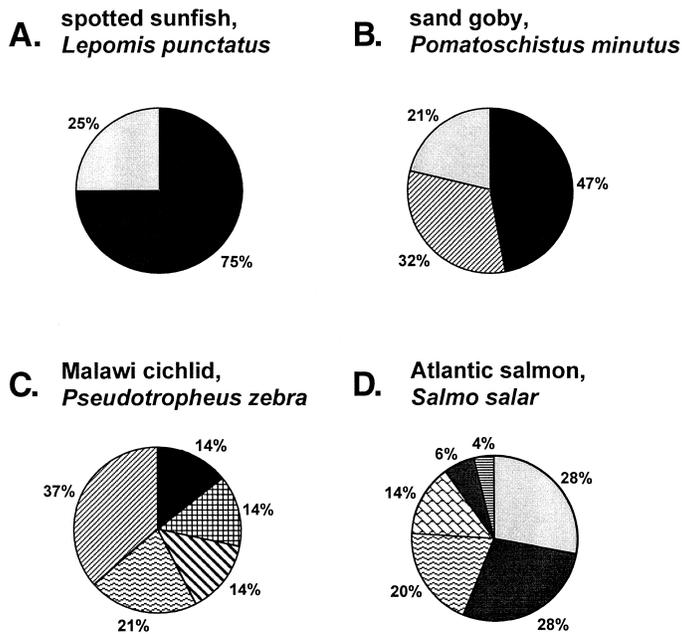


Figure 2. Examples of reproductive skew in a representative nest from each of four fish species (see citations in Tables 1 and 2; the example for Atlantic salmon comes from Moran and Garcia-Vazquez 1998). Each pie diagram summarizes the relative contributions (genetically deduced) of different mothers (A and B) or fathers (C and D) to the pool of embryos within a nest or brood.

that a stolen clutch may prime females to deposit additional eggs in the thief's nest.

Maternity

A male-tended nest may contain embryos from multiple mothers, and in several fish species molecular markers have been employed to address various maternity issues as well.

Number of mothers. The age or appearance of eggs and embryos are inadequate indicators of maternal contributions to a nest (DeWoody et al. 1998, 2000b,c; Kellogg et al. 1998). Thus in recent years biologists have turned to measures of gametic diversity afforded by molecular markers. In addition to providing refined evidence on maternal numbers, molecular markers have been used in some cases to map the microspatial distributions within a nest or brood pouch of embryos from different females (DeWoody et al. 1998; Jones and Avise 1997b; Jones et al. 1999).

The minimum number of mothers who have contributed to a nest can be estimated by counting the maternal gametotypes in the progeny array. Using this method as applied to mitochondrial DNA haplotypes, Crow et al. (1997) reported that about 40% of the nests in the kelp greenling (*Hexagrammos decagrammus*) contained embryos from more than one mother. In microsatellite assays of several other species, on average at least 3.1 females were documented to have spawned

in a typical fish nest (from data in Table 1, excluding Atlantic salmon).

The true incidence of multiple maternity is usually higher because some maternal gametotypes remain undetected in these "face-value" counts (Fiumera et al. 2001). Recent theoretical work has addressed various issues bearing on the genetically deduced number of parents contributing to a progeny array (DeWoody et al. 2000a,e; Harshman and Clark 1998; Kichler et al. 1999). These typically correct the maternal estimates upward. In the spotted sunfish, for example, computer simulations of the empirical genetic data suggest that on average about 7.2 females spawned per nest, whereas the minimum number of mothers based on face-value genotypic counts averaged only 4.4 (DeWoody et al. 2000b).

Extensive sampling of progeny can also reveal the reproductive skew in maternal contributions to a nest. For example, DeWoody et al. (2000e) genotyped nearly all of the 900 embryos from a single nest of the sand goby (*Pomatoschistus minutus*) and found that three mothers had contributed 47%, 32%, and 21% of the eggs subsequently fertilized. Other examples of maternal reproductive skew within a nest are illustrated in Figure 2. In general, reproductive skew can only be estimated when the number of genotyped embryos is very large relative to the number of parents.

Respawning. When a female fails to lay all of her eggs during a single mating bout, she may spawn again in another nest. Genetic detection of such respawning requires, minimally, that a mother's gametotypes are represented in the progeny of multiple nests. The nests must also be spatially consistent with this interpretation, exclusion probabilities must be high, and egg thievery must be considered as a possible alternative explanation. Jones et al. (1998b) and DeWoody et al. (2000d) provide empirical examples in the fifteen-spine stickleback and the largemouth bass, respectively.

A female may also spawn with the same male in temporally discrete bouts. Temporal respawning is evident, for example, when genetic markers reveal that nestmate embryos of two different developmental phases share the same mother. DeWoody et al. (1998, 2000c) provide empirical examples in the redbreast sunfish and tessellated darter (*Etheostoma olmstedii*).

Cuckoldry. Embryos from multiple females are routinely found in nests parented by a single male; such multiple matings may be sequential or simultaneous. In rare cases, simultaneous spawning by multiple females can be thought of as "female cuckoldry" (DeWoody et al. 2000d). In monogamous species with biparental care, females who steal fertilizations from nesters may reap benefits similar to those of cuckold males (discussed above). For example, DeWoody et al. (2000d) found that most (23 of 26) largemouth bass nests were composed of full-sib offspring and thus were parented by a single male and a single female. Only one guardian male was cuckolded, but a very small proportion of offspring in four nests were genetically inconsistent with the primary mother. These maternal exclusions were typically based on multiple loci, and in each case the embryos in question were sired by the guardian male. Thus one possible explanation is that female bass occasionally cuckold one another. We predict that female cuckoldry will eventually be documented in other monogamous fishes that exhibit biparental care.

Pregnant Species and Brooders

Pregnant and brooding species offer logistic advantages in parentage analyses over nest builders by virtue of the fact that progeny arrays come "prepackaged." Hence one biological parent of each brood typically is known unambiguously (Jones

Table 2. Summary of microsatellite-assessed parentage in natural populations of mouthbrooding or pregnant fishes

Species	No. of embryos assayed	No. of broods assayed ^a	Minimum number of mates (SD) per brood ^b	Reference
<i>Gambusia holbrooki</i> (mosquitofish)	823	50	2.0 (0.5)	Zane et al. 1999
<i>Syngnathus scovelli</i> (Gulf pipefish)	838	40	1.0 (0.2)	Jones and Avise 1997a
<i>Syngnathus floridae</i> (dusky pipefish)	924	22	1.9 (0.6)	Jones and Avise 1997b
<i>Syngnathus typhle</i> (Swedish pipefish)	1344	30	3.1 (1.2)	Jones et al. 1999
<i>Hippocampus angustus</i> (western Australian seahorse)	453	15	1.0 (0.0)	Jones et al. 1998a
<i>Pseudotropheus zebra</i> (a Lake Malawi cichlid)	99	7	3.7 (2.0)	Parker and Kornfield 1996
7 species of African cichlids (from Lake Malawi)	203	16	2.1 (1.3)	Kellogg et al. 1995
<i>Protomelas spilopterus</i> (a Lake Malawi cichlid)	200	6	1.7 (0.8)	Kellogg et al. 1998
<i>Poecilia reticulata</i> (guppy)	1812	253	1.5 (0.6)	Kelly et al. 1999 ^c
Total or mean	6699	439	2.0 (0.9)	

^a A brood may include embryos of different developmental phases, so there may be more than one "clutch" per brood.

^b As distinguished from the true number of successful mates (see DeWoody et al. 2000a,e).

^c Unpublished data provided by C. Kelly and J.-G. J. Godin based on Kelly et al. (1999).

and Avise 2001). Several genetic studies have employed molecular markers to estimate biological parentage in species with internal fertilization (Table 2). Our discussion of pregnant and brooding fishes is largely restricted to fishes other than the Syngnathidae [because results for the latter are discussed in detail by Jones and Avise (2001)].

Viviparity

Some of these studies have focused on live-bearing fishes of the family Poeciliidae. These females are internally fertilized, store sperm, brood embryos internally, and give birth to free-swimming young. Chesser et al. (1984) speculated that these features would promote multiple insemination, and they used a panel of three allozyme loci to document multiple paternity in 56% of the mosquitofish (*Gambusia affinis*) females surveyed. Likewise, Travis et al. (1990) found multiple paternity in 52% of pregnant sailfin mollies (*Poecilia latipinna*). As recognized by the authors of both studies, true levels of multiple paternity were likely greater than those detected because of the limited exclusionary power of allozyme markers. Zane et al. (1999) revisited the issue in mosquitofish using more variable microsatellite loci and found levels of multiple paternity approaching 100%.

Some authors have focused on multiple paternity and its correlates. Kelly et al. (1999) reported that guppy (*Poecilia reticulata*) females exposed to high predation

risk were multiply inseminated more frequently than those in low-predation regimes. The authors suggested that the risk of local predation mediates sexual selection through sperm competition. Trexler et al. (1997) found that larger female sailfin mollies were more likely to be multiply inseminated than smaller females, and interpreted this as meaning that larger females are the objects of strong male-male competition. In nest-building fishes, however, there is varied evidence regarding body size and the number of genetically successful mates. Parker and Kornfield (1996) as well as Thomaz et al. (1997) found a significant correlation between these two variables, whereas in other species Jones et al. (1998b, 2001) and DeWoody et al. (2000b) did not.

Another genetic comparison is possible in nesting versus brooding fishes. In species examined to date, the number of genetically deduced mates is significantly higher in nest builders than in brooders (Tables 1 and 2; one-tailed *t* test, $P < .05$). This suggests that opportunities for direct physical unions may be more limited and/or that individual mating choices are more stringent in internal brooders than in species that spawn by releasing gametes into the water. Also, brooders probably are limited by anatomical constraints (e.g., the size of the brood pouch or buccal cavity), whereas nest tenders may be less constrained in terms of how many adults can contribute gametes to a nest. Alternatively, the difference in number of mates may

be related to the fact that most of the viviparous species listed in Table 2 are maternal brooders which do not gain fecundity by multiple mating.

Oviparity

Some oviparous (egg-laying) species also provide parental care, as for example when externally fertilized eggs and the resulting fry are brooded in a female's mouth. Such is the case for *Pseudotropheus zebra*, a cichlid fish from Lake Malawi in which Parker and Kornfield (1996) used microsatellite markers to show that six of seven clutches had been multiply sired and that the mouthbrooding female in each case was the biological mother of all assayed offspring. However, in *Protomelas spilopterus*, another African cichlid, Kellogg et al. (1998) showed that females often brood unrelated young and hence can display alloparental care.

Apart from focused analyses of parentage in particular broods, microsatellite analyses also have found application in extended populational contexts. For example, many mouthbrooding cichlids are social, and adults form shoals. In one such species, the tilapia (*Sarotherodon melanotheron*), Pouyaud et al. (1999) deduced from microsatellite data that aggregations in rivers consisted of unrelated individuals, whereas some schools in lagoons had genetic profiles indicative of full- or half-sib groups. In another microsatellite study, Knight et al. (1998) demonstrated assortative mating by color morphs in three species of Lake Malawi cichlids and suggested that such behaviors probably helped to promote the rapid genetic isolation and speciation that is characteristic of many African cichlids, particularly in lake environments.

Conclusion

Genetic studies compiled in the current review have documented a number of reproductive and social behaviors that had been suspected in various fish species from direct ecological observations. These include kin recognition and schooling behaviors in oviparous species, multiple mating in viviparous species with internal brooding by either males or females, and a variety of behaviors leading to alloparental care, including both male and female cuckoldry in nest-tending species with external fertilization, egg thievery, nest takeovers, and the mouthbrooding of foster young. Beyond documenting the reproductive consequences of such behaviors,

these genetic studies have also provided preliminary quantitative estimates of their relative frequencies of occurrence. Especially when interpreted in conjunction with field data, molecular markers will continue to illuminate otherwise uncertain aspects of fish natural history.

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