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Intensive genetic assessment of the mating system and reproductive success in a semi-closed population of the mottled sculpin, *Cottus bairdi*

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Abstract

Most genetic surveys of parentage in nature sample only a small fraction of the breeding population. Here we apply microsatellite markers to deduce the genetic mating system and assess the reproductive success of females and males in an extensively collected, semi-closed stream population of the mottled sculpin fish, *Cottus bairdi*. In this species, males guard nest rocks where females deposit the eggs for fertilization. The potential exists for both males and females to mate with multiple partners and for males to provide parental care to genetically unrelated offspring. Four hundred and fifty-five adults and subadults, as well as 1259 offspring from 23 nests, were genotyped at five polymorphic microsatellite loci. Multilocus maternal genotypes, deduced via genetic analyses of embryos, were reconstructed for more than 90% of the analysed nests, thus allowing both male and female reproductive success to be estimated accurately. There was no genetic evidence for cuckoldry, but one nest probably represents a takeover event. Successful males spawned with a mean of 2.8 partners, whereas each female apparently deposited her entire clutch of eggs in a single nest (mean fecundity = 66 eggs/female). On average, genetically deduced sires and dams were captured 1.6 and 9.3 metres from their respective nests, indicating little movement by breeders during the spawning season. Based on a 'genetic mark–recapture' estimate, the total number of potentially breeding adults (*c.* 570) was an order-of-magnitude larger than genetically based estimates of the effective number of breeders (*c.* 54). In addition, significantly fewer eggs *per female* were deposited in single than in multidam nests. Not only were perceived high-quality males spawning with multiple partners, but they were receiving more eggs from each female.

Keywords: effective number of breeders, mark–recapture, maternity, parentage analysis, paternity

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Introduction

Genetic analyses of fish reproduction in nature have provided insights into broader aspects of animal mating system evolution (reviewed in Avise 2001; Avise *et al.* 2002). Molecular markers have been used to quantify reproductive success of fishes that exhibit such diverse phenomena as egg thievery (Jones *et al.* 1998), alloparental care of embryos (Munehara & Takenaka 2000) and alternative mating tactics and morphs of breeding adults (DeWoody & Avise 2001; Neff 2001). Genetic parentage analyses in fishes and other taxonomic groups have also been used to estimate population parameters such as gene flow (Kameyama *et al.* 2001), mutation rates and patterns (Jones *et al.* 1999), heritabilities of quantitative traits (King *et al.* 2001) and the nature and intensity of sexual selection (Greene *et al.* 2000; Jones *et al.* 2000, 2001a,b).

Most genetic studies of fish parentage have surveyed relatively open populations and sampled only a small proportion of the potential breeders. In addition, one sex (that which tends the dozens to thousands of embryos in a nest) is often represented disproportionately in the collected

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sample of prospective parents. By contrast, in the current study of the mottled sculpin (*Cottus bairdi*) we have characterized matings and reproductive success for nearly an entire local deme within a typical southern Appalachian stream. Mottled sculpins are good subjects for genetic analyses as they generally have restrictive movement patterns (Downhower & Brown 1979, 1980; Hill & Grossman 1987; Petty 1998) and their highly conspicuous nests are frequently found at moderate to high densities (G. Grossman pers. obs.). These features allowed us to collect and examine a large fraction of nests and putative parents (of both sexes) from a section of stream housing a fairly closed breeding population.

Breeding biology of the mottled sculpin has been studied in the field and aquaria. In early spring, males select and defend cavities under rocks (or other debris) where they court females (Savage 1963). Females probably visit multiple males before spawning (Downhower & Brown 1979), and empirical evidence suggests that each female produces a single clutch per year which she attaches to the underside of the nest-rock of one guardian male (Savage 1963). Larger males are preferred, and multiple females may deposit eggs into a nest (Brown & Downhower 1982a). During incubation, males defend the nest against predation, including that by conspecifics (Bailey 1952).

Here we use microsatellite DNA markers to characterize the genetic mating system and determine paternal and maternal reproductive success in a local population of the mottled sculpin. The genetic parentage data are also employed in a mark-recapture analysis to estimate the local number of potential breeders (N), which in turn is compared to the effective number of breeders (N_b).

Materials and methods

Field collections

Fish were collected from an approximately 150-m section of Shope Fork Creek within Coweeta Hydrological Laboratory in Otto, North Carolina. The population was semi-closed: the downstream point was marked by a pipeline crossing the creek (creating a 0.5-m waterfall), and the upstream end was blocked by a weir about 50 m beyond the study area. To facilitate locating the exact position of each fish and nest, permanent markers were placed at two meter intervals along both banks.

Twenty-three nests and 79 adults were collected between 22 and 25 April, 1999, via snorkelling (27 total hours in the water and three passes through the stream) using aquarium dip-nets. The locations of captured (or missed) individuals and nests were marked with numbered weights, and exact spatial relationships within the stream were reconstructed by triangulation from the bank markers. An additional 376 individuals and one nest were collected using a backpack electro-fisher (two passes on 27 April), and the locations of these samples were recorded to within four stream-metres. Live standard lengths and weights of the fish were measured, individuals were sexed via internal examination and adult tissues were stored in 95% EtOH for DNA analysis.

A nest often contained multiple, partially discrete egg masses. Each fertilized mass was removed from the rock and preserved individually in a solution of 0.25 M EDTA, 20% DMSO and saturated NaCl. For each nest, the number of egg masses and the number of healthy and fungal-infected eggs were recorded.

Microsatellite analysis

A size selected genomic library for *C. bairdi* was constructed according to standard protocols (Choudhary *et al.* 1993) and then screened with multiple di-, tri-, and tetranucleotide probes. Positive clones were sequenced on an ABI 310 automated sequencer and PCR primer pairs were developed from the flanking sequences. In addition, 12 primer sets developed for *Cottus gobio* (Englbrecht *et al.* 1999) were assayed for polymorphism in *C. bairdi*.

From adults and post-hatching juveniles, DNA was extracted using standard phenol/chloroform methods (Maniatis *et al.* 1982); from embryo-containing eggs, DNA extractions were based on a modified fly buffer protocol (Gloor & Engels 1992; DeWoody *et al.* 2000a). PCR amplifications were conducted according to the thermal conditions in Table 1 using 0.1 unit Promega *Taq* polymerase, 1 × PCR buffer, 1.5 mM MgCl$_2$, 0.3 mM of each primer, 0.1 mM of each dNTP and 1 μL DNA template. Loci *Cba*14 and *Cba*51 were multiplexed; all other reactions were performed separately. Markers which proved consistently scorable and polymorphic on radioactive gels were run on an ABI 377 sequencer using fluorescent primers, and genotypes were classified using the associated software. All five loci, distinguishable by their fluorescent colours and nonoverlapping sizes, were assayed together on single gels.

Parentage analyses

For most nests, a total of 48 embryos (equal numbers from each egg mass) were genotyped at the five polymorphic loci. From BROOD simulations (DeWoody *et al.* 2000b), this number of offspring was determined to provide adequate statistical power for detecting the allelic contributions of at least eight females, assuming that they contributed equally to a nest. For nests containing fewer than 48 eggs, all offspring were analysed. In addition, two nests (N015 and N012) were sampled more extensively, with 68 of 355 embryos and 209 of 210 embryos analysed, respectively.

Table 1 Primer sequences and PCR conditions for the five microsatellite loci in mottled sculpins. PCR conditions began with a 2-min denaturing at 94 °C followed by 30 cycles of the respective thermal profile. Thermal profiles represent times (in seconds) spent denaturing at 94 °C, annealing at the appropriate temperature, and extending at 72 °C with a final extension (in minutes) at 72 °C

Primer*	Fluorescent label	Sequence	Thermal profile
*Cba*14F	5′ FAM	5′-TTGGTGGCACACATGTTTGGA-3′	30, 30, 30 @ 55 °C, 2-min extension
*Cba*14R		5′-ACACATCGTCAGTTAGAGCAG-3′	
*Cba*42F	5′ HEX	5′-AAATGGTCGTCTGCTCCCTG-3′	30, 30, 30 @ 60 °C, 2-min extension
*Cba*42R		5′-AGGCAGTGTGGGCATGAAAG-3′	
*Cba*51F	5′ FAM	5′-ATCAACCGGCACTGCAGA-3′	30, 30, 30 @ 55 °C, 2-min extension
*Cba*51R		5′-ATGGAGGGCTACTGAGAATC-3′	
*Cgo*18ZimF	5′ FAM	described in Englbrecht *et al.* (1999)	45, 45, 45 @ 49 °C, 10-min extension
*Cgo*18ZimR		described in Englbrecht *et al.* (1999)	
*Cgo*34ZimF	5′ HEX	described in Englbrecht *et al.* (1999)	45, 45, 45 @ 49 °C, 10-min extension
*Cgo*34ZimR		described in Englbrecht *et al.* (1999)	

*Sequenced clones for *Cba*14, *Cba*42 and *Cba*51 have GenBank accession nos AF519795, AF519796 and AF519797, respectively.

Nest-guarding males were excluded genetically as the biological sire when they were inconsistent with offspring genotypes at multiple loci. For nests at which no guardian male was captured, multilocus paternal genotypes were deduced and matches were made to potential sires in the collection of adults. In most cases, dams were identified by matching reconstructed multilocus maternal genotypes to particular females from the adult population. If multilocus maternal genotypes could not be reconstructed (see below), the minimum number of dams contributing to a nest was estimated by directly counting the number of distinct, maternally derived alleles in the progeny array (Parker & Kornfield 1996).

Mark–recapture estimates of population size

Data from parentage studies can be utilized in 'genetic mark–recapture' analyses to estimate the contemporary size of a breeding population (for details see Jones & Avise 1997; Pearse *et al.* 2001). By applying a corrected Lincoln-Peterson estimator (Pollock *et al.* 1990), that population size is calculated as:

$$\hat{N} = \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)}$$

where n_1 is the number of reconstructed multilocus parental genotypes (i.e. the number of marked individuals), n_2 is the number of adults subsequently captured, and m_2 is the number of matches of captured adults to the genetically deduced parents (i.e. the number of recaptures). The variance of this estimate (Pollock *et al.* 1990), used to generated the 95% confidence limits, is given by:

$$Var(N) = \frac{(n_1 + 1)(n_2 + 1)(n_1 - m_2)(n_2 - m_2)}{(m_2 + 1)^2(m_2 + 2)} .$$

Only individuals two years or older (based on otolith ageing; Grossman *et al. unpublished*) were included in the count of n_2, as they represent the potential breeders.

Estimates of the effective number of breeders

The effective population size, N_e, can often be used as surrogate for the effective number of breeders, N_b (Scribner *et al.* 1997). In this study we used discrete generation N_e estimators to calculate the effective number of breeders (N_b). It is important to recognize that the adult-to-progeny measure of N_b that we have calculated here is not the same as a single generation N_e estimate because *C. bairdi* maintains overlapping generations. For consistency, we have presented the formulae as they appear in the original publications. Here, N_e was estimated in two ways. In the first approach, based on variances in reproductive success among individuals, N_{em} and N_{ef} are the effective numbers of breeding males and females, respectively (Kimura & Crow 1963). In the present study, the effective number of males was computed as

$$N_{em} = \frac{N_m\bar{k}_m - 1}{\bar{k}_m - 1 + \dfrac{V_{km}}{\bar{k}_m}} ,$$

where N_m is the census number of breeding males, $\bar{k}_m$ is the mean number of offspring produced per male (deduced from the genetic paternity analyses in conjunction with census numbers of eggs per nest), and V_{km} is the variance in male reproductive success; an analogous formula yields the effective number of breeding females. Then, N_e was estimated as:

$$N_e = \frac{4N_{em}N_{ef}}{N_{em} + N_{ef}} .$$

Table 2 Characteristics of the 23 analysed nests of the mottled sculpin

Nest	Guarding male present?	No. egg masses	Total no. healthy eggs (per mass)	No. fungal-infected eggs in each egg mass
N003	yes	3	331 (164, 95, 72)	0, 0, 0
N012	yes	4	210 (81, 46, 45, 38)	0, 0, 0, 0
N015	nearby	3	355 (230, 80,45)	0, 0, 0
N017	yes	3	279 (161, 98, 20)	$\approx$ 30, 0, 90
N027	nearby	1	48	0
N033	yes	3	240 (139, 63, 38)	0, 0, 0
N034	nearby	2	191 (102, 89)	6, 8
N041	yes	1	30	13
N046	no	4	263 (116, 51,50, 46)	4, 1, 6, 0
N058	nearby	1	135	6
N059	nearby	1	89	4
N063	yes	1	40	0
N064	no	1	105	0
N065	yes	3	127(62, 35, 30)	$\approx$ 35, 25, 2
N067	no	2	85 (48, 37)	3, 4
N070	nearby	3	158 (77, 54, 27)	2, 20, 1
N071	nearby	2	262 (153, 109)	2, 1
N078	nearby	4	345 (122, 103, 83, 37)	7, 5, 4, 18
N083	yes	1	120	2
N095	no	1	53	0
N100	yes	2	173 (118, 55)	0, 0
N101	no	3	273 (155, 60, 58)	0, 0, 0
N108	no	2	220 (156, 64)	12, 3

In the second approach, N_e was estimated from temporal changes in allele frequency (Nei & Tajima 1981; Pollak 1983; Waples 1989), in this case from the parental to the offspring generation. The standardized variance in allele frequency change between generations (F_k) is given by:

$$\hat{F}_k = \frac{1}{K-1} \sum_{i=1}^{k} \frac{(x_i - y_i)^2}{(x_i + y_i)/2}$$

where K is the number of segregating alleles at a locus and x_i and y_i are the frequencies of the ith allele in the adults and progeny, respectively (Pollak 1983; Waples 1989). Allele frequencies in the progeny were estimated as the weighted average from within each nest. For multiple loci, the weighted mean of the single locus values is calculated according to:

$$\hat{F}_k = \sum (K_j - 1)\hat{F}_{kj} / \sum (K_j - 1),$$

for K_j alleles at the jth locus (Tajima & Nei 1984; Waples 1989). Under sampling plan I as defined by Waples (1989), the estimated effective population size (i.e. the effective number of breeders) is:

$$\hat{N}_e = \frac{t}{2[\hat{F}_k - 1/(2S_0) - 1/(2S_t) + 1/N]}$$

where t is the number of generations traversed (one, in our case), S_0 and S_t are the sample sizes at times 0 and t (here, the adult and offspring generations), and N is the census size in the generation previous to the earliest sampled (assumed to be the same as the potential adult breeders, as estimated from the genetic mark–recapture analyses; see above). Confidence intervals were generated as described in Waples (1989).

Results

Population and nest characteristics

Of the 455 free-swimming individuals captured, 117 were less than 2 years old (based upon otolith ageing; Grossman *et al.* unpublished) and, thus, probably were not yet reproductively mature. Among the 338 adults, the sex ratio was 1.3:1 (females:males).

The DNA in embryos from one nest failed to amplify. Characteristics of the remaining nests are summarized in Table 2. For nine of the 23 nests, a male was captured tending the nest rock. In eight additional nests, a potential sire was captured within 1 m of the nest rock at the same time as the nest was discovered (i.e. in the immediate vicinity of the nest rock). All embryos were 'eyed' and showed little variation in size, either within or between nests. The mean

Table 3 Marker polymorphism in mottled sculpins. Shown are the number of alleles, expected (H_E) and observed (H_O) heterozygosities and exclusion probabilities (under the 'neither parent known' or 'one parent known' models)

Locus	No. alleles	H_E	H_O	P_E (neither known)*	P_E (1 known)†
Cba14	9	0.77	0.81	0.39	0.57
Cba42	4	0.57	0.58	0.16	0.30
Cba51	16	0.73	0.64	0.37	0.56
Cgo18Zim	23	0.88	0.85	0.62	0.76
Cgo34Zim	8	0.75	0.74	0.36	0.54
mean	12	0.74	0.72	0.92‡	0.99‡

*Calculated using equation 2a in Jamieson & Taylor (1997).
†Calculated using equation 1a from Jamieson & Taylor (1997).
‡Cumulative exclusion probability across all loci, calculated using equation 4 from Jamieson & Taylor (1997).

number of 'distinct' egg masses was 2.2 (± 0.23 SE; range 1–4). The mean number of healthy eggs per nest was 179.7 (± 21.2 SE; range 30–355), and the mean number of healthy eggs per mass was 79.5 (± 6.4 SE; range 27–230). Finally, 13 of the nests (57%) contained at least one fungal-infected egg, and the mean number of infected eggs per nest was 13.6 (± 5.7 SE; range 0–120).

Microsatellite markers

Fifteen positive clones were sequenced, 11 of which proved to contain microsatellite repeats [nine with $(GT)_n$, one with $(CT)_n$, and one with both $(GT)_n$ and $(CT)_n$ repeats]. Three of these loci, and two developed by Englbrecht *et al.* (1999) were consistently scorable and polymorphic (Table 3). For the 455 adult and subadult individuals surveyed, the number of alleles per locus ranged from 4 to 23, and expected heterozygosities from 0.57 to 0.88. The cumulative exclusion probabilities were 0.92 (neither-parent-known model) and 0.99 (one-parent-known model).

Locus Cba51 showed a significant deficit of heterozygotes relative to Hardy–Weinberg expectations (Raymond & Rousset 1995; Rousset & Raymond 1995), suggesting the presence of a null allele. Using the technique of Chakraborty *et al.* (1992), the frequency of such an allele was estimated to be 0.075. Given this allele frequency, about three of the 455 genotyped adults and subadults would be expected to be null homozygotes, yet every individual in the population amplified at locus Cba51. However, the deduced genotypes of three unsampled parents were reconstructed as null heterozygotes. Based on the reconstructed multilocus genotypes it was estimated that *de novo* mutations occurred in 13 of the 1259 sampled offspring. As these offspring were genotyped at 5 loci, this would yield a rough estimate of the mutation rate at 2.1×10^{-3} per locus per meiosis, which is consistent

with published estimates for other microsatellite loci (see Ellegren 2000).

Paternity

A total of 1259 embryos were analysed from the 23 nests (Table 4). In 22 of those nests (96%), all the progeny were consistent with having been sired by a single male. The one exception (N059) registers a probable nest takeover event. Here, two sires contributed to the nest and each had spawned with a different, unique female. Although neither sire was captured guarding this nest, one of the two males was captured 1 m upstream.

Additionally, the embryos in nest 064 were consistent with being full-sib progeny, except for two analysed individuals who differed at multiple loci. As none of the genetically decided parents of this nest was captured, it could not be deduced whether these two progeny register low-level cuckoldry or a case where one of two females deposited only a few eggs into the nest. Given the complete absence of cuckoldry in the other sculpin nests, in subsequent analyses we assumed provisionally that nest 064 had been sired by a single male (Table 4).

Unique matches for 14 of the 24 genetically deduced males (58%) were identified among the sampled adults and there was no evidence that males spawn in more than one nest. All nine males captured guarding a nest were identified as the true sire of their respective embryos. Thus, no alloparental care was observed.

Maternity

In many (but not all) cases, the genotypes of embryos within each 'discrete' egg mass were consistent with their being full-sibs and, thus, the multilocus genotype of each contributing female could be reconstructed easily. The mean number of genetically deduced dams per nest was 2.8 (± 0.3 SE; range 1–6) (Table 4). Overall, at least 65 females contributed to the sculpin progeny arrays assayed, and often in varying proportions (Table 4). Of these, a total of 59 multilocus maternal genotypes could be reconstructed exactly, and perfect genetic matches were found for 35 of these (59%) in the collection of females from the adult population. In two nests (N017 and N070), however, genotypes of the progeny were distributed more randomly among the egg masses that we had delineated, and multilocus maternal genotypes could not be deduced with confidence. Although the number of egg masses was a useful predictor of the number of dams ($r^2 = 0.7$, $P \ll 0.001$; Fig. 1), it fell significantly below the genetically deduced estimates of females contributing to a nest ($P < 0.001$, paired *t*-test).

It appears probable that each female deposited most or all of her eggs in the nest of a single male. First, in no case

Table 4 Parentage results in mottled sculpins. Shown for each nest are the genetically deduced sire, his distance from the nest when captured (+, upstream; –, downstream), the number of analysed offspring consistent with each deduced male, the number of genetically deduced dams contributing to each nest and the relative contribution of each female

Nest	Genetically deduced sire	Sire captured?	No. of analysed progeny sired by male	No. of genetically deduced dams	Relative contributions of females (%)
N003	CB003	on nest	48 of 48	4	50, 22, 19, 9
N012	CB012	on nest	209 of 209	4	40, 22, 20, 18
N015	MM015	not captured	48 of 48	6	48, 20, 13, 11, 4, 4
N017	CB017	on nest	68 of 68	minimum of 3*	not deduced
N027	CB248	–11 m away	48 of 48	1	100
N033	CB033	on nest	48 of 48	4	47, 26, 16, 11
N034	CB039	+1 m away	48 of 48	3	46, 34, 20
N041	CB041	on nest	30 of 30	1	100
N046	CB321	–9 m	48 of 48	4	37, 26, 19, 18
N058	MM058	not captured	48 of 48	2	62, 38
N059†	CB005	+1 m away	15 of 48	1	100
	MM059	not captured	33 of 48	1	100
N063	CB063	on nest	40 of 40	1	100
N064	MM064	not captured	48 of 48‡	2‡	96, 4‡
N065	CB065	on nest	48 of 48	4	28, 24, 24, 24
N067	MM067	not captured	48 of 48	2	56, 44
N070	CB182	0 m away, not guarding	48 of 48	minimum of 3*	not deduced
N071	MM071	not captured	48 of 48	3	41, 32, 27
N078	MM078	not captured	48 of 48	4	30, 29, 22, 19
N083	CB083	on nest	48 of 48	2	54, 46
N095	MM095	not captured	48 of 48	1	100
N100	CB100	on nest	48 of 48	3	48, 32, 20
N101	MM101	not captured	48 of 48	3	56, 25, 19
N108	MM108	not captured	48 of 48	3	56, 29, 15

*The multilocus female genotypes could not be reconstructed, so the number of females was estimated via the single-locus minimum method (Parker & Kornfield 1996).

†The nest was composed of two, full-sib progeny arrays. Male CB005 mated to female CB125 and male MM059 mated to female CB155. Neither male was observed guarding the nest.

‡The nest consistent with a full-sib progeny array, excepting two offspring (at multiple loci). Neither the deduced male(s) nor the female(s) were captured and, thus, cuckoldry could not be definitively invoked. The nest was assumed to have been sired by a single male and dammed by two females.
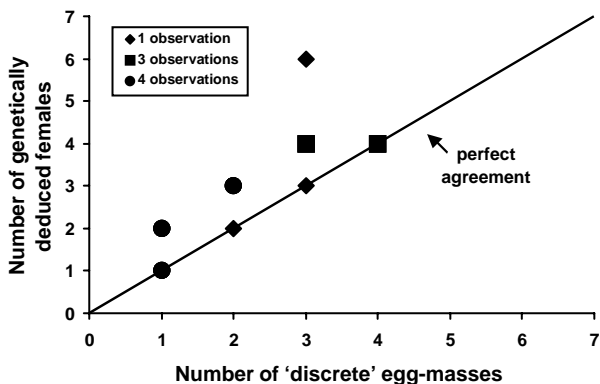

Fig. 1 Number of 'discrete' egg masses in each nest vs. the number of genetically deduced dams. The line represents what would be a perfect agreement between the genetic and face-value estimates.

was the same reconstructed multilocus maternal genotype present in more than one nest. Second, a plot of total embryo numbers in each nest against the deduced number of dams demonstrates that estimates of genetically deduced female fecundity agree well with those from ovarian counts (Fig. 2). The mean number of embryos contributed by each dam in this study was 66 ($\pm$ 4.6 SE; range 4–168 eggs/female) compared to 71 ($\pm$ 2 SE; range 9–166) calculated using ovarian counts from Grossman *et al.* (2002).

Interestingly, significantly more eggs *per female* were deposited in multidam nests (70 $\pm$ 4.9 SE) than in nests with only a single mother (46 $\pm$ 2.8 SE) (Fig. 3). Male guardians of single- vs. multidam nests did not differ significantly in body size, but mothers of the single-dam nests were smaller than those of multidam nests ($P = 0.004$; one-tailed *t*-test assuming unequal variances, total $n = 57$). The
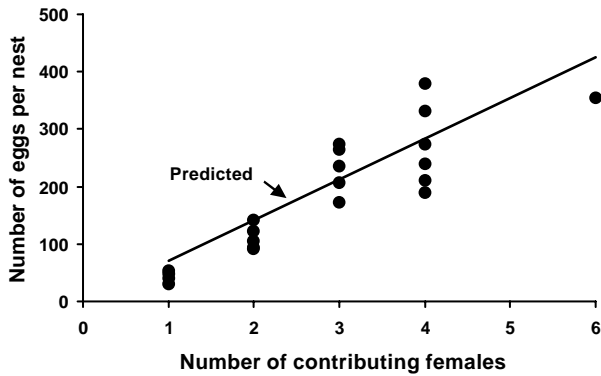
Fig. 2 Total number of eggs per nest as a function of the number of genetically deduced dams. The line represents the predicted number of eggs (assuming that each female deposited all her eggs in one nest) based on a mean of 71 eggs per female from direct ovarian counts (Grossman *et al*. 2002).
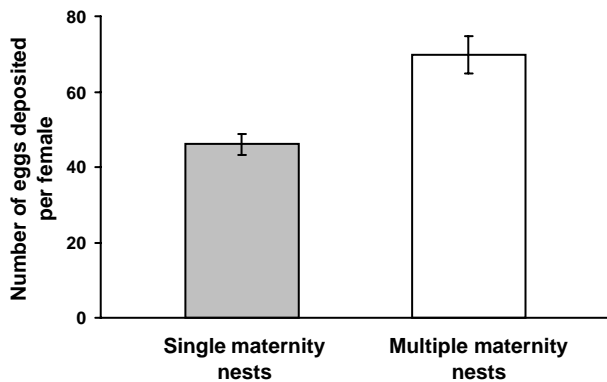

Fig. 3 Mean number of eggs deposited per female in single- vs. multidam nests. Bars show standard errors. This difference between these two classes of nests is significant ($P < 0.001$; one-tailed *t*-test assuming unequal variances).
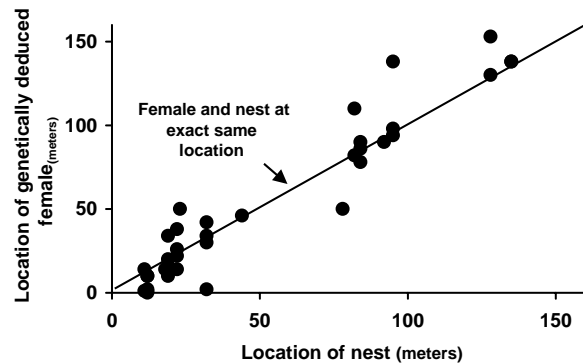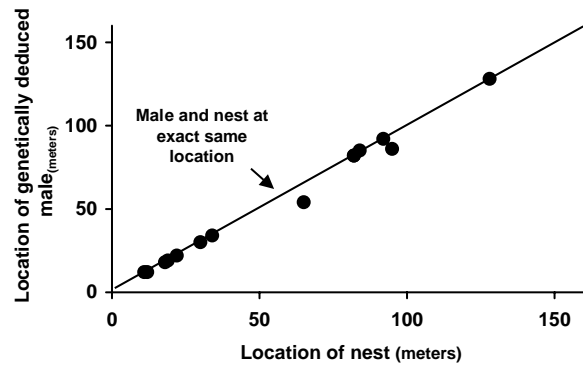

Fig. 4 Locations of mottled sculpin nests (in stream-metres from the starting point) vs. the location of the genetically deduced sires (above) and dams (below). The lines represent what would be observed if the nest and parent were from exactly the same site. For specimens captured via electrofishing, distance from the starting point was calculated as the midpoint of the 4-m section (see text).

mean standard lengths of females were 53 (± 1.0 SE) mm and 59 (± 1.3 SE) mm, respectively, for nests with single and multiple maternity. In addition, mating pairs appeared to be random with respect to the standard body length ($r^2 = 0.06$, $P > 0.1$) and weight ($r^2 = 0.12$, $P > 0.1$).

Relative locations

As expected, given that males in this species tend nests, there was an almost perfect correlation ($r^2 = 0.99$, $P << 0.001$) between the nest location and the collection locale of the genetically deduced sires (Fig. 4). Although females were never captured guarding a nest, there was also a strong correlation ($r^2 = 0.92$, $P << 0.001$) between nest sites and the location of the genetically deduced dams (Fig. 4). On average, sires and dams were captured only 1.6 (± 1.0 SE) m and 9.3 (± 1.8 SE) m from their respective nests.

Adults did not reside preferentially either upstream or downstream from the nest ($\chi^2 = 0.11$, $P > 0.1$), nor did the distance from the nest differ for upstream vs. downstream captures ($P > 0.1$; two-tailed *t*-test). In addition, nest sites for which the genetically deduced parents were not captured were apportioned randomly between the centre and the boundaries of the study area ($P > 0.1$ for both males, and females, $\chi^2 < 1.1$, d.f. = 1), suggesting that failed captures were not the result of individuals moving out of the study area.

Population size and effective number of breeders

Based on the mark–recapture calculations, there were 243 potentially breeding males (95% confidence limits, 172–314) and 323 potentially breeding females (95% confidence limits, 264–382) in the local population, or about 566 adults total. By contrast, the estimated effective number of breeders (N_b) was an order-of-magnitude lower. The 'variance in reproductive success' approach estimated 60 effective individuals (18 males and 99 females) whereas the 'temporal method' estimated 48 effective individuals (95%

confidence limits, 32–68). Thus, the estimated ratio of the effective number of breeders to the 'census size' of the adult population was between 0.08 and 0.11.

Discussion

Previous behavioural research has addressed the reproductive biology of the mottled sculpin, *Cottus bairdi*. Here we employed microsatellite DNA markers to characterize the genetic mating system of an extensively sampled, semi-closed natural population. Such thorough sampling allowed us to estimate accurately the census size of the breeding population as well as the contemporary effective number of breeding individuals, parameters that are usually elusive in large, open populations. These genetic analyses support many of the behavioural observations, but also provide new insights into the sculpin mating system and potential factors influencing mate choice.

Microsatellite markers

Mills *et al.* (2000) suggested using the mean probability of identity (*PI*) to determine the utility of markers for genetic mark–recapture studies. The *PI* was indeed suitably low in the current study (4.6×10^{-6}). Given the number of possible pairwise comparisons (162 165) between the estimated 570 potential sculpin breeders, fewer than one such pair might be expected to share a multilocus genotype by chance and, empirically, no instances of genotypic sharing were observed for the adult males and females sampled. However, the reconstructed genotype of one dam did match a male (CB449) in the collection, an outcome that is almost certainly spurious. For the nest in question (N100), the guardian male (CB100) was consistent genetically with all the offspring. If we assume instead that CB449 sired the embryos, this would require that male CB100 took over nest N100, and would further require that male CB449 spawned with a female whose genotype was identical to that of the guarding male (CB100).

Although a null allele was probably present at locus *Cba*51, it did not compromise the parentage analyses in this study. When many offspring have been analysed, null alleles give the appearance of nonMendelian segregation in a progeny array (see Jones & Avise 1997). In the three cases where the reconstructed multilocus genotype of a parent sculpin was a null heterozygote, matches were simply sought among potential parents who were scored as homozygous for the amplified allele.

Reproductive biology

Mottled sculpin males in this population were polygynous, on average spawning successfully with 2.8 females. These findings are consistent with a previous study in which individual sires spawned with a mean of 1.5–4.0 females in various sculpin populations (Brown & Downhower 1982a). Those latter estimates, however, might need to be adjusted upward somewhat because they came from egg-mass counts, which (as we have shown) can underestimate the true number of dams.

We found no evidence for allopaternal care (via either cuckoldry or nest takeovers) among the collected sires that were actively guarding nests. In addition, we documented no cuckoldry events even among nests from which a guardian male was not captured. However, one nest (N059) did represent a potential nest takeover, but neither of the genetically deduced sires was captured tending the embryos (although male CB005 was within 1 m of the nest). Overall, 96% of the nests were sired by a single male and 98% of the offspring analysed were consistent with a single sire per nest. This latter level of paternity assurance is higher than documented in several other cavity-spawning species: 85% in the sand goby (Jones *et al.* 2001a,b), 86% in the tessellated darter (DeWoody *et al.* 2000a) and 84% in the striped darter (Porter *et al.* 2002). Such high assurances of paternity may justify the extended parental care observed in these species.

Two aspects of our results are consistent with the prediction by Savage (1963) that female mottled sculpins deposit all of their eggs into one nest. First, each reconstructed multilocus female genotype was present in only one nest. Second, average fecundity (66 eggs/female) based on reconstructed multilocus maternal genotypes was consistent with the mean number of eggs in the ovaries of adult females (71; Grossman *et al.* submitted).

Previous studies have suggested that mottled sculpins are sedentary during the nonbreeding season (Brown & Downhower 1982b), and have estimated home ranges of less than 20 m (Hill & Grossman 1987; Petty 1998). Here we demonstrate restrictive travels during the breeding season as well. When captured, genetically deduced sires and dams were on average only 1.6 and 9.3 m away from their respective nests. Such strong correlations provide additional compelling evidence that the multilocus maternal genotypes were accurately reconstructed. Limited excursions by males are expected, because sires tend the nest-rock and provide offspring care. Given that females probably visit multiple males before spawning (Downhower & Brown 1979), the nests visited must often be proximate.

Effective number of breeders

All else being equal, small populations tend to lose genetic diversity more rapidly than large populations. The effective number of breeders (N_b) is a useful measure of differential reproductive success among adults, yet only recently have genetic parentage assessments been applied

to its estimation either in managed populations (Perez-Enriquez *et al*. 1999; Hasen *et al*. 2000) or in nature (Scribner *et al*. 1997; Garant *et al*. 2001).

This study is among the first to estimate N_b for a natural fish population based on genetic parentage data. The genetically deduced effective number of breeders ($N_b \cong 54$) in the mottled sculpin population was much smaller than the census size of potential breeders ($N \cong 570$). Mottled sculpins have overlapping generations, so this discrete-time estimate of N_b (from adults to progeny) is not equivalent to the effective population size (N_e) across a single generation (see Nunney 1993; Waite & Parker 1996; methods section). Nonetheless, the small N_b, combined with the observation that adults have limited movements, imply that genetic drift could rapidly produce genetic heterogeneity of sculpin populations even among nearby streams.

It is important to recognize that multiple factors could influence the estimate of the effective number of breeders. First, a failure to sample some nests could yield underestimates of N_b. Given that *C. bairdi* nests are fairly conspicuous (G. Grossman, pers. obs.), and given that we completed three snorkelling passes through the stream with only one nest being located on the final pass, we are confident that most nests were sampled. Second, an extended breeding season, coupled with limited temporal collections, also could lead to reductions in the calculated N_b. Mottled sculpins, however, tend to have very short spawning seasons (Downhower & Brown 1980), a conclusion supported further by the lack of developmental variation observed among embryos of the surveyed nests.

Finally, the time at which the estimates of N_b are made can affect the calculated value. In this study we have analysed genetically late-stage eggs from each nest to determine: (a) the allele frequencies among the offspring, and (b) the variance in progeny number among reproductive adults. Short of spawning observations on marked fish, this may be the earliest tractable time to estimate N_b. Time, and specifically natural selection operating from the point of egg-laying to the age at maturation, may make estimates of N_b calculated with older age classes very different from those presented in this study.

Indeed, such deviations, if they could be accurately quantified, might yield valuable insights into forces influencing selection in this species. Consider the following simplified example. If N_b estimates calculated using older individuals are larger than those using younger individuals, this might suggest the operation of either balancing or frequency dependent selection. If, however, the converse were true, this might suggest strong directional selection favouring only a small subset of families. Although the theoretical framework for such comparisons is currently lacking, such approaches may enlighten future research endeavors.

Polygyny and fitness

From the genetic maternity data, guardian males who had spawned with multiple females sired more embryos (i.e. received more eggs) *per female* than did sires who spawned with only one dam. Also, females who deposited eggs in multidam nests were larger, on average, than those who deposited eggs in single-maternity nests, and this finding is consistent with the positive correlation between standard length and fecundity previously reported for *C. bairdi* (Grossman *et al*. 2002). This finding is also consistent with the observation that smaller females tended to spawn with bachelor males (Downhower *et al*. 1987). In contrast, we did not find evidence for positive size assortative mating as did Downhower *et al*. (1983, 1987).

Females of many fish species prefer to mate with males whose nests already contain eggs (Constantz 1985; Marconato & Bisazza 1986; Unger & Sargent 1988). This observation has been proposed as an adaptive explanation for allopaternal care (Constantz 1985; Unger & Sargent 1988), as well as for the evolution in some species of male morphological features (such as knobs on the tips of fins) that mimic eggs in appearance (Knapp & Sargent 1989; Page & Bart 1989; Porter *et al*. 2002). However, for mottled sculpins, Downhower & Brown (1981) did not find a female preference for males who were guarding eggs. These results are consistent with the notion that females may sometimes prefer to spawn with a bachelor. Although established nests may be reflective of quality sires (Sargent 1988), additional considerations may well behove a female to weigh all potential costs and benefits in her final choice of spawning partner.

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