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Breeding success of male brook trout (*Salvelinus fontinalis*) in the wild

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Abstract

Competition for females generally results in some males adopting alternative reproductive tactics to acquire matings. For fish, the ecological and evolutionary consequences of these tactics are not well understood because of an inability to link directly the interactions of individuals on the breeding grounds with genetic data. This study combines behavioural observations with genetic estimates of male reproductive success within an intensively studied wild population of lacustrine brook trout (*Salvelinus fontinalis*). Male brook trout exhibit a conditional reproductive strategy with small males adopting a peripheral position to that of larger dominant males in their proximity to spawning females. Parentage analysis of eggs collected from wild redds confirmed the reproductive success of individual males. Males relegated to peripheral positions during spawning participated frequently in spawning events, but in most cases the first male to spawn was the sole contributor, and no more than two males contributed successfully to a single brood. While behavioural observations of salmonines suggests that reproduction is partitioned among males in a manner dependent upon body size and proximity to spawning females, the genetic evidence from this study suggests a more limited distribution of reproductive success in the field. The genetic contributions of male brook trout are highly skewed towards larger males for this population. A review of the salmonine literature suggests little difference in individual reproductive success for males exhibiting size-related tactics within a conditional mating strategy vs. precocial maturation. Collectively, these genetic studies provide new insights on the evolution of alternative life histories among salmonines.

Keywords: mating system, paternity analyses, reproductive success, salmonid

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Introduction

Alternative breeding tactics used by males when competing for access to females is based commonly on differences in behaviour and status stemming from differences in body size (Gross 1996). The conditional strategy of fighting to establish dominance as a way of gaining access to females may be the clearest manifestation of body size as a determinant of alternative mating tactics in males. Relying solely on behavioural observations to assess reproductive success results in surrogate measures, such as time spent in close proximity to females (e.g. Fleming & Gross 1994; Coltman *et al*. 1999) or secondary sexual characters related to territory size used in mating (Lebas 2001), to name a few. However, recent molecular genetic data from a variety of breeding systems provide a more complete picture of the limitations of surrogate measures of male reproductive success (e.g. Coltman *et al*. 1999).

To date, most research comparing 'social' vs. 'genetic' patterns of mating has focused primarily on birds, mammals and social insects (Hughes 1998). Fish, however, exhibit an enormous array of breeding patterns (Breder & Rosen 1966), and serve as some of the best-known examples of alternative reproductive strategies and tactics (Gross 1984, 1996; Taborsky 1994). The underrepresentation of this group to contributions in behavioural ecology has been noted by others (Avise *et al*. 2002), and is due

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largely to the inability to observe the behaviours of individual fish under natural conditions. Field studies have provided genetic evidence to confirm observed breeding behaviours in fish, such as cuckoldry in bluegill sunfish (*Lepomis macrochirus*; Philipp & Gross 1994), sneaked fertilization and egg thievery by male sticklebacks (*Gasterosteus* sp.; Rico *et al*. 1992; Jones *et al*. 1998a) and monogamy in seahorses (*Hippocampus angustus*; Jones *et al*. 1998b). Thus, similar to avian and mammalian studies, genetic investigations have provided evidence to confirm suspected breeding behaviours in fish (DeWoody & Avise 2001). However, this shift in research focus to the genetic contributions of various mating behaviours has occurred mainly without corresponding behavioural observations and, as such, limits the ability to interpret the ecological and evolutionary significance of alternative mating tactics.

One of the most widely studied groups of fishes is the subfamily Salmoninae (salmon, trout and charr). Although breeding patterns are variable within this group they are best characterized as having a site-based competitive mating system with competition among females for access to spawning sites and competition among males for access to females (Fleming 1998; de Gaudemar 1998). Females construct a nest and deposit eggs that are fertilized by one or more males. Because male reproductive success is related to spawning frequency, body size and secondary sexual characters such as hump height are favoured traits for males (Fleming & Gross 1994; Quinn & Foote 1994). Larger males tend to be most often associated with females and also closest to a female (i.e. dominant) in the male hierarchy that typically surrounds a spawning female. The male closest to a female generally fertilizes the greatest proportion of eggs (reviewed in Blanchfield & Ridgway 1999). Smaller male salmonines attempt to circumvent this size-related disadvantage by sneaking fertilizations. The expression of this behaviour ranges from a status-dependent conditional strategy based on competition among males of different size (e.g. Kitano 1996) to the evolution of a distinct alternative life history strategy that involves precocial maturation – known as 'parr' in Atlantic salmon, *Salmo salar* (Jones & King 1952) and 'jacks' in Pacific salmon, *Oncorhynchus* sp. (Gross 1984). To date, the application of molecular techniques to salmonine mating systems has focused primarily on the reproductive success of males exhibiting alternative life history strategies, the success of hatchery vs. wild fish and the estimation of effective population size (see Blanchfield & Jones 2000). In most cases, these studies occur under seminatural conditions and involve a predetermined number of individuals.

The objective of this study was to examine the reproductive success of males under natural conditions for a salmonine mating system. Here we capitalized on previous intensive studies of the breeding behaviour of a closed, lacustrine population of brook trout (*Salvelinus fontinalis*). Females construct nests over sites of upwelling groundwater located in shallow areas, allowing for observations of a high proportion of the breeding population (Blanchfield & Ridgway 1997; Ridgway & Blanchfield 1998). Breeding in this lake environment involves extensive male movement among females to determine female readiness to spawn (Blanchfield 1998). Similar to other salmonines, large body size infers a competitive advantage to male brook trout. Duration on the spawning grounds, movement among females, size of home range during the breeding season and proportion of time in a dominant position when associated with a spawning female increase with body size for males (Blanchfield & Ridgway 1997; Blanchfield 1998). Dominant males are those closest to a spawning female and attempt to deter other males from spawning with that female through aggressive behaviours such as lateral displays and chases (Blanchfield & Ridgway 1999). Whether or not a male becomes dominant or peripheral depends on the size structure of the group of males present around a spawning female. In general, all males will attempt take up the role as the dominant male if no larger males are present; thus the alternative mating tactic of being peripheral is not an extreme in a continuum, but is plastic and status-dependent (Gross 1996). Spawning females are usually accompanied by three males (range 1–9), and roughly half of all observed egg deposition events involved sperm release by multiple males (Blanchfield & Ridgway 1999). In this study we paired behavioural observations of breeding events with genetic assignment of paternity to determine male partitioning of reproductive success. We also compare paternity of unobserved spawnings to determine whether male hierarchical position close to the time of spawning is indicative of breeding success. Further, we examine whether pair relatedness is linked to mate choice in this system.

Materials and methods

Field observations

Almost all the wild breeding brook trout population in Scott Lake, Algonquin Provincial Park, Ontario (Fig. 1a) were captured and marked individually in the autumn of 1998. Fish were captured on the spawning grounds with a trapnet, anaesthetized with MS-222, measured and individually tagged. Measurements included fork length (mm) and weight (g). Tagging involved the insertion of a dorsal PIT-tag as well as an individually coded disk tag (detailed in Blanchfield & Ridgway 1997). The adipose fin of each fish was removed and preserved in 95% ethanol for subsequent genetic analysis.

We monitored via underwater observation, and collected eggs from spawning events in one section of the main spawning area at Scott Lake (area b in Fig. 1a) during the peak spawning period (29 October–11 November

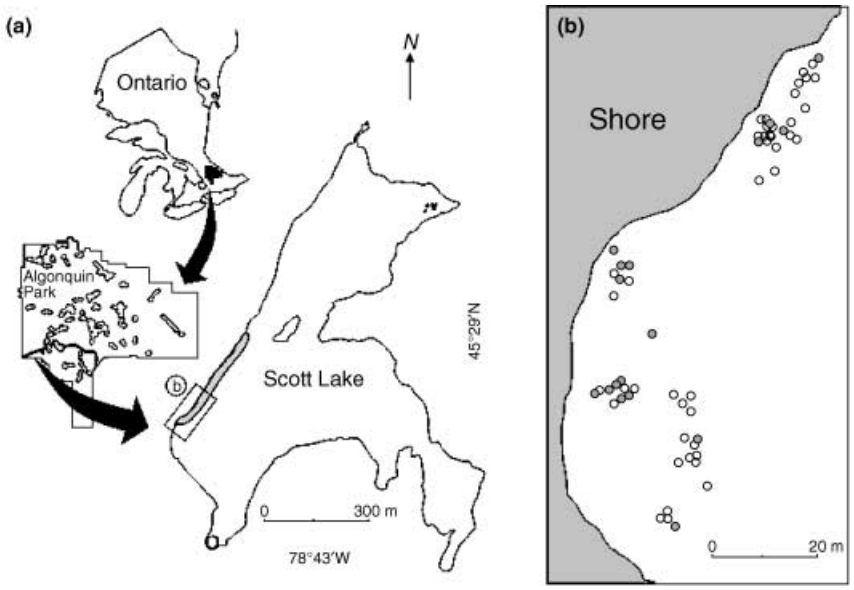

Fig. 1 Map of study site showing the (a) general locations of brook trout spawning activity (shaded areas) within Scott Lake, Ontario, and (b) the distribution of all known spawning sites in our study section. Spawning sites in which eggs were collected for genetic analyses are shown (shaded).

1998). This section represents roughly half the total spawning area in Scott Lake, but contains greater than 80% of all known spawning sites (Ridgway & Blanchfield 1998). In total, we collected eggs from 20 spawning events that occurred at 17 different spawning sites (Fig. 1b). Six multiple-male spawnings were recorded on videotape (Sony® Hi-8 video camera with Amphibico® underwater housing) or observed by swimmers. The remaining 14 spawning events were not observed. For unobserved spawning events, we collected only broods in which eggs were readily visible in the egg pocket. Because female brook trout start to cover eggs immediately after spawning, and the covering of eggs to an extent where they are no longer visible takes roughly 10 min (Blanchfield, unpubl.), we believe these spawning events occurred within 5 min prior to our collection of the eggs. For observed spawnings, we used the behavioural criteria of a previous study (gaping, quivering, presence of milt; see Blanchfield & Ridgway 1999) to determine which males participated in a spawning event. The timing of nest entry for males participating in a spawning event in relation to when the first male commenced spawning was determined by analyses of videotapes with the OBSERVER® software (Blanchfield & Ridgway 1999). In addition, we observed several single-male spawnings (n = 5) that are not included in our genetic analyses, but are included in population-level estimates of reproductive success. We collected eggs with a bucket and hose assembly that created a gentle suction and allowed for the 'vacuuming' of the spawning site (egg pocket) and the removal of all eggs. Eggs were placed in 1-L jars and incubated in the lake during the day and in a refrigerator overnight. Fresh lake water was added to the eggs the following morning, prior to their transport to the Ontario Ministry of Natural Resources research hatchery.

Paternity analyses

Prior to the field spawning observations, four maternal half-sib family crosses were made from wild-caught fish to determine the sensitivity of microsatellite DNA markers for assessing parentage in this system. Using ripe fish captured in trapnets, eggs were stripped from one female and subdivided into four aliquots, which were each fertilized with sperm from a different male. Fry from the test-crosses and each sampled wild spawning event were reared separately at the OMNR research hatchery (one family per incubator tray; sac fry were transferred to individual family tanks upon hatching) under ambient conditions (6–8 °C without feeding) until their yolk sacs were resorbed just prior to swim-up (~80 mg). A sample of 30 fry per brood were then sacrificed and extracted using the same methods as were used for the adult fin clips (see below).

DNA was extracted from the adult adipose fin biopsies using a lysis buffer of STE, SDS and proteinase K, followed by organic (phenol/chloroform) extraction (Bardakci & Skibinski 1994). Samples were precipitated with two volumes of 95% EtOH, resuspended in 100 µL of TE and stored at 4 °C.

Multilocus genotypes of individual adult fish were characterized for seven microsatellite loci. The loci used were Sfo8, Sfo12, Sfo18 and Sfo23 (Angers *et al*. 1995) and SfoC28, SfoD75 and SfoD2.1 (King & Burnham-Curtis, unpubl.). Initial microsatellite amplification conditions were modified from Angers *et al*. (1995), with one primer at each locus labelled with ^{33}P. Following amplification optimization for individual loci, loci were coamplified in two 10 µL multiplex reactions, using 30 ng of genomic DNA, 100 nM dNTPs, 2–5 pmol of each primer per locus, 1 µL of 10× polymerase chain reaction (PCR) buffer (QiaGen, Inc.),

1.5 mM Mg^{2+}, and 0.2 units of *Taq* polymerase. Both multiplex reactions used thermal cycling conditions of initial denaturation at 92 °C for 2 min, followed by 30 cycles of 92 °C for 30 s, 60 °C for 1 min and 72 °C for 1 min, followed by a final extension step at 72 °C for 30 min. PCR products were visualized by electrophoresis on 6% denaturing polyacrylamide gels followed by autoradiography, and allele sizes determined by comparison to a M13 sequence ladder. Allele frequencies and observed and expected heterozygosity were generated in FSTAT (Goudet 2001). Microsatellite loci were tested for deviation from Hardy–Weinberg expectations using GENEPOP 3.3 (Raymond & Rousset 1995). Parentage of test-cross and wild-collected eggs/progeny was evaluated using the PROBMAX program (Danzmann 1997), which allowed explicit testing of potential mating pairs based on field observations. PROBMAX allows for varying degrees of stringency in identifying possible parents (Danzmann 1997). To minimize Type II error, individual fry were assigned potential parentage only provisionally from a particular pair of adults if all alleles within the multilocus progeny genotype could result from possible recombination of parental gametes. Any mismatches (i.e. less than 100% concordance) resulted in parental exclusion. For the purposes of our analysis, it was assumed that no mutation of microsatellite alleles occurred within the scope of the experiment, and that no alleles were misscored. Based on these assumptions, exact matches between progeny genotypes and potential genotypic arrays from adult pairs were required in order for those adults not to be excluded as parents. In some cases, allele scoring errors did occur; the resulting conflicts between offspring and putative parental genotypes were resolved by re-amplifying problem loci from individual fry and adults and running side-by-side comparisons on the same gel. In cases where matings were not observed directly, potential parentage testing was expanded to include all tagged adults.

Pairwise relatedness among adult brook trout was calculated with the RELATEDNESS 5.0.8 software package (Queller & Goodnight 1989), using a symmetric relatedness measure (r) as calculated for all sampled wild adults and individual spawning groups. Relatedness values between spawning pairs were tested against the population mean and distribution by t-test of a sample against a mean (Sokal & Rohlf 1981). Within spawning groups, relatedness of successful males to mated females was tested against r-values of other males that were present.

Results

Microsatellite results

Despite the relatively small size of the lake (< 30 ha) and limited adult population (80–150 individuals), genetic diversity among the sampled adults was quite high. All seven microsatellite loci were highly polymorphic, with allelic richness ranging from six to 11 alleles per locus among the 72 wild adults sampled (Table 1). Six of the seven loci conformed to Hardy–Weinberg expectations and showed comparable observed and expected heterozygosity values (Table 1). Locus SfoD2.1 showed substantially lower observed than expected heterozygosity and significant deviation from Hardy–Weinberg expectations, and was therefore excluded from parentage analyses to avoid null allele complications. The number of eggs collected for each spawning event ranged from 57 to 1160 (mean = 338).

Table 1 Allelic frequencies for seven microsatellite loci [Sfo8, Sfo12, Sfo18 and Sfo23 (Angers *et al*. 1995), and SfoC28, SfoD75 and SfoD2.1 (King & Burnham-Curtis, unpubl.)] among sampled adult brook trout in Scott Lake, Algonquin Park. H_E and H_O indicate respective expected and observed heterozygosity for each locus

Sfo8		Sfo12		Sfo18		Sfo23		SfoC28		SfoD75		SfoD2.1	
allele	freq	allele	freq	allele	freq	allele	freq	allele	freq	allele	freq	allele	freq
203	0.03	197	0.28	173	0.02	153	0.06	173	0.02	178	0.34	153	0.01
223	0.09	253	0.02	177	0.24	161	0.13	177	0.19	198	0.22	167	0.01
225	0.10	265	0.15	179	0.05	167	0.10	179	0.16	202	0.05	179	0.01
239	0.14	269	0.04	183	0.55	169	0.03	181	0.08	206	0.13	181	0.01
255	0.24	271	0.15	185	0.11	173	0.08	185	0.08	210	0.05	193	0.01
257	0.12	273	0.05	227	0.03	179	0.03	189	0.40	214	0.06	197	0.18
271	0.08	275	0.31			185	0.24	193	0.06	218	0.07	201	0.05
281	0.01					203	0.04			222	0.01	209	0.08
315	0.19					205	0.22			226	0.07	217	0.25
						213	0.08					221	0.10
												223	0.28
H_E	0.86	H_E	0.78	H_E	0.64	H_E	0.85	H_E	0.77	H_E	0.81	H_E	0.81
H_O	0.90	H_O	0.83	H_O	0.67	H_O	0.87	H_O	0.80	H_O	0.84	H_O	0.25

On average, 26% of eggs died prior to the swim-up stage, with little mortality after this developmental stage was reached. The lower egg mortality (8%) observed for test-crosses suggests that egg deposition in nature and our egg collection methodology may increase mortality.

Parentage results

All progeny from the four maternal half-sib test-crosses were reliably assigned to their correct parentage (data not shown). A sample size of 10 offspring per family was sufficient for detection of all parental alleles in the test-crosses. To provide reasonable statistical power, however, 30 fry per brood (i.e. spawning event) were examined to resolve the relative contributions of potential parent fish. This sample size enabled 95% confidence of detecting at least one offspring from a mating event with a contribution of 10% or more to a brood, based on the expression $\beta(\text{beta}) = 1 - (1 - P)^n$, with P set to 0.10 and $n = 30$.

Based on the observed allelic diversity among microsatellite loci, power analysis of multilocus genotypes (Bernatchez & Duchesne 2000) confirmed that individual fry could be assigned with high probability to their true parents with only three loci (data not shown). Sac fry from individual wild redds were therefore typed with four loci (Sfo8, Sfo12, Sfo18 and Sfo23) to provide sufficient assignment resolution. In cases where putative parents could not be reliably resolved based on allelic similarity and/or progeny genotypes, loci SfoC28 and SfoD75 were also used.

Parentage of all fry from observed matings were confirmed by genetic assignment testing with PROBMAX. Using the subset of adults identified near redds where mating was not observed directly also resulted in assignment of all brood members to potential parents. Calculation of global assignment probabilities, excluding mating observations and using genotypic data from all trapnetted adults, resulted in a much lower success rate, with 38% of the collected eggs potentially originating from two or more potential adult crosses. Reproductive contributions of adults to families where the actual moment of spawning was not observed represent estimations based on adults present immediately after the spawning events. In several cases, it was not possible to identify both parents from progeny genotype arrays, based on adults observed at the redd and pooled data from all adults sampled during pre-spawning trapnetting. For these families, we were able to reconstruct the genotype of the missing parent based on the genotypes from the collected progeny and adults observed at the redd. Three adults (one male and two females), which contributed to collected broods, had not been captured in the original trapnetting and so were not fin-clipped or tagged. For each fish, it was possible to reconstruct their multilocus genotype from progeny and mate allelic arrays (Tables 2 and 3; families B, E, F, S and T). All three individuals were observed spawning and were confirmed as untagged fish (Table 2).

Table 2 Summary of observed multiple-male spawning events and genetic identification of contributing adults

Spawn event	Female	Dominant male	Peripheral male 1	Peripheral male 2
A	B7	X5 (100)	Y1†	ac
		(0.0)	(1.9)	(2.6)
B	C1	ut-1* (77)	Y2 (23)	X1
		(0.0)	(0.9)	(2.5)
C	D2	X5 (100)	Q1†	
		(0.0)	(1.4)	
D	B5	X4 (100)	Z8†	
		(0.0‡)	(‡)	
E	ut-1*	X8 (77)	Y1 (23)	
		(0.0)	(1.1)	
F	ut-2*	Y4†	W3 (100)	tt†
		(0.7)	(0.0)	(1.4)

Alphanumeric designations represent visual identification tags for spawning adults. For unidentified fish: ac = adipose clipped; tt = t-tag only is present; ut = untagged. Contributions of spawning males are shown in parentheses. Percentage is contribution by males based on analysis of 30 fry per brood. The times of nest entry (in seconds) of the second and third males after the first male started spawning (gaping behaviour observed) are shown in parentheses beneath individual males. †Sperm release observed, but did not result in fertilization of eggs; ‡spawning observed but not on video; *parents identified by DNA.

No evidence of retrieving eggs from multiple broods within a redd was observed. In one instance where some eggs from one redd were spilled inadvertently into a second egg jar in the field, fry from the two families were discriminated readily based on their differing genotypes, which matched predictions from the genotypes of adults observed at each redd (data not shown).

Observed patterns of male participation in spawning events were similar during the 1998 season to previous years for this population, with respect to the presence of peripheral males around spawning females (60% occurrence) and the fact that not all peripheral males release sperm during actual egg deposition (see Blanchfield & Ridgway 1999). However, in contrast to previous observations of Scott Lake brook trout, in which peripheral males released sperm in about two-thirds of witnessed spawnings (gaping and milt observed; Blanchfield & Ridgway 1999), all peripheral males present rushed into the nest as spawning was occurring and at least one peripheral male released sperm in each observed spawning event in 1998 ($n = 6$; Table 2). In one instance, while the dominant male was occupied chasing a peripheral male away from the redd, a different peripheral male spawned prior to the

Table 3 Summary of the genetic contributions of male brook trout for individual spawning events that were not observed. See Table 2 for an explanation of symbols. Numbers in parentheses show percentage of the contribution by males based on analysis of 30 fry per brood

Spawn event	Female	Dominant male	Peripheral male 1	Peripheral male 2	Peripheral male 3	Peripheral male 4
G	B8*	Y4 (100)				
H	D1	X9 (100)	Y5	Y1		
I	A7	Y4 (100)	ut	other		
J	B6	Y8 (57)	Z4 (43)			
K	C5	X5 (100)	Y7	Z8		
L	C1	X4	Z2	Q1* (100)		
M	C2	X2	X6† (100)	other		
N	C5	X8	W4	Y7 (100)		
O	D1	tt	Z4 (100)			
P	D3	Y4 (100)	W6	X1		
Q	C8	X3 (93)	Z5 (7)	X2	tt	tt
R	B9	X6	Y2 (100)			
S	ut-1*	X5 (100)	other			
T	ut-2*	Y4 (100)	Y7	W3	Z9	tt

†Males that were not observed directly at the spawning site, but contributed to progeny;
*parents identified by DNA.

dominant male and fertilized all eggs (spawning F; Table 2). Observational data on timing of nest entry paired with genetic analyses revealed that there is a clear first male mating advantage. The first male to spawn sired over 90% (92.3 ± 11.9%) of a given brood (Table 2). A minimum of two males released sperm in all observed spawning events, which resulted in shared paternity for one-third ($n = 2$) of these attempts (spawning B and E; Table 2). When successful, the second male to spawn fertilized a much smaller proportion of a given brood (23%). Peripheral males that entered the nest at the time of spawning but were not observed to release gametes arrived in the nest approximately 2.5 s after the first male had commenced spawning (Table 2). Dominant males from observed spawning events were always larger than the peripheral males present, and on average were 145 mm longer (range = 12–239 mm) than the mean size of peripheral males.

Genetic determination of male mating success from broods in which the actual moment of spawning was not observed ($n = 14$) were not in complete agreement with observations of male hierarchy made just after spawning. Paternity analyses indicated that the observed dominant male often (9/14) fertilized a large portion of a given brood, but in other instances peripheral males (4/14) or males not observed at the spawning site (1/14) were found to sire the offspring (Table 3). Because we did not observe these spawning events directly, it is not possible to know whether the patterns of paternity are a result of nest entry. However, the distribution of paternity among males was comparable to observed spawnings, with 96.2% (± 11.5%) of a given brood being fertilized by a single male (Table 3). Also similar to observed spawning events was the low occurrence of multiple paternity within broods (2/14; Table 3).

When observed single-male spawnings, confirmed by video or genetic analyses ($n = 5$), were combined with genetic results, patterns of male reproductive success for the breeding population were apparent. On average, females were accompanied by more than two males (2.5 ± 1.1) when spawning (Fig. 2a), which is similar to previous years of confirmed spawning events in this population (1994 and 1995; $n = 45$; 3.0 ± 1.9 males; Blanchfield & Ridgway 1999). Overall, a single male fertilized the brood successfully for most spawning events (21/25) and, within our data set, no more than two males fathered offspring within a single brood (Fig. 2b).

The number of breeding individuals captured at Scott Lake just prior to spawning in 1998 ($n = 75$) was similar to previous years for this population (Quinn *et al*. 1994; Blanchfield & Ridgway 1997). Based on the number of breeding females ($n = 41$) and previous data on female spawning frequency (1994 and 1995; $n = 58$; 2.7 spawns per female), the broods analysed for paternity and observed solo spawnings together represent almost one-quarter (23%) of all spawning activity occurring at Scott Lake during the 1998 season. It is important to mention that there is some pseudoreplication in our data set. With the formation of dominance hierarchies by highly mobile males and the analysis of nearly a quarter of the spawnings that occurred within this lake population, the potential for documenting repeat male and female spawners is high and typical of behavioural data from small populations. The 25 spawning events in our data set (paternity analyses, $n = 20$; observed single-male spawnings, $n = 5$), involved 15 different females and 14 different dominant males. Reproductive success of male brook trout was highly skewed within our sample set, with over half (52%) of all males making no genetic contributions and only a few males successfully

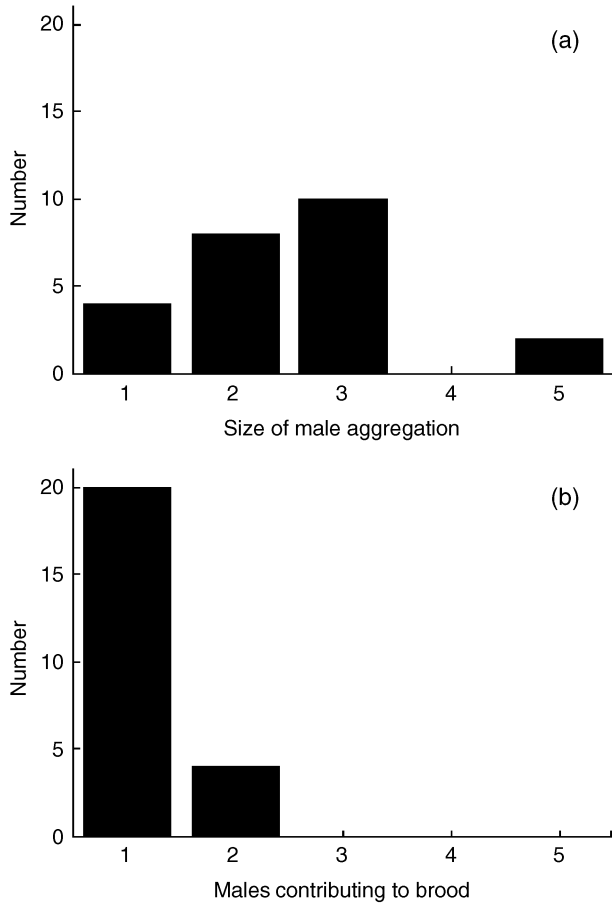

Fig. 2 Comparison of the number of male brook trout (a) present around a spawning female and (b) contributing to a brood.

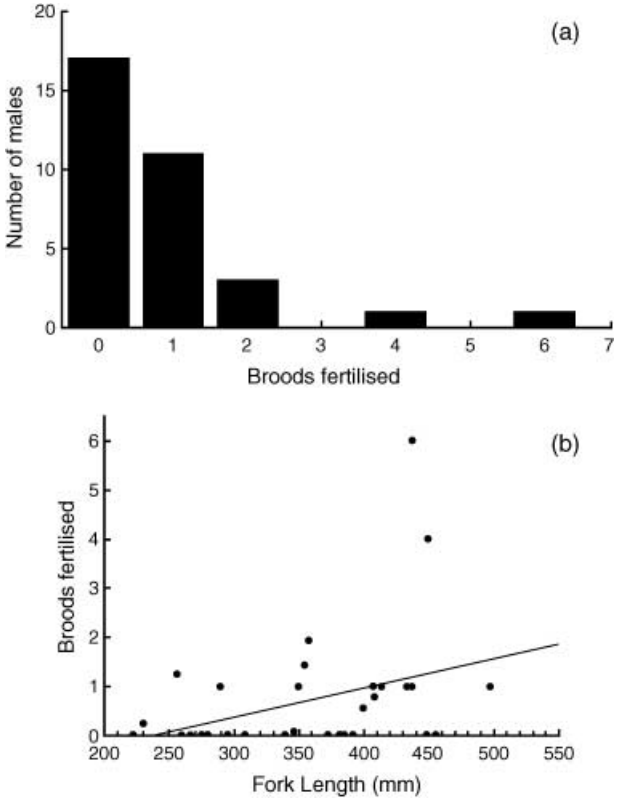

Fig. 3 Male reproductive success was determined from genetic analyses and observed single-male spawnings. Male reproductive success was (a) highly skewed due to many males not making any genetic contributions ('0') and (b) correlated positively to body size.

spawning greater than one time (Fig. 3a). Male body size contributed to reproductive success. The number of broods, or portions of a brood, that individual males made a genetic contribution to increased with male body size ($F_{1,31} = 4.8$, $R^2 = 0.13$, $P = 0.037$; Fig. 3b).

Relatedness results

Distribution of pairwise relatedness coefficients (r) among observed prospective spawning males with females was not significantly different from the trapnetted adult population (two-tailed t-test, $t = -0.87$, $P > 0.05$), suggesting that male brook trout did not seek out or avoid mates based on kinship. Similarly, although the mean relatedness (r) values of successful males with mated females ($r_{sm} = -0.02 \pm 0.25$) was slightly lower than that for unsuccessful males ($r_{um} = 0.09 \pm 0.21$), the difference was not significant with a one-tailed t-test ($t = 1.54$, $P > 0.05$). Comparison of male relatedness to spawning females also failed to detect differences between contributing and unsuccessful males at individual redds. Although a general trend of female preference for unrelated or less closely related males was observed (15 of 21 observations), a Wilcoxon paired-sample test of successful vs. unsuccessful male relatedness values did not detect a significant difference ($T_{05(1),21} = 63$, $P > 0.05$).

Despite the apparent lack of evidence for female mate choice based on kinship, comparison of female relatedness to successful mates vs. female size may indicate that mate choice varies with age or size (Fig. 4). Females with fork lengths less than 400 mm showed no detectable preference based on male relatedness, whereas females with fork lengths greater than 430 mm only mated with unrelated males. Linear regression indicated that this relationship was significant ($F_{1,19} = 6.67$, $R^2 = 0.26$, $P = 0.018$), although this apparent relationship may be driven by matings of one female (C8) with two related males. Removal of these data points, however, still resulted in a significant relationship ($P = 0.046$).

Discussion

The occurrence of alternative male mating tactics is well documented for fish (Taborsky 1994); however, only through

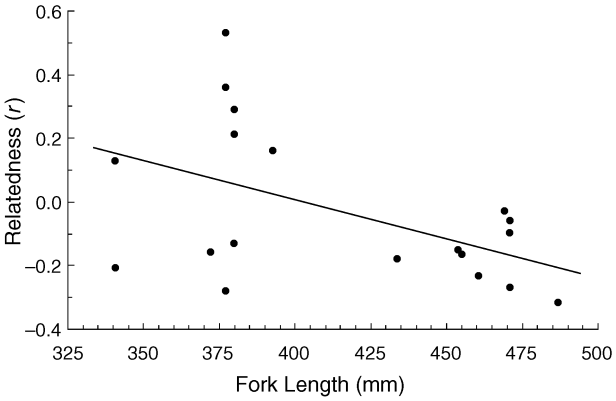

Fig. 4 The relationship between female body size and relatedness of contributing males for brook trout spawning in Scott Lake.

recent molecular techniques have we been able to assess the reproductive success of various tactics. For brook trout (*Salvelinus fontinalis*) spawning in Scott Lake, we observed a major discrepancy between field observations and molecular data. Although multiple males released sperm in all observed spawning events, genetic analyses revealed that, in most cases, only the first male to spawn was successful. When multiple paternity did occur, the largest proportion of the brood was sired by the first male to spawn based on the directly observed spawning events. Within our sample set, no more than two males were detected genetically as contributing to an individual brood. Although the sample sizes used in this study limited the resolution power to 95% certainty of detecting parental contributions of 10% or more, our findings differ significantly from the common assumption of partitioning of paternity among successive males participating in spawning events. This assumption was adopted in the larger literature on salmonine fishes (e.g. Fleming & Gross 1994), but was based on early data from few staged trials that included more than one peripheral male spawning (Schroder 1981, $n = 1$; Chebanov *et al*. 1983, $n = 1$). Recent studies of salmonine breeding behaviour would suggest that male timing of nest entry (Mjølnerød *et al*. 1998) or distance from the breeding pair (Foote *et al*. 1997) are not necessarily consistent predictors of fertilization success.

Prior to the use of genetic markers to estimate paternity, the proportion of time spent as 'dominant' relative to a spawning female has been used as a surrogate of male reproductive success for salmonines (e.g. Fleming & Gross 1994). Male body size and the expression of secondary sexual characters are the strongest predictors of dominance (e.g. Quinn & Foote 1994). For male brook trout, we have previously shown that large body size results in a greater amount of time spent closest to a spawning female ('dominant'; Blanchfield 1998). The main finding of this study is that dominance is a strong predictor of male mating success. For observed spawnings, dominant males generally spawned first and that there was a distinct first-male advantage in the fertilization of eggs. It is important to note that although large body size conferred a reproductive advantage for males, it explained little of the variation in male reproductive success (13%). This finding is similar to population-level estimates of male reproductive success in Atlantic salmon (16%; Garant *et al*. 2001), and suggests that a variety of factors influence male success in salmonines.

An interesting finding of this study was the discrepancy between 'genetic' and 'observed' dominance for spawnings that were not witnessed, but had probably occurred just minutes prior to our arrival at the site. We believe the lower predictive ability of our behavioural observations for unobserved spawnings is due to the high mobility of males. In contrast to the static nature of large or 'dominant' Pacific salmon males (genus *Oncorhynchus;* Healey & Prince 1998), this brook trout mating system is characterized by extensive size-related movement of males throughout the breeding grounds (Blanchfield 1998) and changing dominance hierarchies around females (Blanchfield & Ridgway 1999). The greater mobility of large males may have resulted in them appearing and taking a dominant position around a female just after a spawning event, or leaving a female soon after successfully fertilizing the eggs in search of other females. Both these scenarios could explain the mismatch between the observed dominance hierarchies and genetic findings for unobserved spawning events. For mammals, the ability to predict paternity results from behavioural observations is often dependent on the stability of male dominance hierarchies around breeding females (see Coltman *et al*. 1999). Here we show that for unobserved spawning events, there is only partial agreement between observed male dominance hierarchies and genetic paternity for an externally fertilizing species. The ability of peripheral males to gain first access to spawning females has been observed in other studies (Foote *et al*. 1997; Mjølnerød *et al*. 1998), and may be the predominant avenue by which smaller males fertilize eggs. However, based on the frequency by which this occurred in our observed spawning events (1/6), it is likely that a combination of first-male spawning by peripheral males and hierarchy changes resulted in the observed paternity results.

Genetic studies of parents and offspring often confirm previously known behaviours, but the lack of concomitant behavioural observations does not allow for a full mating system perspective. Our data encompassed roughly one-quarter of all breeding events that occurred during a season and is highly representative of the reproductive output of the entire population. The limited ability of status-dependent peripheral males to gain paternity, despite frequent sperm release, promotes extreme skews in reproductive success in this population. Few male brook trout produced a large proportion of young — a pattern seen in other mating systems — with roughly half the male breeding

Table 4 Summary of paternity studies with genetic estimates of male reproductive success within the subfamily Salmoninae. Studies including the reproductive success of hatchery-reared fish were not included in this summary. The range in the proportion of broods fertilized by peripheral males and the mean (in parentheses) are shown

Species	Method	Spawning observed	Broods analysed[a]	Reproductive success (%) of peripheral males[b]	Peripheral males present	Source
Precocial males not present						
Oncorhynchus keta	Viewing chamber	yes	6	0–47 (28.3)	1–2	Schroder 1981
Oncorhynchus nerka	Ponds	yes	2	10–48 (28.8)	1–2	Chebanov *et al*. 1983
Salmo salar	Experimental channel	yes	10	0–100 (41.2)	1–2	Mjølnerød *et al*. 1998
Salmo salar	Experimental channel	no	6	0 (0)	2	Martinez *et al*. 2000
Salvelinus fontinalis	Wild	yes	6	0–100 (26.8)	1–2	This study
Salvelinus fontinalis	Wild	no	13	0–43 (3.8)	1–4	This study
Precocial males present						
Oncorhynchus nerka[c]	Stream pens	yes	17	3–100 (43.4)	1–2	Foote *et al*. 1997
Salmo salar	Stream tank	yes	15	2–29 (15.5)	1–20	Hutchings & Myers 1988
Salmo salar	Enclosed channel	no	5	1–28 (11.0)	NA	Jordan & Youngson 1992
Salmo salar	Experimental channel	no	3	25–89 (51.2)	1–12	Moran *et al*. 1996
Salmo salar	Wild	no	1	72	6[d]	Moran & Garcia-Vazquez 1998
Salmo salar	Wild	no	8	0–55 (28.9)	0–4[d]	Thompson *et al*. 1998
Salmo salar	Experimental tributary	no	7	17–87 (68.2)	21	Martinez *et al*. 2000
Salmo salar	Wild	no	2	64–80 (71.8)	6–8[d]	Martinez *et al*. 2000
Salmo salar	Experimental raceway	no	18	0–100 (29.8)	20[d]	Jones & Hutchings 2002
Salvelinus malma miyabei	Pens	yes	8[e]	0–43 (18.8)	1–2	Maekawa & Onozato 1986

[a]Includes only broods for which data was obtained, > five progeny analysed, and peripheral males were present. [b]For observed spawnings percentage of success equals the sum of contributions by all males present minus the contribution of the first male to spawn; for unobserved spawnings, success of peripheral males is assumed to be the sum of all contributions minus the largest contribution. [c]Estimates include contributions of jack and kokanee peripheral males. [d]A minimum estimate based on the number of males identified as contributing to a brood. [e]Includes spawnings in which dominant males are lake-run and stream-resident.

population not making any genetic contribution during the period of this study. While the behavioural observations of spawning male brook trout indicate prevalent partitioning of fertilizations within broods, the genetic evidence from this study suggests a much more limited distribution of reproductive success in the field. This discrepancy may, in part, be accounted for by the fact that most previous studies have focused on the reproductive success of males of differing life histories (Table 4). Presumably, discrepancy among sizes leads to life history diversification within the salmonines (Gross 1985). Interestingly, for this brook trout system, we find a large skew in male reproductive success with a more equitable size distribution than the sharp differences in size categories of salmonines that exhibit alternative life history types. Although not investigated here, this difference may be due in part to the more iteroparous breeding strategy of charr species compared to other salmonines.

From the salmonine literature, we compared the reproductive success of status-dependent peripheral males to

that of peripheral males exhibiting an alternative life history ('parr' or 'jack'). Although there are large differences among studies in the numbers of peripheral males present and the conditions under which these studies took place (Table 4), there are some interesting comparisons to be made with respect to the genetic contributions of secondary males. For individual spawning events, males that matured precocially tended to be much smaller (355 ± 155 mm; $n = 46$) relative to the dominant male than status-dependent peripheral males (98 ± 88 mm; $n = 17$). On average, status-dependent peripheral males fertilize about 20% of eggs (19.4% ± 30.6%; $n = 43$; Fig. 5a), whereas peripheral males exhibiting alternative life histories are almost twice as successful, fertilizing 35% of eggs (34.5% ± 31.5%; $n = 84$; Fig. 5b). Interestingly, although the success of true precocial peripheral males is greater than that of status-dependent males, the number of eggs fertilized is shared among greater numbers of precocial males (Table 4). Thus, the genetic contributions of peripheral males sneaking fertilizations by using alternative tactics within a conditional strategy may not differ greatly from precocially maturating males as an alternative life history strategy (Fig. 5a,b). This summary of the genetic contributions of peripheral males within the subfamily Salmoninae provides some interesting data that should lead to a better understanding of the occurrence of alternative life history types within this group of fish and warrants a much more thorough examination.

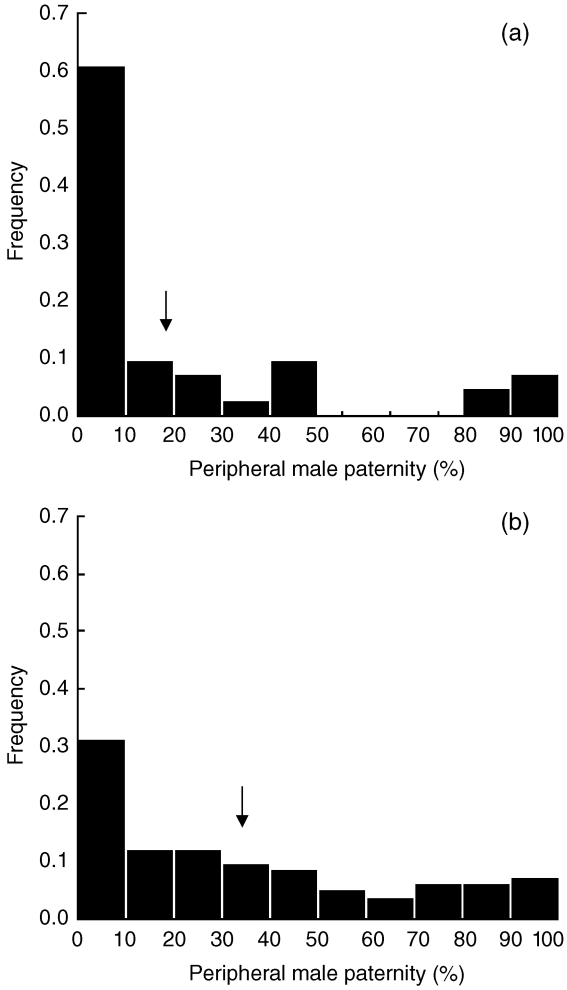

Fig. 5 Summary of partitioning of paternity for multiple-male spawnings with the presence of (a) status-dependent peripheral males and (b) alternative life-history males within the Salmoninae subfamily. The solid arrows show the mean proportion of eggs fertilized by peripheral males. Individual reproductive success was calculated as the proportion of brood fertilized by all peripheral males, divided by the number of peripheral males present. When the numbers of peripheral males were not known, we used the number of males contributing to the brood as our estimate of number of peripheral males present. Data are from the studies listed in Table 4.

Relatedness

Very little is known about the role of relatedness or kin recognition in mate selection in salmonids. It is well established that juvenile salmonids are able to recognize kin and alter their behaviour accordingly (Quinn & Busack 1985; Olsén *et al*. 2002, and references therein). Whether this kin recognition plays a role in adult mate choice, however, is still unknown. In reconstructing mating events in Atlantic salmon from genotyping adults and subsequent offspring in a closed system, Landry *et al*. (2001) found no evidence of inbreeding avoidance based on microsatellite DNA.

The difference in mate choice between smaller and larger females may be related to more selective choice by experienced older females, or may simply be an artefact of fewer older or larger males being present in the population (Blanchfield, unpubl.). If size differences may be inferred to represent younger vs. older females, the observed differences in mate choice may reflect greater choosiness by more experienced females. If large or old males are in relatively short supply, however, random mating within size-assortative boundaries could produce the same results. Natural attrition of cohorts would result in fewer siblings persisting with increasing age, and could produce the same pattern in the absence of mate choice. This 'false positive' association would be strengthened if male and female brook trout exhibit different growth or maturation schedules. Male brook trout tend to become reproductively mature at age 2, a year earlier than females (Blanchfield, unpubl.). To evaluate accurately whether kin avoidance in brook trout mating is apparent or real, it would be desirable to construct an empirical null model with male and female growth rates, maturation schedules and survivorship, and superimpose these values on expected relatedness among mature fish by size class. Such a

model is beyond the scope of this paper, but could provide useful insights into the factors underlying mate choice in iteroparous fish species.

Evidence of size-assortative mating in this population indicates that male size plays an important role in female mate choice (Blanchfield & Ridgway 1999), and may heighten the skew in mating success of larger males. Of the 10 broods produced by females greater than 430 mm in length, only two males less than 430 mm were successful contributors. The remaining eight broods were fathered by three large males. Although all the estimated pairwise relatedness values between these males and the large females were lower than the population mean, this may be an artefact of fewer males reaching large sizes, through either differential mortality or alternate resource allocation such as investment in reproduction vs. growth. Further study will be needed to resolve the importance of relatedness in mate selection in brook trout, and fish in general.

The ability to provide social and genetic breeding data under natural conditions is common practice in mammalian and avian mating systems, yet remains largely unexplored in fish despite their incredible diversity of mating patterns. However, this approach is fundamental to an understanding of the realized fitness of various behavioural strategies and tactics (Taggart *et al*. 2001). In summary, the high variance in fertilization success of peripheral males within this group of fishes (Table 4) suggests that the partitioning of reproductive success among males is difficult to predict from behavioural observations. In this study, the marked difference between estimates of reproductive success generated by behavioural observations and those generated by genetic data for male brook trout highlights the complex and dynamic nature of salmonine mating systems and the need for continued research into the fitness consequences of alternative reproductive behaviours.

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Paul Blanchfield conducted this study while with the University of Toronto Aquatic Ecology Group and Ontario Ministry of Natural Resources. Previous detailed behavioural observations of a brook trout mating system have laid the foundation to address individual- and population-level questions of reproductive success from a genetic perspective. The work here represents a continuation of the author's research of salmonine mating systems in the wild. Mark Ridgway studies the breeding behaviour, ecology and life history of North American fishes. Chris Wilson uses genetic tools to investigate issues relating to the management and conservation of aquatic species.
