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Kinship analysis of brook trout *Salvelinus fontinalis* during their breeding migration

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Microsatellite markers were used to test whether groups of pre-spawning adult brook trout *Salvelinus fontinalis* from the same population and captured at the same location during their breeding migration comprised kin. Only weak evidence for kin associations was found at the onset of breeding: the proportion of kin captured at the same location was low and similar to the proportion found across all locations and the average relatedness of *S. fontinalis* captured at the same location was low. A dilution of kin associations from the feeding to breeding phase is hypothesized to stem from mainly natural mortality that reduces family size by the adult stage. The results illustrate the dynamic nature of kin associations between consecutive life stages, even within the same fish population.

Key words: population; salmonid; sex bias; social organization.

INTRODUCTION

Evolutionary explanations for genetically non-random group associations in different organisms vary widely, including active kin selection (Hamilton, 1964; Olsen, 1989), active familiarity (Magurran *et al*., 1994; Griffiths, 2003) and orientation towards common environmental cues (Pfennig, 1990; Fraser *et al*., 2005). In fishes, several studies have documented contradictory evidence that social groups may comprise genetically related individuals at specific life stages (Fontaine & Dodson, 1999; Russell *et al*., 2004; Fraser *et al*., 2005; Carlsson, 2007; Palm *et al*., 2008; Piyapong *et al*., 2011; O'Farrell *et al*., 2012). A multitude of factors appear to influence the extent to which fishes will associate and orient with related individuals. For instance, kin associations may arise or be more favourable when (1) the habitat is temporally stable and restricts dispersal from natal sites, (2) there is a greater degree of hatching synchrony, (3) population sizes are sufficiently small to provide ample opportunities for related individuals to interact and (4) when high population densities favour cooperative behaviours (Fontaine & Dodson, 1999; Griffiths, 2003; Carlsson *et al*., 2004; Carlsson, 2007). As fishes at different life stages face contrasting environmental pressures that affect survival and mating, an understudied

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but likely possibility is that social group interactions may also change over the lifecycle.

In a previous study of migratory brook trout *Salvelinus fontinalis* (Mitchill 1814) populations inhabiting a large post-glacial lake (Mistassini Lake, Quebec, Canada), Fraser *et al*. (2005) documented that during the feeding migration, schools of *S. fontinalis* regularly comprised individuals from the same population and the same family. They suggested that such kin associations were manifested in juveniles in river environments and were carried on in sub-adults and adults in feeding areas. Whether or not these kin associations might extend through to the onset of breeding was not studied at that time, but merits investigation for three reasons. First, the potential benefits of kin associations to individuals in juvenile and sub-adult stages, such as enhanced homing to natal streams before breeding (Fraser *et al*., 2005), may not apply to adults. Instead, associating with kin during the breeding season may increase the likelihood of inbreeding and potentially inbreeding depression. Second, the potential advantages of not orienting with kin may vary with sex, although sex differences in kin associations were not detected during feeding migrations (Fraser *et al*., 2005). Indeed, lower relatedness among males than females might be expected within social groups just prior to breeding, because male *S. fontinalis* must actively compete for access to females at this time, and this may explain the repeated observation of greater mobility and dispersal in males than females in this species (Hutchings & Gerber, 2002; Blanchfield *et al*., 2003; Fraser *et al*., 2004). Third, a demonstration of kin associations during the breeding migration might reinforce the traditional practice of only partially harvesting *S. fontinalis* social groups and rotating fishing areas by local First Nations fishers to maintain family diversity within the population (Fraser *et al*., 2006).

Using microsatellite markers and known capture locations, the main objective of this study was therefore to examine the kinship of adults from one population of Mistassini *S. fontinalis* during their breeding migration. Specifically, the aim was to contrast the kinship during breeding migration with Fraser *et al*.'s (2005) kinship analysis during the feeding migration of these *S. fontinalis*, to test whether kin associations persist to the onset of breeding and whether these are less prevalent in males.

MATERIALS AND METHODS

SAMPLING

One hundred and seventy pre-spawning *S. fontinalis* were captured *via* angling from 17 spatial locations within the Pepeshquasati River, Quebec, Canada (Fig. 1), between 14 and 20 September 2011. The distance separating these locations ranged from *c*. 200 m to 14 km but several locations were re-sampled on multiple days (Fig. 1). Each location, whether defined by space or time, was treated separately for relatedness analysis. The numbers of *S. fontinalis* captured per location ranged from one to 25, with eight individuals captured alone (*i.e*. 28 locations had multiple individuals). Sampling consisted of determining the sex, age and total length (L_T, mm) of each *S. fontinalis*, as well as collecting a small piece of adipose fin tissue for DNA analysis. Males and females were easily distinguished on the basis of morphological characteristics. Age was assessed from standard scale analysis and defined as the number of completed winter seasons, *e.g*. age 3+, 4+, 5+ or 6+ in Pepeshquasati. Most *S. fontinalis*

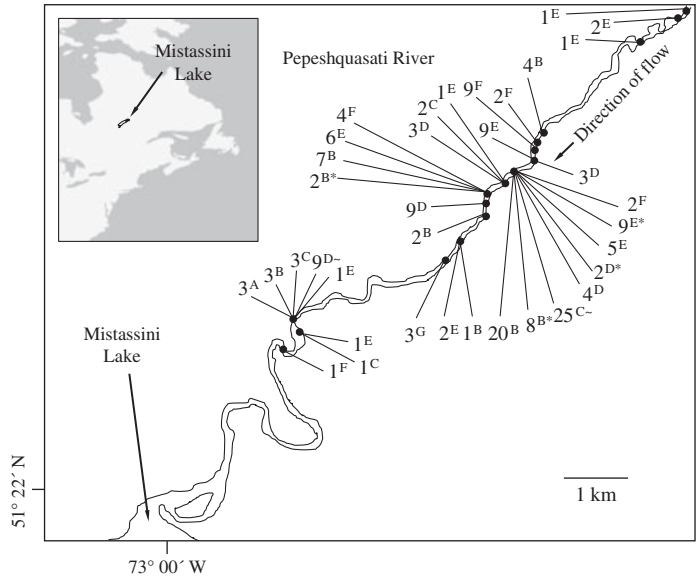

FIG. 1. Map of the sampling locations (●) within the Pepeshquasati River, a tributary of Mistassini Lake, Quebec, Canada. Numbers indicate how many *Salvelinus fontinalis* were captured at each location; letters represent the day of capture (A, 14 September 2011; B, 15 September 2011; C, 16 September 2011; D, 17 September 2011; E, 18 September 2011; F, 19 September 2011; G, 20 September 2011). *, same spatial location sampled at two times on the same day; ∼, high proportion of kin dyads detected.

were released unharmed following sampling; the remainder were killed for consumption by local First Nations fishers.

MOLECULAR GENETIC ANALYSES

Total genomic DNA from adipose fin tissue taken from each *S. fontinalis* was extracted following the procedure described by Fraser *et al*. (2004) and genotyped at 15 polymorphic microsatellite loci (Table I). The within-population genetic structure of the Pepeshquasati was temporally stable in earlier research from 2000 to 2002, based on non-significant F_{ST} and the low number of loci having significantly different allele frequency distributions between years (Fraser *et al*., 2004). Furthermore, Fraser *et al*. (2004) found no evidence for sub-population structure in these three separate years within the Pepeshquasati using standard population genetic clustering analyses (STRUCTURE 2.1; http://pritch.bsd.uchicago.edu/software/structure2_1.html/; Pritchard *et al*., 2000). These same results were obtained with the 2011 dataset, when either comparing it to 2000–2002 for the same loci (10 of 15) used in the study of Fraser *et al*. (2004) or when running the 2011 dataset in STRUCTURE using all 15 loci. Thus, any potential effects of cohort or sub-population structure on interpretations of relatedness were probably minimal. PCR profiles followed specific loci protocols as described by Fraser *et al*. (2004) and Belmar-Lucero *et al*. (2012), with PCR products separated electrophoretically using either a LICOR 4200 global IR2 system or an Applied Biosystems 3500 automated sequencer (Life Technologies; www.lifetechnologies.com); allele sizes were scored based on a fluorescently labelled size standard as in these two studies. Expected heterozygosities, potential deviations from Hardy–Weinberg equilibrium (HWE) at each locus and tests of linkage disequilibrium between each locus pair were quantified using procedures implemented in GENEPOP 3.1 (Raymond & Rousset, 1995).

TABLE I. Microsatellite loci used for simulating and calculating relatedness using pair-wise and likelihood approaches within the Pepeshquasati *Salvelinus fontinalis* breeding population, as well as the number of alleles (A) resolved per locus and individual locus expected (H_e) and observed (H_o) heterozygosities

Locus	A	H_e	H_o
Sco218[a]	7	0·79	0·79
Sco220[a]	10	0·84	0·81
Ssa407[b]	10	0·77	0·76
Ssa408[b]	9	0·78	0·77
SalE38[c]	15	0·89	0·86
Sfo18[d]	4	0·31	0·35
SfoB52[e]	5	0·74	0·65
SfoC28[e]	6	0·77	0·67
SfoC86[e]	4	0·51	0·56
SfoC88[e]	6	0·41	0·40
SfoC113[e]	6	0·50	0·50
SfoC129[e]	3	0·61	0·55
SfoD75[e]	11	0·83	0·92
SfoD91[e]	11	0·62	0·64
SfoD100[e]	9	0·74	0·75

Loci references: [a]DeHaan & Ardren (2005); [b]Cairney *et al*. (2000); [c]McGowan *et al*. (2004); [d]Angers *et al*. (1995); [e]T. L. King (unpubl. data).

KINSHIP ANALYSES

Two approaches were used to explore trends in the overall relatedness of individual *S. fontinalis* captured at the same and different locations during their breeding migration, as well as in relation to their age and sex. With both approaches, distinguishing between pairs of *S. fontinalis* (dyads) that were unrelated and those with at least a half-sib relationship was focussed on, because the species has a promiscuous breeding system (Blanchfield *et al*., 2003).

The first approach calculated relatedness between all individual dyads using Queller & Goodnight's (1989) estimator r_{xy}, similar to that reported for assessing relatedness in adults during the feeding migration (Fraser *et al*., 2005). Ten thousand dyads of unrelated, half-sib and full-sib individuals were randomly generated based on allele frequencies observed in the Pepeshquasati population across all individuals to obtain simulated distributions of r_{xy} for each relatedness category. Observed r_{xy} values of *S. fontinalis* dyads from the same and different locations were compared with simulated data to statistically discriminate unrelated individuals from kin (half- or full-sibs) using a 1% threshold of type I error in KINGROUP 2.0 (Konovalov *et al*., 2004). At this level of type I error (*i.e*. the chance that a half- or full-sib dyad was incorrectly assigned as an unrelated dyad), the type II error was 52%, reducing the possibility that unrelated dyads would be classified as kin.

The second approach used the maximum full-likelihood method implemented in COLONY 2.0 (Wang, 2004) to similarly partition dyads as half- or full-sibs. To be classified at either of these relatedness categories, and be analogous with the first approach, dyads had to have at least a 99% probability of being kin (*i.e*. a probability threshold of 99% was applied). For this analysis, a conservative 5% genotyping error was allowed and COLONY's multinomial sibship prior was implemented. Indeed, the latter is recommended when both sexes are polygamous as in *S. fontinalis*, because it reduces the chance that unrelated or loosely related individuals are falsely inferred to be siblings (Wang, 2004).

Using both approaches, the number of kin dyads at the same and different locations was quantified, as well as the number of kin dyads within locations assigned to the same or different

age cohorts. The low number of kin dyads sharing the same age precluded age comparisons of kin association ($n = 7$ with each approach), so this was assessed by comparing r_{xy} between the three age cohorts with sufficient sample sizes (4+, 5+, 6+) for all possible within-location dyads using Mann–Whitney U-tests for each pair. Similarly, the kinship of *S. fontinalis* captured at the same location in relation to sex was assessed. First, χ^2 tests were used to compare the proportions of male–male, female–female and male–female dyads designated as kin within locations using both general approaches. Second, r_{xy} was compared between the same three sex categories for all possible within-location dyads using Mann–Whitney U-tests for each pair.

RESULTS

GENETIC DIVERSITY

Allelic diversity at the 15 microsatellite loci ranged from three to 15 alleles per locus ($7{\cdot}7 \pm 3{\cdot}3$; mean $\pm$ 1 S.D.), with expected heterozygosities of 0·31–0·89 ($0{\cdot}66 \pm 0{\cdot}17$) (Table I). Following Bonferroni correction, no loci deviated from HWE ($\alpha = 0{\cdot}05/15$), nor did the number of significant pair-wise linkage disequilibrium tests between loci deviate from random (three out of 105 tests, $\alpha = 0{\cdot}05$): these results supported random mating within the Pepeshquasati population and independence of the loci employed.

TRENDS IN KINSHIP AT DIFFERENT RIVER LOCATIONS

With the two adopted approaches for identifying kin, 17·9 and 28·6% of locations included kin dyads. With pooling across all locations, the proportion of individuals within locations that were implicated in kin dyads was 15·3 and 17·1% using the respective approaches (Table II). A high proportion of individuals implicated in these kin dyads (12 of 26 using KINGROUP and 20 of 29 using COLONY) were found at the same two specific locations using both approaches (denoted $25^{C\sim}$ and $9^{D\sim}$ in Fig. 1). With both approaches the proportion of within-location *v*. between-location kin dyads did not differ (all χ^2 test, $\chi^2_{(1)} \leq 1{\cdot}54$, all $P > 0{\cdot}05$) (Table II), suggesting that kin were no more likely to be found together than apart. Similar results were obtained when the analyses were carried out on half- and full-sibling kin dyads separately. Furthermore, although mean r_{xy} value among individuals within-locations was significantly higher than those between-locations (Mann–Whitney U-test: $U_{(790,13575)} = 5{\cdot}4 \times 10^6$, $P < 0{\cdot}05$), r_{xy} values of each category were very close to unrelated (being near zero: $0{\cdot}009 \pm 0{\cdot}005$ *v*. $-0{\cdot}005 \pm 0{\cdot}004$; mean $\pm$ S.E.), again suggesting a lack of clear group aggregation based on genetic relatedness.

Within locations, most designated kin dyads (76·5–78·6%) were determined to be of the same age or separated by only 1 year (Table II). Family size, inferred as the number of individuals connected in kin relationships (whether captured at the same or different location), ranged from two to five individuals. Mean $\pm$ S.E. r_{xy} values for the same aged-dyads did not differ among the three cohorts with adequate data (age 4+: $0{\cdot}010 \pm 0{\cdot}022$, 134 dyads; age 5+: $0{\cdot}045 \pm 0{\cdot}016$, 129 dyads; age 6+: $0{\cdot}042 \pm 0{\cdot}058$, 9 dyads; Mann–Whitney U-tests: all $U_{(9,129)}$, $U_{(9,134)}$ and $U_{(129,134)} \leq 1{\cdot}5 \times 10^5$, $P > 0{\cdot}05$).

Little evidence was found for sex differences in kin relationships of migrating *S. fontinalis*. Proportions of within-location kin dyads that were male–male,

TABLE II. The numbers and proportions of *Salvelinus fontinalis* kin dyads observed within and across locations, the proportion of individuals within all locations implicated in kin dyads, as well as the statistics in relation to sex and age, within the Pepeshquasati River population, using KINGROUP and COLONY

	KINGROUP	COLONY 99%
Within locations		
Number of kin dyads (total dyads = 790)	14	17
Proportion of locations with kin dyads	0·286 (8/28)	0·179 (5/28)
Proportion of individuals implicated in kin dyads	0·153 (26/170)	0·171 (29/170)
Proportion of female–female kin dyads (total dyads = 266)	0·023	0·008
Proportion of male–female kin dyads (total dyads = 384)	0·013	0·031
Proportion of male–male kin dyads (total dyads = 140)	0·021	0·021
Kin dyads with the same age or 1, 2 or 3 years apart	7, 4, 3, 0	7, 6, 3, 1
Between locations		
Number of kin dyads between locations (total dyads = 13 575)	191	368

NA, not applicable.

female–female and male–female did not differ from one another using either approach (using COLONY: all χ^2 tests, $\chi^2_{(1)} \leq 3{\cdot}01$, $P > 0{\cdot}05$; using KINGROUP: $\chi^2_{(1)} \leq 0{\cdot}77$, $P > 0{\cdot}05$). Mean ± S.E. r_{xy} values in female–female dyads ($0{\cdot}028 \pm 0{\cdot}011$, 266 dyads) were significantly higher than male–female dyads ($-0{\cdot}002 \pm 0{\cdot}009$, 384 dyads; Mann–Whitney U-test: $U_{(266,384)} = 4{\cdot}5 \times 10^5$, $P < 0{\cdot}05$), but r_{xy} values were close to zero (*i.e.* unrelated) in both cases. There were no differences in r_{xy} values between either male–female *v.* male–male dyads ($0{\cdot}006 \pm 0{\cdot}014$, 140 dyads) or female *v.* male only dyads (Mann–Whitney U-tests: both $U_{(140,384)}$ and $U_{(140,266)} \leq 1{\cdot}7 \times 10^5$, $P > 0{\cdot}05$).

DISCUSSION

Most *S. fontinalis* from the Pepeshquasati population were not found orienting with kin during their breeding migration. Average genetic relatedness between individuals captured at the same location was close to unrelated. It was also substantially lower than previously estimated among co-schooling Pepeshquasati *S. fontinalis* captured during their feeding migration within Mistassini Lake, using a comparable amount of genotypic data ($r_{xy} = 0{\cdot}077 \pm 0{\cdot}01$; Fraser *et al.*, 2005). Moreover, the proportion of designated kin dyads within locations was lower during the breeding than feeding migration based on a similar methodology (1·8–2·2% *v.* 6·8% from Fraser *et al.*, 2005). The proportion of locations having kin dyads was also lower during the breeding migration than for co-schooling *S. fontinalis* captured during the feeding migration (17·9–28·6% *v.* 34·9%), as was

the proportion of sampled individuals implicated in kin dyads (15·3–17·1% *v*. 23·2%).

Collectively, within the Pepeshquasati population, kin associations that are probably manifested by juveniles and maintained through sub-adults (Fraser *et al*., 2005) ultimately appear to be diluted by the onset of breeding. Two explanations may account for this dilution. First, exploitation or natural mortality within Mistassini Lake may increase the likelihood that few family individuals survive to adult stages to facilitate kin associations closer to the breeding period (Carlsson *et al*., 2004). Second, given that associating with kin as pre-breeding adults could increase the probability of inbred matings, a reduction of such behaviour may be favoured over time. Interestingly, a reduced tendency to associate with kin at the onset of breeding might not be a ubiquitous occurrence within this population, as two locations harboured family groups of up to five related individuals using both approaches to quantify genetic relatedness.

Additionally, very little evidence was found that Pepeshquasati males and females differed in their frequency of association with related individuals during their breeding migration, as there were similar proportions of same-sex kin dyads and low same-sex relatedness estimates within locations. This was unexpected because differences in mating costs can lead to sex-specific grouping behaviour (Griffiths & Magurran, 1998) and competition between *S. fontinalis* males has been suggested to explain higher male than female mobility among locations during the pre-breeding and breeding period (Hutchings & Gerber, 2002; Blanchfield *et al*., 2003; Fraser *et al*., 2004). Similarly, no indication was found that breeding adults differed in their degree of kin association based on age. The trend for a considerable number of kin dyads to be designated between individuals of differing ages further suggested that a considerable portion of adults in the Pepeshquasati are iteroparous, a not unexpected occurrence for this species (Power, 1980; Hutchings, 1993).

Resolution for discerning genetic relatedness was high within the dataset. The unavoidable partial sampling of locations and potential sampling of mixed social groups, however, may have made discerning true group composition difficult at some locations. Field sampling also took place during the peak period of the Pepeshquasati population breeding migration (Fraser *et al*., 2006) but did not cover the entire breeding migration, and so other interesting elements of kinship could not be studied. For example, in steelhead trout *Oncorhynchus mykiss* (Walbaum 1792), Bentzen *et al*. (2001) found that the frequency of genetically-related individuals carrying out their breeding migration <1 week apart was higher than expected at random across the entire breeding migration, suggesting a genetic basis to run-timing.

In conclusion, this study, combined with the results of Fraser *et al*. (2005), is one of a few illustrating how the extent of kin associations may shift from one life stage to the next within the same fish population (Carlsson *et al*., 2004). The ultimate consequences of such dynamic social interactions for individual fitness and population-level processes merit further investigation, but may have relevance for understanding population structure and how genetic diversity is retained within populations.

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