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Migratory charr schools exhibit population and kin associations beyond juvenile stages

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Abstract

Few studies have critically investigated the genetic composition of wild fish schools. Yet, such investigations may have profound implications for the understanding of social organization and population differentiation in both fundamental and applied research. Using 20 microsatellite loci, we investigated the composition of 53 schools (total n = 211) of adult and subadult migratory brook charr (*Salvelinus fontinalis*) sampled from the known feeding areas of two populations inhabiting Mistassini Lake (Québec, Canada). We specifically tested whether (i) school members originated from the same population, (ii) individuals from the same population within schools were kin (half- or full-siblings), and (iii) kin schooling relationships differed between sexes. Randomization tests revealed a tendency for most schools to be population specific, although some schools were population mixtures. Significantly more kin were found within schools than expected at random for both populations (≈ 21–34% of the total number of school members). This result, combined with the observed size range of individuals, indicated that stable associations between kin may occur beyond juvenile stages for up to 4 years. Nevertheless, a high proportion of school members were non-kin (≈ 66–79%). No differences were detected between sexes in the propensity to school with kin. We discuss the hypothesis that the stable kin groups, rather than arising from kin selection, may instead be a by-product of familiarity based on individual selection for the maintenance of local adaptations related to migration (natal and feeding area philopatry). Our results are noteworthy because they suggest that there is some degree of permanence in the composition of wild fish schools. Additionally, they support the hypothesis that schools can be hierarchically structured (from population members down to family groups) and are thus nonrandom genetic entities.

Keywords: familiarity, kin selection, migration, relatedness, salmon, schooling behaviour, sex-specific behaviour, shoaling

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Introduction

Living in groups can provide fitness benefits to individuals and is a common attribute of diverse animal taxa (Krause & Ruxton 2002). In fishes, schooling is a ubiquitous behaviour (Krause *et al*. 2000). The direct fitness benefits of schooling (e.g. cooperative foraging, swimming efficiency, predator avoidance) are assumed to be largely responsible for the organization of fish schools (Weihs 1973; Mininski 1987; Pitcher & Parrish 1993). However, it has been suggested that by schooling with kin, individuals could also gain inclusive fitness benefits by increasing the probability that genes identical to their own would be passed on to the next generation (Hamilton 1964; Quinn & Busack 1985; Olsen 1989; Brown & Brown 1996). For instance, schooling with relatives might improve natal homing to breeding areas in migratory species (Quinn & Busack 1985; Olsen 1989). Consequently, kin-associated schooling has several implications for the understanding of social organization and population differentiation, in both fundamental and applied research. Surprisingly, though, the genetic composition of wild fish schools and the relative importance of kin-associated schooling remain largely enigmatic.

Consider the few and equivocal studies that have evaluated whether kin-associated schooling exists in the wild. Although kin-associated schooling should vary among

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species due to differences in habitat, dispersal and population density (Herbinger *et al*. 1997; Krause *et al*. 2000), methodological issues have limited genetic studies of schooling. Some studies did not sample independent schools (Naish *et al*. 1993; Gerlach *et al*. 2001). Earlier studies using allozymes or mitochondrial DNA (mtDNA) had low resolution (unavoidably) to estimate relatedness with precision (Ferguson & Noakes 1981; Avise & Shapiro 1986; Dowling & Moore 1986; Naish *et al*. 1993; Peukhuri & Seppa 1998). Likewise, recent works used too few microsatellite DNA loci (three to six completely genotyped: Pouyaud *et al*. 1999; Gerlach *et al*. 2001; Russell *et al*. 2004) to accurately distinguish between critical relatedness levels (e.g. between pairs of unrelated individuals and siblings: see Blouin *et al*. 1996; Blouin 2003). Recent works also either did not distinguish kin-associated schooling from underlying population differentiation (Pouyaud *et al*. 1999; Gerlach *et al*. 2001), or only considered average within- and between-school relatedness as an indicator of kin structuring (Russell *et al*. 2004). The latter may fail to reveal subsets of highly related individuals within schools and thus ignore underlying dynamics of schooling behaviour. To the best of our knowledge, no study has overcome all of these difficulties thus far. Collectively, there is uncertainty about the extent and importance of kin-associated schooling in the wild.

Migratory salmonid fishes are exemplary models to test for kin-associated schooling in the wild. Juveniles of several species discriminate and associate preferentially with kin in laboratory studies (Brown & Brown 1996; Hiscock & Brown 2000). Salmonids may be in close proximity to kin upon hatching in the wild (Elliott 1987), and there is some evidence for kin-associated distributions among juveniles in natural river environments (Hansen *et al*. 1997; Carlsson *et al*. 2004). Individuals may also form schools when migrating to ocean or lake feeding areas (Wood *et al*. 1993), with experimental evidence suggesting but not confirming that this could occur among kin (Olsen *et al*. 2004). However, it remains to be tested whether kin-associated schooling occurs in the wild in salmonid fishes, and whether it might persist into adulthood. Indeed, feeding areas of migratory salmonid life cycles are difficult to observe at the population level in nature (e.g. within expansive and complex ocean feeding areas: McKinnell *et al*. 1997). Nevertheless, there are systems where this can be more feasibly undertaken (e.g. self-contained lakes). To fully understand the potential significance of kin-associated schooling, feeding areas must be considered because they may present novel environmental conditions that facilitate the behaviour (e.g. new predation, habitat and foraging regimes).

Another under-explored possibility is that males and females may differ in kin-schooling behaviour (but see Griffiths & Magurran 1998; Russell *et al*. 2004). Migratory salmonid fishes exhibit several sex-specific behaviours that may occur at life cycle stages other than breeding time (Holtby & Healey 1990; Mjølnerod *et al*. 1999; Theriault & Dodson 2003). Within oceanic or lake feeding areas, for example, females may experience greater mortality than males since they show more intensive foraging behaviour, leading to faster growth rates but seemingly at the expense of enhanced predation risk (Holtby & Healey 1990; Spidle *et al*. 1998; Hard & Heard 1999; Tamate & Maekawa 2004). Indeed, reproductive success in female salmonids is primarily limited by body size and the number of eggs they produce, whereas male reproductive success is restricted by competition with other males for access to females (Hutchings & Gerber 2002). Thus, if cooperative behaviours orientated towards kin translate into more efficient growth (Brown & Brown 1996), salmonid females might be expected to gain more advantages from orienting towards kin than males.

We recently documented the presence of three genetically distinct breeding populations of migratory brook charr (*Salvelinus fontinalis*) inhabiting Mistassini Lake (2150 km^2), Québec, Canada (50°25′N, 73°53′W) (Fraser *et al*. 2004). Juveniles spend 1–2 years in natal rivers before migrating to feeding areas in the lake as maturing adults (an additional 1–4 years). Sexually mature fish migrate back to breeding areas in natal rivers to complete their life cycle. Estimates of effective population size (N_e) suggest a moderate abundance of a few to several thousand fish in each breeding population (Fraser *et al*. 2004). A prominent behavioural feature observed within feeding areas is the schooling of charr. These are typically small groups (n = 3–12), and they can be seen easily in the shallow, clear waters of the littoral zone where charr frequently forage (Cree First Nations Trapper's Association, Mistissini, Québec, personal communication; D. Fraser, personal observations). The attributes of this system therefore provide a good opportunity for testing hypotheses regarding free-ranging schooling relationships in migratory salmonids beyond juvenile stages.

In this study, we use 20 microsatellite loci and take advantage of detailed knowledge on the population genetic structure and feeding areas of two populations to address three questions concerning their schooling behaviour beyond juvenile stages. (i) Do individuals within schools originate from the same population? (ii) Are individuals from the same population within schools related at the level of kin (half- or full-siblings) more than expected at random? (iii) Do the sexes differ in kin-schooling behaviour?

Materials and methods

School sampling

A total of 53 schools of foraging brook charr (total n = 211) were sampled via angling in the first 2 weeks of June in 2001 (17) and 2002 (36) from Mistassini Lake (Figs 1 and

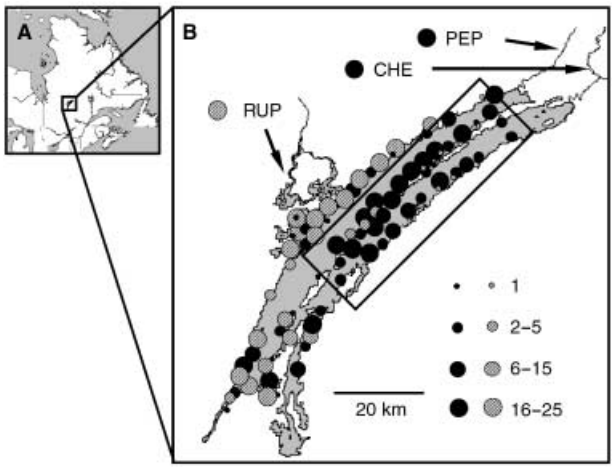

Fig. 1 (A) Geographical location of Mistassini Lake in Québec, Canada. (B) Summer spatial distribution of the three breeding populations in feeding areas (Rupert, RUP; Pepeshquasati, PEP; Cheno, CHE; modified from Fraser & Bernatchez 2005). The rectangle outlined in black represents the sampling area of schools in the present study. PEP and CHE are both denoted in black because their spatial distributions did not differ from one another; the spatial distribution depicted is for the year 2001, but similar results were obtained for year 2000 (Fraser & Bernatchez 2005). The size of circles within the legend is proportional to the number of individuals assigned to populations at each sampling location.

2A). Most charr were killed for consumption by local First Nations fishers. Schools were sampled within the known feeding areas of two breeding populations (Pepeshquasati, PEP; Cheno, CHE; Fig. 1). Attempts to obtain sufficient schools from the feeding area of the third breeding population (Rupert, RUP; Fig. 1) were unsuccessful. Within our sampling area of schools, 94% of brook charr captured over two consecutive summers (2000, 2001) were allocated to PEP and CHE (other 6% to RUP) based on individual assignment methods (Fraser & Bernatchez 2005). We sampled schools only in shallow (< 2.5 m) waters of the littoral zone to ensure sampling of individual cohesive schools (defined as a group of visually detected brook charr foraging in the same area within 50 m of shoreline). Sample sizes from each school ranged from 2 to 10. Some schools were partially sampled, but resampling of the same schools was unlikely because sampled schools were generally separated by several kilometres. We measured the total length (mm) of each charr for 32 schools. We also determined the sex of individuals within 40 schools based on external or internal morphology.

Molecular genetic analyses

Total genomic DNA from adipose fin tissue samples taken from each school individual was extracted following Fraser *et al*. (2004), and genotyped at 20 polymorphic microsatellite loci (Table 1; Fig. 2A). To characterize independently

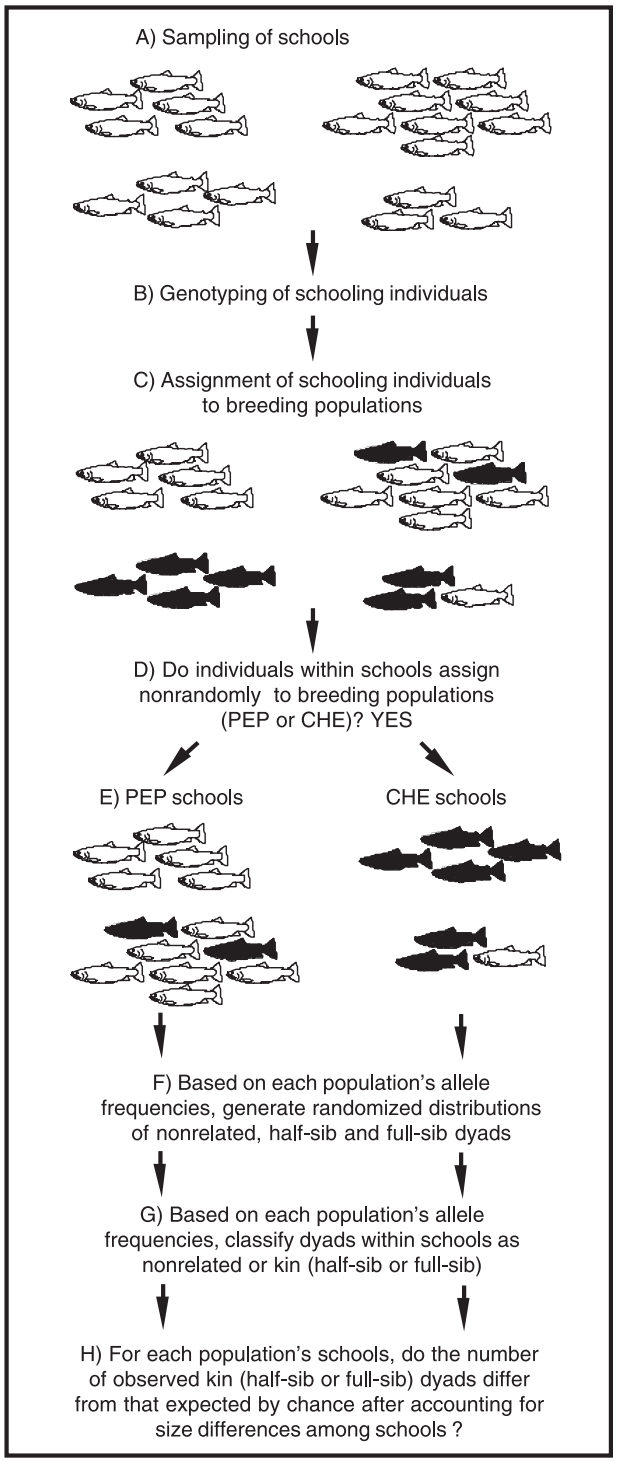

Fig. 2 Flow chart outlining the study of school composition and determination of kin relationships in Mistassini Lake brook charr breeding populations (Pepeshquasati, PEP; Cheno, CHE).

derived population allele frequencies for subsequent relatedness analyses of school individuals, breeding population individuals captured on spawning grounds in PEP and CHE were also genotyped at the 20 loci. This data

Table 1 Microsatellite loci used for simulating and calculating r_{xy} values within the PEP (Pepeshquasati) and CHE (Cheno) breeding population individuals data set and schools of brook charr, as well as the total number of alleles (A) resolved per locus (between PEP and CHE) and individual locus expected (H_E) and observed (H_O) heterozygosities

Locus	A	$H_{E(PEP)}$	$H_{O(PEP)}$	$H_{E(CHE)}$	$H_{O(CHE)}$	Locus	A	$H_{E(PEP)}$	$H_{O(PEP)}$	$H_{E(CHE)}$	$H_{O(CHE)}$
**Mst85*[a]	9	0.72	0.71	0.71	0.73	**SfoC86*[d]	4	0.45	0.44	0.48	0.52
Sfo8[b]	9	0.26	0.29	0.28	0.31	*SfoC88*[d]	5	0.40	0.31	0.57	0.63
**Sfo12*[b]	5	0.55	0.56	0.55	0.51	*SfoC113*[d]	6	0.50	0.56	0.45	0.41
**Sfo18*[b]	6	0.30	0.31	0.26	0.23	*SfoC115*[d]	4	0.51	0.50	0.49	0.56
**Sfo23*[b]	14	0.79	0.82	0.78	0.78	**SfoC129*[d]	5	0.60	0.61	0.59	0.53
Sfo122[c]	5	0.75	0.73	0.72	0.83	**SfoD75*[d]	11	0.84	0.86	0.82	0.84
**SfoB52*[d]	8	0.74	0.68	0.68	0.68	**SfoD91*[d]	10	0.69	0.61	0.63	0.55
SfoC24[d]	3	0.20	0.22	0.14	0.16	**SfoD100*[d]	8	0.79	0.82	0.78	0.75
SfoC28[d]	6	0.75	0.73	0.77	0.77	*SfoD105*[d]	41	0.96	0.89	0.90	0.85
SfoC38[d]	3	0.40	0.44	0.42	0.43	*Ssa197*[e]	3	0.42	0.39	0.38	0.30

Loci references[a–e]: [a]Presa & Guyomard (1996); [b]Angers *et al*. (1995); [c]GML Perry *et al*. Laval University, unpublished; [d]TL King, US Geological Survey, unpublished; [e]O'Reilly *et al*. (1996). *Loci genotyped in Fraser *et al*. (2004).

set, hereafter referred to as 'PEP and CHE breeding population individuals', was composed of (i) all individuals genotyped at 10 of the 20 loci and that were sampled over 3 years in a previous study (2000–2002: PEP n = 185; CHE n = 137; Fraser *et al*. 2004) and (ii) a subset of these individuals genotyped in this study at the other 10 loci (n = 60 each for PEP and CHE, of which $n \approx 20$ came from each sampling year between 2000 and 2002). Note that the population genetic structure of these populations was temporally stable over three sampling years (including the same years that schools in this study were sampled; details in Fraser *et al*. 2004). Consequently, any potential effects of cohort structure within schools on interpretations of genetic relatedness were likely minimal. Polymerase chain reaction (PCR) profiles followed the protocol in Fraser *et al*. (2004), with PCR products separated electrophoretically using either an ABI 377 automated sequencer (Perkin-Elmer) or an FMBIO-200 (Hitachi) fluorescent imager. 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 version 3.1 (Raymond & Rousset 1995).

Are schools composed of individuals from the same population?

Our ability to accurately classify school individuals to breeding populations was assessed using individual assignment tests in three steps. First, to determine whether any school individuals originated from the third breeding population (RUP) within our sampling area of PEP and CHE feeding areas, a preliminary assignment of school individuals to their most likely of the three breeding populations of origin was conducted using the 10 loci from Fraser *et al*. (2004). Two individuals comprising one of the 53 schools were assigned to RUP with this test (Table 2). This school was thus abandoned from PEP and CHE relatedness analyses below (note that there was > 97% correct assignment rate between RUP vs. PEP and CHE; Fraser & Bernatchez 2005). Second, the 'leave-one-out' method and Bayesian assignment test of GENECLASS (Cornuet *et al*. 1999) was employed on PEP and CHE breeding population individuals. Each individual was removed from the data set, allele frequencies were recalculated, and the individual was then assigned to a breeding population. This method correctly reassigned 77% of PEP and 80% of CHE breeding individuals (reassignment rate did not improve when conducting the assignment only on individuals genotyped at all 20 loci). Thus, despite a good rate of reassignment between breeding populations, we expected that some schools would appear to be a mix of populations from PEP and CHE even if all individuals in that school originated from one sole population. In a third step, we assigned each school individual to breeding populations using the PEP and CHE breeding population individual data set (Fig. 2C). We next designed and conducted a 'population signal test' to determine whether individuals within schools were more likely to originate from the same population than under a random model given the size of schools (using 50 000 randomizations; see Appendix A; Fig. 2D). Worksheets for this population signal test, as well as a 'kin signal test' (Appendix B) were coded using MAPLE 7 (Waterloo Maple 2001) and are available upon request.

Individuals from the same population within schools: are they kin?

Because of the same-population membership tendency within most schools (see Results), it was important to define

Table 2 Summary of school sizes, *n*, assignment of individuals to breeding populations (P, Pepeshquasati; C, Cheno), classification of school (Pop, i.e. from P, Pepeshquasati or C, Cheno), and numbers and relationships of kin. Kin relationships represented by three consecutive individuals (e.g. 1–2–3) illustrate triads of individuals where all dyads were kin (i.e. 1–2, 1–3, 2–3)

		Individual assigned to											Type I error	
School	*n*	1	2	3	4	5	6	7	8	9	10	Pop	2.5%	1%
1	8	C	C	C	P	P	C	P	P			P, C	P: 1–8, 2–3, 2–8, 3–5 C: none	P: 1–8, 2–8 C: none
2	3	P	P	P								P	2–3	
3	4	C	P	P	P							P	2–4	2–4
4	6	C	C	C	P	P	C					C	2–3, 3–5	2–3
5	4	C	P	P	P							P	2–3	2–3
6	3	P	C	P								P		
7	4	P	P	P	P							P	3–4	3–4
8	2	P	P									P		
9	6	C	C	C	P	P	P					P, C		
10	2	C	C									C	1–2	
11	10	P	C	P	P	P	P	C	P	P	C	P	4–5	
12	9	C	C	C	P	P	C	P	C	C		C	1–8	
13	2	P	P									P		
14	7	C	P	P	C	P	P	C				P	2–3, 3–4, 2–5	2–3
15	4	P	P	P	P							P	2–3	2–3
16	3	C	P	C								C	1–2	1–2
17	5	P	P	P	P	C						P		
18	2	P	P									P		
19	3	P	P	P								P		
20	3	P	P	P								P		
21	5	P	P	P	P	P						P	2–3, 2–5, 3–4, 3–5	2–3, 3–5
22	2	P	P									P		
23	2	P	P									P		
24	2	P	P									P		
25	3	C	C	P								C		
26	2	P	P									P	1–2	
27	2	P	P									P		
28	2	P	P									P		
29	3	P	C	C								C		
30	2	P	C									P, C		
31	2	C	C									C	1–2	1–2
32	2	Both individuals assigned to the Rupert River (see Materials and methods for details)												
33	3	C	P	P								P	2–3	
34	2	P	C									P, C		
35	6	C	P	P	P	P	P					P	1–2–3, 3–4	1–3, 3–4
36	4	P	P	P	P							P		
37	3	P	P	P								P	1–2	
38	2	C	C									C		
39	8	P	C	P	P	C	P	C	C			P, C	C: 2–3–4, 2–5, 2–7, 4–5, 4–6 P: 2–3–4, 2–5, 2–7, 4–5, 4–6, 7–8	C: 2–5, 3–4, 4–5 P: 2–5, 2–7, 3–5, 4–5
40	9	P	P	P	C	P	P	C	C	C		P	2–5, 3–6, 6–7	3–6, 6–7
41	7	P	P	P	P	C	P	P				P	1–4–7	1–4–7
42	2	P	P									P		
43	5	P	P	P	P	P						P		
44	3	P	P	C								P		
45	4	P	C	P	P							P	2–4	2–4
46	6	P	P	P	C	P	P					P		
47	3	P	P	P								P		
48	2	P	P									P		
49	5	P	P	C	P	C						P	1–3, 2–3	1–3, 2–3
50	2	C	C									C		
51	9	P	P	P	P	P	P	P	P	P		P	1–3, 2–7, 8–9	1–3, 8–9
52	3	P	C	P								P	1–2	1–2
53	4	C	C	P	P							P, C		

schools as being from PEP or CHE (Fig. 2E) in order to treat relatedness among each population's school members separately, according to that population's independently derived allele frequency distributions (estimated from the PEP and CHE breeding population individual data set). Among the 52 PEP/CHE schools, 24 were composed of purely PEP- (20) or CHE-assigned (4) individuals. Most of the remaining schools (22/28) were disproportionately composed of PEP (17) or CHE (5) (Table 2); these schools were considered as having originated from the most-represented population. The six remaining schools had equal proportions of PEP- and CHE-assigned individuals. We considered these schools twice as if they had originated from both populations, yielding a total of 43 and 15 schools used in subsequent and separate analyses of relatedness for PEP and CHE schools, respectively (total $n = 239$). In general, such a method for defining schools as either PEP or CHE was conservative: if some school members actually came from another population than the one attributed to the school, there would also be more interpopulation dyads (pairs) (i.e. PEP × CHE). Such dyads are less likely to be classified as kin than intrapopulation dyads, thereby weakening the school's kinship signal.

Based on allele frequencies observed in each population (i.e. from the PEP and CHE breeding population individual data set), we calculated relatedness between all individual dyads of charr within PEP and CHE schools separately from Queller & Goodnight's (1989) estimator r_{xy}. Brook charr are promiscuous breeders (Blanchfield *et al*. 2003), so some individuals were likely related at the level of half-sib. Thus, in each population, we randomly generated 10 000 dyads of unrelated, half-sib and full-sib individuals to obtain simulated distributions of r_{xy} for assigning individual dyads within PEP and CHE schools into these three categories of relatedness (Fig. 2F, G). Simulations and estimates of r_{xy} were conducted using KINSHIP 1.2 (Goodnight & Queller 1999).

We next used an *ad hoc* threshold approach to determine the likelihood that a dyad of one r_{xy} category would be misclassified as another r_{xy} category in each population (*sensu* Blouin *et al*. 1996), using the midpoint between the means of any two simulated distributions of r_{xy} categories in a population (e.g. unrelated vs. full-sib). This analysis yielded mean r_{xy} values of randomly generated pairs of unrelated, half-sib and full-sib PEP and CHE individuals consistent with theoretical distributions (−0.0017, 0.247, 0.495 and −0.0022, 0.249, 0.495, respectively; Queller & Goodnight 1989) (Fig. 3). Type I and II errors (estimated from simulations) associated with misclassifying unrelated dyads as full-sibs or full-sibs as unrelated dyads at threshold values [$r_{xy} = 0.246$ (PEP); 0.247 (CHE)] were both ≈ 5% (PEP: 5.81%, 4.44%; CHE: 6.49%, 5.21%, respectively). Type I and II errors associated with misclassifying unrelated dyads as half-sibs or half-sibs as unrelated dyads at threshold values [$r_{xy} = 0.123$ (PEP); 0.124 (CHE)] were higher [21.71%, 20.68% (PEP); 22.39%, 20.58% (CHE), respectively]. Consequently, we reduced type I error to a value of 2.5% by adopting thresholds of $r_{xy} = 0.310$ (PEP) and $r_{xy} = 0.315$ (CHE), and to a value of 1% with thresholds of $r_{xy} = 0.370$ (PEP) and $r_{xy} = 0.368$ (CHE) (Fig. 3). Our cut-off values for controlling type I error at 2.5% and 1% were very strict given that they also increased type II errors to values of 10 to three times these values (range: 26.65–33.68%; Table 2; Fig. 3). This greatly reduced the possibility that unrelated dyads would be classified as kin. Note also that our focus was not to discriminate between half-sibs and full-sibs, but to distinguish between nonrelated dyads and dyads with r_{xy} values of at least a half-sib relationship. Furthermore, our approach based on defining relationship categories from distributions of r_{xy} facilitated the use of a threshold of r_{xy} necessary for our 'kin signal test' that we used separately for PEP and CHE after partitioning schools into each population (Fig. 2H; Appendix B). Notably, this kin signal test compared the proportion of within-school dyads classified as kin (half-sibs or full-sibs) to that expected at random (50 000 randomizations) based on each type I error threshold (i.e. 2.5% and 1%) and the size characteristics of individual schools of the particular population (Appendix B).

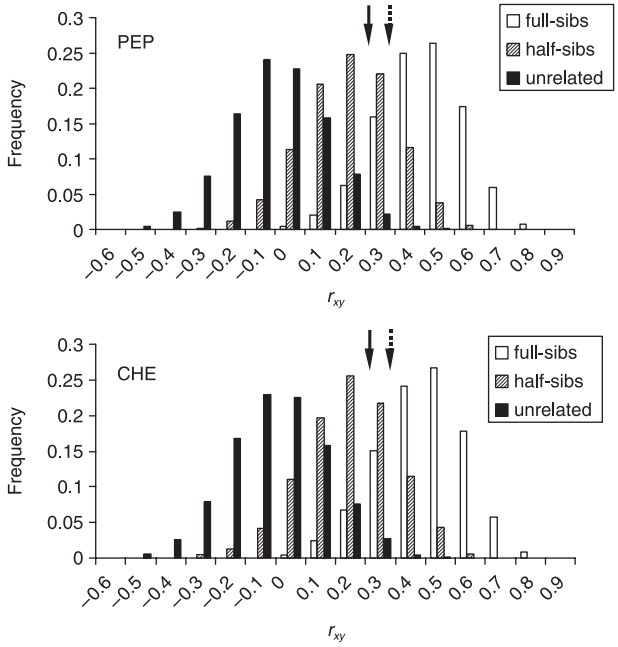

Fig. 3 Distribution of relatedness (r_{xy}) values for 10 000 randomly generated pairs of full-sib, half-sib and unrelated individuals in Pepeshquasati (PEP) and Cheno (CHE) populations. Respective means and standard deviations: PEP (0.495 ± 0.141, 0.247 ± 0.151, –0.0017 ± 0.158); CHE (0.495 ± 0.144, 0.249 ± 0.154, –0.0022 ± 0.161). Solid- and dashed-line arrows represent cut-off points for designating kin dyads based on type I error thresholds of 2.5% and 1%, respectively.

Do the sexes differ in kin schooling relationships?

Using χ^2 tests, we first compared the proportions of male–male, female–female and male–female dyads designated as kin within schools at type I error thresholds of 2.5% and 1%. To improve statistical power of these tests, sexed kin dyads were pooled from both populations' schools (PEP and CHE) because of the low numbers of such dyads in CHE (see Results), after removing from the six schools designated as originating from both populations. A different way to approach our question was to consider actual relatedness values of all males and females. Therefore, we also compared r_{xy} between the same three-dyad categories for all possible within-school dyads, using a one-way analysis of variance (ANOVA; factor: dyad category; again, after removing the six schools that had been designated as originating from both populations).

Results

Genetic diversity

Allelic diversity at the 20 microsatellite loci ranged from 3 to 41 alleles (mean 8.3 ± 1.8, ± 1 SE) in the PEP and CHE breeding population individual data set, with expected heterozygosities of 0.14 to 0.96 (mean 0.58 ± 0.03, ± 1 SE; Table 1). No loci deviated from HWE in PEP and CHE, nor did the number of significant pairwise linkage disequilibrium tests between loci deviate from random (12 out of 380): these results supported random mating within populations and independence of the loci employed.

Are schools composed of individuals from the same population?

The P value obtained with the population signal test when considering the 52 schools was 0.086; with the addition of the one school attributed to RUP (for a total school of $n = 53$), $P = 0.028$. We take $P = 0.086$ to mean the following: if there was no connection between schooling and PEP or CHE population membership, i.e. if the same population tags were randomly distributed among the schools, the P value would take equal or larger values than the observed value about 8–9% of the time. We contend that this constitutes evidence for population-associated schooling; specifically, individuals were assigned predominantly to PEP or CHE in the majority of schools (41/52 with ≥ 66.7% assigned to one population, 32/52 with ≥ 75%; Table 2). However, we are fully aware that this is a probabilistic statement. Given the results of the power test, i.e. much lower P values for homogeneous (monopop) schools (Appendix A), it is very unlikely that all schools were in fact monopop, i.e. that heterogeneous mixture in some schools was only an artefact of misallocation.

Do individuals from the same population school with kin?

Kin signal tests conducted separately on PEP and CHE schools revealed that in each population's schools, the observed proportion of kin-classified dyads (half- or full-sib) was higher than with randomized permutations of specimens among schools for the population in question, at both thresholds of type I error (2.5%, 1% PEP: $P < 0.001$; 2.5% CHE: $P = 0.001$; 1% CHE: $P = 0.016$; Table 3). These

Table 3 Type I error thresholds and their associated thresholds of r_{xy} for classifying within-school dyads as kin for Pepeshaquasati (PEP) and Cheno (CHE) breeding populations. Type II error for each type I error threshold are also listed, as are the number of kin dyads observed within schools and their associated significance values (P) following random permutations (Appendix B), the proportion of schools with kin dyads, and the proportion of school individuals implicated in kin dyads

Threshold type I error	2.5%	1%
Pepeshquasati		
Type II error	27.03%	33.68%
r_{xy} threshold (for defining kin as half- or full-siblings)	0.310	0.370
Number of kin dyads within schools (total dyads = 382)	45	26
Significance value (P)	< 0.001	< 0.001
Proportion of schools with kin dyads	0.465 (20/43)	0.349 (15/43)
Proportion of school individuals implicated in kin dyads	0.356 (63/177)	0.232 (41/177)
Cheno		
Type II error	26.65%	32.75%
r_{xy} threshold (for defining kin as half- or full-siblings)	0.315	0.368
Number of kin dyads within schools (total dyads = 143)	13	6
Significance value (P)	0.001	0.016
Proportion of schools with kin dyads	0.400 (6/15)	0.267 (4/15)
Proportion of school individuals implicated in kin dyads	0.274 (17/62)	0.161 (10/62)
Total proportion of schools with kin dyads	0.448 (26/58)	0.328 (19/58)
Total proportion of school individuals implicated in kin dyads	0.335 (80/239)	0.213 (51/239)

results demonstrated that more kin were found within schools of each population than expected at random. At type I error thresholds of 2.5% and 1%, 45% and 33% of schools (PEP and CHE combined) included kin dyads, respectively (Table 3). The proportion of the total number of individuals within all schools that were implicated in kin dyads was 34% and 21%, respectively (Table 3). Both populations showed similar patterns for each of these statistics when considered separately (Tables 2 and 3). Another indication that school individuals in each population were composed of related members was the higher mean r_{xy} (± 1 SE) between all individuals within schools than the mean r_{xy} between all individuals among schools, although the former was much less than the half- or full-sib level of relatedness (PEP $r_{xy} = 0.077 \pm 0.010$; vs. $r_{xy} = 0.024 \pm 0.001$; Mann–Whitney *U*-test: $U_{382,15576} > 3 \times 10^6$, $P < 0.001$; CHE $r_{xy} = 0.065 \pm 0.015$; vs. $r_{xy} = 0.018 \pm 0.004$; $U_{143,1748} > 2 \times 10^5$, $P < 0.001$).

Individuals within schools were also assorted by length (one-way ANOVA; factor: school, $F_{31,85} = 7.40$, $P < 0.001$; this test and subsequent length difference tests below combined PEP and CHE schools to increase statistical power, after removing the six schools designated as originating from both populations; see Materials and methods). Mean proportional length differences among school members (the difference in length between individuals in a dyad divided by their mean length) were also small: these were lower between kin than non-kin dyads, but the difference was not significant (0.055 ± 0.008 vs. 0.067 ± 0.005; Mann–Whitney *U*-test: $U_{38,183} = 3772$, $P = 0.21$; 2.5% type I error). Since size strongly correlates with age in fish, this is suggestive that kin dyads were more likely to be of the same cohort than non-kin dyads. No significant differences were detected between the mean length of charr implicated in kin and non-kin dyads (463.5 ± 6.2 mm vs. 462.1 ± 4.6 mm; Mann–Whitney *U*-test: $U_{44,118} = 3639$, $P = 0.85$; 2.5% type I error). The length range of charr classified as kin (366–513 mm) was consistent with 2+ to 4+ age classes for these populations (D. Fraser, unpublished). Of the 38 kin dyads where length was measured (2.5% type I error), in only two cases did the individuals differ in length by > 60 mm, the lowest mean difference in length between separate cohorts in PEP and CHE (D. Fraser, unpublished).

Do sexes differ in kin schooling relationships?

We found no evidence for sex differences in kin-schooling relationships. The proportions of within-school male–male, female–female and male–female dyads designated as kin did not differ at both thresholds of type I error (2.5%: $\chi^2_2 = 0.06$, $P = 0.97$; 1%: $\chi^2_2 = 0.11$, $P = 0.95$; PEP and CHE schools combined; see Material and methods). Likewise, the three dyad categories did not differ in r_{xy} (one-way ANOVA, factor: dyad category; $F_{2,346} = 1.91$, $P = 0.15$). Mean r_{xy} values were: 0.070 ± 0.029 (male–male), 0.104 ± 0.016 (female–female), 0.074 ± 0.015 (male–female). However, the power of the ANOVA was estimated at 0.40 using $\alpha = 0.05$ (Zar 1999), indicating there was a 60% chance of not detecting a significant difference when there was one.

Discussion

Population- and kin-associated schooling

Our study provided evidence for population- and kin-associated schooling beyond juvenile stages in migratory brook charr. Clearly, however, some schools were population mixtures, and a higher proportion of school members were unrelated than were kin. Nevertheless, within a considerable number of schools from each population, small groups of kin were found, and more often than expected at random. Although the duration of kin associations prior to capture is unknown, the migratory life cycle of these charr (see Introduction) strongly implies that such associations are manifested in juvenile river environments and are carried on into adult feeding areas. A main feature of our data is thus the demonstration that stable associations between kin may persist for up to 4 years (based on the size range of individuals) despite the possibility of movement among different schools.

Insufficient data on juvenile stages in Pepeshquasati and Cheno rivers currently prevent us from distinguishing the degree to which different factors may lead to kin associations. In other salmonid fishes, the high density and low dispersal characteristics of small streams are thought to provide more opportunities for juvenile kin associations than larger, more turbulent rivers, especially given the potential asynchrony (despite close spatial proximity) of salmonid hatching (Fontaine & Dodson 1999; Carlsson *et al*. 2004). Additionally, interactions within populations between breeding individual numbers, habitat availability, family size variance and density-dependent mortality may play a role (Fontaine & Dodson 1999; Carlsson *et al*. 2004).

Overall, the demonstration that social groups (schools) of migratory brook charr are a mixed composition of kin and non-kin parallels recent work in diverse taxa (e.g. Gardner *et al*. 2001; Hatchwell *et al*. 2001; Parsons *et al*. 2003). Similar examinations of schooling behaviour have not been conducted in other migratory salmonids, or other fish species, so the generality of our findings in fish is unknown. However, some degree of stable kin-associated schooling may be widespread. McKinnell *et al*. (1997) reported a significant number of tagged steelhead (*Oncorhynchus mykiss*) released as juveniles at similar times and locations that were captured together up to 3 years later in commercial fisheries. In addition, Olsen *et al*. (2004) used an experimental stream channel to show that Atlantic salmon (*Salmo salar*) siblings migrated downstream more

closely in time to both familiar and unfamiliar kin than to unrelated individuals. Stable school association between certain individuals (of unknown relatedness) has also been recently documented in diverse fish species (Klimley & Holloway 1999; Hay & McKinnell 2002; Ward *et al*. 2002).

Proximate and ultimate explanations for population- and kin-associated schooling

Kin selection theory has motivated adaptive explanations for schooling with kin in relation to increased inclusive fitness (Quinn & Busack 1985; Olsen 1989; Pitcher & Parrish 1993). Since kin-associated schooling in this study likely persists over extended periods, kin could disproportionately and effectively orientate cooperative behaviours towards one another. Why then, are schools not composed entirely of kin? Other ecological factors may require schools to be larger than the number of available kin that outweigh the costs of cooperating with non-kin (Aviles *et al*. 2004). Few or no kin may sometimes be available to associate with since early mortality is high in other brook charr populations (Hutchings 1993).

A high level of relatedness between group-living individuals does not, however, mean that kin selection has been necessarily important in shaping the social system with respect to fitness benefits (Grafen 1990; Pfennig 1990; Griffin & West 2002). In our study, more school members were unrelated than were kin. Yet, schools were still length assorted and there was a tendency for schools to be population specific. Schooling choice may thus relate to the direct fitness benefits of schooling with similar phenotypes and/or familiar population members, regardless if they are kin or not. For several reasons, we believe this is an equally parsimonious alternative explanation for the observed population and kin associations.

Studies on juvenile salmonid fishes have shown individual preferences for associating with kin based on olfactory cues (Brown & Brown 1996), but kin were often reared together. Preferences may therefore have been due to familiarity of odours rather than an innate kin-recognition mechanism (Griffiths & Magurran 1999; Krause *et al*. 2000). In fact, studies addressing this issue have found that individuals reared with kin and non-kin did not discriminate between them (Quinn & Hara 1986), and that individuals reared in isolation were unable to discriminate kin from non-kin (Winberg & Olsen 1992). Several other fishes can also recognize individuals on the basis of familiarity alone (Van Havre & Fitzgerald 1988; Dugatkin & Wilson 1992; Magurran *et al*. 1994), and schooling with familiar fish would accrue many of the same direct fitness benefits as schooling with kin (e.g. enhanced predator avoidance: Griffiths *et al*. 2004). Thus, preferential association with familiar individuals in juvenile environments, whether kin or non-kin, is a parsimonious explanation for population- or kin-associated schooling into adult feeding areas.

A familiarity-based explanation for the observed behaviours is also plausible in light of the migratory salmonid life cycle. A plethora of research on salmonids has documented the adaptive significance of traits related to natal philopatry, migration timing and feeding-area spatial distributions (Riddell & Leggett 1981; Quinn & Dittman 1990; Taylor 1991; Quinn *et al*. 2000; Bentzen *et al*. 2001; Ruzzante *et al*. 2004; Waples *et al*. 2004), including in Mistassini Lake brook charr populations (Fraser *et al*. 2004; Fraser & Bernatchez 2005). To the extent that these traits have an environmental or genetic basis, individuals may orientate with familiar conspecifics (kin or other population members) because they (i) were exposed to common environmental cues learned early in ontogeny (O'Hara & Blaustein 1982; Quinn & Hara 1986; Grafen 1990; Pfennig 1990) and/or (ii) have a genetic predisposition to migrate to similar places at similar times (Bentzen *et al*. 2001; Olsen *et al*. 2004; Fraser & Bernatchez 2005). Migrations can also be far ranging in salmonids. Preferential long-term association with familiar conspecifics might also improve the capacity of individuals to locate either natal breeding areas (Olsen 1989) or feeding areas (i.e. habitats) where they are locally adapted. Collectively, population- and kin-associated schooling may not be at all related to kin selection. Instead, the behaviour may arise merely as a by-product from orienting to familiar cues that synchronize and maintain local adaptations throughout the migratory life cycle.

Sex and schooling

Asymmetries in mating costs may ultimately lead to sex-specific schooling behaviour (Griffiths & Magurran 1998). However, we found no evidence that male and female brook charr differ in their propensity to school with kin. This suggests that there are no advantages for females to preferentially associate with related members of the same sex in feeding areas than males (see Introduction). These results must be interpreted with some caution because of the limited resolution to detect statistical differences among sex-dyad categories (see Results). Additionally, if our sampling had been done closer to the breeding period in September, we might have found higher relatedness among females than males within schools (with the lowest relatedness exhibited between females and males), but for an alternative reason: increased male mobility among schools. Indeed, male-biased dispersal ('straying') has been detected between Pepeshquasati and Cheno river breeding populations, and mate competition among males has been invoked to explain this bias (Fraser *et al*. 2004). Elevated levels of male mobility have also been observed just prior to breeding time in other brook charr populations (Hutchings & Gerber 2002; Blanchfield *et al*. 2003).

Caveats of the study

We outlined above our justification for designating schools as being from either Pepeshquasati ('P') or Cheno ('C') (see Materials and methods, Results). Nevertheless, six schools were equivalent mixtures of P and C assigned individuals. Also, the number of P and C individuals only differed by one in another 10 schools. The unavoidable partial sampling of some schools and the misallocation of some P and C individuals on spawning grounds (even with 20 microsatellite loci) led to two difficulties from this standpoint. First, the population designation of these schools might have changed if one or two more charr were captured from these schools. Second, our sampling scheme (angling) assumed equal capture probabilities for individuals. If, however, individuals from the same populations or families have more similar feeding preferences, nonrepresentative sampling of schools might have occurred. Combined with the misallocations between P and C, this makes it difficult to assess whether mixed schools truly were mixed or whether the aforementioned schools designated as population-specific were actually mixed. In general, if having multiple school members from one population designated to the other population led to an upward bias in kin dyad estimates, we would expect a large number of kin dyads within schools to be between individuals classified to another population than the one attributed to the school. This, however, was not the case: only six (2.5% type I error) or three (1% type I error) of 49 such dyads were kin dyads (eight of nine of these were from school 39), which represents only 9–10% of all kin dyads at either type I error threshold (Table 2).

Another potential anomaly was the considerable portion of kin dyads composed of individuals assigned to different populations (19 of 58 with 2.5% type I error), given that estimates of gene flow between P and C populations are not nearly this high (mean migration rate *sensu* Wright $m = 0.015$; Fraser *et al*. 2004). We contend that this is quite expected. Suppose the 58 dyads were indeed monopop (either PP or CC), then we seek the expected proportion of those dyads that appeared mixed (PC or CP). We found that 77% of P's and 80% of C's sampled on spawning grounds were correctly reassigned. So, the probability that true PP and CC dyads appear mixed was $2 \times (0.77) \times (1 - 0.77) = 0.354$ and $2 \times (0.80) \times (1 - 0.80) = 0.32$, respectively. To obtain the global proportion of false mixed dyads, the two above probabilities need to be weighed according to the relative frequencies of PP and CC. The expected relative frequencies of P's and C's based on the number of P and C assigned individuals in the sampling area of schools were 0.69 and 0.31, respectively (see Appendix for details). From this we derive the monopop dyad expected (relative) frequencies: $\text{Freq(PP)} = (0.69 \times 0.69)/[(0.69 \times 0.69) + (0.31 \times 0.31)] = 0.832$; $\text{Freq(CC)} = (1 - 0.832) = 0.168$. Thus, the expected global (either PP or CC) proportion of monopop dyads that appear mixed is $0.832 \times 0.354 + 0.168 \times 0.32 = 0.348$. Assuming that all 58 kin dyads are indeed monopop dyads then we would expect: $58 \times 0.348 = 20.2$ apparently mixed dyads, which is indeed very close to the observed value (19). Thus, the observed number of mixed kin dyads may be due only to the misallocation of some P and C individuals.

Finally, we note that stringent type I error thresholds may have actually led to some kin dyads being classified as unrelated dyads (i.e. greater type II error). In several instances, individual dyads 'A-C' and 'B-C' within schools were classified as probable full-sibs (i.e. $r_{xy} > 0.40$: data not shown), but not the dyad 'A-B' (e.g. 1% type I error: schools 1, 21, 35, 39, 40, 49; Table 2). Biologically, many of these 'dyad-triads' would likely be all full-sibs (see also Fontaine & Dodson 1999). In fact, most individuals shared a kin relation with at least one other individual from another kin dyad in schools having multiple kin dyads (Table 2). These results imply that kin within schools originated from the same family group.

Study implications

There has been little prior evidence that fish schools exhibit kin associations, other degrees of nonrandom genetic relatedness (e.g. population associations), or that particular individuals may stay together within schools over extended periods (e.g. beyond juvenile stages) (Helfman 1984; Hilborn 1991; Naish *et al*. 1993; Peukhuri & Seppa 1998; Hoare *et al*. 2000; Griffiths 2003; Russell *et al*. 2004). Our research on migratory charr shows that subgroups of kin may persist in wild fish schools for several years, and that schools may be hierarchically structured (from populations down to family groups). This opens several new interesting questions. Are non-kin associations within schools more or less (or equally) temporally stable than kin associations? Do schools comprised of kin components differ (if at all) in any fitness component from those with only non-kin components? Do the advantages of familiarity alone explain schooling associations between kin or individuals from the same population? Such behaviours, if more common in other systems, may also have consequences for the conservation of exploited species such as salmonids. The higher within-school than between-school relatedness and presence of small kin groups within schools in Mistassini Lake argues against harvesting entire schools because this might contribute to the erosion of genetic variability within populations. Many salmonid populations face increased risks of extirpation or extinction (Nehlsen *et al*. 1991; COSEWIC 2002), and the importance of addressing how conservation practices may be affected by natural behaviours has been stressed (McKinnell *et al*. 1997; Sutherland 1998). Clearly, additional investigation into the genetic composition of wild fish schools, and of migratory social groups in other species, is merited.

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This study is part of DJ Fraser's PhD thesis on the evolution of population diversity within brook charr. All three authors are interested in integrating ecological and evolutionary studies of natural populations for applications in biodiversity conservation.

Appendix

(A) Population signal test

Define each Pepeshquasati- or Cheno-assigned individual within schools as a 'P' or a 'C', respectively. Let $S_{monopop}$ represent the sum of the most frequent number of individuals assigned to the same population (monopop) within each school. For instance, for the schools 'PPPC' and 'CCCCCCP', $S_{monopop} = 3$ and 6, respectively. Take the sum of these numbers over all schools as $\sum S_{monopop}$. For a particular group of schools, the maximum value of $\sum S_{monopop}$ equals the total number of individuals over all schools: the more schools are monopop, the larger $\sum S_{monopop}$ will be. Thus, $S_{monopop}$ is simply a measure of category homogeneity within each school, and does not assume school-based designation in any way. P's and C's are then randomized and restructured according to the size characteristics of the observed group of schools. For each randomization, $\sum S_{monopop}$ is recalculated: the significance value (P) is obtained by determining the proportion of randomizations equal to or exceeding the observed $\sum S_{monopop}$. Consequently, the population signal test follows standard permutation test logic. It consists essentially of eliminating connections between some aspect/quality as measured by a meaningful statistic, while preserving all other structural properties such as the sizes of schools and the number of P and C individuals, which we did.

Note that the permutation procedure of the population signal test did not ensure the quality of the test. Additionally, the power to detect a monopop trend will be reduced by misallocations. We therefore designed and ran a power test with two objectives in mind: (i) to measure the capacity of the test to detect monopop structuring under the same circumstances (i.e. same rates of correct allocations and same sizes of schools) and (ii) to better ascertain the meaning of the P value we obtained from the real collection of schools. Groups of monopop schools were formed based on size characteristics of the observed schools. A population was randomly chosen and attributed to each school with a probability proportional to the proportion of individuals originating from each population. The ratio of PEP- to CHE-assigned individuals within the geographical area wherein all the schools were sampled was 0.69:0.31 (Fraser & Bernatchez 2005). Then, supposing a school was attributed to PEP, each artificial specimen of the school was randomly tagged as either PEP or CHE with respective probabilities equal to the probabilities of identifying a PEP specimen correctly as a PEP or incorrectly as a CHE, based on expected individual assignment success. An analogous procedure was used when the school was attributed to CHE. Significance (P) of the value of $\sum S_{monopop}$ of each fully monopop group of schools was calculated as with real groups of schools (see above).

Our results showed that the choice of the statistics and the permutation procedure detected monopop structuring in every case with a very strong signal (P value = 0). This confirmed the power of the test. It also demonstrated that the relatively weak monopop signal we obtained (P value = 0.086) could not be easily explained by a lack of power. Although we believe that our PEP/CHE estimate was reasonable, we also ran the power test with PEP/CHE = 1.5 and 3.0 in order to assess the sensitivity of the power test to PEP/CHE variations, and as reasonable upper and lower bounds for the PEP/CHE estimate. In both cases, the power was found to be 1.0 (i.e. 100% of all 100 iterations produced P values much smaller that 0.05). This shows that the observed P value does not signal purely monopop schools.

(B) Kin signal test

Within-school kin dyads are first determined according to allele frequencies in each population and type I error thresholds. Let S_{kin} represent the observed sum of within-school kin dyads detected in each population. Individuals are then randomly redistributed among schools while respecting the size characteristics of the observed group of schools in each population. For each randomization, S_{kin} is recalculated: the significance value (P) is obtained by determining the proportion of randomizations equal to or exceeding the observed S_{kin}.