

## Population and family structure of brown trout, *Salmo trutta*, in a Mediterranean stream

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**Abstract.** The physical arrangement of closely related individuals is expected to significantly influence the pattern of population genetic structure. For example, if related individuals are non-randomly distributed and included in samples, this may lead to exaggerated conclusions about genetic differentiation. In the present study, we compared population structure *v.* family relationships of brown trout (*Salmo trutta* L.) along a Mediterranean stream (Pyrenees) by using eight microsatellite loci. Results showed low levels of genetic ( $F_{ST}$ ) differentiation between collections in a 6.5-km transect along the stream, and a significant correlation between genetic and geographical distance matrices, indicating a weak population structure associated with spatial distribution. Our data also indicated that geographical proximity of related individuals in the youngest (0+, 1+) cohorts probably was associated with limited dispersal of younger brown trout from spawning redds. Family relationships provided evidence, however, for movement of adult trout over distances of a few kilometres that probably contributed to the low observed differentiation. Dispersal of adult Mediterranean trout contrasts with the clustering of related older trout observed for some north European rivers and could be related to the reduced productivity in southern stream basins.

**Additional keywords:** family reconstruction, fish movement, isolation by distance.

### Introduction

Advances in molecular genetics and statistical techniques have improved tools for analysis of populations beyond merely detecting genetic differentiation. Highly polymorphic microsatellite loci can provide the fine-scale discrimination required to address questions at the within-population level, including describing family relationships and relatedness among individuals (Ritland 2000; Carlsson *et al.* 2004; Buston *et al.* 2007). In salmonids (Bentzen *et al.* 2001; García-Vázquez *et al.* 2001; Seamons *et al.* 2004) and other species (Hain and Neff 2007; Neff *et al.* 2008), mating strategies and relative individual reproductive success have been examined via parentage assignment. Studies on family relationships have been also used to evaluate stocking impact on native salmonid populations (Ruzzante *et al.* 2001) and to examine kinship histories of individuals to avoid deleterious effects of inbreeding in breeding programs (Hansen and Jensen 2005; McLean *et al.* 2008). Few studies have, however, used family relationships to investigate the spatial distribution of individuals at a microgeographical

scale and to understand individual dispersal behaviour (Hansen *et al.* 1997; Carlsson and Carlsson 2002; Carlsson *et al.* 2004).

Since Gerking's (1959) theory on 'the restricted movement of resident fish populations', several studies have supported the notion that resident individuals of stream salmonids show limited movement, whereas others have detected substantial movement of individuals (review in Gowan *et al.* 1994; Knouft and Spotila 2002; Höjesjö *et al.* 2007). These studies also assessed the mechanisms that cause stream salmonids to move and showed that, apart from passive dispersal of fry with flow, movements are often associated with individual behaviour. A complete understanding of individual behaviour within any animal group requires understanding the kin structure of a group. Many species, including some fishes, form groups composed of kin (Möller *et al.* 2006; Hain and Neff 2007). Several studies of artificially bred sibling groups of juvenile salmonids, including brown trout (*Salmo trutta* L.), have shown evidence for kin-biased aggressive behaviour and preference for sibling groups of juveniles (Brown and Brown 1996; Olsén

1999). Nevertheless, studies undertaken in natural settings have revealed variable patterns of kin-biased behaviour in both stream- and lake-dwelling salmonids (Carlsson and Carlsson 2002; Carlsson *et al.* 2004; Fraser *et al.* 2005).

In addition, kin-biased distribution may have consequences when sampling freshwater fish populations to analyse population differentiation. In species exhibiting a non-random distribution of closely related individuals, sampling of a few families may lead to overestimation of the observed pattern of structure (Hansen *et al.* 1997). The age composition of samples may also influence this pattern of genetic population structure if young and adult individuals show different dispersal patterns.

In the present study, we investigated family relationships of brown trout populations to determine whether they consist of aggregates of close relatives or alternatively display random distributions of related individuals. Comparisons of population structure v. family relationships were used to infer the extent of brown trout dispersal patterns. Such comparisons were made for each cohort to evaluate whether distribution and movement patterns of related individuals depend on age.

## Material and methods

### Samples

The Vallferrera stream is a tributary of the Noguera Pallaresa River (42°35'N, 1°19'E; Ebre drainage), and is situated in the eastern Pyrenees (Spain). As in other Mediterranean drainages,

this stream shows strong inter-annual flow variation, with peak flow during spring following snowmelt, followed by severe drought periods during summer. Small populations of resident brown trout occupy this and other Mediterranean streams. A total of 140 brown trout was caught from the Vallferrera stream by electrofishing in 2002 and 2003. Individuals were collected from four sections of the stream measuring 100 m, regularly distributed along 6.5 km of the river and located 1.3 km downstream of an impassable dam (VF1,  $n = 16$ , all individuals collected in 2002; VF2,  $n = 58$ , 18 individuals collected in 2002 and 40 in 2003; VF3,  $n = 66$ , 36 individuals collected in 2002 and 30 in 2003; and VF4,  $n = 14$ , all individuals collected in 2002) (Fig. 1). For each individual, a small piece of adipose fin was removed for DNA extraction. Age of all fish captured was analysed from scales, and seven age classes were defined among all wild fish captured, ranging from 0+ to 6+.

A sample of 40 adult individuals from the Bagà hatchery stock, used for stocking native populations in the sampled area, was also analysed to detect introgression of hatchery genes into wild populations and to exclude hatchery individuals from the analysis.

### Genotypic analyses

DNA was extracted from adipose fin tissue by the Chelex-proteinase K procedure (Estoup *et al.* 1996). The following eight microsatellite loci were analysed: *Str15*, *Str73* (Estoup *et al.*

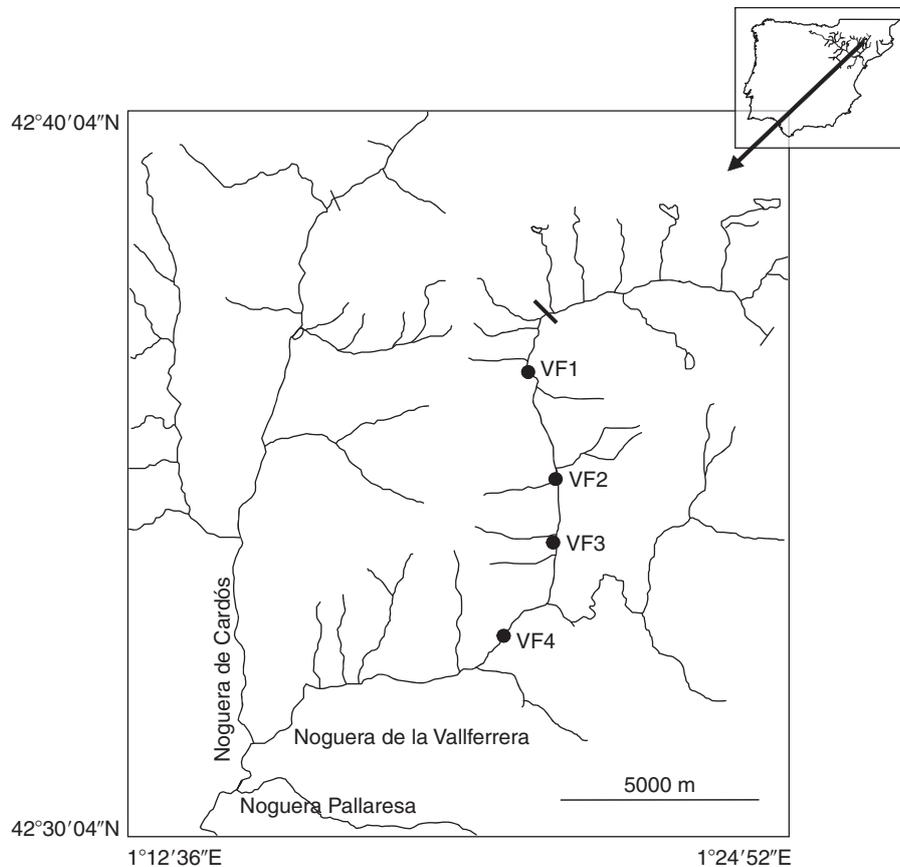


Fig. 1. Geographical locations of brown trout samples. Sample codes are provided in the text.

1993), *T3–13* (Estoup *et al.* 1998), *Ssa85*, *Ssa197* (O'Reilly *et al.* 1996), *SsHaeIII14.20* (J. L. Goodier, unpubl. data, GenBank Accession number U10050), *SsoSL417* (Slettan *et al.* 1995) and *SsoSL438* (Slettan *et al.* 1996). Details on the PCR conditions used are provided in Hansen (2002). For each locus, allele size was determined using Allele Locator software (Amersham Pharmacia Biotech, Piscataway NJ, USA).

#### *Gene diversity within and among locations*

For each collection, a Bayesian Markov Chain Monte Carlo-based approach method was implemented using STRUCTURE v2.1 software (Pritchard *et al.* 2000) to assess the extent of hatchery gene introgression by estimating individual admixture coefficients. This estimates the proportion of an individual's genome derived from native *v.* hatchery trout, respectively. Estimates were calculated incorporating data from the Bagà sample as the hatchery baseline and assuming an admixture model with two populations (hatchery and native), where hatchery individuals were forced to be non-admixed, and where allelic frequencies in wild and hatchery trout were independent. Individuals with a proportion of hatchery genome greater than 90% were considered pure hatchery fish and excluded from the subsequent analyses.

Analysis of genetic variation and population structure were investigated from the 2002 collections. Estimates of genetic variation in samples included mean unbiased expected heterozygosity ( $H_S$ ) and allelic richness ( $A$ ) using pop100 gene (<http://www.ensam.inra.fr/URLB/pop100gene/pop100gene.html>, verified May 2010) and FSTAT (Goudet 1995) software. For each collection, genotypic distributions were tested for conformance to Hardy–Weinberg expectations by using GENEPOP v3.3 (Raymond and Rousset 1995). Significance levels were adjusted by applying the sequential Bonferroni technique (Rice 1989).

Fisher exact tests for genetic differentiation per locus and between all sample pairs were computed using GENEPOP v3.3. In all cases of multiple tests, significance levels were adjusted by using the sequential Bonferroni technique. Population structure was inferred by estimating the number of populations ( $K$ ) over all present genotypes by using a Bayesian approach implemented in the STRUCTURE v2.1 software. STRUCTURE was tested with  $K = 1–4$  populations by using an admixture model with correlated allele frequencies ( $F_{ST} = 0.01$ ). The number of populations represented by the four wild collections was estimated according to Garnier *et al.* (2004). Nei's estimators of gene diversity and differentiation were used to assess genetic differentiation among samples. In addition, Weir and Cockerham estimators with 1000 permutations evaluated  $F_{ST}$  values and their significance for all population pairs. All estimates were computed by using FSTAT. The genetic relationships among pairs of samples were further assessed with Cavalli–Sforza chord distance (Cavalli–Sforza and Edwards 1967). A Mantel's test with 1000 random permutations from the NTSYSpc 2.11 software (Rohlf 1993) was used to test for isolation by distance. For that, we compared geographical distance among pairs of samples, with genetic differences estimated by pairwise  $F_{ST}$  values and Cavalli–Sforza chord distance.

#### *Family structure*

To study family relationships, all samples collected in 2002 and 2003 were pooled and individuals grouped into cohorts (from

cohort 1998 to cohort 2002). Additional older cohorts were excluded from analyses because of the reduced number of individuals. A hierarchical analysis of molecular variance (AMOVA) was used to quantify the degree of differentiation among cohorts related to geographical differences among locations, using Arlequin 2.000 software (Schneider *et al.* 2000).

Family relationships among individuals within cohorts were estimated by calculating a 'two-genes' relatedness coefficient ( $r$ ) and a 'four-genes' relatedness coefficient ( $h$ , fraternity coefficient, Wang 2002) with SPAGEDi v1.2 software (Hardy and Vekemans 2002). All individuals were pooled to determine the baseline. Following Hansen and Jensen (2005), we compared the distribution of observed pairwise relationship coefficients to that expected in a sample of simulated unrelated individuals from a population with the same allele frequencies. For each cohort, this was achieved by simulating 250 unrelated individuals from all genotypes with HYBRIDLAB v1.0 software (Nielsen *et al.* 2006). Comparisons between real and simulated pairwise coefficients were undertaken using a Mann–Whitney  $U$ -test implemented in the SPSS 15.0 package.

The randomness of relative distributions among locations was assessed for each cohort. Thus, relationship coefficients for individual pairs from the same location (intra) were compared with coefficients estimated between individuals from different locations (inter) by using a Mann–Whitney  $U$ -test. Additionally, we used the permutation program PERM 1.0 (Duchesne *et al.* 2006) to test for a correlation between population structure and family distribution. This program is designed to detect grouping factors or correlates statistically by randomly permuting specimens across groups within a collection of groups while preserving all original data as well as group sizes. We ran the program with 100 permutations and 10 iterations to estimate the  $P$ -value.

COLONY 1.2 software (Wang 2004) was used to infer family relationships among individuals. All sampled individuals were partitioned into full-sib families nested within half-sib families, without information on parental genotypes. We assumed an error rate of 2% for all loci, caused by allelic drop-outs and erroneous sizing of alleles. In this case, the random distribution of full-sib and half-sib families among locations was tested using the Fisher's exact test in SPSS 15.0.

## **Results**

### *Hatchery exclusion, gene diversity and population structure*

Estimates of introgression based on the admixture coefficient in STRUCTURE were not significant ( $q < 0.01$ ) for most collections, except for VF3 ( $q = 0.026$ ). A single individual from this VF3 showed a genome proportion from the hatchery higher than 0.90 ( $q = 0.995$ ) and was considered to be of first-generation hatchery origin and therefore excluded from further analyses. Estimates of gene diversity ranged from  $H_S = 0.634$  in VF2 to  $H_S = 0.664$  in VF3. Estimates of allelic richness were similar in all samples, with values ranging from 5.252 in VF1 to 5.605 in VF3. Comparable estimates of genetic diversity have been reported in other Mediterranean brown trout populations (Poteaux *et al.* 1999; Sanz *et al.* 2009). Significant deviations from Hardy–Weinberg equilibrium at the 5% significance level were observed in five of 32 tests. Only three tests were significant after Bonferroni correction, two at the *Str73* locus

(in VF1 and VF3,  $P < 0.001$  for both) and the third at the *SsoSL417* locus (in VF3,  $P < 0.001$ ). Of 28 gametic disequilibrium tests conducted, only one in VF1 ( $P = 0.001$ ) and a second in VF2 ( $P < 0.001$ ) remained significant after Bonferroni correction, involving different pairs of loci.

Nei's estimators of gene diversity and population differentiation indicated that only 1.8% ( $G_{ST} = 0.018$ ) of total gene diversity ( $H_T = 0.659$ ) was due to differences among locations. Low  $F_{ST}$  values were observed between most pairs of samples (range: 0.009–0.033), with only very small significant ( $P = 0.049$ ) differentiation evident between VF2 and VF4. Isolation by distance was not detected when geographical distance was compared with pairwise  $F_{ST}$  values in a Mantel's test. Similarly, STRUCTURE suggested a single genetically homogeneous group ( $K = 1$ , mean  $\ln P = -1872$ ;  $K = 2$ , mean  $\ln P = -1912$ ;  $K = 3$ , mean  $\ln P = -2175$ ) when all 2002 collections were pooled. Fisher's exact tests of genetic differentiation, however, detected significant ( $P = 0.004$ ) differences between VF1 and VF4 as well as between VF1 and VF3 ( $P = 0.007$ ) that persisted after Bonferroni correction. These minor genetic differences resulted in a matrix of Cavalli-Sforza genetic distance that reflected significant, albeit low, spatial differentiation among sampled populations (Table 1). In this case, isolation by distance was confirmed by significant correlation between genetic and geographical distance ( $r = 0.932$ ,  $P = 0.021$ ) in a Mantel's test.

#### Family relationships

Hierarchical analysis of molecular variance (AMOVA) assigned most genetic differentiation to differences among locations within cohorts ( $F_{LC} = 3.48\%$ ,  $P = 0.013$ ), with no significant differences detected among cohorts ( $F_{CT} = 0\%$ ,  $P = 0.435$ ).

Relatedness coefficients ( $r$ , Wang 2002) among individuals within cohorts were significantly ( $P < 0.001$ , in all cases) lower than those expected among simulated unrelated individuals in all cohorts, except the oldest cohort ( $P = 0.053$ ) (1998). In contrast, fraternity coefficients ( $h$ ) were significantly (in all cases  $P < 0.001$ ) higher between real than between simulated individuals in all cohorts, except for the oldest cohort ( $P = 0.088$ ) (Fig. 2).

Results of a Mann-Whitney  $U$ -test and the permutation program PERM to test correlations between family and population structure were similar and showed few significant differences between intra- and inter-location pairwise relatedness ( $r$ ) and fraternity ( $h$ ) coefficients. In all significant cases, except for

VF3, differences reflected higher average relatedness coefficients between individuals within locations compared with between individuals from different locations (Table 2).

Family reconstruction with COLONY 1.2 was consistent among replicates. The oldest cohorts (1998 and 1999) showed a similar family structure, with 14–15 full-sib families nested within nine half-sib families, whereas younger cohorts (2000, 2001 and 2002) showed a family structure composed of 27 or 28 full-sib families nested within 12–14 half-sib families. Family sizes were small, ranging from one to three individuals, with larger families observed in the youngest cohorts (Fig. 3). Analysis of variance failed to detect significant differences among cohorts, however, when the average number of full-sibs ( $K_{FS}$ ) and half-sibs ( $K_{HS}$ ) were compared ( $P = 0.344$ ,  $P = 0.127$ , respectively). Given that sample sizes of the oldest cohorts were lower, this may imply that some family groups were lost rather than a reduction in all family sizes over time. A Fisher's exact test indicated that families of older age classes were distributed randomly among the sampled locations (in all cases  $P > 0.05$ ). Nevertheless, this test also supported a non-random geographical distribution of full- and half-sib families in the 2001 cohort ( $P = 0.047$ ,  $P = 0.042$ , respectively) and for the half-sib families in the 2002 cohort ( $P = 0.001$ ).

## Discussion

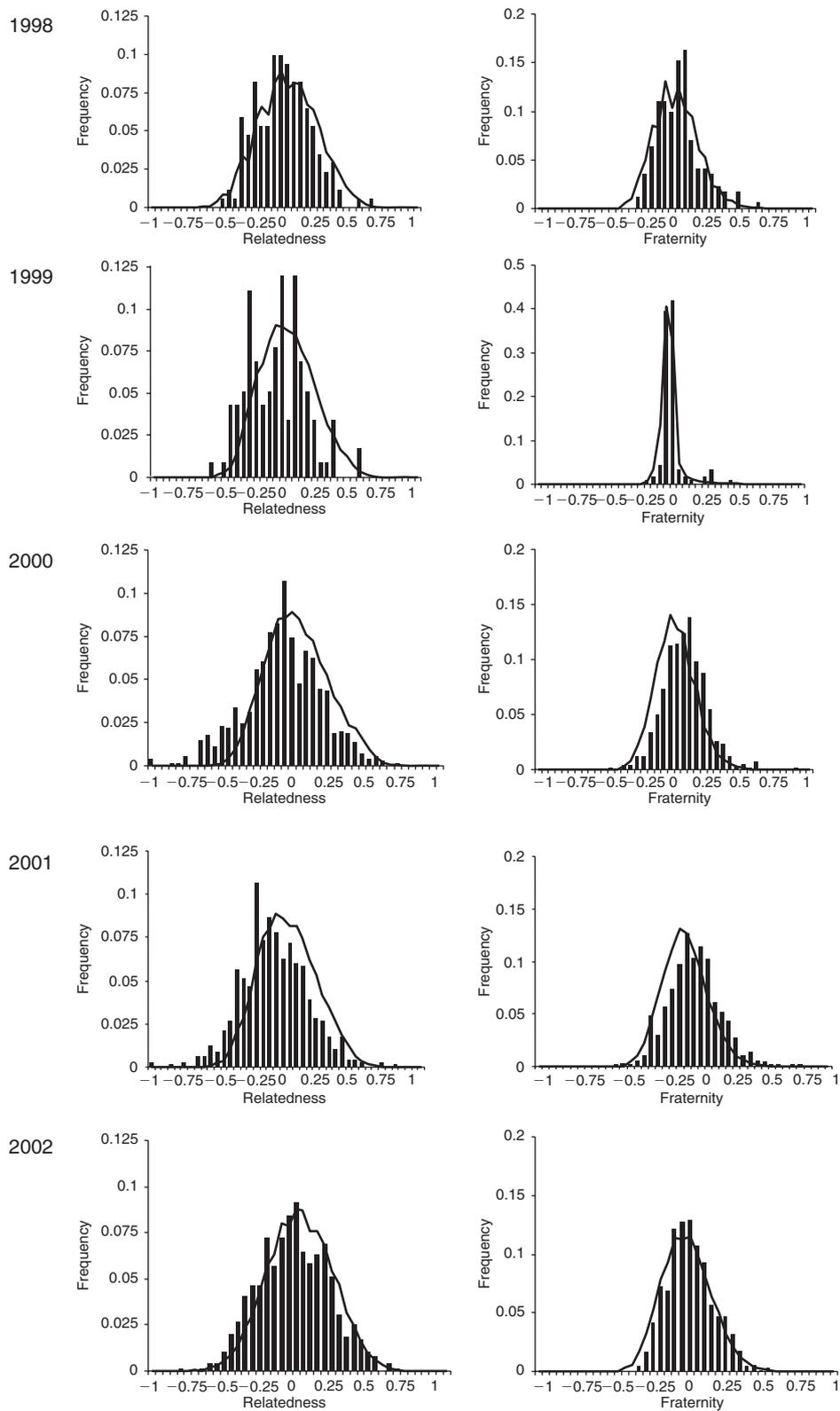
### Limited dispersal of brown trout

Analyses of population structure indicated a low degree of spatial genetic differentiation. Nevertheless, weak isolation by distance was evident among populations. This suggests that individual dispersal distances of brown trout in the Vallferrera streams are limited. In salmonids, patterns of isolation by distance are common at large geographical scales (Sanz *et al.* 2000) and have been also observed at micro-geographical scales (Carlsson *et al.* 1999). Thus, genetically differentiated populations can coexist even without any apparent physical barriers to dispersal. For example, sympatric populations of brown trout have been found in Lough Melvin, Ireland (Ferguson and Taggart 1991). Several studies have indicated that in these cases, specific fish behaviours, including preferences for distinctive spawning habitats or different life-history forms, can produce the reproductive isolation necessary to maintain discrete breeding units (Ferguson and Taggart 1991; Olsén *et al.* 1996; Thériault *et al.* 2007). Thus, some correlations between different behavioural traits and genetic structure can be expected (Carlsson *et al.* 1999). In our study area, lack of waterfalls or other physical barriers to dispersal by brown trout in the target stream suggests that other mechanisms are likely to be responsible for the limited structure observed. Although the distributions of related individuals along the Vallferrera stream were random in the oldest cohorts, our data also showed that related individuals in the youngest cohorts were found in closer proximity than expected by chance. A wide range of fishes has been reported to show kin-biased behaviours, including reduced aggressiveness and increased food-sharing among relatives. In brown trout, kin-biased behaviour has been reported in experimental stocks of young brown trout (Olsén *et al.* 1996), and in Atlantic populations under natural conditions at any age class (Carlsson *et al.* 2004).

**Table 1. Genetic distance and multilocus  $F_{ST}$  estimates between pairs of samples**

Above diagonal: Cavalli-Sforza chord distance. Below diagonal:  $F_{ST}$  estimates with significant values over 1000 permutations, \* $P < 0.05$

	VF1	VF2	VF3	VF4
VF1	–	0.0290	0.0332	0.0393
VF2	0.0225	–	0.0258	0.0355
VF3	0.0094	0.0224	–	0.0299
VF4	0.0141	0.0333*	0.0110	–



**Fig. 2.** Frequency distributions of pairwise relatedness and fraternity coefficients between individuals (Wang 2002). The curves denote frequency distributions of pairwise values between 250 simulated unrelated individuals, whereas bars denote the frequencies of observed relationship values.

**Table 2. Average pairwise relatedness and fraternity coefficients (Wang 2002) between individual pairs from the same location (intra) and between individual pairs from different locations (inter) estimated for each cohort and location**

*n*, sample size. Correlation between population and family distribution was estimated with the Mann–Whitney *U*-test/permutation program PERM (Duchesne *et al.* 2006); n.s., not significant; \**P* < 0.05; \*\*\**P* < 0.001

Cohort locality ( <i>n</i> )	Relatedness coefficient			Fraternity coefficient		
	Intra	Inter	Correlation	Intra	Inter	Correlation
1998	0.0391	−0.0182	n.s./n.s.	0.0187	0.0341	n.s./n.s.
VF1 (5)	0.054	−0.025	n.s./n.s.	0.130	0.052	n.s./n.s.
VF2 (4)	−0.061	−0.049	n.s./n.s.	0.058	0.002	n.s./n.s.
VF3 (8)	0.059	0.004	n.s./n.s.	−0.033	0.038	n.s./n.s.
VF4 (2)	−0.068	−0.007	n.s./n.s.	0.109	0.042	n.s./n.s.
1999	−0.0034	−0.0968	n.s./n.s.	0.0546	0.0802	n.s./n.s.
VF1 (6)	−0.056	−0.070	n.s./n.s.	0.062	0.086	n.s./n.s.
VF2 (5)	0.101	−0.127	n.s./*	0.030	0.077	n.s./n.s.
VF3 (3)	−0.089	−0.077	n.s./n.s.	0.099	0.093	n.s./n.s.
VF4 (1)	–	–	–	–	–	–
2000	−0.0848	−0.0919	n.s./n.s.	0.0886	0.0813	n.s./n.s.
VF1 (3)	−0.100	−0.110	n.s./n.s.	0.262	0.109	*/*
VF2 (15)	0.021	−0.087	***/*	0.074	0.088	n.s./n.s.
VF3 (15)	−0.197	−0.109	***/*n.s.	0.105	0.090	n.s./n.s.
VF4 (6)	−0.035	−0.059	n.s./n.s.	0.038	0.039	n.s./n.s.
2001	−0.1075	−0.0841	n.s./n.s.	0.0993	0.0672	n.s./*
VF1 (2)	−0.150	−0.086	n.s./n.s.	0.043	0.032	n.s./n.s.
VF2 (12)	0.065	−0.081	***/**	0.194	0.082	***/**
VF3 (19)	−0.179	−0.094	***/*n.s.	0.066	0.073	n.s./n.s.
VF4 (4)	0.025	−0.063	n.s./n.s.	0.005	0.035	n.s./n.s.
2002	−0.0306	−0.0506	n.s./n.s.	0.0351	0.0322	n.s./n.s.
VF1 (0)	–	–	–	–	–	–
VF2 (18)	−0.012	−0.051	n.s./n.s.	0.006	0.032	n.s./n.s.
VF3 (19)	−0.047	−0.051	n.s./n.s.	0.061	0.032	n.s./n.s.
VF4 (0)	–	–	–	–	–	–

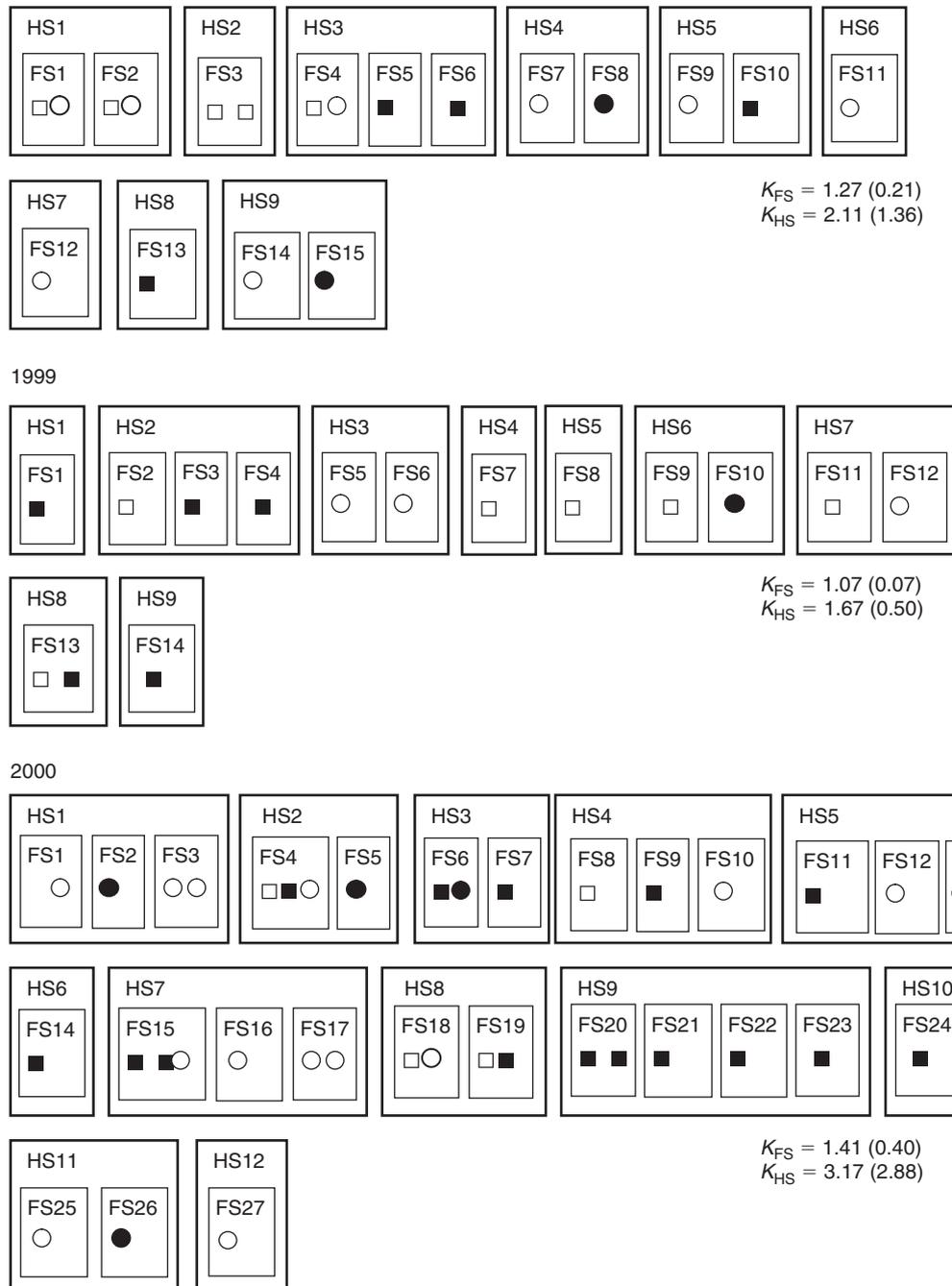
The association observed here is probably too weak to be a consequence of kin-biased behaviour; otherwise, we would expect kin-biased association also in adult individuals. There will obviously be a very high association of kin among eggs and fry in spawning redds. After emergence from redds, this association is expected to decay over time as juveniles disperse. This is the null hypothesis against which kin-biased behavioural association (i.e. hypotheses invoking kin selection) should be tested (Carlsson *et al.* 2004). Limited dispersal from spawning redds, leading to a high relatedness among neighbouring juvenile brown trout, has been observed in Atlantic brown trout populations (Hansen *et al.* 1997; Carlsson *et al.* 2004). Our data confirm a similar phenomenon in Mediterranean brown trout, where the observation of kin association in younger, but not older, trout conforms with the patterns expected under a scenario of continuous dispersal from spawning redds.

#### *Environmental conditions shaping brown trout dispersal patterns*

The spatial distribution of individual salmonids in wild streams, with respect to relatedness, is likely to be influenced by individual age. On hatching and until 1 year or more of age, little up- or downstream movement of fry is expected in brown trout (Elliott 1994). Older fish, however, show strong density-dependent mortality and these result in high individual competition and a reduction in the numbers of surviving individuals from each

family. Furthermore, fish tend to move to new and perhaps deeper parts of the river (Elliott 1994; Gowan *et al.* 1994). The relatively low numbers of individuals detected in the oldest cohorts (1998–1999) and the random distribution of related individuals in these cohorts reflect this demographic process.

Studies using mark–recapture and telemetry methods have demonstrated that most stream-dwelling salmonids, including brown trout, are relatively sedentary, with the limited movement within locations associated with competition for profitable foraging sites (Knouft and Spotila 2002; Höjesjö *et al.* 2007; Lobón-Cerviá 2009). For instance, Knouft and Spotila (2002) concluded that a high percentage of resident stream brown trout remained in the same 800 m of the stream throughout the year. Within-location movement of less than 100 m was reported by Höjesjö *et al.* (2007) for adult resident brown trout in a Swedish river. No published studies are available for movement patterns of brown trout in the Mediterranean region. Data from mark–recapture methods in the study region (A. Almodóvar, unpubl. results), however, show that 79% of recaptured fish were caught within 600 m of sites where they were originally stocked and showed a maximum displacement of 600 m upstream and 6200 m downstream from this point. Mean rate of trout movement coincides with sedentary behaviour observed in other rivers (Cresswell 1981; Hesthagen and Johnsen 1989), where the movement direction was predominantly downstream (Helfrich and Kendall 1982).



**Fig. 3.** Distribution of individuals into full-sib families (FS) nested with half-sib families (HS), by using the software COLONY 1.2 (Wang 2004). □, ■, ○ and ● symbols indicate individuals from VF1, VF2, VF3 and VF4 locations, respectively. For each cohort,  $K_{FS}$ ,  $K_{HS}$ , the number of full-sibs and the number of half-sibs, with its variance in the parentheses, are indicated.

In the current study, the extensive and largely random distribution of sibling individuals in different locations of the stream suggests that adult brown trout often move several kilometres from their natal site. These results contrast with those found in a river in Sweden, where associations were detected among related older trout (Carlsson and Carlsson 2002). Greater movement by Mediterranean brown trout than some Atlantic stocks may be due to extreme habitat conditions that promote

high competition among adult individuals, forcing them to disperse to find profitable feeding areas. Ecological studies of the Vallferrera stream detected a very low primary productivity in this river, and indicated that food was a limiting resource for brown trout (Rocaspana *et al.* 2006). Such environmental factors may also be responsible for small family sizes and the relatively low number of individuals detected in our Mediterranean populations, compared with equivalent populations in Denmark

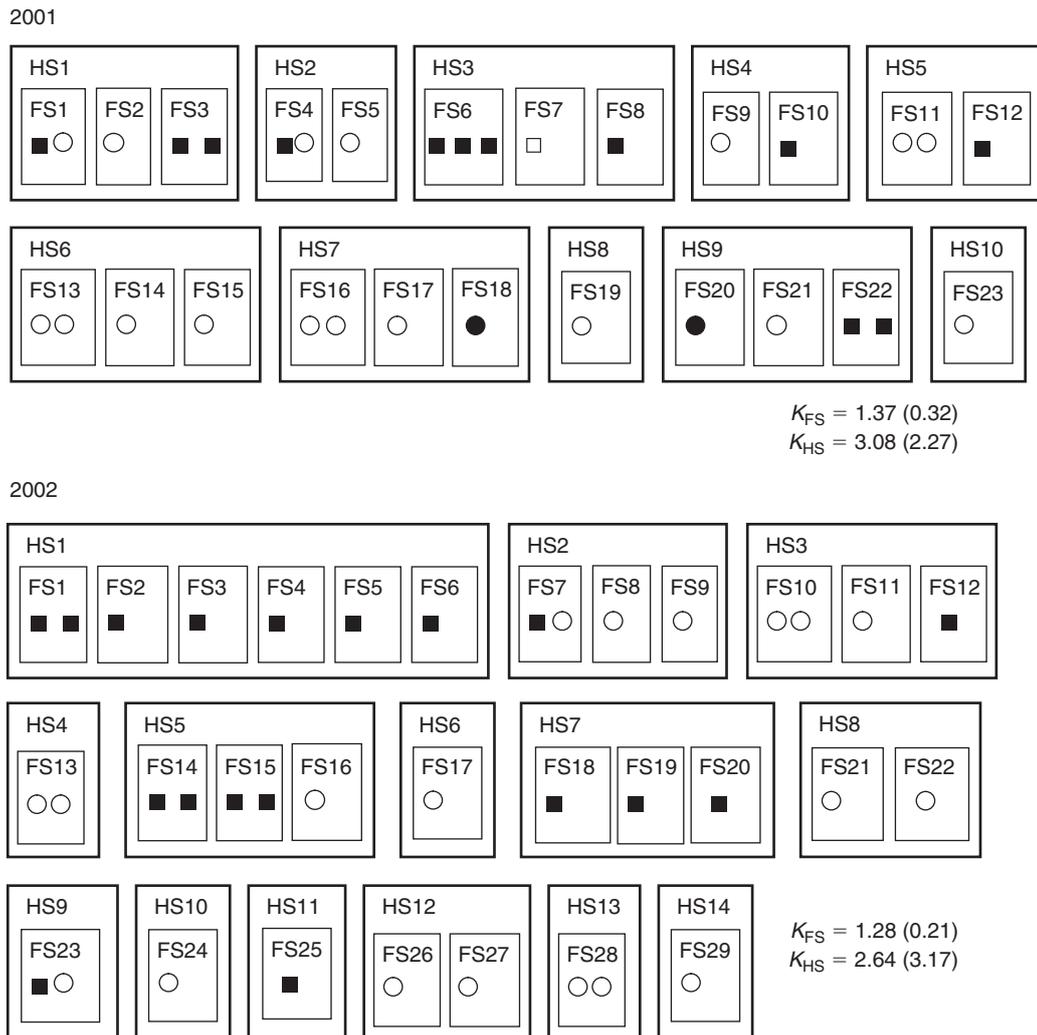


Fig. 3. (Continued)

(Hansen and Jensen 2005). Extensive movement by individuals throughout the river may therefore produce low levels of genetic differentiation among sites. At the same time, more sedentary behaviour by younger individuals may explain the limited pattern of isolation by distance observed here.

These observations also imply that if a population is sampled by catching fish only from a short river stretch and if this sample contains a large proportion of fry, then this involves a considerable risk of sampling individuals representing only one or a few families. Consequently, estimates of allele frequencies of the population may be biased, leading to exaggerated or even erroneous conclusions regarding genetic differentiation. Thus, the recommendation is to sample adult individuals or, alternatively, to sample juveniles over longer river stretches (Hansen *et al.* 1997).

#### Mating system inferred from individual relationships

Pairwise relatedness ( $r$ ) and fraternity ( $h$ ) coefficients provide valuable information on populations' mating systems. In an outbreeding population,  $r$  and  $h$  are expected to be 0.5 and 0,

respectively, for parents and offspring, 0.5 and 0.25, respectively, for full-sibs and 0.125 and 0, respectively, for half-sibs. In our study, pairwise relationship coefficients estimated with SPAGEDi software indicated an excess of low relatedness values ( $r$ ) among real individuals compared with values from unrelated simulated genotypes. Relatedness of offspring strongly depends on the inbreeding relationship of their parents. Low values of relationship are expected when parents are not related and higher values are expected under a high inbreeding scenario. Thus, low relatedness values in cohorts from the Vallferrera stream indicate that parents are unrelated and may reflect the presence in the samples of adult immigrants from different populations from non-studied areas.

Alternatively, results of pairwise fraternity coefficients ( $h$ ) were significantly higher between real rather than simulated genotypes in all but the oldest cohort, suggesting the presence of some full-sib individuals, and this was supported by results obtained using COLONY. Additionally, the presence of several half-sib families detected within cohorts by using COLONY may have resulted from multiple paternities, as described in other brown

trout populations where more than a single male had fertilised the eggs of individual females (García-Vázquez *et al.* 2001).

## Conclusions

In the Vallferrera stream, kin-biased aggregation of juvenile brown trout was observed. This result implies that analysis of genetic population structure inferred from highly polymorphic markers (microsatellites), at least in part, can be influenced by family relationships. Family relationships also provided evidence for greater movement by adult trout that occurs over distances of at least a few kilometres. This is likely to contribute to the observed low spatial genetic differentiation. Dispersal of adult Mediterranean trout contrasts with observations in some rivers from northern Europe for the same species and could be related to lower primary productivity in southern basins. Finally, our results show that combining genetic structure and family relationship data can be a useful approach for assessing movement patterns of brown trout and other species in streams and could complement information obtained using mark–recapture and radio-telemetry methods.

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