

Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations

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Abstract

We examined the long-term temporal (1910s to 1990s) genetic variation at eight microsatellite DNA loci in brown trout (*Salmo trutta* L) collected from five anadromous populations in Denmark to assess the long-term stability of genetic composition and to estimate effective population sizes (N_e). Contemporary and historical samples consisted of tissue and archived scales, respectively. Pairwise θ_{ST} estimates, a hierarchical analysis of molecular variance (AMOVA) and multidimensional scaling analysis of pairwise genetic distances between samples revealed much closer genetic relationships among temporal samples from the same populations than among samples from different populations. Estimates of N_e using a likelihood-based implementation of the temporal method, revealed $N_e \geq 500$ in two of three populations for which we have historical data. A third population in a small (3 km) river showed $N_e \geq 300$. Assuming a stepping-stone model of gene flow we considered the relative roles of gene flow, random genetic drift and selection to assess the possibilities for local adaptation. The requirements for local adaptation were fulfilled, but only adaptations resulting from strong selection were expected to occur at the level of individual populations. Adaptations resulting from weak selection were more likely to occur on a regional basis, i.e. encompassing several populations. N_e appears to have declined recently in at least one of the studied populations, and the documented recent declines of many other anadromous brown trout populations may affect the persistence of local adaptation.

Keywords: effective population size, gene flow, historical samples, local adaptation, microsatellite DNA, *Salmo trutta*

Received 12 April 2002; revision received 13 August 2002; accepted 13 August 2002

Introduction

Developments in statistics and molecular genetic analysis in population genetics have improved the possibilities for analysing genetic differentiation among populations (Luikart & England 1999). A deeper understanding of the biological significance of the genetic structure of populations, beyond the mere demonstration of genetic

differentiation, requires, however, information on the relative roles of mutation, random genetic drift, gene flow and selection (e.g. Nagylaki & Lucier 1980; Slatkin 1985a; Adkison 1995) and on the relationships between genetic differentiation and geological and geographical factors (e.g. Castric *et al.* 2001).

Salmonid fish, including brown trout (*Salmo trutta*) are characterized by strong genetic differentiation among populations inhabiting different rivers (Altukhov *et al.* 2000). While in some cases (e.g. resident populations) the genetic differentiation is the result of complete reproductive isolation (e.g. Ryman 1983; Bouza *et al.* 1999), populations potentially linked by gene flow, such as anadromous salmonids, have also been found to be genetically differentiated

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(reviewed in Altukhov *et al.* 2000). Genetic differentiation among anadromous populations of salmonid fish is facilitated by restricted gene flow as a result of homing behaviour, i.e. the propensity to return to spawn in natal rivers (Stabell 1984).

The observed genetic differentiation in salmonid populations, based on studies of presumably selectively neutral genetic markers, raises the question whether this is merely a reflection of limited gene flow and genetic drift, or if populations are likely to be adapted to local conditions (Elo 1993; Adkison 1995). A direct approach for addressing this question is to demonstrate local adaptations in the wild. Several examples are available of differences in traits among salmonid populations that may be ascribed to local adaptation (e.g. Taylor 1991; Palm & Ryman 1999). In many cases, however, a definitive proof of local adaptation is lacking, as there are few studies demonstrating that differences in traits among populations are maintained by different selection pressures in different environments. A notable exception is a recent study by Koskinen *et al.* (2002a). They used a combination of presumably neutral microsatellite markers and analysis of quantitative traits and showed that differences in life history traits among introduced grayling (*Thymallus thymallus*) populations, derived from a common source population, must be the result of strong selection.

Overall, however, demonstration of selection and adaptation in nature remains a complicated task (Endler 1986) and primarily provides information on the specific traits under study. A more general evaluation of the possibility for local adaptation may be obtained by estimating population genetic parameters from neutral genetic markers and then use these parameters to predict the scale and extent to which local adaptation is likely to occur (e.g. Elo 1993; Adkison 1995). Knowledge of three related factors is particularly central for making such an evaluation, i.e. information on the temporal stability of the genetic composition of populations, estimates of gene flow and estimates of effective population size, N_e , a key parameter in population genetics, which determines the extent of genetic drift and inbreeding. N_e is nearly always considerably lower than the census population size because of differences in the number of males and females contributing to reproduction, variance in reproductive success and family size, and variance of N_e over several generations (Frankham 1995). Consequently, even if census population size estimates are available it may be difficult to assess N_e because of the number and complexity of factors affecting it.

DNA extracted from archived fish scales and otoliths has proven highly useful for studying genetic differentiation in time and space (Miller & Kapuscinski 1997; Nielsen *et al.* 1997; 1999a; 1999b; Tessier & Bernatchez 1999; Adcock *et al.* 2000; Ruzzante *et al.* 2001a; Heath *et al.* 2002; Koskinen *et al.* 2002b). Archived fish scales have been used to show

that the genetic structure of Atlantic salmon populations has remained stable over time spans of up to 80 years (Nielsen *et al.* 1999b; Tessier & Bernatchez 1999). Analysis of historical samples may also facilitate estimation of effective population size, N_e . N_e can be estimated from demographic data based on direct observations (e.g. Kelly 2001), but more precise estimates may be obtained using genetic markers (reviewed by Beaumont 2001). Among the latter methods, the so-called temporal method has proven particularly useful (e.g. Waples 1989; Jorde & Ryman 1995; Laikre *et al.* 1998). The basic principle is to sample a population at two or more points in time separated by a specified number of generations. Based on the changes in allele frequencies that have occurred during the interval it is then possible to estimate the variance effective population size. The precision of the estimates improves considerably with the number of generations between temporal collections. Historical samples are particularly useful for this purpose, and this approach has been used by Miller & Kapuscinski (1997) and Heath *et al.* (2002) for estimating N_e in northern pike (*Esox lucius*) and steelhead trout (*Oncorhynchus mykiss*), respectively.

In the present study we examined the variability at eight microsatellite loci in anadromous brown trout sampled during the 1910s, 1950s (archived scales) and 1990s (contemporary tissue samples). We first assessed whether the genetic composition remained stable over time. Second, we estimated N_e using a likelihood-based implementation of the temporal method. Finally, this information was used to examine the possibility and extent of local adaptations in anadromous brown trout populations.

Materials and methods

Populations studied

We analysed brown trout from five Danish rivers, the Karup (abbreviation: KAR), Kovads (KOV), Odder (ODR), Vejle (VEJ) and Kolding (KOL) Rivers (Fig. 1). The river KOV is a 3-km tributary to the larger (approx. 50 km) Lindborg River system. The four other rivers range in size from approximately 15 km (ODR) to approximately 60 km (KAR). The historical and contemporary spawning census population sizes are not known accurately. In the 1950s the river KOV had an annual spawning run of anadromous trout of at least 100 (Larsen 1993), whereas the runs in the other rivers have probably exceeded 1000. All the rivers contain resident trout, including precocious males, which may significantly increase the total spawning population (Martinez *et al.* 2000). The rivers VEJ, KOL and KAR have previously been stocked with non-native domesticated trout, whereas KOV and ODR have not. Despite intensive stocking with domesticated trout in the past, their genetic contribution in the KAR population has

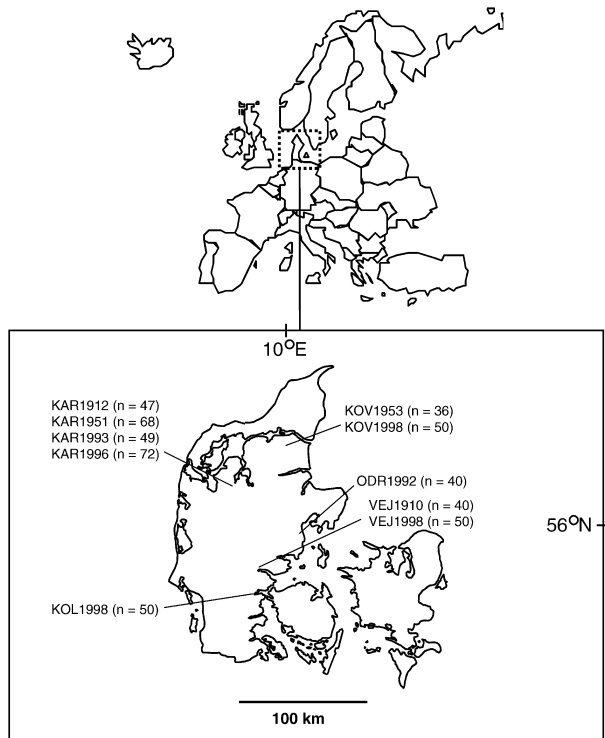


Fig. 1 Map showing the sampling localities for brown trout in Denmark along with information on sample sizes from different years.

been shown to be very small (Hansen 2002). Currently KAR, VEJ and KOL are subject to supportive breeding, i.e. stocking with offspring of indigenous wild-caught trout.

Contemporary samples were collected from all five rivers by electrofishing. Archived scale samples were available from three of these five populations. Sample sizes and year of sampling are listed on Fig. 1. For the historical samples, year of sampling denotes the median year of sampling as not all individuals were collected in the same year. All samples (both historical and contemporary) from KAR, VEJ and KOL consisted of anadromous spawners, whereas all samples from KOV and ODR consisted of juveniles (primarily age classes 1+ and 2+).

Microsatellite analysis

DNA was extracted from scale samples using phenol-chloroform extraction and micro-concentrators (see Nielsen *et al.* 1999a). Extraction of DNA from contemporary samples used either phenol-chloroform extraction (Taggart *et al.* 1992) or proteinase K/chelex extraction (Estoup *et al.* 1996). One tetranucleotide and seven dinucleotide microsatellite loci were analysed: Str 15, Str 60, Str 73 (Estoup *et al.* 1993), Ssa 85, Ssa 197 (tetranucleotide microsatellite) (O'Reilly *et al.* 1996), SsoSL 417 (Slettan *et al.* 1995), SsoSL

438 (Slettan *et al.* 1996) and T3-13 (Estoup *et al.* 1998). Details of polymerase chain reaction conditions are given in Hansen (2002). Work with historical samples was kept separated from work with contemporary samples to avoid contamination of historical DNA. The reproducibility of results obtained from historical samples was confirmed by performing a second round of polymerase chain reaction amplification and scoring for subsamples of individuals (between 10 and 40% reanalysed for each locus). The microsatellites were analysed on a Pharmacia ALFlex-press automated sequencer, according to the manufacturer's recommendations.

Statistical treatment

Deviations from Hardy-Weinberg equilibrium were tested both by 'conventional' exact tests (Guo & Thompson 1992) and by tests assuming an alternative hypothesis of heterozygote deficiency (Rousset & Raymond 1995), using GENEPOP 3.1c (Raymond & Rousset 1995). Genetic differentiation among all populations and between pairs of populations was estimated with pairwise θ_{ST} estimates (Weir & Cockerham 1984) and 95% confidence intervals were determined by bootstrapping over loci. The program FSTAT 2.9.1 (Goudet 1995) was used for these analyses. Furthermore, to quantify the degree of differentiation among temporal samples from the same populations relative to differentiation among geographically different populations we performed a hierarchical analysis of molecular variance (AMOVA), using the software ARLEQUIN 2.000 (Schneider *et al.* 2000). The first level of the hierarchy consisted of the three populations VEJ, KOV and KAR, and the second level consisted of the temporal samples from each of these populations.

Genetic differentiation between pairs of populations was also tested by permuting genotypes between samples using a test by Goudet *et al.* (1996). The genetic relationships among samples were further analysed by multi-dimensional scaling analysis (MDSA) of a matrix of Nei's (1978) unbiased genetic distance between populations. The program VISTA 5.6.3 (Young 1996) was used for MDSA.

We estimated gene flow from the relationship F_{ST} (or θ_{ST}) = $1/(1 + 4N_e m)$ (Wright 1931), where N_e denotes effective population size and m denotes migration rate. We also estimated $N_e m$ using the private allele method by Slatkin (1985b) as implemented in GENEPOP 3.1c (Raymond & Rousset 1995). Both methods depend on a number of assumptions that are unlikely to be fulfilled in real populations (Whitlock & McCauley 1999), and we treat this issue further in the discussion.

To estimate effective population size per generation, N_e , we applied a new implementation of the temporal method (Berthier *et al.* 2002), which estimates effective population size based on two samples from the same population

separated by a given number of generations. This likelihood-based method relies on coalescence theory, which provides a good approximation to a case with overlapping generations as in brown trout, and uses Markov chain Monte Carlo (MCMC) simulations for generating a posterior distribution of N_e values. It incorporates Bayesian prior information by making the user specify a maximum N_e ($N_{e\max}$). We based our estimates on 100 000 MCMC replicates and report the results as the median, 5% and 95% quantiles of the posterior distributions of N_e . In most cases we assumed an $N_{e\max}$ of 1000, but also tried other values to assess the influence of the prior information on the posterior distribution of N_e . The method requires knowledge of the number of generations between consecutive samples. The average generation length of anadromous trout in a typical Danish river was estimated to be 3.8 years using data from Frier (1994). However, resident trout, including precocious males, were not taken into account in the estimate, and because precocious males may spawn at 2 years of age, we assumed an average generation length of 3.5 years. Should the true average generation length be shorter than 3.5 years, more generations would have elapsed between the temporal samples in our study than accounted for in the N_e calculation, and N_e would thus be underestimated. Conversely, if the generation length exceeds 3.5 years N_e will be overestimated. As a result of the presence of precocious males (see above) we find it more likely that N_e has been underestimated.

For assessing the potential for local adaptation we focused on the stepping-stone model of genetic population structure (Kimura & Weiss 1964), where gene flow primarily occurs between neighbouring populations. This appears to be the most realistic model accounting for patterns of gene flow in anadromous salmonids (Adkison 1995). This includes anadromous brown trout, for which several recent studies, some dealing with trout populations elsewhere (e.g. Morán *et al.* 1995; Bouza *et al.* 1999) and some dealing with the same Danish trout populations as in the present study (Hansen & Mensberg 1998; Ruzzante *et al.* 2001b), have demonstrated isolation by distance. We used the approach of Adkison (1995), based on formulas in Nagylaki & Lucier (1980). Briefly, two conditions must be fulfilled before local adaptation can occur. First, $\beta \gg 1$, where β is the ratio of the strength of selection to the strength of random genetic drift and is defined as $\beta = 2\sqrt{2msN_e}$. Here m denotes migration rate, s denotes the strength of local selection and N_e denotes effective population size. Second, $k \gg 1$, where k represents the ratio of the geographical scale at which selection is the same relative to the length of the cline determined by gene flow and selection. k is defined as $k = j/l_c$ where j denotes the spatial scale of selection (i.e. the number of populations over which local selection is the same; if $j = 1$, then local selection occurs at the level of individual populations,

whereas if $j > 1$ then local selection is the same over several populations) and l_c denotes Slatkin's (1973) 'characteristic length' of a cline, basically the length of a cline determined by gene flow and selection, where $l_c = \sigma/\sqrt{s}$ [σ is the standard deviation of dispersal distances (here measured in numbers of populations or 'stepping-stones')]. If $\beta < 1$, then random differentiation is predicted for the specific locus. If $\beta \gg 1$ and $k < 1$, then genetic homogeneity is predicted (i.e. lack of strong differentiation, though weak differentiation may occur), and, finally, if both $\beta \gg 1$ and $k \gg 1$, then local adaptation is predicted.

We evaluated the magnitudes of β and k using the estimates of effective population size and gene flow obtained in this study, and then defined different scenarios using different values of j and s and assuming two different patterns of gene flow, i.e. a strict stepping-stone model where gene flow occurs only between neighbouring populations, and a relaxed model, where dispersal also occurs over longer distances. To represent the latter we assumed that 60% of gene flow occurred between neighbouring populations and the remaining part occurred between populations separated by two (30%) and three (10%) population units or 'stepping-stones'.

Results

Observed and expected heterozygosity values and numbers of alleles per locus in individual samples and in total are listed in Table 1. Four deviations from Hardy-Weinberg equilibrium were observed in a total of 80 tests (see Table 1). The tests for deviations from Hardy-Weinberg equilibrium, assuming an alternative hypothesis of heterozygote deficiency, yielded a significant outcome in the same four cases. Three of the four significant deviations were observed in the two oldest samples, VEJ1910 and KAR1912, and were thus associated with heterozygote deficiencies. These significant results could reflect technical artefacts such as allelic drop-outs as a result of strongly degraded DNA. However, given the observed temporal stability of genetic composition of populations (see below) we assume that such factors have not seriously influenced the results.

Exact tests for genetic differentiation revealed significant differentiation in all pairwise comparisons, except between the samples from KAR from 1912 and 1951 (KAR1912-KAR1951) and between those from VEJ from 1910 and the contemporary sample from the neighbouring KOL (VEJ1910-KOL1998; see Table 2 below diagonal). Quantification of genetic differentiation by θ_{ST} (Table 2 above diagonal), however, showed that differentiation was very small between temporal samples from the same populations (θ_{ST} from 0 to 0.016; average 0.008), whereas differentiation between samples from different populations ranged from 0.010 to 0.071 (average 0.037). This pattern was also evident

Table 1 Summary of observed number of alleles per locus (per population and in total), allele size ranges for the loci, *P* values of exact tests for deviations from expected Hardy–Weinberg proportions (H–W test; Guo & Thompson 1992), expected (H_E) and observed heterozygosity (H_O) and sample sizes (*n*) of the studied populations. Tablewide significance levels were applied, using the sequential Bonferroni technique (Rice 1989) (initial *k* = 80)

Locus		VEJ 1910	VEJ 1998	KOV 1953	KOV 1998	KAR 1912	KAR 1951	KAR 1993	KAR 1996	KOL 1998	ODR 1992
Str 15	No. alleles	6	6	4	4	6	6	5	5	4	4
Total no.	H–W. test	0.000***	0.816	0.399	0.982	0.000***	0.554	0.741	0.191	0.211	0.239
alleles: 7	H_E	0.657	0.699	0.629	0.604	0.734	0.747	0.710	0.737	0.635	0.591
Size range:	H_O	0.324	0.760	0.556	0.620	0.535	0.662	0.694	0.681	0.580	0.700
212–230 bp	<i>n</i>	37	50	36	50	44	68	49	72	50	40
Str 60	No. alleles	2	4	3	2	3	5	3	3	2	2
Total no.	H–W. test	0.692	0.657	0.699	0.751	0.123	0.655	0.915	0.427	0.709	0.107
alleles: 5	H_E	0.399	0.432	0.509	0.447	0.435	0.454	0.541	0.508	0.368	0.309
Size range:	H_O	0.436	0.500	0.500	0.420	0.426	0.463	0.510	0.542	0.400	0.225
95–105 bp	<i>n</i>	39	50	36	50	47	67	49	72	50	40
Str73	No. alleles	3	4	5	4	5	4	3	4	4	4
Total no.	H–W. test	0.494	0.407	0.860	0.834	0.187	0.033	0.942	0.088	0.287	0.342
alleles: 5	H_E	0.581	0.607	0.644	0.717	0.499	0.518	0.505	0.567	0.579	0.619
Size range:	H_O	0.590	0.600	0.676	0.740	0.457	0.441	0.510	0.556	0.600	0.500
141–151 bp	<i>n</i>	39	50	34	50	46	68	49	72	50	40
SsoSL 417	No. alleles	10	9	9	7	10	7	8	7	10	8
Total no.	H–W. test	0.000***	0.426	0.265	0.678	0.004	0.014	0.698	0.644	0.204	0.100
alleles: 13	H_E	0.852	0.833	0.778	0.700	0.815	0.813	0.760	0.789	0.825	0.809
Size range:	H_O	0.459	0.720	0.750	0.700	0.705	0.632	0.755	0.833	0.820	0.650
167–215 bp	<i>n</i>	37	50	36	50	44	68	49	72	50	40
SsoSL 438	No. alleles	5	7	4	4	4	5	5	4	5	5
Total no.	H–W. test	0.560	0.077	0.029	0.128	0.138	0.368	0.001*	0.184	0.061	0.653
alleles: 7	H_E	0.674	0.745	0.561	0.561	0.567	0.591	0.594	0.593	0.703	0.660
Size range:	H_O	0.744	0.640	0.765	0.560	0.617	0.603	0.735	0.597	0.600	0.725
103–113 bp	<i>n</i>	39	50	34	50	47	68	49	72	50	40
Ssa 85	No. alleles	7	7	6	6	7	6	6	6	6	5
Total no.	H–W. test	0.586	0.799	0.818	0.080	0.084	0.559	0.544	0.108	0.777	0.025
alleles: 8	H_E	0.771	0.721	0.671	0.726	0.604	0.671	0.748	0.708	0.703	0.488
Size range:	H_O	0.816	0.700	0.629	0.680	0.565	0.735	0.796	0.611	0.680	0.425
104–120 bp	<i>n</i>	38	50	35	50	46	68	49	72	50	40
Ssa 197	No. alleles	10	11	10	10	10	9	10	9	8	8
Total no.	H–W. test	0.146	0.288	0.003	0.028	0.004	0.003	0.037	0.342	0.021	0.701
alleles: 14	H_E	0.816	0.798	0.716	0.804	0.823	0.810	0.746	0.786	0.788	0.830
Size range:	H_O	0.744	0.880	0.563	0.880	0.809	0.824	0.776	0.861	0.740	0.825
118–162 bp	<i>n</i>	39	50	32	50	47	68	49	72	50	40
T3–13	No. alleles	21	16	13	16	19	19	15	16	15	12
Total no.	H–W. test	0.324	0.077	0.865	0.845	0.801	0.250	0.314	0.129	0.926	0.806
alleles: 26	H_E	0.922	0.893	0.858	0.887	0.903	0.912	0.906	0.902	0.884	0.870
Size range:	H_O	0.973	0.840	0.971	1	0.909	0.896	0.918	0.944	0.920	0.949
177–227 bp	<i>n</i>	37	50	35	50	44	67	49	72	50	39

See Fig. 1 for sample abbreviations.

*Significant at the 5% level, *** significant at the 0.1% level.

from the hierarchical analysis of molecular variance (AMOVA), which showed that the largest percentage of variance (3.2%; $F_{CT} = 0.032$, $P < 0.001$) was distributed among populations, whereas a much smaller part of the variance (0.6%; $F_{SC} = 0.006$, $P < 0.001$) was distributed among temporal samples within populations.

The close genetic relationships among temporal samples from the same populations were also illustrated by the multidimensional scaling plot of dimensions 1 (explaining 49.9% of the variance) and 2 (explaining 26.2% of the variance; Fig. 2). All four samples from KAR, ranging from 1912 to 1996, showed close genetic relationships. Also, the

Table 2 Above diagonal: θ_{ST} values between pairs of samples with 95% confidence limits in parentheses, determined by bootstrapping 1000 times over loci using the program F-STAT (Goudet 1995); below diagonal: tests for genetic differentiation between samples, using the test procedure by Goudet *et al.* (1996)

	VEJ1910	VEJ1998	KOV1953	KOV1998	KAR1912	KAR1951	KAR1993	KAR1996	KOL1998	ODR1992
VEJ1910		0.009 (0.002–0.017)	0.036 (0.023–0.050)	0.043 (0.011–0.073)	0.028 (0.016–0.039)	0.030 (0.019–0.043)	0.029 (0.015–0.044)	0.023 (0.013–0.035)	0.010 (0.001–0.017)	0.027 (0.006–0.048)
VEJ1998	***		0.053 (0.039–0.066)	0.053 (0.025–0.089)	0.029 (0.014–0.047)	0.028 (0.012–0.044)	0.033 (0.017–0.050)	0.024 (0.010–0.039)	0.002 (0.000–0.007)	0.040 (0.021–0.062)
KOV1953	***	***		0.016 (0.007–0.023)	0.034 (0.016–0.059)	0.041 (0.020–0.066)	0.040 (0.012–0.070)	0.034 (0.017–0.051)	0.041 (0.026–0.060)	0.037 (0.025–0.055)
KOV1998	***	***	**		0.053 (0.019–0.099)	0.056 (0.022–0.100)	0.060 (0.019–0.107)	0.052 (0.022–0.086)	0.049 (0.017–0.083)	0.039 (0.022–0.055)
KAR1912	***	***	***	***		0.000 (0.000–0.001)	0.013 (0.005–0.024)	0.004 (0.000–0.008)	0.028 (0.015–0.040)	0.050 (0.025–0.088)
KAR1951	***	***	***	***	NS		0.012 (0.007–0.017)	0.003 (0.000–0.006)	0.028 (0.017–0.039)	0.053 (0.032–0.089)
KAR1993	***	***	***	***	***	***		0.003 (0.000–0.010)	0.034 (0.014–0.057)	0.071 (0.043–0.102)
KAR1996	***	***	***	***	***	***	**		0.024 (0.012–0.037)	0.051 (0.026–0.082)
KOL1998	***	NS	***	***	***	***	***	***		0.041 (0.012–0.069)
ODR1992	***	***	***	***	***	***	***	***	***	

Above diagonal, numbers in bold denote θ_{ST} values between temporal samples from the same population.

Below diagonal, tablewide significance levels were applied, using the sequential Bonferroni technique (Rice 1989) (initial $k = 45$).

ns, not significant; **Significant at the 1% level, *** significant at the 0.1% level.

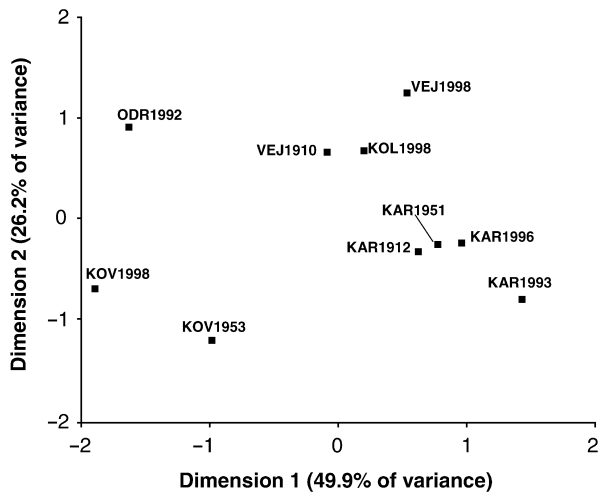


Fig. 2 Multidimensional scaling analysis of the matrix of pairwise Nei's (1978) genetic distances between populations. Dimension 1 explains 49.9% of the variance and dimension 2 26.2% of the variance.

two KOV samples (KOV1953 and KOV1998) were closely related. The sample VEJ1910 showed close relationships with both the contemporary VEJ1998 sample and that from the neighbouring KOL1998.

Genetic differentiation among all five populations was estimated based on the most recent samples (KAR1996, KOV1998, ODR1992, VEJ1998 and KOL1998) and yielded a θ_{ST} value of 0.037 (95% CI 0.023–0.052, determined by bootstrapping over loci). Other studies of anadromous trout populations on comparable geographical scales employing allozymes or microsatellites have yielded F_{ST} values of approximately the same magnitude (Moran *et al.* 1995, $F_{ST} = 0.077$; Knutsen *et al.* 2001, $F_{ST} = 0.054$; Ruzzante *et al.* 2001b, $F_{ST} = 0.049$). The observed θ_{ST} value corresponds to a gene flow estimate of $N_e m = 6.5$ (4.6–10.6). A congruent result was obtained using Slatkin's (1985b) private allele method, which yielded a $N_e m$ estimate of 5.1.

Long-term estimates of N_e were high in VEJ (VEJ1910–VEJ1998) and KAR, at least until the 1950s (KAR1912–KAR1951), with 90% posterior probability intervals of N_e in both populations ranging from approx. 500–980, assuming $N_{emax} = 1000$ (Table 3). N_e estimates involving either KAR1912 or KAR1951 and one of the contemporary samples, KAR1993 and KAR1996, tended to be lower. A recent decline of N_e in KAR was clearly suggested by the low estimates involving the 1993 and 1996 samples (90% posterior probability intervals ranging from 35 to 761 and 24 to 91, assuming $N_{emax} = 1000$ and 100, respectively). Finally, the population in the small Kovads River (KOV) exhibited a substantial N_e with a 90% posterior probability interval ranging from 296 to 924.

In Bayesian analysis it is a matter of concern if the posterior distribution merely reflects the prior assumptions.

Table 3 Estimates of effective population size

Samples	T	N_{emax}	Median	90% posterior probability interval
VEJ1910–VEJ1998	25	2000	1087	607–1837
VEJ1910–VEJ1998	25	1000	795	537–980
KOV1953–KOV1998	13	1000	547	296–924
KAR1912–KAR1951	11	1000	799	489–980
KAR1912–KAR1993	23	1000	614	378–930
KAR1912–KAR1996	24	1000	671	417–949
KAR1951–KAR1993	12	1000	456	253–856
KAR1951–KAR1996	13	1000	530	301–897
KAR1993–KAR1996	0.85	1000	84	35–761
KAR1993–KAR1996	0.85	100	49	24–91

N_e , using the temporal method by Berthier *et al.* (2002). T denotes the number of generations between temporal samples, N_{emax} denotes the prior information about the upper limit of N_e , and finally the median and 90% posterior probability intervals of N_e are listed.

In the case of estimation of N_e , the influence of the prior information on the lower (5%) percentile must be considered critical, as in a conservation context the primary interest is to ensure that N_e is not below a certain threshold. However, assuming different N_{emax} values caused only a minor change of the lower value of the 90% posterior probability intervals, whereas the upper limits were more strongly affected (as examples, see the N_e estimates from VEJ1910–VEJ1998 and KAR1993–KAR1996 in Table 3 using different N_{emax} values).

To assess the potential for local adaptation we focused on three N_e values, 500 and 1000, to represent relatively large Danish anadromous trout populations as those in the KAR and VEJ populations (cf. Table 3) and 30, to represent populations experiencing recent declines of N_e such as the contemporary KAR population (Table 3). If N_e is in the order of 500–1000, then $N_e m$ estimates of 4.6–10.6 should correspond to migration rates, m , of 0.005–0.02. However, given the problems with estimating gene flow from F_{ST} values (Whitlock & McCauley 1999) we also did the calculations by assuming a higher migration rate, m , of 0.05, roughly corresponding to estimates of straying rates among brown trout populations obtained by tagging (e.g. Altukhov *et al.* 2000). Assuming N_e of 30 and m of 0.01, local adaptation was predicted only to occur in the case of strong selection ($s = 0.1$; Table 4, scenario A and B). Assuming N_e of 500 and m of 0.01, local adaptation was predicted in individual populations if selection was strong ($s > 0.01$; scenario C and E), but if the spatial scale of selection encompassed several population ($j = 4$), then local adaptation was possible for smaller values of s (D and F). Assuming a higher m of 0.05 resulted in qualitatively similar

Scenario	N_e	m	Mode of gene flow	j	$s = 0.001$	$s = 0.01$	$s = 0.1$
A	30	0.01	a	1	R	R	A
B	30	0.01	a	4	R	R	A
C	500	0.01	a	1	H	H-A	A
D	500	0.01	a	4	A	A	A
E	500	0.01	b	1	H	H	A
F	500	0.01	b	4	H	A	A
G	500	0.05	a	1	H	H	A
H	500	0.05	a	4	H	A	A
I	1000	0.005	a	1	H	A	A
J	1000	0.005	a	4	A	A	A
K	1000	0.005	b	1	H	H	A
L	1000	0.005	b	4	H-A	A	A

Table 4 Assessment of the potential for local adaptation, based on the approach and formulas from Nagylaki & Lucier (1980) and Adkison (1995)

N_e denotes effective population size and m is migration rate. 'Mode of gene flow' denotes the spatial pattern of gene flow: a, where all gene flow occurs exclusively between neighbouring populations; and b, where 60% of gene flow occurs between neighbouring populations, 30% between populations two 'stepping-stones' apart and 10% between populations three 'stepping-stones' apart. j denotes the number of populations over which selection regimes are the same, and s denotes the strength of selection. H denotes scenarios where genetic homogeneity is predicted, R denotes scenarios where random differentiation is predicted and, finally, A denotes situations where the prediction is local adaptation. H-A denotes situations intermediate between homogeneity and adaptation. See Materials and methods for a more detailed description of the approach.

predictions (G and H). As expected, assuming even higher N_e (1000) and lower m (0.005) resulted in increased possibilities for local adaptation (I-L). The mode of gene flow, i.e. whether gene flow occurred exclusively between neighbouring populations or could occur also over longer distances, had a significant effect on the potential for local adaptation (C-D vs. E-F and I-J vs. K-L), and assuming gene flow over longer distances decreased the potential for local adaptation approximately as much as increasing m from 0.01 to 0.05 (E-F vs. G-H).

Discussion

Spatiotemporal genetic structure of populations

Several studies have documented that brown trout are subdivided into genetically distinct populations at the level of individual rivers (e.g. Ryman 1983; Ferguson 1989; Bouza *et al.* 1999; Ruzzante *et al.* 2001b; Laikre *et al.* 2002). However, except for some studies testing the temporal stability of genetic composition on a timescale of a few years (e.g. Hansen & Loeschcke 1996; Carlsson *et al.* 1999; Laikre *et al.* 2002) the long-term stability of the observed genetic structure has not been examined. The present study revealed statistically significant differentiation between most temporal samples from the same populations, but the magnitude of differentiation, measured as θ_{ST} was small (Table 2). Furthermore, the hierarchical analysis of

molecular variance (AMOVA) and the multidimensional scaling plot of genetic distances (Fig. 2) showed much closer genetic relationships among temporal samples from the same populations compared to samples from different populations. It is remarkable that even though statistically significant differentiation was observed between the VEJ1910 and VEJ1998 samples, the N_e estimate based on the same samples was above 500 (Table 3). This stresses the point made by Waples (1998) and Hedrick (1999) that statistically significant but biologically insignificant differentiation may easily occur at hypervariable markers such as microsatellites. We therefore conclude that the genetic composition of the anadromous brown trout populations examined has essentially been stable over the time-span of the last 50–100 years. Thus, neither drift nor migration from genetically divergent populations (including stocked domesticated trout) has strongly altered the genetic composition of populations over such time-spans. This result corresponds well with previous studies of the spatio-temporal genetic structure of Atlantic salmon, where microsatellite analysis of historical samples documented temporal stability of genetic population structure over several decades (Nielsen *et al.* 1999b; Tessier & Bernatchez 1999).

The concept of metapopulations, usually defined as systems of populations linked by limited gene flow and experiencing extinction–recolonization events (Hanski & Gilpin 1991), has been increasingly applied in population

genetics, including studies targeted at salmonid fish (e.g. Garant *et al.* 2000). However, the present results from brown trout, along with the results by Nielsen *et al.* (1999b) and Tessier & Bernatchez (1999) from Atlantic salmon, demonstrate long-term stability of populations. This suggests that natural (as opposed to anthropogenic) population turn-over does not occur frequently in salmonid populations, except, perhaps, in populations inhabiting highly unstable environments (e.g. small rivers experiencing frequent droughts or geologically unstable areas where landslides occur; Heath *et al.* 2002). This has important implications for the potential for local adaptation, as a high rate of turn-over of populations is expected to result in random genetic divergence at the expense of adaptation (e.g. Adkison 1995).

Effective population sizes in anadromous brown trout

Our results show that N_e is high in the studied anadromous trout populations, above approximately 500 in VEJ and in the historical KAR samples (i.e. involving the samples KAR1912–KAR1951), and above approximately 300 in the KOV population. Consequently, N_e values clearly exceed 50, the minimum short-term N_e suggested for avoiding inbreeding problems, and at least in VEJ and historical KAR N_e is also above approximately 500, the minimum long-term N_e required for maintaining the evolutionary potential of populations (Franklin 1980). It could be questioned whether KOV trout represent a distinct population, as this river is a tributary to a larger river system. Slight, but statistically significant differentiation has been observed among samples from different tributaries to the system (Ruzzante *et al.* 2001b), but we cannot entirely rule out that the two temporal samples from KOV represent the gene pool of the total river system rather than a distinct KOV population.

The estimated N_e values represent the harmonic mean of N_e over the generations spanned by the samples used for the estimation. Thus a few generations with low N_e will have a large negative influence on total N_e . While the historical samples, particularly those taken in the 1910s, represent populations prior to serious human disturbance, the contemporary samples were collected at a time where exploitation and environmental degradation is known to have caused population declines of brown trout in Denmark and elsewhere in Europe (Laikre 1999). The four temporally spaced samples from KAR allow for further insight into this issue. N_e appeared to be higher from 1912 to 1951 compared to estimates involving the contemporary samples, and the N_e estimates involving KAR1993–KAR1996 strongly suggested that a recent population decline had taken place. We do not know the exact reason for the low N_e estimate in the contemporary KAR population. However, the most likely explanation is a general population

decline because of habitat degradation combined with the use of too few parent fish for supportive breeding, as it is known that in some years in the late 1980s and early 1990s as few as 10–15 males and 20–30 females were used. In the short term this may lead to a much reduced total N_e (Ryman & Laikre 1991), though recent studies have also shown that in the long term the consequences are not necessarily so severe if supportive breeding is successful in increasing census population sizes (Wang & Ryman 2001; Duchesne & Bernatchez 2002).

Another possible explanation of the low N_e estimate in KAR from 1993 to 1996 involves gene flow because of stocking with non-native domesticated trout, which could have resulted in different genetic contributions of hatchery-derived alleles in the two temporal samples. However, we find this explanation less likely, as Hansen (2002) observed small and quite similar admixture proportions of domesticated trout in the two samples. In general, as both KAR and VEJ have been stocked with non-native domesticated trout, there is the possibility that a minor degree of introgression by the stocked fish may have contributed to the small divergence between the historical and contemporary samples from these populations. If so, the expected outcome is for N_e to have been underestimated.

Overall, there are still relatively few estimates available of N_e in wild salmonid populations, based on the temporal method, and most studies have been aimed at small, endangered populations (e.g. Waples 1990). Heath *et al.* (2002) obtained N_e estimates ranging from 92 to 560 in steelhead populations in British Columbia. Jorde & Ryman (1996) estimated N_e in four Swedish resident brown trout populations. Compared to the present study N_e was relatively small (point estimates from 52 to 480). Laikre *et al.* (2002) used mitochondrial DNA analysis for estimating female effective size of anadromous brown trout populations in small rivers on the Baltic island of Gotland. The estimates were generally low, ranging from 7 to 177, and presumably also correspond to low total effective population sizes. The most obvious explanation for the higher N_e estimates obtained for the Danish populations is that these populations inhabit larger rivers than those studied by Laikre *et al.* (2002) and that they are anadromous, compared to the resident populations studied by Jorde & Ryman (1996). The fact that a large proportion of the population migrates between the river and the sea means that the census population size of adult trout becomes less dependent on the availability of resources such as food and space in the river (e.g. Elliott 1994).

Potential for local adaptation

Before discussing specifically the potential for local adaptation it is necessary to evaluate critically the estimates of migration rate, m , used for the calculations in

Table 4. Most methods available for estimating gene flow from molecular data, including those by Wright (1931) and Slatkin (1985b), are based on unrealistic assumptions (Whitlock & McCauley 1999). Thus, it could be argued that migration–drift equilibrium has not yet been reached during the time elapsed since the last glaciation (approx. 10–13 000 years before the present), in which case m may have been overestimated. F_{ST} will be close to equilibrium if $1/t \ll 1/2N_e + 2m$ (Crow & Aoki 1984), where t denotes number of generations. If we assume a t of 2800 trout generations (corresponding to approximately 10 000 years), then even if we assume a high N_e (1000) and a low m (0.005) the migration–drift equilibrium is expected to have been attained. However, a more severe problem concerns the fact that the estimates of gene flow assume an island model, whereas a stepping-stone model is more realistic for our study. In this case m has probably been underestimated (Whitlock & McCauley 1999). The smallest m -value (0.005) assumed in Table 4 should therefore be considered a minimum estimate, whereas the highest m -value (0.05; corresponding to estimates of straying rates based on tagging data and assuming equal reproductive success of immigrants and indigenous fish) should be considered a maximum estimate.

Elo (1993) and Adkison (1995) treated the issue of local adaptation by considering a wide range of N_e and m -values. The results of the present study allowed us to focus on the most realistic range of values of these parameters in existing anadromous brown trout populations. Given the values of N_e (500–1000) and m (0.005–0.05) assumed in this study, local adaptation is indeed likely to occur (Table 4). However, and in accordance with Adkison (1995), local adaptation involving traits subject to weak selection ($s = 0.01$ and 0.001) is more likely to be present on a regional basis, i.e. encompassing more rivers and populations, than at the scale of individual rivers/populations. This becomes even more evident when the mode of gene flow is taken into account. A strict stepping-stone model, where gene flow occurs exclusively between neighbouring populations, is unlikely to be valid in anadromous brown trout. Thus, tagging data by Svärdsön & Fagerström (1982) show that even though straying primarily occurs between geographically proximate populations, some straying over long distances also takes place. Scenarios E–F and K–L in Table 4 are therefore probably the most realistic, a result that again stresses the conclusion that local adaptation at the scale of individual populations (scenarios E and K) is only likely to occur if selection is strong, whereas adaptations resulting from weaker selection are more likely to occur at a higher hierarchical level encompassing several populations sharing similar selection regimes (scenarios F and L).

What then are the expected extent and geographical scale of local adaptation in the anadromous trout populations

studied? There is presumably no simple answer, as it depends on the strength of selection, which is expected to vary considerably among traits (Endler 1986). One important factor concerns whether the trait in question is polygenic or encoded by a single or few loci. Thus, in the case of quantitative traits where several loci have an effect on the trait, the selection acting on individual loci may be quite weak, even if the trait itself is subject to strong selection (Lynch 1984). There are some examples of single locus 'traits' possibly subject to differential local selection in salmonids, such as the major histocompatibility complex (MHC) class II B locus (Landry & Bernatchez 2001) and the malic enzyme-2 (*MEP-2**) locus (Verspoor & Jordan 1989) in Atlantic salmon. However, most morphological, behavioural and life history traits potentially involved in local adaptation are expected to be quantitative traits affected by several loci. It follows that unless selection is very strong, local adaptation involving such traits is primarily expected to occur at the scale of more populations experiencing similar selection regimes.

Is it realistic to assume that neighbouring populations share the same selection regimes? In Denmark, all rivers flow through lowland landscapes and there are no dramatic shifts in geochemical properties. Consequently, some similarity of selection regimes, e.g. determined by water chemistry and temperature, at the scale of several neighbouring rivers is not an unreasonable assumption. This need not necessarily be the case, however, in regions exhibiting more geological variation than Denmark. For instance, among rivers with neighbouring outlets into the sea some could be derived from mountainous areas with low water temperatures, whereas others could represent lowland drainages with higher water temperatures, thus potentially resulting in highly differing selection regimes.

A spatial scale of selection of four rivers/populations, as assumed in some of the scenarios in Table 4, would in practical terms in Denmark correspond to rivers with outlets into the sea separated by waterway distances of approximately 40–75 km. For instance, at a distance of ≤ 40 km from the outlet of the Karup River (KAR) there are five other rivers of comparable size, along with some other considerably smaller rivers. The fact that neighbouring rivers flow into similar marine environments, such as the same or neighbouring fjords, raises the issue of whether adaptation to the local marine environment could not also be important in addition to adaptation to riverine environments. Both Utter (2000) and Hansen *et al.* (2000) have suggested that stocked non-native salmonids perform better adopting a resident life history than if they become anadromous. This could imply that adaptation to the marine environment, or perhaps the transition from fresh water to the marine phase during smoltification, is important. Tagging data by Svärdsön & Fagerström (1982) also suggest adaptation to the local marine environment, as

brown trout populations from different parts of the Baltic Sea undertook very different kinds of feeding migrations and in most cases retained their migratory behaviour when translocation experiments were conducted.

In a conservation context the recent decline and extirpation of many anadromous brown trout populations may compromise local adaptation. Effective population sizes may be brought down to a level at which adaptations within single populations are lost. However, population declines and extirpations also have consequences for the overall genetic structure of populations. Extirpation of populations is equivalent to removal of migrational stepping-stones and may reduce the number of populations sharing similar selection regimes, thereby causing loss of local adaptations occurring at the scale of several neighbouring populations. Consequently, even though limited resources may necessitate prioritization of some populations for conservation over others (e.g. Allendorf *et al.* 1997), it should be taken into consideration that maintaining the genetic structure of populations is also important. Otherwise, giving lower priority to some populations may in fact have negative consequences also for the populations that are highly prioritized for conservation.

Acknowledgements

We thank Louis Bernatchez, Jens Carlsson and two anonymous reviewers for comments on a previous version of the manuscript, Gordon Luikart and Mark Beaumont for help and advice on estimating effective population size, and the anglers from the Kolding, Vejle and Karup Rivers for assistance in collecting samples.

References

- Adcock GJ, Ramirez JHB, Hauser L, Smith P, Carvalho GR (2000) Screening of DNA polymorphisms in samples of archived scales from New Zealand snapper. *Journal of Fish Biology*, **56**, 1283–1287.
- Adkison M (1995) Population differentiation in Pacific salmon: Local adaptation, genetic drift or the environment? *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 2762–2777.
- Allendorf FW, Bayles D, Bottom DL *et al.* (1997) Prioritizing Pacific salmon stocks for conservation. *Conservation Biology*, **11**, 140–152.
- Altukhov YP, Salmenkova EA, Omelchenko VT (2000) *Salmonid Fishes. Population Biology, Genetics and Management*. Blackwell Science Ltd, Oxford.
- Beaumont MA (2001) Conservation Genetics. In: *Handbook of Statistical Genetics* (eds Balding DJ, Bishop M, Cannings C), pp. 779–812. John Wiley, New York.
- Berthier P, Beaumont MA, Cornuet J-M, Luikart G (2002) Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. *Genetics*, **160**, 741–751.
- Bouza C, Arias J, Castro J, Sanchez L, Martinez P (1999) Genetic structure of brown trout, *Salmo trutta* L., at the southern limit of the distribution of the anadromous form. *Molecular Ecology*, **8**, 1991–2002.
- Carlsson J, Olsén HK, Nilsson J, Øverli Ø, Stabell OB (1999) Microsatellites reveal fine-scale genetic structure in stream-living brown trout. *Journal of Fish Biology*, **55**, 1290–1303.
- Castric V, Bonney F, Bernatchez L (2001) Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution*, **55**, 1016–1028.
- Crow JF, Aoki K (1984) Group selection for a polygenic behavioral trait: Estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences USA*, **81**, 6073–6077.
- Duchesne P, Bernatchez L (2002) An analytical investigation of the dynamics of inbreeding in multi-generation supportive breeding. *Conservation Genetics*, **3**, 45–58.
- Elliott JM (1994) *Quantitative Ecology and the Brown Trout*. Oxford University Press, Oxford.
- Elo K (1993) Gene flow and conservation of genetic variation in anadromous Atlantic salmon (*Salmo salar*). *Hereditas*, **119**, 149–159.
- Endler JA (1986) *Natural Selection in the Wild*. Princeton University Press, Princeton.
- Estoup A, Presa P, Krieg F, Vaiman D, Guyomard R (1993) (CT)_n and (GT)_n microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). *Heredity*, **71**, 488–496.
- Estoup A, Largiadier CR, Perrot E, Chourrout D (1996) Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Molecular Marine Biology and Biotechnology*, **5**, 295–298.
- Estoup A, Rousset F, Michalakis Y *et al.* (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology*, **7**, 339–353.
- Ferguson A (1989) Genetic differences among brown trout, *Salmo trutta*, stocks and their importance for the conservation and management of the species. *Freshwater Biology*, **21**, 35–46.
- Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genetical Research, Cambridge*, **66**, 95–107.
- Franklin IR (1980) Evolutionary change in small populations. In: *Conservation Biology: an Evolutionary-Ecological Perspective* (eds Soulé ME, Wilcox BA), pp. 135–150. Sinauer, Sunderland.
- Frier JO (1994) Growth of anadromous and resident brown trout with different life histories in a Danish lowland stream. *Nordic Journal of Freshwater Research*, **69**, 58–70.
- Garant D, Dodson JJ, Bernatchez L (2000) Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **9**, 615–628.
- Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.
- Goudet J, Raymond M, deMeeus T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.
- Guo SW, Thompson EA (1992) Performing the exact test for Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.
- Hansen MM (2002) Estimating the long-term effects of stocking domesticated trout into wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. *Molecular Ecology*, **11**, 1003–1015.
- Hansen MM, Loeschcke V (1996) Temporal variation in mitochondrial DNA haplotype frequencies in a brown trout (*Salmo trutta* L.) population that shows stability in nuclear allele frequencies. *Evolution*, **50**, 454–457.

- Hansen MM, Mensberg K-LD (1998) Genetic differentiation and relationship between genetic and geographical distance in Danish sea trout (*Salmo trutta* L.) populations. *Heredity*, **81**, 493–504.
- Hansen MM, Ruzzante DE, Nielsen EE, Mensberg K-LD (2000) Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Molecular Ecology*, **9**, 583–594.
- Hanski I, Gilpin M (1991) Metapopulation dynamics: brief history and conceptual domain. In: *Metapopulation Dynamics: Empirical and Theoretical Investigations* (eds Gilpin M, Hanski I), pp. 3–16. Academic Press, London.
- Heath DD, Busch C, Kelly J, Atagi DY (2002) Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). *Molecular Ecology*, **11**, 197–214.
- Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.
- Jorde PE, Ryman N (1995) Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics*, **139**, 1077–1090.
- Jorde PE, Ryman N (1996) Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change of allele frequencies. *Genetics*, **143**, 1369–1381.
- Kelly MJ (2001) Lineage loss in Serengeti cheetahs: Consequences of high reproductive variance and heritability of fitness on effective population size. *Conservation Biology*, **15**, 137–147.
- Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–576.
- Koskinen MT, Haugen TO, Primmer CR (2002a) Contemporary fisherian life-history evolution in small salmonid populations. *Nature*, **419**, 826–830.
- Koskinen MT, Sundell P, Piironen J, Primmer CR (2002b) Genetic assessment of spatiotemporal evolutionary relationships and stocking effects in grayling (*Thymallus thymallus*, Salmonidae). *Ecology Letters*, **5**, 193–205.
- Knutsen H, Knutsen JA, Jorde PE (2001) Genetic evidence for mixed origin of recolonized sea trout populations. *Heredity*, **87**, 207–214.
- Laikre L, ed. (1999) *Conservation genetic management of brown trout (Salmo trutta) in Europe. Report by the Concerted action on identification, management and exploitation of genetic resources in the brown trout (Salmo trutta), 'TROUTCONCERT'; EU FAIR CT97-3882*. <http://www.dfu.min.dk/ffi/consreport/index.htm>.
- Laikre L, Jorde PE, Ryman N (1998) Temporal change of mitochondrial DNA haplotype frequencies and female effective size in a brown trout (*Salmo trutta*) population. *Evolution*, **52**, 910–915.
- Laikre L, Järvi T, Johansson L *et al.* (2002) Spatial and temporal population structure of sea trout (*Salmo trutta*) at the island of Gotland, Sweden, delineated from mitochondrial DNA. *Journal of Fish Biology*, **60**, 49–71.
- Landry C, Bernatchez L (2001) Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). *Molecular Ecology*, **10**, 2525–2539.
- Larsen K (1993) The sea trout spawning run into Danish streams 1900–60. III. Northern Jutland including streams debouching into the Limfjord. *Meddelelser fra Ferskvandsfiskerilaboratoriet*, **1/91**. (Report from the Danish Institute for Fisheries Research, in Danish.)
- Luikart G, England PR (1999) Statistical analysis of microsatellite DNA data. *Trends in Ecology and Evolution*, **14**, 253–256.
- Lynch M (1984) The selective value of genes underlying polygenic traits. *Genetics*, **108**, 1021–1033.
- Martinez JL, Moran P, Perez J, De Gaudemar B, Beall E, Garcia-Vazquez E (2000) Multiple paternity increases effective size of southern Atlantic salmon populations. *Molecular Ecology*, **9**, 293–298.
- Miller LM, Kapuscinski AR (1997) Historical analysis of genetic variation reveals low effective population size in a northern pike (*Esox lucius*) population. *Genetics*, **147**, 1249–1258.
- Morán P, Pendás AM, García-Vázquez E, Izquierdo JI, Lóbon-Cervía J (1995) Estimates of gene flow among neighbouring populations of brown trout. *Journal of Fish Biology*, **46**, 593–602.
- Nagylaki T, Lucier B (1980) Numerical analysis of random drift in a cline. *Genetics*, **94**, 497–517.
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
- Nielsen EE, Hansen MM, Loeschcke V (1997) Analysis of microsatellite DNA from old scale samples of Atlantic salmon: a comparison of genetic composition over sixty years. *Molecular Ecology*, **6**, 487–492.
- Nielsen EE, Hansen MM, Loeschcke V (1999a) Genetic variation in time and space: Microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution*, **53**, 261–268.
- Nielsen EE, Hansen MM, Loeschcke V (1999b) Analysis of DNA from old scale samples: Technical aspects, applications and perspectives for conservation. *Heredity*, **130**, 265–276.
- O'Reilly PT, Hamilton LC, McConnell SK, Wright JM (1996) Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2292–2298.
- Palm S, Ryman N (1999) Genetic basis of phenotypic differences between transplanted stocks of brown trout. *Ecology of Freshwater Fish*, **8**, 169–180.
- Raymond M, Rousset F (1995) GENEPOP (Version 1.2): a population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Rousset F, Raymond M (1995) Testing heterozygote excess and deficiency. *Genetics*, **140**, 1413–1419.
- Ruzzante DE, Taggart CT, Doyle RW, Cook D (2001a) Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics*, **2**, 257–269.
- Ruzzante DE, Hansen MM, Meldrup D (2001b) Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. *Molecular Ecology*, **10**, 2107–2128.
- Ryman N (1983) Patterns of distribution of biochemical genetic variation in salmonids: Differences between species. *Aquaculture*, **33**, 1–21.
- Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective population size. *Conservation Biology*, **5**, 325–329.
- Schneider S, Kueffer J-M, Roessli D, Excoffier L (2000) *Arlequin*, Version 2000. A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva.
- Slatkin M (1973) Gene flow and selection in a cline. *Genetics*, **75**, 733–756.

- Slatkin M (1985a) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.
- Slatkin M (1985b) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.
- Slettan A, Olsaker I, Lie Ø (1995) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics*, **26**, 277–285.
- Slettan A, Olsaker I, Lie Ø (1996) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL438, SSOSL439 and SSOSL444 loci. *Animal Genetics*, **27**, 57–64.
- Stabell OB (1984) Homing and olfaction in salmonids: a critical review with special reference to the Atlantic salmon. *Biological Reviews of the Cambridge Philosophical Society*, **59**, 333–388.
- Svärdson G, Fagerström Å (1982) Adaptive differences in the long-distance migration of some trout (*Salmo trutta* L.) stocks. *Report. Institute of Freshwater Research, Drottningholm*, **60**, 51–80.
- Taggart JB, Hynes RA, Prodöhl PA, Ferguson A (1992) A simplified protocol for routine total DNA isolation from salmonid fishes. *Journal of Fish Biology*, **40**, 963–965.
- Taylor EB (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, **98**, 185–207.
- Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **8**, 169–179.
- Utter FM (2000) Patterns of subspecific anthropogenic introgression in two salmonid genera. *Reviews in Fish Biology and Fisheries*, **10**, 265–279.
- Verspoor E, Jordan WC (1989) Genetic variation at the *Me-2* locus in the Atlantic salmon within and between rivers: evidence for its selective maintenance. *Journal of Fish Biology*, **35A**, 205–213.
- Wang J, Ryman N (2001) Genetic effects of multiple generations of supportive breeding. *Conservation Biology*, **15**, 1619–1631.
- Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, **121**, 379–391.
- Waples RS (1990) Conservation genetics of Pacific salmon III. Estimating effective population size. *Journal of Heredity*, **81**, 277–289.
- Waples RS (1998) Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity*, **82**, 117–125.
- Wright (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.
- Young FW (1996) *ViSta: The Visual Statistics System. Research Memorandum 94-1 (B)* 2nd edn. L.L.Thursone Psychometric Laboratory, University of North Carolina, Chapel Hill, NC.

This paper represents one of the major common interests of the authors, i.e. the use of historical samples to address problems in conservation and population genetics. Previous work of the authors using microsatellite analysis of historical samples has focused on species such as Atlantic salmon, brown trout, European grayling (*Thymallus thymallus*), cod (*Gadus morhua*) and otter (*Lutra lutra*).
