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Genetic differentiation among Danish brown trout (*Salmo trutta*) populations

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Brown trout (*Salmo trutta* L.) from twelve locations in small rivers in Jutland, Denmark, were examined by allozyme electrophoresis. Seven of the locations are tributaries to the small (3.3 km^2) Lake Hald. These and two other locations are assumed to have been mainly inaccessible to gene flow from outside for hundreds of years because of impassable dams. The levels of polymorphism indicated that little or no loss of genetic variation had occurred in these isolated populations compared to populations open to gene flow. In Lake Hald significant genetic differentiation among the tributary populations was detected. Intensive stocking with trout from a hatchery strain directly into the lake was shown to have had little or no effect on the genetic composition of the original populations. In contrast, transplantation of trout from one tributary to another within the lake system was successful. The geographical distribution of genetic variation indicated that the Lake Hald populations are genetically divergent from the other populations and for that reason special care concerning management practices in the lake is recommended.

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In Denmark, as in other countries, many brown trout habitats and spawning sites have been destroyed by various anthropogenic effects. In order to compensate for the decline in natural production of trout traditionally intensive stocking of hatchery-reared brown trout has been undertaken. About 2.1 mill. trout are stocked each year in Denmark (Peter Geertz-Hansen, Inland Fisheries Laboratory, Silkeborg, Denmark, pers. commun.) and probably the majority of natural populations have been affected by this.

Stocking activity may result in a decline of genetic variance among stocks and, perhaps, breaking up of stock-specific co-adapted groups of genes (NELSON and SOULÉ 1987). Negative effects connected with genetic interaction of wild and reared fish have been reviewed by HINDAR et al. (1991). Consequently, there is a need to identify and conserve the remaining unaffected stocks. Many of these stocks in Denmark may be found in watercourses where dams in connection with millponds have made upstream migration impossible for centuries.

Lack of gene flow in connection with a finite population size will eventually result in loss of genetic variation due to genetic drift, the extent of which depends on the effective population size (FRANKEL and SOULÉ 1981). One aim of the present study was therefore to investigate if isolated populations, where insignificant or no stocking activity has taken place, have suffered a significant loss of genetic variation. Further, we were interested in the question whether genetic differentiation among the tributary populations of one of the study areas (the small Lake Hald) could be observed. In the same location we wanted to investigate the genetic effects of some previous stocking and transplantation activities. Finally, as the Lake Hald trout is one of the very few remnants of natural lake-dwelling brown trout populations in Denmark, we wanted to determine the genetic relationships of the Lake Hald trout to other Danish trout populations.

The study areas

The approximate location of the main sample localities are shown in Fig. 1. The abbreviations designating the sample localities are listed in Table 1.

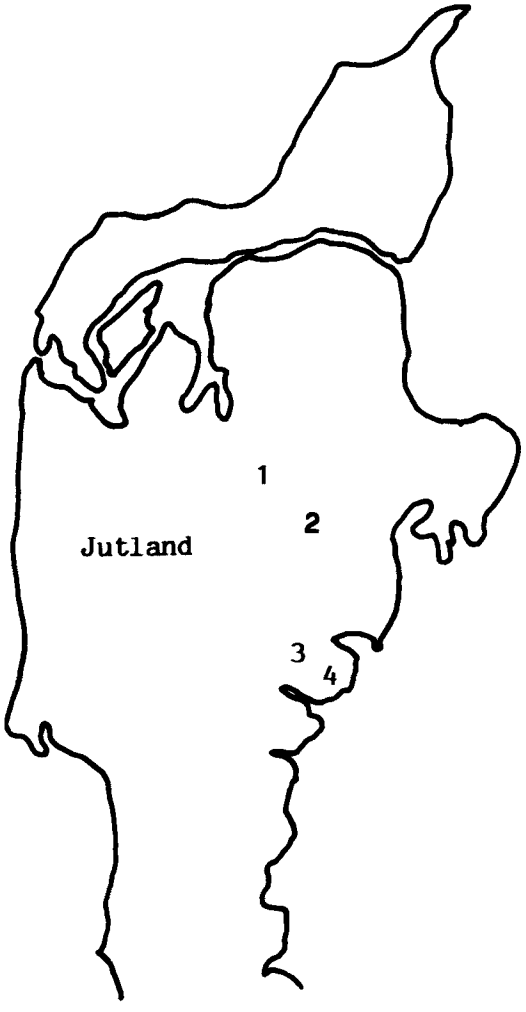

Fig. 1. Approximate location of the main sampling areas in Jutland, Denmark: (1) Lake Hald, (2) Brandstrup bæk, (3) Klokkedals å and (4) Tirsbæk.

Table 1. Sample locality abbreviations

Sample location	Main area	Abbreviations
Brandstrup bæk	Gudenå River	BRA
Tirsbæk, above dam	Tirsbæk	TOF
Tirsbæk, below dam	–	TNF
Klokkedals å, above dam	Klokkedals å	KOF
Klokkedals å, below dam	–	KNF
Krobæk	Lake Hald	KRO
Bisballe bæk	–	BIS
Kilde v. Dollerup bæk	–	KDO
Dollerup bæk	–	DOL
Kapeldal bæk	–	KAP
Gjelbæk	–	GJB
Dollerup Møllebæk	–	DMB

Tirsbæk is a small shallow river with a total length of about 3 km. 1 km from the outlet in the sea, a millpond has been established, and for that reason upstream migration by trout is impossible. The age of the dam is unknown but it has been built in connection with an old manor from the 16th century. It is therefore likely that the dam itself is several centuries old. The population below the dam is composed of resident and anadromous trout while obviously only resident trout are found in the isolated part of the river. No stocking has taken place to our knowledge.

Klokkedals å is very similar to Tirsbæk. It has a total length of about 6 km. About 2 km from the outlet in the sea an impassable dam has been built in connection with a millpond. This dam has with certainty existed since 1910 but is probably considerably older. Both resident and anadromous trout are found in the lower part of the river while only resident trout are found in the isolated part. No stocking with non-local fish has taken place to our knowledge, but in a few cases spawning trout have been transplanted from below to above the dam (LARSEN 1987).

Brandstrup bæk is about 5 km long and is a shallow tributary to the major Gudenå river. No dams are present. The spawning population is composed of resident and anadromous trout. No stocking has taken place in Brandstrup bæk itself but the Gudenå river is heavily stocked.

Lake Hald (Fig. 2) constitutes an upper part of the Gudenå river drainage. It covers an area of 3.3 km^2 and has a maximum depth of 34 m. The only outlet is Non Mølleå, where dams have made upstream migration by trout impossible since the 15th century. Lake-dwelling trout spawn in the small and shallow tributaries. After 1–3 years the offspring smoltify and migrate into the lake (LARSEN 1984). About 70 % of the males mature as parr (LARSEN 1984), but only very few larger resident trout have been observed. Dollerup bæk and Dollerup Møllebæk are of special interest, as in 1983 they were both devoid of trout owing to destruction of spawning places by fish farm waste (LARSEN 1984). This pollution was later stopped and suitable spawning conditions were reestablished by a local anglers' club. Thus, in 1989, when sampling took place, both rivers contained fish in number and density comparable to the other rivers. 1247 trout (comprising both 0+ and older fish) were transplanted from Krobæk to Dollerup Møllebæk in 1983 while colonization of Dollerup bæk has with certainty taken place in a natural

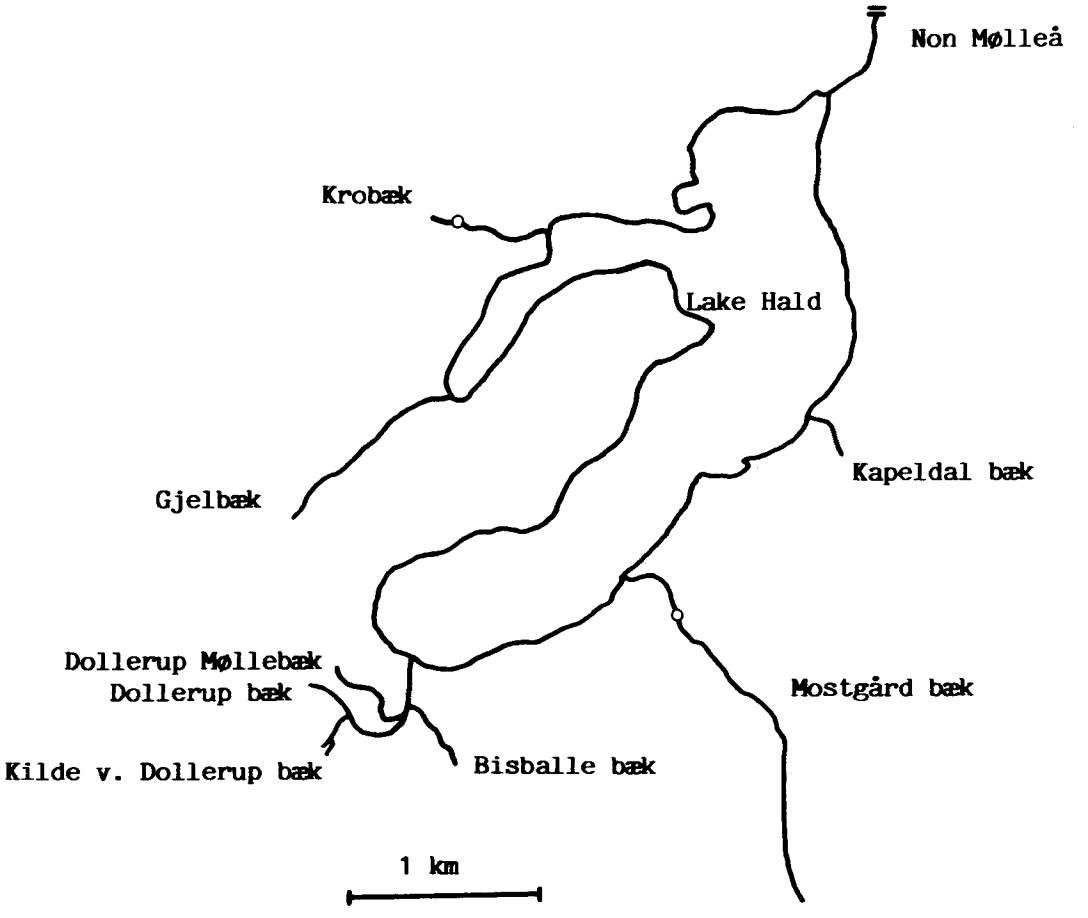

Fig. 2. Lake Hald, its tributaries and the outlet, Non Mølleå, which is impassable to fish. Mostgård bæk was considered to be devoid of trout.

way. There are no other records of stocking activity in the inlets, but in the late 70's 3000 trout of age 2+ were stocked directly into the lake. The stocked fish originated from a hatchery strain where isozyme variation has been studied (SIMONSEN and RASMUSSEN 1989).

Materials and methods

Preparation of samples and isozyme electrophoresis

Fish were caught from August to December 1989 by electrofishing. Ages of fish were determined from both scale readings and length-frequency distributions. Fish were stored at −20°C. Prior to electrophoresis they were gently thawed, and tissue samples from liver, heart, brain, muscle and eye were taken. A homogenization buffer (pH 7.4) was added and tissue samples were frozen at −60°C. Upon rethawing, samples were homogenized using a glass rod. The electrophoretic method was horizontal starch gel electrophoresis (e.g., FERGUSON 1980), and the buffers have been described by RIDGWAY et al. (1970) (electrode buffer pH 8.1, gel buffer pH 8.5, used for CK, GPI, G3PDH, LDH, and SOD), CLAYTON and TRETIAK (1972) (pH 6.1, used for AAT, DIA, FUM, IDHP, MDH, MEP, and PGM), and AYALA et al. (1972) (pH 7.0, used for MPI and PGDH). Gels were stained for (E.C. numbers and presumptive loci in parentheses): Aspartate aminotransferase (2.6.1.1) (*$_s$AAT-1,2**, *$_s$AAT-4**), creatine kinase (2.7.3.2) (*CK-A1**, *CK-A2**), diaphorase (1.6.2.2) (*DIA-1**), fumarase (4.2.1.2) (*FUM-1,2**), glucosephosphate isomerase (5.3.1.9) (*GPI-A1**, *GPI-A2**,

*GPI-B1**), glycerol-3-phosphate dehydrogenase (1.1.1.8) (*G3PDH-2**), isocitrate dehydrogenase (1.1.1.42) (*$_s$IDHP-1**, *$_s$IDHP-2**), lactate dehydrogenase (1.1.1.27) (*LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**), malate dehydrogenase (1.1.1.37) (*$_s$MDH-A1**, *$_s$MDH-A2**, *$_s$MDH-B1,2**), malic enzyme (1.1.1.40) (*MEP-1**, *MEP-2**), mannose-6-phosphate isomerase (5.3.1.8) (*MPI-1**), phosphoglucomutase (5.4.2.2) (*PGM-1**), phosphogluconate dehydrogenase (1.1.1.44) (*PGDH-1**), and superoxide dismutase (1.15.1.1) (*SOD-1**). This makes a total of 28 loci. A less extensive screening for variation in the eye-specific lactate dehydrogenase locus (*LDH-C1**) was also undertaken. The investigated loci were chosen in accordance with other brown trout studies and thus probably include the more variable ones in this species. Enzyme nomenclature is according to SHAKLEE et al. (1990).

Statistical treatments

Tests for deviations from expected Hardy-Weinberg proportions were carried out using Fisher's Exact Test (e.g., SOKAL and ROHLF 1981). In cases where the frequency of the most common allele did not exceed 0.8, F_{is} (WRIGHT 1951) was calculated.

Average expected heterozygosity of the samples were calculated according to NEI (1978). Both monomorphic and polymorphic loci were included.

Homogeneity of allelic frequencies between age classes within the DOL (based on *$_s$AAT-1,2**, *G3PDH-2**, and *$_s$MDH-A2**), KRO (based on *$_s$AAT-1,2**, *$_s$MDH-A2**, and *MPI-1**), and BRA (based on *DIA-1**, *$_s$IDHP-1**, *$_s$MDH-A2**, and *MPI-1**) samples, between pairs of samples and among samples in the main sample areas were tested using WORKMAN and NISWANDER's (1970) genic chi-square contingency test.

Genetic differences between the sampled populations were analyzed by NEI's (1978) genetic distance (D) and NEI and CHESSER's (1983) gene diversity analysis. Samples were divided into the two major groups: *Localities within Lake Hald* and *localities outside Lake Hald* in order to carry out a hierarchical gene diversity analysis.

In all cases of multiple tests "table-wide" significance levels were employed using the sequential Bonferroni test (RICE 1989).

The genetic relationships among populations were summarized by constructing a phylogenetic tree with the restricted maximum likelihood method. This was done with the program CONTML from the PHYLIP package (version 3.4) (FELSENSTEIN 1989).

Results

Ages of fish were determined to be 0+–3+. All samples contained several age classes. The following variant alleles were found (the most common allele in each locus was given a relative mobility of 100): *$_s$AAT-1,2*140*, *$_s$AAT-4*74*, *CK-A1*115*, *DIA-1*90*, *G3PDH-2*50*, *GPI-A1*90*, *$_s$IDHP-1*160*, *LDH-A1*n* (null allele), *$_s$MDH-A2*152*, *MPI-1*105*, *PGDH-1*85*, *GPI-A2*n*, *FUM-1,2** (mobility undetermined), *$_s$MDH-B1,2*75* and *$_s$MDH-B1,2*125*. No variation was found in *LDH-C1**, and side-by-side comparison with samples from Atlantic salmon (*Salmo salar* L.) suggested that the trout were fixed for the "novel" brown trout allele *LDH-C1*90* (FERGUSON 1989). Based on the relative mobilities apparently all variant alleles, except *GPI-A1*90*, have been described previously (TAGGART et al. 1981; FERGUSON 1989), although *GPI-A2*n* has been found in only very few populations (FERGUSON 1989). Heterozygotes and 140/140 homozygotes in the isoloci *$_s$AAT-1,2** could be distinguished due to a pronounced difference in intensity of heterodimeric and homodimeric bands. In the *LDH-A1** locus n/100 heterozygotes could be distinguished because of lowered intensity of the most cathodal bands on the gel. Random, and perhaps non-genetic, variation of such bands has been observed (TAGGART et al. 1981). However, in Klokkedals å, where this polymorphism was found, n/n homozygotes could easily be identified. The individuals scored as heterozygotes showed consistently lowered intensity of bands so we consider the scoring reliable. A satisfactory interpretation of the variation in *FUM-1,2** could not be given. **100/100* homozygotes and **n/100* heterozygotes could not be distinguished in the locus *GPI-A2**. A similar situation occurred in the isoloci *$_s$MDH-B1,2**. *$_s$MDH-B1,2*100/75* and *$_s$MDH-B1,2*75/75* genotypes and *$_s$MDH-B1,2*100/125* and *$_s$MDH-B1,2*125/125* genotypes, respectively, could not be distinguished, with many artefact bands further complicating interpretation. Inclusion of allelic frequencies calculated from the square root of the frequency of distinguishable homozygotes would present serious statistical problems because of the relatively small sample sizes (BAILEY 1975). These

Table 2. Allelic frequencies and average expected heterozygosities (H) of the samples studied. N denotes sample size. Note: The frequency of *GPI-A2*n* was calculated as the square-root of the frequency of *n/n* homozygotes. This locus is not used in further calculations. In a few cases one or a few individuals could not be scored for a specific locus

Allele	Samples studied											
	BRA	TOF	TNF	KOF	KNF	KRO	BIS	KDO	KAP	GJB	DOL	DMB
*$_s$AAT-1,2*140*	0.286	0.171	0.143	0.200	0.186	0.388	0.389	0.382	0.257	0.614	0.362	0.437
*$_s$AAT-4*74*	0.129	0.057	0.100	0.186	0.086	0.122	0.062	0.043	0.057	0.200	0	0.025
*CK-A1*115*	0	0	0	0	0	0.311	0.400	0.300	0.500	0.286	0.460	0.437
*DIA-1*90*	0.071	0.014	0	0	0	0	0	0	0	0	0.013	0
*G3PDH-2*50*	0.071	0	0.067	0.229	0.206	0.270	0.137	0.029	0.005	0.300	0.039	0.225
*GPI-A1*90*	0	0	0.014	0	0	0	0	0	0	0	0	0
*$_s$IDHP-1*160*	0.200	0.243	0.214	0.086	0.243	0.243	0.175	0.257	0.343	0.257	0.092	0.100
*LDH-A1*n*	0	0	0	0.057	0.229	0	0	0	0	0	0	0
*$_s$MDH-A2*152*	0.236	0.329	0.371	0.186	0.214	0.125	0.125	0.143	0.186	0.371	0.151	0.212
*MPI-1*105*	0.507	0.400	0.414	0.329	0.234	0.526	0.387	0.143	0.471	0.529	0.132	0.437
*PGDH-1*85*	0.007	0	0	0	0	0	0	0	0	0	0	0
*GPI-A2*n*	0?	0?	0?	0?	0?	0?	0.224	0.293	0?	0?	0.279	0?
H	0.082	0.071	0.078	0.084	0.093	0.114	0.097	0.084	0.103	0.131	0.074	0.105
N	35/70[#]	35	35	35	35	37/76[£]	40	35	35	35	38/77[$]	40

[#] N = 70 for *DIA-1**, *$_s$IDHP-1**, *PGDH-1**, *$_s$MDH-A2** and *MPI-1**.
[£] N = 76 for *$_s$AAT-1,2**, *$_s$MDH-A2** and *MPI-1**.
[$] N = 77 for *GPI-A2**, *$_s$AAT-1,2**, *G3PDH-2** and *$_s$MDH-A2**.

loci were therefore discarded. Allelic frequencies of the other variable loci (including *GPI-A2**) and average expected heterozygosity values are listed in Table 2.

No significant deviations at the 5 % level from expected Hardy-Weinberg proportions were found in the 84 tests carried out (data not listed). Of the 47 calculated F_{is} values 23 cases of non-significant excess of heterozygotes were observed, so there was no tendency of excess/deficit of heterozygotes (data not listed).

Tests for homogeneity of allelic frequencies between age classes in three samples did not reveal substantial genetic differences, although in KRO the result of the test was significant at the 5 % level (chi-square value: 10.9, 3 df). In general, the results of pairwise tests for homogeneity of allelic frequencies between samples were highly significant, while genetic distances were small to moderate compared to other studies of this species (FERGUSON 1989) (Table 3). Tests for homogeneity of allelic frequencies at different levels, among all samples, among samples from the Lake Hald area, and among samples outside Lake Hald all yielded highly significant results (Table 4).

Genetic differentiation in terms of G_{ST} was observed among the Lake Hald populations while only moderate differentiation was observed among the other populations (Table 4). A hierarchical gene diversity analysis showed that the major part of genetic differences between populations was distributed between the Lake Hald tributary populations and the populations outside Lake Hald (Table 4).

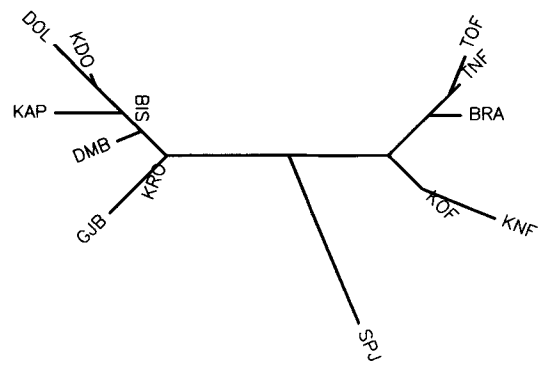

Fig. 3. Restricted maximum likelihood tree, constructed with the program CONTML from the PHYLIP package (FELSENSTEIN 1989), to summarize the genetic relationships among the sampled populations. SPJ denotes the hatchery strain which was used for stocking directly into Lake Hald. The tree is based on allelic frequencies of the loci *$_s$AAT-1,2**, *$_s$AAT-4**, *CK-A1**, *DIA-1**, *G3PDH-2**, *GPI-A1**, *$_s$IDHP-1**, *LDH-A1**, *$_s$MDH-A2**, *MPI-1**, and *PGDH-1**.

The program for constructing maximum likelihood trees was run 30 times and the tree with the maximum likelihood is presented in Fig. 3. The tree supported the dichotomy between populations within and outside the Lake Hald area which was

Table 3. Above diagonal: Nei's (1978) genetic distance between samples. Below diagonal: Pairwise contingency tests for homogeneity of allelic frequencies between samples (degrees of freedom in parentheses)

	KOF	KNF	TOF	TNF	BRA	KRO	BIS	KDO	KAP	GJB	DOL	DMB
KOF		0.002	0.005	0.004	0.003	0.008	0.017	0.024	0.019	0.016	0.029	0.015
KNF	$19.5_{(7)}$		0.005	0.005	0.007	0.012	0.023	0.029	0.020	0.020	0.037	0.021
TOF	$38.6_{(7)}$ ***	$41.1_{(7)}$ ***		0.000	0.001	0.012	0.018	0.018	0.014	0.018	0.025	0.017
TNF	$25.2_{(7)}$ **	$33.0_{(7)}$ **	$1.6_{(5)}$		0.002	0.012	0.018	0.019	0.015	0.017	0.026	0.017
BRA	$29.6_{(8)}$ ***	$45.2_{(8)}$ ***	$17.7_{(7)}$	$15.7_{(7)}$		0.007	0.012	0.011	0.013	0.012	0.019	0.012
KRO	$51.4_{(8)}$ ***	$68.9_{(8)}$ ***	$74.0_{(7)}$ ***	$67.7_{(7)}$ ***	$50.5_{(8)}$ ***		0.002	0.009	0.005	0.004	0.012	0.002
BIS	$77.4_{(8)}$ ***	$96.4_{(8)}$ ***	$79.0_{(7)}$ ***	$75.6_{(7)}$ ***	$61.3_{(8)}$ ***	$14.9_{(7)}$		0.004	0.002	0.009	0.003	0.000
KDO	$102.2_{(8)}$ ***	$114.9_{(8)}$ ***	$72.6_{(7)}$ ***	$77.3_{(7)}$ ***	$63.8_{(8)}$ ***	$48.6_{(7)}$ ***	$42.4_{(7)}$ ***		0.007	0.016	0.001	0.007
KAP	$85.8_{(8)}$ ***	$88.0_{(8)}$ ***	$59.2_{(7)}$ ***	$61.4_{(7)}$ ***	$60.5_{(8)}$ ***	$27.4_{(7)}$ **	$14.2_{(7)}$	$28.2_{(7)}$ **		0.014	0.008	0.005
GJB	$69.5_{(8)}$ ***	$85.9_{(8)}$ ***	$84.2_{(7)}$ ***	$71.3_{(7)}$ ***	$62.6_{(8)}$ ***	$29.8_{(7)}$ **	$41.6_{(7)}$ ***	$67.3_{(7)}$ ***	$54.1_{(7)}$ ***		0.020	0.006
DOL	$132.8_{(8)}$ ***	$156.4_{(8)}$ ***	$108.0_{(7)}$ ***	$113.0_{(7)}$ ***	$93.9_{(8)}$ ***	$79.7_{(7)}$ ***	$28.4_{(7)}$ **	$11.3_{(6)}$	$41.5_{(7)}$ ***	$111.7_{(7)}$ ***		0.006
DMB	$73.4_{(8)}$ ***	$95.3_{(8)}$ ***	$83.1_{(7)}$ ***	$77.2_{(7)}$ ***	$66.8_{(8)}$ ***	$17.3_{(7)}$	$8.4_{(7)}$	$39.0_{(6)}$ ***	$29.8_{(7)}$ **	$33.8_{(7)}$ ***	$40.3_{(6)}$ ***	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 4. Gene diversity analyses and tests for homogeneity of allelic frequencies among samples. G_{ST} denotes the gene diversity distributed between subpopulations within the total. G_{SG} denotes gene diversity distributed between subpopulations within the two major groups: Samples from Lake Hald and samples from outside Lake Hald. G_{GT} denotes gene diversity distributed between the two major groups within the total. Total 1 denotes the result of the hierarchical gene diversity analysis when the geographical groups of populations are given equal weight. Total 2 denotes the result of the same analysis when the two groups are weighted according to the number of populations they contain

Area	G_{ST}	G_{SG}	G_{GT}	χ^2	df
Lake Hald	4.7 %			239.7***	42 #
Outside Lake Hald	2.6 %			124.2***	28 §
Total 1	8.9 %	2.2 %	6.7 %	554.3***	77 £
Total 2	8.9 %	3.6 %	5.3 %		

*** $p < 0.001$

\# Test based on *$_{s}$AAT-1,2**, *$_{s}$AAT-4**, *CK-A1**, *G3PDH-2**, *$_{s}$IDHP-1**, *$_{s}$MDH-A2**, and *MPI-1**

§ Test based on *$_{s}$AAT-1,2**, *$_{s}$AAT-4**, *G3PDH-2**, *$_{s}$IDHP-1**, *LDH-A1**, *$_{s}$MDH-A2**, and *MPI-1**

£ Test based on *$_{s}$AAT-1,2**, *$_{s}$AAT-4**, *CK-A1**, *G3PDH-2**, *$_{s}$IDHP-1**, *$_{s}$MDH-A1**, and *MPI-1**

suggested by the hierarchical gene diversity analysis. Within Lake Hald the topology of the tree was mainly in accordance with the geographical location of the sampled locations.

Discussion

Genetic variation

The isolated populations did not appear to have suffered a loss of heterozygosity compared to populations open to gene flow (Table 2). The populations in the accessible parts of Klokkedals å (KNF) and Tirsbæk (TNF) can be considered representative of the isolated populations (KOF and TOF) in the same rivers *before* the dams were built. In both cases average expected heterozygosities were very similar. Likewise, in the case of Lake Hald heterozygosity values of the same order of magnitude as in the accessible populations were found. It could be argued that the reason for this was a lack of gene flow even to the accessible populations. The brown trout may well exhibit strong genetic differentiation, but in light of the studies of homing and "straying" of trout and other Salmonids (e.g., SVÄRDSON and FAGERSTRÖM 1982) we find this argument less valid.

It could also be argued that heterozygosity is not an optimal indicator of loss of genetic variation as this measure on a short-term basis is relatively insensitive to a lowering of effective population size

(N_e) (FRANKEL and SOULÉ 1981). However, reduction of heterozygosity, presumably caused by low N_e, has been reported for both hatchery strains (e.g., RYMAN and STÅHL 1980) and natural populations (KARAKOUSIS and TRIANTAPHYLLIDIS 1988).

In general the number of variable loci and, in particular, the number of rare alleles is considered a more sensitive indicator of loss of genetic variation (FRANKEL and SOULÉ 1981). The number of variable loci was very constant in all samples (Table 2). Several "rare" alleles (frequency <0.05) were found in the samples, but due to the relatively small sample sizes we do not want to put too much emphasis on this point.

No differences in allelic frequencies between age classes in BRA and DOL were observed. The deviation in KRO was largely due to one locus, *MDH-2**, which may be explained as a type 1 error.

Thus, the N_e's of the isolated populations have obviously been sufficiently high to prevent loss of genetic variation (and, presumably, inbreeding effects) even though the habitats are small and have been isolated for many generations. A plausible explanation of this may be that spawning of mature male parr has kept N_e high (L'ABÉE-LUND 1989) as mature males of age 1+ and 2+ were found in all samples. For *Oncorhynchus nerka* it has been found that small mature males in general exhibit a higher level of heterozygosity and thus act as a genetic reserve for the population (ALTUKHOV 1990). From the present data it was not possible to decide if the same phenomenon applies to brown trout, but if this is the case N_e may well have been low but loss of genetic variation yet may have been avoided. Anyhow, the levels of genetic variation do not indicate a need to introduce further variation in the form of stocked fish, and the isolated populations do not seem to be immediately endangered for genetic reasons.

Genetic differentiation

The dams in Tirsbæk and Klokkedals å could not be shown to have caused any genetic differences between the populations above and below the weirs (Table 3, Fig. 3). The populations do therefore not seem to have diverged much from each other following habitat fractionating. In Klokkedals å this may be partly explained by the occasional transplantation of spawners from below to above the dam.

The Tirsbæk and Brandstrup bæk populations are genetically very similar to each other (Table 3, Fig. 3). The rivers are separated by considerable geographical distance, but lack of correlation between genetic and geographic distance is often found in brown trout (RYMAN 1983).

In spite of the restricted geographical area the tributary populations of Lake Hald obviously did not constitute one panmictic population (Table 4). The pairwise tests for homogeneity of allelic frequencies between samples (Table 3) show that several different stocks were present. Three main groups could be identified: GJB, KDO-DOL, and KRO-BIS-DMB-KAP, although the latter group could not be said to be homogenous (chi-square value: 57.11, 21 df, $p < 0.001$). The G_{ST} value of 4.7 % is rather low compared to most other brown trout studies. Some examples are a G_{ST} value of 31 % from a study of trout in the Lough Neagh catchment, Northern Ireland (CROZIER and FERGUSON 1986), and a G_{ST} value of 11 % from a study of trout in the Limfjord catchment, Denmark (LANDBO and PERSSON 1987). However, these studies comprise much larger geographical areas. Therefore the genetic differentiation among the tributary populations of Lake Hald must be considered rather pronounced. When gene flow was calculated according to the n-Island Model:

$$G_{ST} = 1/(1 + 4N_e m[n/n - 1]^2)$$

(TAKAHATA and NEI 1984), where n denotes number of populations, N_e effective population size, and m migration rate, the result was: $N_e m = 3.7$. As non-significant constellations of samples were not pooled, actual gene flow is probably even less. The result compares well with calculated gene flows from studies of trout in e.g., Lake Lulejaure, Sweden ($N_e m = 3.4$; ALLENDORF 1983) and the Limfjord catchment, Denmark ($N_e m = 3.4$; LANDBO and PERSSON 1987).

Concerning the genetic status of the Lake Hald trout in relation to the other populations, the hierarchical gene diversity analysis (Table 4) and the phylogenetic tree (Fig. 3) suggest a relatively strong divergence. If the two groups of the hierarchical gene diversity analysis were weighted according to the number of populations, this divergence became a little less pronounced. However, though statistically sound this procedure may have less biological relevance in this particular case as the group of populations "outside Lake Hald" is given least weight but covers a much larger geographical area. The possibly special status of Lake

Hald trout is further supported by the high frequencies of *GPI-A2*n* in some of the samples (Table 2).

Transplantation and stocking activities

As mentioned previously intensive stocking has taken place directly into Lake Hald. Perhaps the high levels of genetic variation in the tributaries could be explained by this stocking. In the hatchery strain (Spjarup) used for stocking, the frequencies of *LDH-A1*n* and *DIA-1*90* were 0.193 and 0.098, respectively (SIMONSEN and RASMUSSEN 1989). Only one *DIA-1*90* heterozygote was found in the Lake Hald samples, and *LDH-A1*n* was not present at all. Supposing a genetic contribution of the stocked fish to the native gene pools of 50 %, 10 %, or 5 %, the probability of observing one or zero *DIA-1*90* alleles would be 1.2×10^{-10}, 0.037, and 0.277, respectively, and the probabilities of observing no *LDH-A1*n* alleles would be 1.2×10^{-23}, 4×10^{-5}, and 0.006, respectively. Thus, despite the large number of stocked fish the native populations appear to be almost unaffected although we cannot rule out a very modest rate of introgression. No substantial gametic phase disequilibria could be detected employing the method described by WEIR and COCKERHAM (1979) (data not listed). Major differences in allelic frequencies between stocking material and wild fish would result in gametic phase disequilibria being generated as a result of introgression (CAMPTON 1987). The lack of influence of the stocked fish is further supported by the phylogenetic tree (Fig. 3) where the hatchery strain has been included.

The apparently unsuccessful stocking (or successful from the viewpoint of conservation) directly into the lake could reflect lack of adaptation of the hatchery strain to local conditions combined with the method of stocking. Presumably, the fish are not imprinted to any of the tributaries and, as a consequence, aberrant or ineffective spawning and migratory behavior is expected (HANSEN et al. 1987; JONSSON et al. 1990). Examples of unsuccessful transplantations of stocks of Salmonid species due to the inadaptive nature of transplanted stocks are given by, i.a., ALTUKHOV and SALMENKHOVA (1987) and MORAN et al. (1991) while studies by TAGGART and FERGUSON (1986) and HAUSER et al. (1991) suggest at least some introgression of stocked fish into native gene pools.

From Fig. 3 and Table 3 it appears that DOL has been colonized by spawners from the neighboring river KDO. It also seems that the transplantation of trout from KRO to DMB in 1983 was successful. As KRO, DMB, BIS, and KAP in general are closely related, colonization of DMB by spawners from, in particular, the neighboring river BIS might provide an alternative explanation. However, electrofishing in DMB in 1983, 1984, and 1985 revealed that the transplanted trout were able to survive in the river (Inland Fisheries Laboratory, unpublished results) and therefore probably are the founders of this population. The contrast to the failure of introducing hatchery trout to the lake would suggest that the native trout show some adaptation to local conditions although the different circumstances in connection with the stocking must also be considered.

In conclusion, none of the isolated populations had suffered a detectable loss of genetic variation, and introduction of non-native trout did not seem to be immediately necessary. However, if stocking was to be considered it should not exceed the gene flow that would occur under natural conditions, i.e., about 3–4 trouts per population per generation (e.g., ALLENDORF 1983; LANDBO and PERSSON 1987; this study). In that way the integrity of individual stocks could still be maintained (RYMAN 1991). Further, stocking activity and other sorts of fishery management in isolated areas of restricted size, like Lake Hald, should be subject to much care as native trout may perhaps be adapted to local conditions and as, even in such small areas, genetic differentiation among tributary populations may be found.

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