doi:10.1111/j.1420-9101.2004.00844.x

Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway

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Keywords:

Fst;
gene flow;
gill-rakers;
microsatellites;
niche;
polymorphism;
Qst;
speciation.

Abstract

Combining morphological and genetic analysis, we compared patterns of diversification within and between morphs among sympatric European whitefish (*Coregonus lavaretus* L.) populations in Lake Femund, Norway. Seven external populations, from potential colonization routes into Lake Femund were included. We found that deep-, shallow-, river- and bay spawning populations are distinct morphs in Lake Femund. Within morphs, populations range from being similar genetically (Fst = 0–0.005) among deep-spawning populations to being highly differentiated (Fst = 0.153) between bay-spawning populations. Between morphs, genetic differences ranged from a low (Fst = 0.008–0.022) between deep- and shallow-spawning populations to high difference (Fst = 0.125–0.143) between shallow- and bay-spawning populations. A higher proportion of molecular variance was seen among (3.9%) than within morphs (2.8%). The adaptive gene combinations behind the four morphs seem to have originated within the lake, although the lake could have been colonized from more than one source population.

Introduction

Understanding the mechanisms of species formation is a central theme in evolutionary biology, partitioning the roles of historical contingency and deterministic interactions of evolutionary processes (Losos *et al.*, 1998; Taylor & McPhail, 2000; Mayr, 2001; Turelli *et al.*, 2001; Hendry *et al.*, 2002). Species complexes within the genera *Coregonus*, *Gasterosteus*, *Oncorhynchus*, *Osmerus*, *Prosopium*, *Salmo, Salvelinus* and *Lepomis* frequently display intralacustrine diversification in morphology, life history, habitat and diet (Svärdson, 1979; Robinson & Wilson, 1994; Taylor, 1999; Jonsson & Jonsson, 2001). For some of these species complexes, both scenarios of allopatric and sympatric speciation have been proposed (Pigeon *et al.*, 1997; Taylor, 1999; Taylor & McPhail, 2000). The occurrence of such sympatric populations represents a challenge to taxonomists, population geneticists and management authorities as it may be imperative to sort out the current status of populations and their evolutionary origin prior to implementation of conservation plans (Bernatchez, 1995; Nielsen & Powers, 1995; Schluter, 2000, 2004).

The European whitefish species complex (*Coregonus lavaretus* L.) illustrates the full range of problems associated with the interpretation of morphological diversity. Here, the traditional species designations suggested that numerous whitefish species inhabit European waters (Linnaeus, 1758; Steinmann, 1950a, b, 1951; Svärdson, 1957, 1998; Berg, 1962; Resethnikov, 1968; Himberg & Lehtonen, 1995; Kottelat, 1997). However, the phenotypic species classification poses two fundamental problems (see Felsenstein & Sober, 1986; Humphries & Parenti, 1999). First, a classification may be limited by the available number of traits to assess the phylogenetic relationship between any closely related taxa, and in addition problems arise when assessing the relative importance of these traits. Secondly, comparisons based on traits that are subjected to natural selection could

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erroneously result in the clustering into a monophyletic group when parallelism is more likely. Alternatively, the use of molecular markers such as microsatellite DNA could provide an unbiased record of recent population divergence, while maternally inherited mitochondrial DNA (mtDNA) in nonhybridizing populations reflects evolutionary history, thus contrasting potential selection with the neutral genetical history (Li, 1997; Goldstein & Schlötterer, 1999).

For a given polymorphic whitefish system, at least three nonexclusive, evolutionary scenarios may explain diversity among sympatric populations (Smith & Todd, 1984). First, intra-lacustrine radiation and sympatric speciation may result from an adaptive diversification of a gene-pool into divergent niches (Schliewen *et al*., 1994, 2001; Pigeon *et al*., 1997; Lu & Bernatchez, 1999; Schluter, 2000). Secondly, colonizations by already differentiated genetic lineages could result in persistence of gene-pools, the emergence of new genetic adaptive variants, or introgression and subsequent breakdown of the adaptive gene combinations (Lu *et al*., 2001). Thirdly, phenotypic plasticity in a single gene-pool could stem from environmental induction by e.g. differential habitat preference or by developmental responses to diet (Tåning, 1958; Skúlason & Smith, 1995; Day & McPhail, 1996; Robinson & Pearson, 2002).

Adaptive divergence may evolve with or without gene flow (Haldane, 1948; Slatkin, 1973; Rice & Hostert, 1993; Liou & Price, 1994; Campbell & Bernatchez, 2004). In a divergence with gene flow scenario, adaptive differentiation results from natural selection outweighing the homogenizing effect of gene flow. In contrast, a single population could very rapidly split into two reproductively isolated populations if a chance dispersal into a new spawning area was successful, and homing to spawning sites was perfect. By comparing the quantitative trait divergence with neutral allele frequencies it is possible to indicate which traits that are likely driven by natural selection and which traits that likely have no adaptive value and hence are structured by genetic drift or reflect phenotypic plasticity (Wright, 1951; Spitze, 1993; Schluter, 2000). Given the short evolutionary time in northern lakes since the Weichselian glaciation, populations may still be evolving towards the habitat-specific optimal phenotype (Hendry *et al*., 2002).

In Lake Femund, Norway, local fishermen have long recognized several whitefish forms (Næsje *et al*., 1992). The taxonomy of the polymorphic whitefish in this lake is, however, not clear. Huitfeldt-Kaas (1918) suggested that a large bodied and a small bodied whitefish race co-occurred in the lake, whereas Svärdson (1957) suggested based on gill-raker numbers, that four whitefish species inhabited the lake. Comparing with other Scandinavian whitefish species, he also suggested that two of these species could have introgressed. More recent surveys suggested the presence of at least three whitefish morphs based on gill-raker numbers and growth differences (Sandlund & Næsje, 1989). Næsje *et al*. (1992) observed concordance between groups being classified according to gill-raker counts and the genetic distances, based on allozymes, among 11 spawning populations sampled from rivers, and shallow and deep waters. However, genetic heterogeneity were found within some of these gill-raker groups, implying the presence of further population sub-structuring.

On this background, we performed a combined morphological and genetic study of the whitefish in Lake Femund to quantify divergence and to study the evolutionary origin of populations and morphs. We sampled 11 known spawning populations that are separated by habitat and time of spawning. We describe patterns of differentiation in morphometric, meristic, and life-history traits, and compare these patterns with the distribution of genetic variation using microsatellites. Based on a combination of measures, we suggest the best grouping of populations into morphs inhabiting Lake Femund. In search of the traits most likely structured by natural selection, we compared the level of differentiation based on phenotypic variance of quantitative traits with that of molecular differentiation between the morphs, and between populations within morphs. Finally, to elucidate the most probable origin of whitefish populations and morphs in Lake Femund, we compared morphological and genetical characteristics with the seven externally sampled populations in the four major water drainages that potentially could have supplied founders to Lake Femund.

Material and methods

Study area and sampling

Lake Femund, 662 m a.s.l. and 204 km^2, is located in the southern part of Norway (62°00′N, 11°55′E) (Fig. 1, Table 1). The lake has two basins with maximum depths of 134 and 90 m, respectively. More than half the lake have depths <20 m mainly in large, shallow bays. The lake is ultra-oligotrophic (Løvik & Kjellberg, 1982), and forms the headwater of the catchment area of River Trysilelva/Klarälven, which drains southeast into Lake Vänern in southern Sweden, and further through River Götaälv to the North Sea. The fish fauna comprises European whitefish, Arctic charr (*Salvelinus alpinus*), brown trout (*Salmo trutta*), pike (*Esox lucius*), perch (*Perca fluviatilis*), burbot (*Lota lota*), grayling (*Thymallus thymallus*) and European minnow (*Phoxinus phoxinus*). In September to December 1997, we sampled 11 spawning populations in Lake Femund, which comprised four groups according to spawning time and site (Fig. 1, Table 2).

We also sampled seven whitefish populations (subsequently termed ‘external populations’) from six lakes situated in the four watercourses that might have been

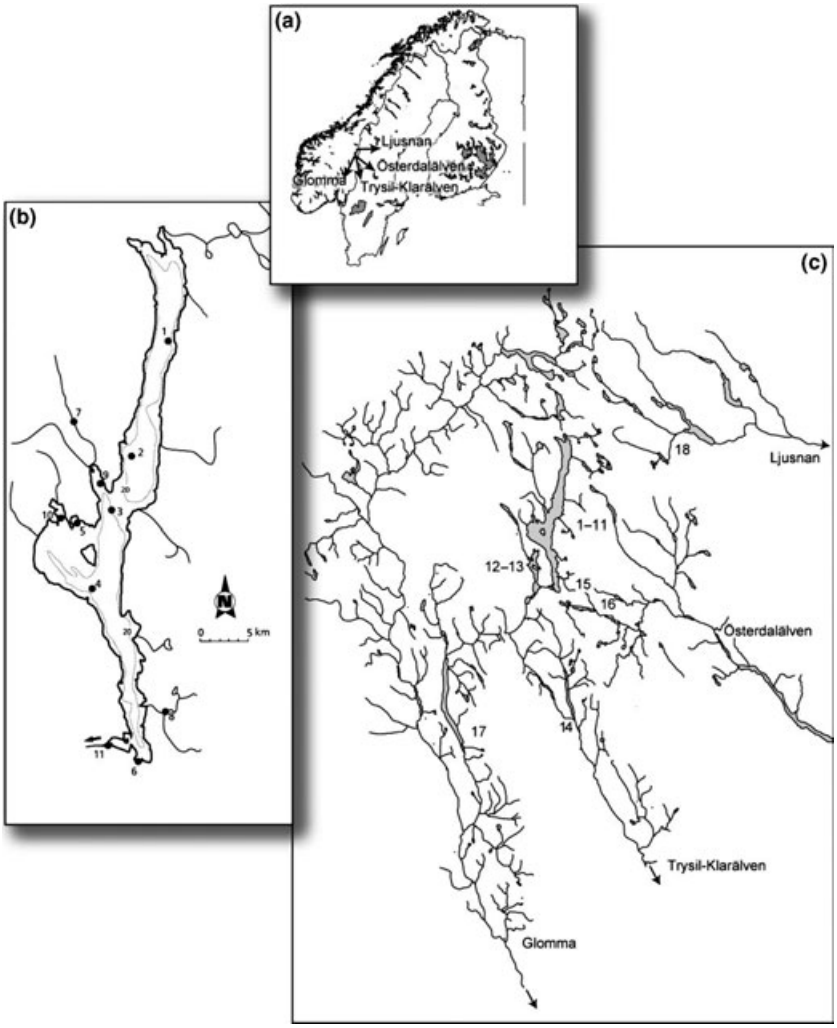

Fig. 1 Geographical information of the 18 studied whitefish populations. (a) Map of Fennoscandia with the four main drainages, (b) location of the eleven spawning populations in Lake Femund, with depth contours (<20 m), (c) the total seven study lakes.

Table 1 Geographical, morphometric and biological data of study lakes (population numbers refer to map code in Fig. 1).

Population-Lake	Main river	*n*	Altitude (m.a.s.l)	Area (km^2)	Maximum depth (m)	St	Tt	Ll	Pf	El	Pp	Sa	Rr	Cp	Ca
1–11. Femund	Trysil-Klarälven	509	662	204.2	134	x	x	x	x	x	x	x	–	–	–
12–13. Isteren	Trysil-Klarälven	60	645	28.9	20	x	x	x	x	x	x	x	–	–	–
14. Engeren	Trysil-Klarälven	45	472	14.0	85	x	x	x	x	x	x	x	–	–	–
15. Drevsjøn	Österdalälven	45	668	0.98	11	x	x	x	x	x	x	x	x	–	–
16. Vurrusjøn	Österdalälven	45	663	4.97	20	x	x	x	x	x	x	x	x	–	–
17. Storsjøen	Glomma	30	251	48.6	309	x	x	x	x	x	x	x	x	x	–
18. Lossnen	Ljusnan	29	540	360	85	x	x	x	x	x	–	–	–	x	x

n, Sample size of whitefish.

Species: St, Brown trout (*Salmo trutta*); Tt, Arctic grayling (*Thymallus thymallus*); Ll, Burbot (*Lota lota*); Pf, Eurasian perch (*Perca fluviatilis*); El, Northern pike (*Esox lucius*); Pp, Minnow (*Phoxinus phoxinus*); Sa, Arctic charr (*Salvelinus alpinus*); Rr, Roach (*Rutilus rutilus*); Cp, Siberian bullhead (*Cottus poecilopus*); Ca, Vendace (*Coregonus albula*).

potential post-glacial immigration routes for fish to Lake Femund (Fig. 1, Table 1). Lakes Isteren and Engeren are downstream in the Trysilelva watercourse. Lake Storsjøen is in River Glomma, Lakes Vurrusjøen and Drevsjøen are in River Dalälven, and Lake Lossnen is in River Ljusnan. The Trysilelva and Glomma watercourses

Table 2 Summary data of the 11 spawning populations from Lake Femund with spawning habitat, depth and nominal spawning time.

Map code	Population	Spawning			*n*
		Habitat	Depth (m)	Time	
1	Storvika	Deep	35	November–December	45
2	Hullet	Deep	60	November–December	36
3	Vestfjorden	Deep	30	November–December	56
4	Joneset	Deep	35	November–December	45
5	Hallsteinvika	Shallow	4–5	October–November	49
6	Femundsenden	Shallow	2–5	October–November	42
7	Tufsinga	River	1–2	September–October	45
8	Sorkelva	River	1–2	September–October	56
9	Tjønnan	River	2–4	September–October	45
10	Kvernvika	Bay	2–4	November–December	45
11	Gløtfossen	Bay	2–5	November–December	45

n, The number of analysed fish.

drain to the North Sea, whereas the rivers Dalälven and Ljusnan drain to the Baltic Sea. Lake Isteren harbours two whitefish morphs separated in body size, whereas the other lakes, to our knowledge, have monomorphic whitefish. The composition of the fish fauna of the lakes is shown in Table 1. All the fish species are natural colonizers (Huitfeldt-Kaas, 1918), and stocking of whitefish had not occurred in these lakes.

Multi-meshed bottom gill nets were used to collect all samples. In Lake Femund and Lake Storsjøen, all fish were sampled on the spawning grounds as sexually mature individuals. In the other locations, fish was sampled in early fall and represent a mixture of maturity stages. The number of fish in each sampled population ranged between 29 and 56, adding up to a total material of 756 fish (Fig. 1, Tables 1 and 2). All fish were frozen individually in plastic bags at −20 °C prior to conducting all the analysis.

Morphology

Scale length was selected as the best measurement of the body length as natural length and fork length were less reliable because of shearing of fins (Fig. 2a). Using a digital caliper, we measured 17 morphometric traits (Fig. 2b–f); head length (HEL), head height (HEH), preorbital length (PRL), orbital length (ORL), orbital height (ORH), post-orbital length (POL), upper jaw length (UPL), lower jaw length (LOL), inter-orbital breadth (INB), snout width (SNW), snout height (SNH), upper jaw width (UPW), upper gill arch length (UGL), lower gill arch length (LGL), length of gill-raker in the angle between upper and the lower gill arch (GIL), and length of intestine (INL). To measure upper and lower mandibula and calculate the difference between upper and lower bite (ULO), we used a mm-scaled cone (60°) inserted into the mouth of the fish (Fig. 2d). Three meristic traits were counted on the left side only (Fig. 2a,e); number of lateral line scales (LAS), number

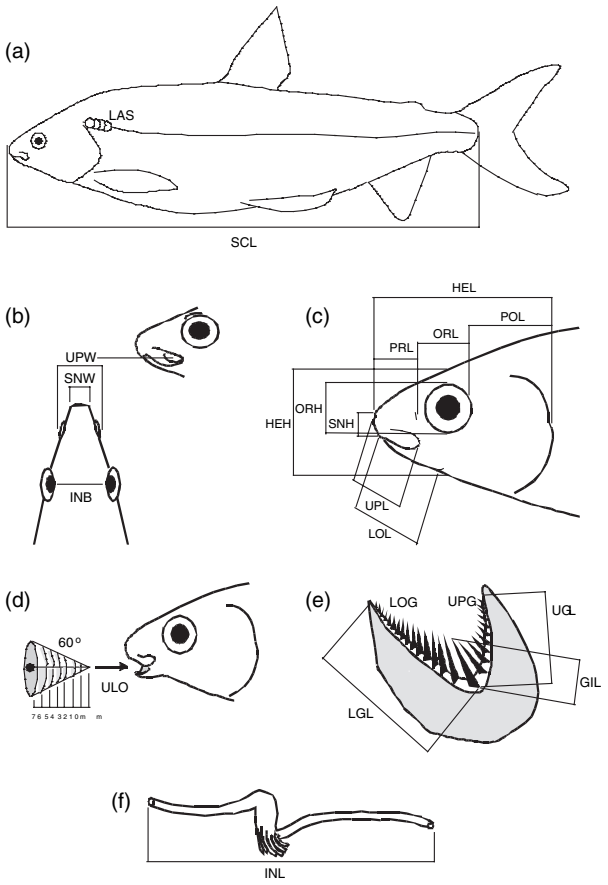

Fig. 2 Scored traits using a digital caliper. In (a), scale length (SCL) was used as body length measure, and lateral line scales (LAS) was counted along lateral line, in (b) upper jaw width (UPW) was measured on the middle of the upper mandibula, in (c) orbital height (ORH) was measured between inner and outer eye socket, whereas orbital length (ORL) included the preorbital skin-fold until end of socket, in (d) a cone of 60° was inserted into the mouth measuring the bite length for the upper and lower mandibula giving upper and lower jaw length (ULO), in (e) upper gill arch length (UGL) and lower gill arch length (LGL) extend from base of gill-raker length (GIL) until end of gill-arch rim, and GIL from base to top of raker, in (f) the measure of intestine length (INL) was taken on a fully thawed dissected and stretched intestine (from the mouth to the anal pore).

of gill-rakers on upper gill arch (UPG), and number of gill-rakers on lower gill arch (LOG).

Life-history

All fish in Lake Femund were aged using otoliths (Skurdal *et al.*, 1985), sexed and classified as mature or immature based on gonad development (Nikolskii, 1963). Mean length-at-age of the 11 Lake Femund populations was described by the vonBertalanffy model (Bagenal & Tesch, 1978). We calculated the mean age of spawners in each of the populations in Lake Femund as an indication of life longevity.

Genetic markers

DNA was extracted from pectoral fins or body muscle using the Phenol/Chloroform procedure (Sambrook *et al.*, 1989). Six microsatellite loci were analysed; *SsBgIIIM.26* (Goodier & Davidson; Genbank acc. no. U10051), *Cocl-23* (Rogers *et al.*, 2004), *C2-157* (Turgeon *et al.*, 1999), *Bwf1* (Patton *et al.*, 1997), *Bwf2* (Patton *et al.*, 1997), and *C1-g* (Turgeon *et al.*, 1999). Unpublished mapping data confirm that these loci are on separate linkage groups (S. Rogers, Université Laval, Québec, Canada, unpublished data). The PCR amplifications of each microsatellite locus were performed in a 12 μL reaction volume of 0.3 units of Taq polymerase, 1.25 μL reaction buffer [19 mM Tris-HCL (pH 9.0), 0.1% TritonX-100, 50 mM KCL], 0.6 μL of 1.5 mM $MgCl_2$, 750 μmol of dNTPs, with 0.03 pmol of each two primers, using 25–50 ng template DNA (2 μl). All loci were run in separate PCR's on a Perkin-Elmer 9600 thermocycler (version 2.01; Perkin-Elmer, Boston, MA, USA) according to the following programme: initial denaturation for 2 min at 95 °C, followed by 30 cycles [1 min at 94 °C, 45 s at locus-specific annealing temperature °C (see original references), 30 s at 72 °C], and 10 min at 72 °C. All loci were genotyped on the ABI 377 sequencer (Perkin Elmer) using the fluorescent dye detection, where one primer of each locus was 5′-labelled with one of three fluorescent dyes, allowing six loci to be scored simultaneously as two loci with the same dye had nonoverlapping allele size. Allele sizes were scored using GENESCAN 2.1 (AppliedBiosystems, 1996a) using an internal size standard (Tamra^{TM} 350 bp), and were compared with a standard sample in each gel. The final scoring of the allelic sizes was performed three times for each individual using GENOTYPER 2.0 (AppliedBiosystems, 1996b).

Data analysis

As most morphometric and meristic traits were significantly related to individual size, traits were allometrically scaled prior to a statistical analysis using scale length (SCL: two measures), head length (HEL: 14 measures), lower gill arch length (LGL: two measures), and upper gill arch length (UGL: one measure) for standardization (Reist, 1985). First, we $\log_{10}$ transformed traits in order to homogenize variance. Then, we estimated the common within-group regression slope between each $\log_{10}$-trait with $\log_{10}$-SCL, $\log_{10}$-HEL, $\log_{10}$-LGL, or $\log_{10}$-UGL using an ANCOVA without interaction. In this step, and the following, all 18 populations were used in the allometric scaling. Slopes were used to correct each fish to the overall comparative standardized mean fish size using the mean values of the $\log_{10}$-transformed SCL (1.47), HEL (1.79), LGL (1.36) or UGL (1.41) as incorporated into the following formula (see Hendry *et al.*, 2002):

$$M_{\mathrm{std}} = M_{\mathrm{o}}(L_{\mathrm{x}}/L_{\mathrm{o}})^{b}$$

where M is the trait size, L is trait used for scaling, b is the ANCOVA slope with the interaction term removed, and subscripts std, o and x refer to standardized, observed and mean values of traits. For the meristic traits, three populations had a significant correlation between LAS × SCL, and four populations had a correlation between UPG × SCL and LOG × SCL, concordantly we size-corrected traits by SCL, LGL and UGL.

Morphological variation was assessed by a canonical linear discriminant analysis of allometrically scaled metric and meristic traits using JMP 5.0 (SAS, 2002). This analysis uses Mahalanobis distance to estimate each individual's distance from the group multivariate means. A stepwise forward variable selection, entering one trait at time, was performed where trait entering order represents discriminating power. This analysis was used to describe pattern of divergence in the 11 Lake Femund populations, and to find the most likely colonizer of Lake Femund, forcing each fish from Lake Femund into the seven external populations from the likely immigration routes using K-means clustering in JMP 5.0 (SAS, 2002).

Life-history variation was documented modelling the population specific growth curves (vonBertalanffy's growth model, Bagenal & Tesch, 1978) for the 11 Lake Femund populations. Populations with nonoverlapping confidence intervals (95% CI) for the asymptotic length (L_{∞}) were considered as significantly different.

Genetic variation was described as microsatellite allele frequency, observed heterozygosity (H_{O}), expected heterozygosity (H_{E}), and by F-statistics using Genepop 3.3 (Raymond & Rousset, 1995). Allelic richness, i.e. allele number corrected for sample size (El Mousadik & Petit, 1996), was quantified using FSTAT 2.9.3 (Goudet, 1995). Deviation from Hardy–Weinberg equilibrium was tested by the exact (probability) test for each locus and population (Guo & Thompson, 1992), and P-values were corrected using the sequential Bonferroni method for each locus ($\alpha = 0.05$; $k = 18$).

Population differentiation was estimated using the log-likelihood based exact test on contingency tables of genotypes (rather than on the alleles as deviation from H-W equilibrium was found in several cases). Tests were made across populations, and between pairs of populations, using Genepop 3.3, and combined across loci using Fisher's combined probabilities. Genetic relationships within Lake Femund were also studied using assignment tests where each individual was assigned to the closest population based on its genetic distance (D_{A}; Nei *et al.*, 1983) to all populations in GeneClass 1.0 (Cornuet *et al.*, 1999). This distance method can be used regardless of deviations from Hardy–Weinberg equilibrium (Cornuet *et al.*, 1999).

The distribution of allelic variation was quantified by intergroup components of total variance by ϕ statistics using a hierarchical analysis of molecular variance, AMOVA, in Arlequin 2.0 (Excoffier *et al.*, 1992; Schneider *et al.*, 2000). In this way, variance components among

morphs, among populations within morphs, and within populations were estimated in Lake Femund.

Genetic relationships between Lake Femund whitefish spawning populations and the external populations were assessed by two methods. First, we used an assignment test to find the most likely origin of the eleven Lake Femund populations, by fitting each individual into one of the surrounding reference populations using the D_A distance. Secondly, we performed a principal component analysis in PCA-GEN 1.2 (Goudet, 1999; http://www2.unil.ch/popgen/softwares/pcagen.htm) to visualize genetic relationships between the Lake Femund whitefish populations and the seven external populations. Significance of the PC1 and PC2 axis was estimated by 1000 randomizations of individuals among populations.

In order to explore the possible role of natural selection in shaping morphological divergence between and within the suggested Lake Femund morphs, we compared the extent of morphological divergence (Qst) with that based on molecular divergence (Fst) using methods from Spitze (1993). These measures should be equal if the underlying loci are selectively neutral, and if the quantitative traits have an additive genetic basis (Wright, 1951; Schluter, 2000; Lópes-Fanjul *et al*., 2003). We used phenotypic variance as a surrogate for the additive genetic variance for phenotypic traits (Merilä *et al*., 1997; Merilä & Crnokrak, 2001), and estimated the phenotypic variance components following procedures in Bernatchez (2004). Qst for each trait was estimated between spawning populations, and also between the suggested morphs. The Qst and Fst values were significantly different if their 95% confidence intervals did not overlap. Confidence intervals for all pairwise Fst values were calculated from the standard error of Fst among all eleven populations, using FSTAT 2.9.3 (Goudet, 1995). We are fully aware that Qst estimates without quantitative genetics information is only tentative (e.g. Lópes-Fanjul *et al*., 2003), but we use this method merely to suggest traits most likely targeted by selection. In this particular case also, comparing morphs within Lake Femund will reduce environmental effects as compared with studies across lakes (Bernatchez, 2004).

Results

Morphological divergence of populations in Lake Femund

There were significant differences for all the 20 studied traits when analysed over all the 18 populations (Appendix 1). In Lake Femund, all 20 traits also differed significantly among eleven spawning populations (ANOVA, $R^2 = 0.06$–0.85, $P < 0.05$). The five most differentiated traits, based on R^2 values, were the number of lower gill rakers (LOG, $R^2 = 0.85$, range of population means: 18.0–28.7), upper gill rakers (UPG, $R^2 = 0.83$, 10.4–13.4), gill-raker length (GIL, $R^2 = 0.76$, 5.29–9.33), lower jaw length (LOL, $R^2 = 0.49$, 22.5–25.4), and upper jaw length (UPL, $R^2 = 0.29$, 16.4–18.3).

The canonical discrimant analysis of the eleven Lake Femund populations was significant with 20 traits combined (Pillai's trace = 2.87, $P < 0.0001$), with only three traits not contributing significantly to this model (Table 3, Fig. 3). Based on the forward stepwise variable selection, the highest discriminating power was found in LOG, GIL, ULO, UGL and SNH (see entering order in Table 3). The first canonical axis (CA1) explained 79.5% of the total variation in the material, where +LOG loaded with −LGL, −ULO, +GIL and −SNH. Thus, separation of populations along CA1 was due to fish with many gill rakers on the gill arch, long gill rakers, small snout height and terminal mouth being separated from fish with fewer rakers on the gill arch, short gill rakers, high snout heights and a subterminal mouth.

Table 3 Eigenvectors in canonical discriminant analysis based on metric and meristic traits for the 11 spawning populations in Lake Femund.

			Population			
Trait	Abbr.	Corr.	Order	CA1	CA2	CA3
1. Head length	HEL	SCL	17	−4.63	−3.83	−20.62
2. Head height	HEH	HEL	20*	1.59	16.38	−8.07
3. Preorbital length	PRL	HEL	16	3.25	−16.76	5.43
4. Orbital length	ORL	HEL	18*	3.08	−14.09	11.33
5. Orbital height	ORH	HEL	7	−10.72	26.90	5.57
6. Postorbital length	POL	HEL	11	11.63	−2.75	25.06
7. Upper jaw length	UPL	HEL	14	−1.61	3.57	−25.79
8. Lower jaw length	LOL	HEL	6	12.78	5.83	−26.09
9. Interorbital breadth	INB	HEL	10	−1.89	25.05	2.67
10. Snout width	SNW	HEL	9	10.33	6.53	29.81
11. Snout height	SNH	HEL	5	−5.42	−7.54	0.63
12. Upper jaw width	UPW	HEL	8	4.49	−27.43	−20.97
13. Upper gill arch length	UGL	HEL	4	1.62	−5.68	3.22
14. Lower gill arch length	LGL	HEL	15	−16.50	4.93	16.32
15. Gill-raker length	GIL	LGL	2	8.13	1.89	−2.75
16. Intestine length	INL	SCL	13	4.55	14.75	5.83
17. U.-L. jaw length 60 °	ULO	HEL	3	−2.04	−1.77	2.46
18. Upper gill arch rakers	UPG	UGL	12	11.69	6.75	0.90
19. Lower gill arch rakers	LOG	LGL	1	18.04	−8.76	6.00
20. Lateral line scales	LAS	SCL	19*	5.42	−5.97	1.17
Eigenvalues				11.49	0.85	0.68
Cumulative per cent explained				79.5	85.4	90.1

Traits (Corr.) used for size correction (Abbr.) in allometric scaling are given. Order refers to entering order in the stepwise variable selection.

*The traits that had a nonsignificant contribution in the model ($P > 0.05$).

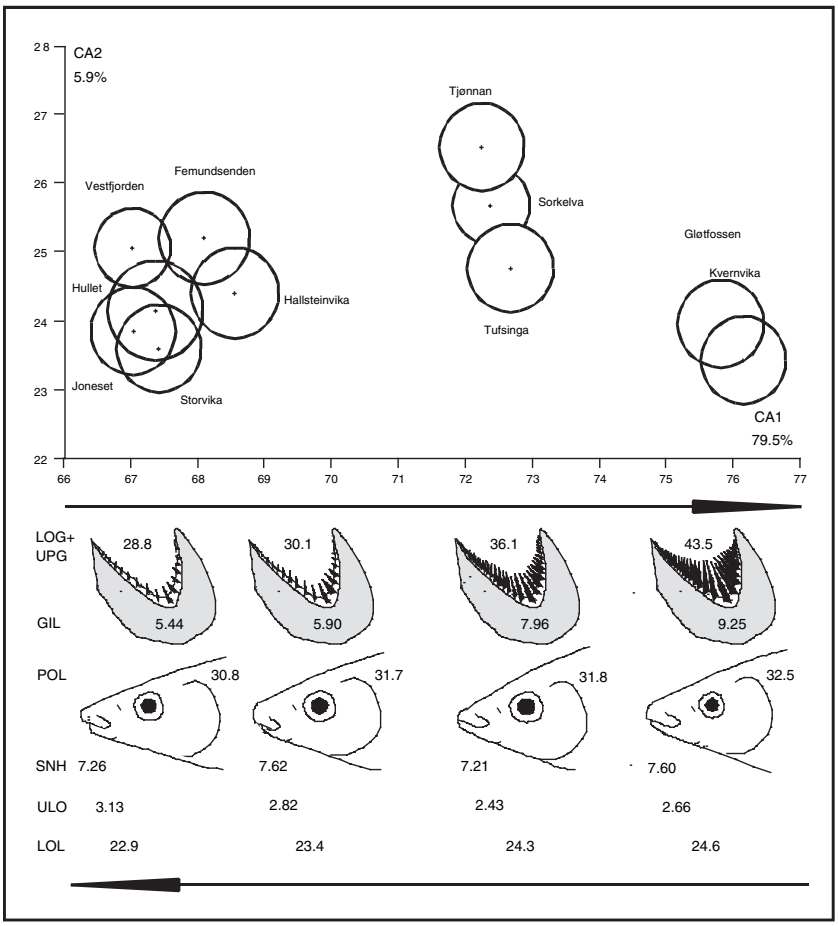

Fig. 3 Canonical discriminant analysis for the 11 Lake Femund populations based on all 20 scored morphological traits (upper section). The multivariate mean with 95% confidence limits is given. Groups that are significantly different tend to have nonintersecting circles. In the lower section, the most important traits separating the four main groups are displayed with mean values (see trait definitions and abbreviations in Table 3, Fig. 2).

A graphical interpretation (and mean values) of the main trait differences between the populations is given in Fig. 3, where four groups are resolved in Lake Femund: (I) Joneset, Vestfjorden, Hullet and Storvika, all being deep-spawning populations (see Table 2), (II) Hallsteinvika and Femundsenden, both spawning in shallow locations, (III) Tufsinga, Tjønnan and Sorkelva, spawning in rivers including a river mouth and (IV) Kvernvika and Gløtfossen, spawning in semi-isolated bays. The deep spawning populations were significantly different from the shallow spawning populations, whereas these two were both significantly different from river spawners, and the two bay spawning populations. Finally, the river spawners were also significantly different from the bay spawners.

Life-history divergence of populations in Lake Femund

All estimated length-at-age growth models for the 11 populations were significant ($P < 0.05$) (Fig. 4). Asymptotic body-length (L_∞) in the populations varied from 28.9 cm (Storvika) to 38.8 cm (Kvernvika). The four deep-spawning populations (Joneset, Vestfjorden, Hullet and Storvika) did not differ from each other in L_∞, but were significantly different from all other populations. A second group (based on overlapping confidence intervals for L_∞) consisted of the two shallow-spawning populations (Hallsteinvika and Femundsenden), and the three river-spawning populations (Tufsinga, Tjønnan and Sorkelva). They were different, however, from a third group of populations with the largest L_∞, the bay-spawning populations (Kvernvika and Gløtfossen). The mean age of spawners in each population was 8.5–9.5 years in the bay morph (Kvernvika and Gløtfossen), 11–12 years in the river morph (Tufsinga, Tjønnan and Sorkelva), 14–15 years in the shallow morph (Hallsteinvika and Femundsenden) and 12.5–16.5 years in the deep morph (Joneset, Vestfjorden, Hullet and Storvika) (see Fig. 4).

Genetic differentiation of populations in Lake Femund

In all but one sample, the expected heterozygosity was higher than the observed heterozygosity (i.e. $F_{is} > 0$).

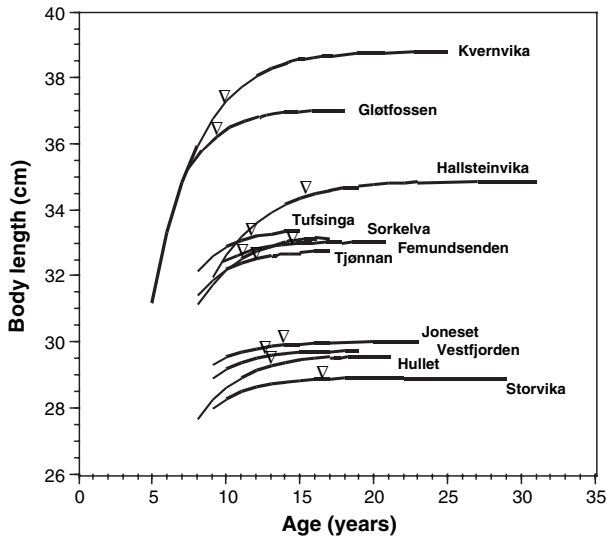

Fig. 4 Life-history parameters based on vonBertalanffy growth model in the 11 Lake Femund populations. Length-at-age is given for each population. Triangles denote the mean population age. Scale length (Fig. 2; SCL) is used as a measure for body-length.

Consequently, significant deviations from Hardy–Weinberg proportions were observed in a number of cases (Appendix 2). Most deviations were associated with the locus *C1-g*. This condition was not caused by a significant difference between cohorts (tested among age groups in a combined sample of all deep-water whitefish), nor by one specific homozygote being dominant in all the populations. Rather, different homozygotes tended to be over-represented in different populations. We therefore believe that a null allele in *C1-g* is the most likely cause for deviations from Hardy–Weinberg proportions. Alternatively, Wahlund effects cannot entirely be ruled out as fish on spawning grounds could have strayed from another population.

The six microsatellites were variable in all populations, with 10–29 alleles per locus in Lake Femund (Appendix 2). Allelic richness, the mean number of alleles per locus and population adjusted to $n = 27$, varied little among populations. The lowest mean values (6.0–6.3) were found in Gløtfossen and Kvernvika, and the highest value (7.6) in Hallsteinvika and Vestfjorden. The average observed heterozygosity within populations varied from 0.481 (Kvernvika) to 0.580 (Tufsinga), whereas the average gene diversity estimate varied from 0.561 (Storvika) to 0.655 (Tufsinga).

Five genetically distinct gene pools were resolved within Lake Femund; the four distinct morphs, as well as a distinction between the two populations of the bay morph (Table 4). Thus, pairwise tests of population heterogeneity showed that the populations of Hullet, Storvika, Vestfjorden and Joneset were not significantly differentiated (Table 4). The Tufsinga river population was not significantly different from Sorkelva or Tjønnan, although the latter two were significantly different from each other. Hallsteinvika differed neither from Femundsenden nor from Storvika, Vestfjorden or Joneset. Kvernvika and Gløtfossen populations were significantly different from all the other populations, as well as from each other. Pairwise Fst values among populations ranged from 0.005 or less between the four deep-spawning populations (Storvika, Hullet, Vestfjorden and Joneset), and between the two shallow-spawning populations (Femundsenden and Hallsteinvika), to 0.153 between the bay-spawning populations (Kvernvika and Gløtfossen). The three river-spawning populations (Tufsinga, Tjønnan and Sorkelva) had Fst values of approximately 0.01.

A hierarchical analysis of molecular variance (AMOVA; all $P < 0.0001$) showed that 3.9% of the genetic variation was found between the four morphs, 2.8% was found among populations within morphs and 93.3% within populations. Excluding the two highly genetically divergent bay morph populations (Kvernvika and Gløtfossen)

Table 4 The summary statistics of the pairwise population heterogenity tests (*P*-values, above diagonal), and the genetic divergence as found by Fst (below diagonal).

Population	1. Stor	2. Hull	3. Vest	4. Jone	5. Hall	6. Femu	7. Tufs	8. Sork	9. Tjøn	10. Kver	11. Gløt
1. Storvika		0.215*	0.104*	0.472*	0.003*	0.001	0.001	0.001	0.001	0.001	0.001
2. Hullet	0.000		0.019*	0.085*	0.001	0.001	0.001	0.001	0.001	0.001	0.001
3. Vestjorden	0.004	0.005		0.395*	0.007*	0.001	0.001	0.001	0.001	0.001	0.001
4. Joneset	0.003	0.004	0.000		0.009*	0.001	0.001	0.001	0.001	0.001	0.001
5. Hallsteinvika	0.016	0.013	0.008	0.015		0.290*	0.001	0.001	0.001	0.001	0.001
6. Femundsenden	0.022	0.015	0.016	0.019	0.001		0.001	0.001	0.001	0.001	0.001
7. Tufsinga	0.046	0.037	0.042	0.034	0.029	0.036		0.007*	0.145*	0.001	0.001
8. Sørkelva	0.022	0.029	0.030	0.023	0.033	0.039	0.011		0.001	0.001	0.001
9. Tjønnan	0.044	0.037	0.043	0.032	0.048	0.057	0.006	0.012		0.001	0.001
10. Kvernvika	0.120	0.121	0.124	0.105	0.125	0.143	0.132	0.130	0.121		0.001
11. Gløtfossen	0.130	0.129	0.124	0.100	0.136	0.141	0.093	0.095	0.088	0.153	

*Not significant after multiple alpha-correction.

from the analysis (AMOVA; all $P < 0.0001$) caused the between morphs component to increase relative to the among populations within morphs component (2.6% vs. 0.6%, respectively), whereas the majority of variation was found within populations (96.8%).

Taking into consideration spawning site, spawning time, morphology, life history and genetic structure, the eleven spawning populations in Lake Femund were naturally grouped into; (I) Deep morph (Storvika, Hullet, Vestfjorden and Joneset), (II) Shallow morph (Hallsteinvika and Femundsenden), (III) River morph (Tufsinga, Tjønnan and Sorkelva) and (IV) Bay morph (Kvernvika and Gløtfossen). The two bay morph populations of Kvernvika and Gløtfossen were genetically different, yet similar in morphology and life-history traits. In the following, they are viewed as two genetically differentiated populations of the same morph.

Comparison of morphological and genetic divergence in Lake Femund

Between populations within the same morph, there was little evidence that the genetic variation underlying morphological traits differed from the pattern of variation in microsatellites (Fig. 5). The four deep-spawning populations (panel A) did not differ significantly in their Qst–

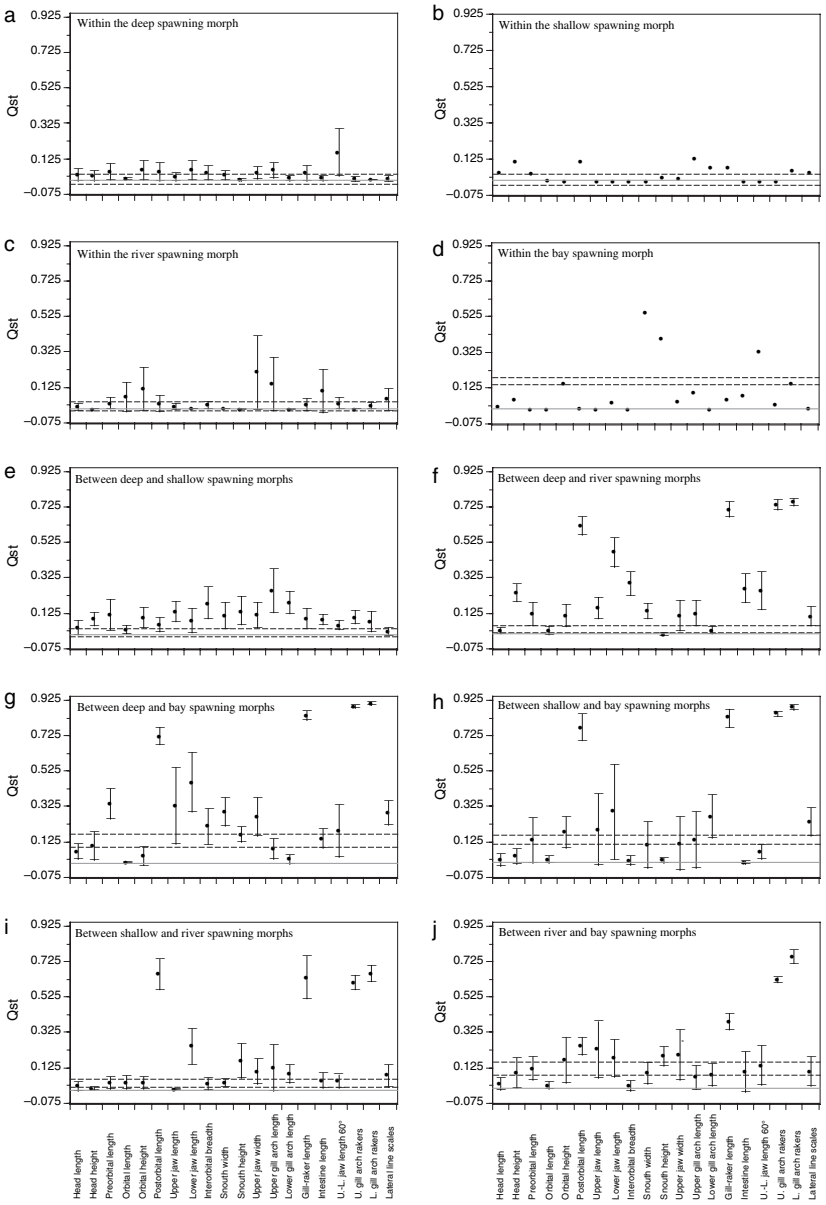

Fig. 5 Qst–Fst values for all the within- and between-morph comparisons with means and the 95% confidence intervals for Qst (bars), and 95% CI for Fst values (stippled lines). Zero is denoted by a grey dotted line.

Fst values as the 95% CI for Fst encompassed all the Qst values. The same general result held true for the three river-spawning populations (panel C), and most likely also for the two shallow-spawning populations (panel B) where all Qst values were small and close to Fst. The two bay populations were harder to evaluate, as they had a high Fst and featured traits having both higher, and lower Qst value (panel D). Also, we could not evaluate whether the Qst values are significantly different from Fst as we only have one pairwise comparison for each trait in panels B and D. Yet, it is noteworthy that 15 of 20 traits have a smaller Qst value than Fst in the bay morph populations.

In contrast to within-morph comparisons, in all comparisons of populations belonging to different morphs, we found traits showing significantly higher Qst values than Fst (Fig. 5e–j). The number of gill rakers (UPG, LOG) showed the highest Qst/Fst ratio and were significantly different in five (LOG) or all (UPG) of the six inter-morph comparisons. Gill-raker length had the second highest Qst/Fst ratio being different in four of six comparisons. Post-orbital length showed significantly higher Qst in all comparisons except between populations in the deep and shallow morph. Other head morphology traits, especially jaw and snout measurements, showed significantly higher Qst than Fst in some of the comparisons.

In the comparisons involving the bay-spawning populations, the lower Fst confidence limit was well above zero [Fig. 5g (0.094), h (0.110) and j (0.084)]. Hence, for these comparisons it is possible to test whether some Qst values were significantly smaller than the neutral expectation. The deep and bay morph showed significantly small Qst values for orbital length and lower gill-arch length (panel G), the shallow and bay morph showed significantly small Qst for several head measurements and for intestine length (panel H), whereas the river and bay morphs showed a significantly small Qst for three head measurements (panel J).

Morphological relationships of Lake Femund vs. nearby lakes

When we applied the canonical discriminant analysis (Pillai's trace 3.89, $P < 0.001$) on all phenotypic traits, extracting 59.8% of the total variation on CA1 and 24.8% on CA2, the four Lake Femund morphs clustered together with the normal morph from Lake Isteren and Lake Storsjøen (Fig. 6). Lake Engeren also appeared to be quite close morphologically, whereas Lossnen, Vurrusjøen, Drevsjøen and the Lake Isteren dwarfs were clearly much more distant. The closely localized Lake Femund and Lake Isteren, both in the Trysil-Klarälven water drainage, separated only by a short stretch of river, encompass all the variation found in all the 18 populations.

When treating each of the 11 Lake Femund populations as unknown samples forced into the seven external populations using the K-means clustering, the assignment was highest to Storsjøen. With only one exception (Hullet), all the Lake Femund populations were closest to Lake Storsjøen, then Engeren and Lake Isteren-normal. Very few individuals were assigned to Lake Lossnen or Drevsjøen, and none was assigned to Lake Isteren-dwarf. Moreover, the three Lake Femund populations with the highest assignment to Lake Storsjøen (a river spawning population) were the three Lake Femund river spawning populations of Tufsinga, Tjønnan and Sorkelva.

Genetic relationships of Lake Femund vs. nearby lakes

Among the seven external populations, we found that the six microsatellites ranged between 3 and 26 alleles

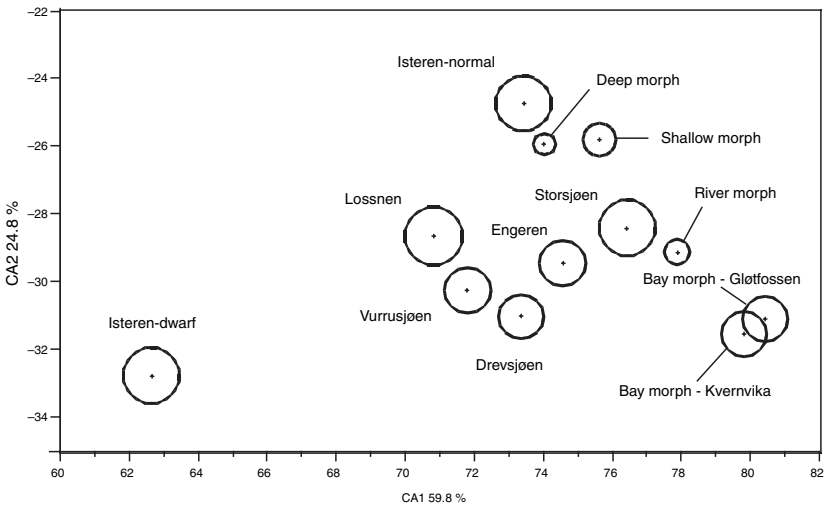

Fig. 6 Canonical discriminant analysis for the four Lake Femund morphs (the bay morph has been partitioned into Gløtfossen and Kvernvika populations) and seven external populations using 20 traits. Multivariate means with 95% confidence limit are given.

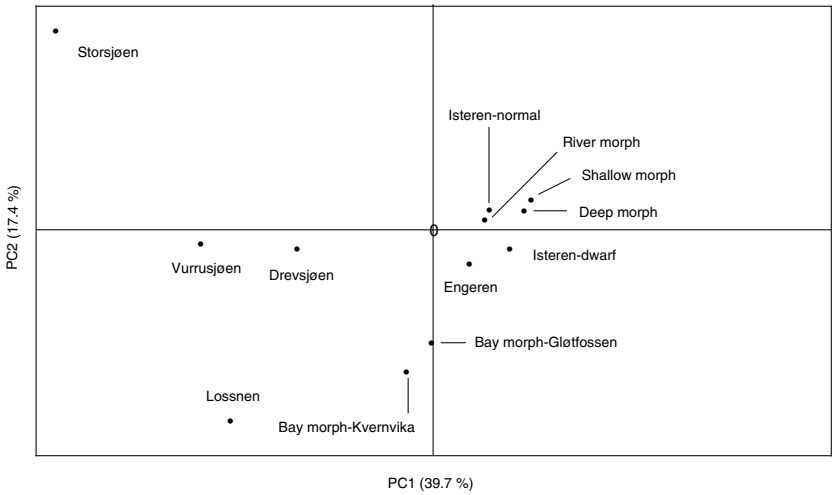

Fig. 7 Principal component analysis on multilocus microsatellites from four Lake Femund morphs (with the bay morph partitioned into Gløtfossen and Kvernvika populations) compared with the seven external populations.

per locus (Appendix 2). Adjusted for sample size (n = 27), allelic richness varied from 5.7 (Isteren-normal) to 12.7 (Vurrusjøen). The average observed heterozygosity within populations varied between 0.528 (Lake Storsjøen) and 0.793 (Vurrusjøen), whereas the average expected heterozygosity (or gene diversity) ranged from 0.599 (Isteren-normal) to 0.856 (Lake Vurrusjøen, Appendix 2). Most values were therefore on the high side, or above, those found in the Lake Femund populations.

Using pairwise tests of population heterogeneity, all the seven external populations were significantly different from each other, as well as from the eleven Lake Femund populations (i.e. $P < 0.05$ after Bonferroni correction). Pairwise Fst values ranged from 0.031 (Vestfjorden vs. Isteren-normal) to 0.356 (Storvika vs. Lake Storsjøen). The sympatric dwarf and normal morphs in Lake Isteren had Fst value of 0.085 ($P < 0.001$).

The relationships between Lake Femund whitefish and the external populations were also assessed by assigning individuals from Lake Femund to one of the external populations. For eight of the 11 Lake Femund populations (all deep-water populations, two river populations, both shallow-water populations), more than 95% of the fish were assigned to Isteren or Engeren. Moreover, assignment to Isteren was to both the dwarf and normal morph, irrespective of Lake Femund morph. For the remaining three populations in Lake Femund, assignment was in addition to Lossnen (<26% for Gløtfossen, Tjønnan, Kvernvika) and to Lake Drevsjøen (<3% for Tjønnan, Kvernvika). No individuals were assigned to Lake Vurrusjøen or Storsjøen. Thus, for each of the 11 Lake Femund populations at least 71% were assigned to jointly Lake Isteren (dwarf + normal) and Lake Engeren in the Trysil-Klarälven river.

A principal component analysis explained 39.7 and 17.4% of variation for PC1 axis ($P = 0.002$) and PC2 axis ($P = 0.34$), respectively (Fig. 7). All Lake Femund samples (seen as morphs in Fig. 7) clustered with the morphs from Lake Isteren (normal and dwarf), and with Lake Engeren. Thus, the external populations bearing the closest genetic relationship to Lake Femund populations come from other lakes in the same river system.

Discussion

Our results showed that the four whitefish morphs (deep, shallow, river and bay) inhabiting Lake Femund constitute discrete units based on morphological, life-history and genetic traits, and spawn at different times in segregated environments. In contrast, populations within these morphs were remarkably similar, as opposed to divergence in traits across morph categories, except for the two bay morphs which are highly genetically differentiated, but very similar in terms of morphology and life-history. Potential determinants behind intralacustrine divergence are discussed below.

Trait differentiation in Lake Femund compared with other coregonid systems

In Lake Femund, the four morphs were mainly separated by the number and length of gill rakers, lower jaw length and mouth position. Differences at these traits are commonly found to separate European whitefish morphs (Rufli, 1978; Kirchhofer, 1990; Amundsen *et al.*, 2004), as well as other coregonids, e.g. Lake Baikal Omul (*C. autumnalis migratorius*) (Smirnov, 1992; Bronte *et al.*, 1999), North American lake whitefish (*C. clupeaformis*) (Bodaly, 1979; Lu & Bernatchez, 1999), North American ciscoes (*Coregonus* spp.) (Todd & Smith, 1992; Turgeon *et al.*, 1999), and European vendace (*C. albula*) (Svärdson, 1979; Schulz & Freyhof, 2003). In laboratory experiments, variation in such traits has been shown to influence prey-handling ability (Malmquist, 1992; Day & McPhail, 1996; Adams & Huntingford, 2004).

Life-history traits of the four Lake Femund morphs differed with respect to juvenile growth rate, age at sexual maturation, growth trajectories and longevity (see Sandlund *et al*., 1995). Jensen (1985) found that these traits are related in the lake whitefish, and that changes in age at maturity and survival of immature fish had a large effect on the net reproductive rate of whitefish populations. Such life history differentiation is commonly seen in whitefish systems both in Europe and in North America (Bodaly, 1979; Kirkpatrick & Selander, 1979; Svarvar & Müller, 1982; Kirchhofer & Tschumi, 1986; Lehtonen & Niemelä, 1998; Svärdson, 1998; Lu & Bernatchez, 1999; Kahilainen & Lehtonen, 2002). It seems to be a general trend that sympatric whitefish populations differing in gill-raker numbers also display different growth trajectories. In North America, however, a much less pronounced divergence in gillraker numbers is associated with similar body size difference as seen in Europe.

The Fst values between pairs of the four Lake Femund morphs ranged from 0.008 to 0.141. This covers a large part of the range of within-lake genetic differentiation reported from *Coregonus* spp. and other salmonids (Hindar, 1994; Hendry & Stearns, 2004). In the European whitefish, Douglas *et al.* (1999) found an average Fst of 0.049 between sympatric morphs in eight Alpine lakes. In lake whitefish, Fst values between sympatric pairs within the same mtDNA lineage ranged from 0.01 to 0.084, whereas comparisons between pairs representing different mtDNA lineages gave Fst values from 0.020 to 0.256 (Kirkpatrick & Selander, 1979; Bodaly *et al*., 1992; Lu & Bernatchez, 1999). In four morphologically-defined cisco taxa (*Coregonus* spp.) in Lake Nipigon, Turgeon *et al.* (1999) found Fst values from 0.001 to 0.029. Thus, the maximum Fst values between the Lake Femund morphs are very high, and close to values in contact zones between divergent mtDNA lineages in lake whitefish (Lu & Bernatchez, 1999; Lu *et al*., 2001).

Apparently, sympatric populations of *Coregonus* are generally (but not always) characterized by rather small Fst values despite a pronounced divergence in morphology, life-history and ecology. Furthermore, the generality of trait differentiation between sympatric populations suggests that a similar suite of evolutionary forces may be acting in promoting divergence across the different species and environments.

Are phenotypic traits in Lake Femund morphs associated with use of niches?

If trait differences between the Lake Femund morphs reflect foraging specialization, we would expect an association between trait value and habitat use, prey selection, and behaviour. It is commonly seen that whitefish in monomorphic stocks have a wider niche than individual morphs living in sympatry (e.g. Sandlund *et al*., 1995; Amundsen *et al*., 2004). In dimorphic stocks, the most densely-rakered morph usually utilizes the pelagic habitat feeding on zooplankton, whereas the low-rakered morph to a larger extent feeds epibenthically on zoobenthos (Amundsen *et al*., 2004). Differentiation along a similar gradient is seen in stocks with three (Kahilainen *et al*., 2004) or four morphs (Bergstrand, 1982). In light of these observations, habitat use and diet of the whitefish morphs in Lake Femund are somewhat unusual. Here, adults of the densely-rakered morphs utilize the pelagic zone to a lesser extent than the low-rakered morphs, feeding on zooplankton in late summer and autumn (Næsje *et al*., 1998; Saksgård *et al*., 2002). The deep and shallow morphs are distributed in all epibenthic depth ranges (0–60 m) as well as the pelagic zone during summer, whereas the river and bay morphs are more restricted to epibenthic shallow waters (<20 m). However, in line with other studies, it seems that deep- and shallow-morphs have a wider niche than river- and bay-morphs, suggesting a more opportunistic foraging behaviour of low-rakered morphs in Lake Femund. In this ultra-oligotrophic lake, zooplankton densities are very low, even during peak densities in August–October (Løvik & Kjellberg, 1982). Thus, improved ability to use zooplankton may not be of great adaptive value to adults. Diet-related selection pressures may rather be related to the epibenthic resource in juvenile stages.

Less data are available in the literature with regard to a potential association between trait and foraging behaviour. Experiments suggest that trait variation is associated with prey-handling abilities (e.g. Day & McPhail, 1996). Thus, it seems reasonable to expect adaptive behavioural differences in foraging between morphs. Three lines of argumentation suggest that behavioural differences in foraging acquisition between morphs are likely and adaptive. First, behaviour modification will be a more rapid event associated with the use of a new niche than modification of structural traits (Dill, 1983). Secondly, there are probably functional constraints with regard to optimal foraging on a given prey item, with densely-rakered fish being more effective feeding on small prey than sparsely-rakered fish (e.g. Larson, 1976; Bentzen & McPhail, 1984). Thirdly, behaviour may be heritable (Klemetsen *et al*., 2002). For example, whitefish hybrids seem to be intermediate in diet choice (Svärdson, 1957; Voloshenko, 1973). Also, Rogers *et al.* (2002) recently documented a genetically based phenotype–environment association for swimming behaviour in two morphs of the lake whitefish. Finally, in Lake Muddusjärvi, three whitefish morphs show different foraging behaviour and prey efficiencies when experimentally consuming chironomid larvae (K. Kahilainen, personal communication, Department of Biological and Environmental Sciences, University of Helsinki, Finland). Although information on feeding behaviour is lacking in Lake Femund, it is reasonable to assume an association between adaptive traits and foraging behaviour. Also, similarity of morphs in these northern oligotrophic lakes

suggests a potential for convergence in adaptive behavioural traits.

Sympatric origin of the divergent morphs in Lake Femund?

Our results suggest that an intra-lacustrine origin of the four whitefish morphs in Lake Femund is a more likely event than allopatric origin. Huitfeldt-Kaas (1918) assumed that all whitefish colonized Lake Femund via the Swedish River Österdalälven through Lake Vurrusjøen-Drevsjøen, and suggested that these lakes were connected with the southern end of Lake Femund by a historical river course. Huitfeldt-Kaas (1918) assumed that the Trysil-Klarälven River rapids were too steep to allow upstream migration. In contrast, our study suggests that colonization of Lake Femund is likely to have occurred along Trysil-Klarälven River. First, microsatellite data suggest a closer genetic relationship with other populations in this river system than with any other population in neighbouring river systems. Secondly, the fish fauna (Table 1) and also mtDNA data (Bodaly *et al.*, 1991; Bernatchez & Dodson, 1994; K. Østbye, unpublished data) suggest a historical relationship between Lake Femund and other lakes in the Trysil-Klarälven River. In particular, immigration through Lake Vurrusjøen-Lake Drevsjøen seems unlikely, as these lakes harbour fish species, mtDNA haplotypes, and microsatellite alleles not seen in Lake Femund.

The morphological groups found in Lake Femund differed from the nearby Lake Isteren normal and dwarf morph. Lake Isteren normal and dwarf morphs are highly different in body size ($R^2 = 0.98$, $P < 0.0001$, $n = 60$, with mean ± SD being 32.6 ± 2.1 vs. 14.5 ± 1.2 cm), and not very different in gill-raker counts ($R^2 = 0.29$, $P < 0.0001$, $n = 60$, mean ± SD of 25.5 ± 1.9 vs. 27.9 ± 1.8 gill-rakers). The fact that Lake Femund and Lake Isteren populations differ significantly from each other suggests that the current whitefish morphs in Lake Femund is not a subset (or combination) of populations in other lakes. This is further supported by the fact that all the Lake Femund populations are most phenotypically similar to, but genetically divergent from, the geographically remote Lake Storsjøen. Apparently, the morphological resemblance originates without genetic relationship, implying either parallel selection or phenotypic plasticity.

We cannot exclude that more than one colonization of Lake Femund took place, as suggested by assignment of some individuals of the bay morph to populations in other drainages. However, genetic drift in the numerically small bay populations (Sandlund & Næsje, 1989) could also be responsible for the genetically divergent bay morphs. At any rate, it is likely that the adaptive gene combinations that have produced the four Lake Femund morphs have originated within this lake, although the genes themselves could have arrived from more than one source population. This conclusion corroborates the results of Douglas *et al.* (1999) who suggested that sympatric whitefish morphs in the central European Alps, being divergent in morphology, spawning site, and spawning time, likely had an intra-lacustrine origin.

Could traits separating Lake Femund morphs be altered by natural selection?

The observed differences of traits that may have an adaptive basis between the four morphs in Lake Femund suggest that their divergence may have been driven by natural selection. Alternatively, differences could reflect (non)adaptive phenotypic plasticity where trait expression is controlled by cues from a given environment (Via & Lande, 1985; Robinson & Pearson, 2002; Price *et al.*, 2003). Analysing several geographical samples of spawning populations within morphs gave us the possibility to compare traits and suggest responsible mechanisms.

High heritability (h^2) values for meristic traits, and lower values for metric and life-history traits have commonly been reported (Mousseau & Roff, 1987). Crossing experiments in European whitefish and other fish suggest a high additive genetic variance component of gill-raker numbers with h^2-estimates between 0.19 and 0.84, and for metric traits, similar to the ones scored in Lake Femund, with h^2-values between 0.21 and 0.84 (Lindroth, 1957; Svärdson, 1957, 1979; Hagen, 1973; Lavin & McPhail, 1987; Leary *et al.*, 1992; Hatfield, 1997; Foote *et al.*, 1999; Hermida *et al.*, 2002). In a study of adaptive head shape differences in cichlids, Albertson *et al.* (2003) suggested that head traits may collectively be inherited together because of pleiotropy in the genetic architecture. Further, Schluter *et al.* (2004) observed parallel inheritance of genetic differences in adaptive traits in two independent lineages of threspine sticklebacks, suggesting that an ancestral trait in close relatives would follow the same developmental pathway when exposed to similar selection pressures. These results suggest an additive genetic basis for the analysed traits in Lake Femund implying they could be structured by selection.

Indeed, the Qst–Fst results suggest that natural selection have shaped divergence of morphs in Lake Femund, especially in traits related to foraging. Using a Qst/Fst ratio, we found that the between-morph comparison of upper and lower gill-raker numbers were 5.7–21.1 and 7.6–23.7, respectively. These ratios are much higher than those reported by Bernatchez (2004) for North American lake whitefish sympatric pairs, which suggests more pronounced selective pressures exerted on gill-rakers in Lake Femund. Thus, if natural selection has been important in driving the differentiation of morphs, we should observe a higher degree of differentiation between spawning morphs than within morphs reflecting directional selection. In addition, we should observe

stabilizing selection within populations of the same morph as a manifestation of phenotypic optimum. This pattern seems in general to hold true, especially for traits related to the gill-raker apparatus which were much more different among morphs than within. Similar observations were made in comparative analysis of sympatric morphs in smelt (*Osmerus mordax*) (Saint-Laurent *et al.*, 2003). The most distinct morph with regard to these traits are the bay morph which have the highest number of gill-rakers, longest rakers and the most dense gill-raker apparatus. These are also the two populations (Gløtfossen and Kvernvika), when compared within morphs, that have the highest number of Qst trait values below Fst, suggesting stabilizing selection may be involved in maintaining similarity at these traits between populations of the same morph.

As an alternative to a selection-mediated divergence, phenotypic plasticity could be induced by environmental cues such as temperature (Tåning, 1958). However, such effects on gill-raker numbers is at best small (Todd, 1998). On body-proportion traits, Svärdson (1950) demonstrated an effect of phenotypic plasticity through growth-rate related change in metric values. In the threespined stickleback, Day *et al.* (1994) showed that plasticity accounted for 0% (gill-raker number) up to 58% (head depth) when comparing five foraging-related traits in two morphs that were fed the natural diet of the other morph. Such phenotypic placticity induced by feeding experience has also been reported in other fish (Meyer, 1987; Wimberger, 1992; Skúlason *et al.*, 1999; Alexander & Adams, 2004).

However, the most divergent traits between the four morphs in Lake Femund, gill-raker number and length, show high heritability in many studies, and are associated with foraging ability implying that differentiation of these four morphs are likely to have been driven by natural selection.

Conclusion

The European whitefish in Lake Femund feature four morphs, with distinct biology, being most divergent in traits related to foraging. Two or more populations exist within each of the four morphs being phenotypically similar despite the micro-geographical separation. Genetic divergence between morphs is moderate to large, with values being generally small between populations within morphs. A higher proportion of molecular variance is also found between than within morphs. The evolutionary origin of the four morphs seems to be intralacustrine, where divergent selection has been sufficiently strong to maintain phenotypic differentiation in spite of gene flow. Apparently, the adaptive gene combinations that have produced the four Lake Femund morphs have originated within this lake, although the genes themselves could have arrived from more than one source population outside the lake.

Acknowledgments

This work was funded by the Research Council of Norway, the Norwegian Directorate for Nature Management, and by our host institutions. We are grateful to O. Elgåen from Femund Fiskerlag for collecting the samples. The following persons were invaluable during laboratory work; T. Balstad, K. Kvaløy, C. Landry, L. Papillon, M. Valcourt, R. Saint-Laurent and C. Potvin. Fruitful discussions with J. Blais, G. Lu, O. Ugedal, D. Garant, R. Saint-Laurent, D. Fraser, M. Schulz, V. Castric, J. Turgeon, E. Østbye, and constructive comments from O. Seehausen, A. Vøllestad, and an anonymous referee greatly improved the manuscript.

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Received 1 July 2004; revised 29 September 2004; accepted 4 October 2004

Appendix 1 The mean (SD) values of $\log_{10}$ transformed and allometrically scaled traits in all the 18 surveyed populations.

Code	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	R^2	*P*-value
HEL	1.795	1.784	1.789	1.789	1.781	1.789	1.786	1.785	1.790	1.780	1.786	1.828	1.775	1.794	1.799	1.805	1.804	1.774	0.33	<0.0001
	(0.024)	(0.011)	(0.019)	(0.015)	(0.013)	(0.014)	(0.011)	(0.012)	(0.011)	(0.012)	(0.011)	(0.016)	(0.013)	(0.022)	(0.023)	(0.014)	(0.013)	(0.013)		
HEH	1.418	1.418	1.424	1.415	1.433	1.427	1.432	1.435	1.432	1.419	1.430	1.329	1.415	1.416	1.405	1.395	1.429	1.390	0.65	<0.0001
	(0.016)	(0.014)	(0.014)	(0.016)	(0.024)	(0.017)	(0.013)	(0.012)	(0.011)	(0.017)	(0.012)	(0.020)	(0.010)	(0.017)	(0.017)	(0.022)	(0.012)	(0.016)		
PRL	1.206	1.196	1.189	1.204	1.220	1.201	1.215	1.219	1.205	1.224	1.234	1.041	1.181	1.174	1.183	1.175	1.195	1.129	0.74	<0.0001
	(0.019)	(0.020)	(0.018)	(0.023)	(0.022)	(0.023)	(0.018)	(0.021)	(0.027)	(0.026)	(0.020)	(0.039)	(0.016)	(0.022)	(0.030)	(0.024)	(0.016)	(0.026)		
ORL	1.234	1.229	1.236	1.227	1.241	1.237	1.231	1.230	1.239	1.230	1.233	1.197	1.249	1.244	1.229	1.236	1.259	1.219	0.26	<0.0001
	(0.017)	(0.016)	(0.016)	(0.019)	(0.019)	(0.017)	(0.017)	(0.014)	(0.015)	(0.021)	(0.015)	(0.025)	(0.017)	(0.015)	(0.020)	(0.029)	(0.014)	(0.021)		
ORH	1.157	1.156	1.166	1.147	1.171	1.169	1.158	1.174	1.175	1.149	1.156	1.106	1.184	1.167	1.157	1.167	1.170	1.120	0.41	<0.0001
	(0.018)	(0.026)	(0.021)	(0.020)	(0.024)	(0.016)	(0.015)	(0.019)	(0.019)	(0.015)	(0.016)	(0.023)	(0.017)	(0.016)	(0.018)	(0.029)	(0.025)	(0.018)		
POL	1.483	1.486	1.494	1.490	1.502	1.501	1.506	1.498	1.504	1.514	1.509	1.503	1.416	1.484	1.473	1.462	1.493	1.468	0.71	<0.0001
	(0.003)	(0.002)	(0.001)	(0.003)	(0.002)	(0.002)	(0.002)	(0.001)	(0.002)	(0.001)	(0.001)	(0.002)	(0.003)	(0.002)	(0.003)	(0.002)	(0.002)	(0.002)		
UPL	1.227	1.223	1.216	1.220	1.238	1.237	1.243	1.237	1.235	1.227	1.262	1.098	1.211	1.223	1.207	1.203	1.227	1.160	0.71	<0.0001
	(0.019)	(0.024)	(0.022)	(0.015)	(0.025)	(0.015)	(0.015)	(0.016)	(0.019)	(0.025)	(0.011)	(0.023)	(0.018)	(0.019)	(0.028)	(0.025)	(0.011)	(0.018)		
LOL	1.365	1.360	1.362	1.352	1.371	1.365	1.386	1.388	1.384	1.379	1.405	1.310	1.372	1.367	1.366	1.371	1.374	1.340	0.56	<0.0001
	(0.014)	(0.014)	(0.018)	(0.012)	(0.020)	(0.014)	(0.011)	(0.014)	(0.013)	(0.016)	(0.016)	(0.015)	(0.015)	(0.022)	(0.019)	(0.024)	(0.017)	(0.016)		
INB	1.240	1.244	1.254	1.254	1.262	1.266	1.268	1.267	1.274	1.261	1.270	1.149	1.248	1.245	1.244	1.223	1.281	1.208	0.63	<0.0001
	(0.021)	(0.021)	(0.016)	(0.019)	(0.022)	(0.013)	(0.012)	(0.020)	(0.014)	(0.023)	(0.014)	(0.034)	(0.018)	(0.022)	(0.026)	(0.029)	(0.015)	(0.022)		
SNW	1.044	1.055	1.048	1.042	1.076	1.052	1.063	1.063	1.066	1.083	1.066	0.858	1.060	1.024	0.997	0.995	1.066	0.981	0.79	<0.0001
	(0.022)	(0.022)	(0.022)	(0.023)	(0.031)	(0.021)	(0.022)	(0.022)	(0.019)	(0.027)	(0.017)	(0.030)	(0.022)	(0.023)	(0.030)	(0.030)	(0.021)	(0.023)		
SNH	0.861	0.863	0.861	0.861	0.892	0.872	0.857	0.863	0.854	0.879	0.883	0.631	0.836	0.841	0.814	0.786	0.871	0.772	0.78	<0.0001
	(0.022)	(0.016)	(0.020)	(0.020)	(0.032)	(0.032)	(0.021)	(0.025)	(0.025)	(0.030)	(0.024)	(0.036)	(0.022)	(0.036)	(0.030)	(0.043)	(0.025)	(0.029)		
UPW	1.191	1.192	1.175	1.182	1.205	1.191	1.212	1.189	1.180	1.207	1.215	1.015	1.188	1.173	1.146	1.126	1.169	1.104	0.74	<0.0001
	(0.027)	(0.024)	(0.024)	(0.022)	(0.026)	(0.016)	(0.024)	(0.017)	(0.022)	(0.023)	(0.017)	(0.040)	(0.026)	(0.022)	(0.032)	(0.035)	(0.024)	(0.022)		
UGL	1.150	1.135	1.138	1.148	1.173	1.159	1.170	1.146	1.156	1.160	1.150	1.019	1.186	1.128	1.107	1.108	1.162	1.104	0.68	<0.0001
	(0.014)	(0.021)	(0.020)	(0.020)	(0.027)	(0.024)	(0.020)	(0.019)	(0.025)	(0.021)	(0.015)	(0.037)	(0.016)	(0.022)	(0.025)	(0.029)	(0.021)	(0.016)		
LGL	1.366	1.366	1.371	1.371	1.388	1.380	1.374	1.374	1.372	1.367	1.360	1.251	1.379	1.357	1.334	1.346	1.369	1.332	0.71	<0.0001
	(0.014)	(0.016)	(0.015)	(0.014)	(0.018)	(0.016)	(0.014)	(0.015)	(0.019)	(0.019)	(0.012)	(0.028)	(0.015)	(0.016)	(0.022)	(0.020)	(0.016)	(0.017)		
GIL	0.758	0.723	0.729	0.732	0.768	0.773	0.917	0.897	0.891	0.970	0.962	0.396	0.666	0.796	0.754	0.717	0.878	0.621	0.84	<0.0001
	(0.060)	(0.067)	(0.055)	(0.071)	(0.047)	(0.058)	(0.031)	(0.050)	(0.047)	(0.037)	(0.043)	(0.063)	(0.046)	(0.048)	(0.056)	(0.076)	(0.045)	(0.050)		
INL	1.475	1.462	1.474	1.465	1.488	1.485	1.496	1.484	1.506	1.493	1.480	1.359	1.510	1.457	1.441	1.433	1.492	1.443	0.56	<0.0001
	(0.029)	(0.025)	(0.021)	(0.031)	(0.031)	(0.030)	(0.027)	(0.020)	(0.018)	(0.030)	(0.015)	(0.027)	(0.023)	(0.038)	(0.037)	(0.035)	(0.017)	(0.028)		
ULO	0.529	0.388	0.503	0.540	0.456	0.445	0.428	0.365	0.369	0.509	0.342	0.253	0.291	0.305	0.282	0.299	0.382	0.364	0.42	<0.0001
	(0.062)	(0.111)	(0.104)	(0.087)	(0.194)	(0.141)	(0.112)	(0.094)	(0.101)	(0.116)	(0.116)	(0.101)	(0.06)	(0.026)	(0.113)	(0.001)	(0.095)	(0.086)		
UPG	1.020	1.026	1.021	1.031	1.049	1.050	1.126	1.127	1.121	1.204	1.187	1.028	0.981	1.086	1.109	1.083	1.078	1.062	0.78	<0.0001
	(0.031)	(0.029)	(0.029)	(0.034)	(0.031)	(0.035)	(0.027)	(0.025)	(0.030)	(0.031)	(0.024)	(0.033)	(0.037)	(0.026)	(0.040)	(0.036)	(0.036)	(0.031)		
LOG	1.262	1.257	1.261	1.260	1.282	1.271	1.356	1.362	1.353	1.458	1.434	1.247	1.199	1.330	1.333	1.290	1.315	1.258	0.81	<0.0001
	(0.031)	(0.034)	(0.029)	(0.032)	(0.033)	(0.036)	(0.021)	(0.023)	(0.026)	(0.029)	(0.024)	(0.031)	(0.037)	(0.033)	(0.045)	(0.048)	(0.020)	(0.032)		
LAS	1.935	1.930	1.931	1.929	1.939	1.924	1.936	1.947	1.947	1.957	1.954	1.952	1.965	1.956	1.979	1.993	1.945	1.958	0.52	<0.0001
	(0.019)	(0.018)	(0.017)	(0.011)	(0.017)	(0.019)	(0.015)	(0.017)	(0.016)	(0.020)	(0.016)	(0.016)	(0.017)	(0.017)	(0.021)	(0.016)	(0.019)	(0.016)		

The population and trait codes are found in Tables 1–3 and Figs 1,2. In last two columns test statistics for univariate ANOVA is given.

Appendix 2 Allelic variability at six microsatellite loci in the 18 studied populations estimated as the number of alleles at each locus (A), allele richness (Ar), the range of allele size in base pairs (R), the observed heterozygosity (Ho: proportion of heterozygous individuals per sample), and genetic diversity (He; Nei, 1987). All the locus deviating from the Hardy–Weinberg equilibrium is marked as * after a tests of sequential Bonferroni adjustments. The overall column gives the populations measure.

Location-population	N tot		SsBglIIM.26	Cocl-23	Bwf2	C1-g	C2-157	Bwf1	Overall
1. Storvika	45	A	10	9	5	17	5	5	51
		Ar	8.2	7.4	4.2	13.7	4.5	4.4	7.1
		R	155–219	268–294	151–161	198–254	121–153	209–233	121–294
		Ho	0.822	0.444	0.467	0.644	0.556	0.267	0.533
		He	0.715	0.529	0.434	0.864	0.524	0.299	0.561
2. Hullet	36	A	9	9	5	10	7	6	46
		Ar	8.2	7.9	4.9	9.3	6.6	5.3	7.0
		R	155–221	266–288	149–161	206–248	121–155	209–231	121–288
		Ho	0.806	0.472	0.500	0.543*	0.611	0.361	0.549*
		He	0.784	0.506	0.563	0.849	0.556	0.364	0.603
3. Vestfjorden	56	A	8	8	5	20	11	4	56
		Ar	7.1	6.5	4.2	15.9	8.2	3.5	7.6
		R	155–219	268–288	149–161	200–248	117–161	207–229	117–288
		Ho	0.585	0.396	0.574	0.642*	0.815	0.259	0.545*
		He	0.671	0.488	0.522	0.906	0.642	0.322	0.591
4. Joneset	45	A	7	7	5	16	8	4	47
		Ar	6.4	6.0	4.6	14.1	6.4	3.6	6.9
		R	155–219	268–288	149–161	200–248	121–155	209–231	121–288
		Ho	0.622	0.533	0.533	0.591*	0.578	0.244	0.517*
		He	0.727	0.562	0.511	0.874	0.649	0.359	0.613
5. Hallsteinvika	49	A	8	7	7	17	10	6	55
		Ar	6.7	6.0	5.6	14.3	8.5	4.5	7.6
		R	155–219	268–288	151–163	200–248	121–161	207–229	121–288
		Ho	0.702	0.208*	0.500	0.625*	0.563	0.313	0.484*
		He	0.713	0.441	0.563	0.915	0.620	0.446	0.616
6. Femundsenden	42	A	8	8	3	15	10	4	48
		Ar	7.2	6.6	3.0	12.7	7.9	3.8	6.9
		R	155–219	268–286	155–161	200–252	115–153	207–229	115–286
		Ho	0.805	0.279*	0.605	0.744	0.395	0.419	0.539*
		He	0.763	0.353	0.521	0.893	0.576	0.435	0.589
7. Tufsinga	45	A	8	9	5	15	8	5	50
		Ar	6.7	8.1	4.1	13.3	7.4	3.1	7.1
		R	155–221	268–286	155–165	206–254	121–155	207–229	121–286
		Ho	0.809	0.532*	0.426	0.553*	0.761	0.404	0.580*
		He	0.764	0.597	0.441	0.865	0.825	0.444	0.655
8. Sorkelva	56	A	7	7	5	15	8	5	47
		Ar	6.3	6.2	3.9	12.3	6.7	3.4	6.5
		R	155–219	268–286	155–161	206–252	117–161	207–229	117–286
		Ho	0.745	0.582	0.357	0.519*	0.607	0.286	0.512*
		He	0.707	0.611	0.338	0.87	0.712.	0.351	0.595
9. Tjønnan	45	A	10	8	5	17	8	4	52
		Ar	8.7	7.9	4.2	14.1	7.1	3.2	7.5
		R	155–223	268–286	155–175	198–250	121–155	207–233	121–286
		Ho	0.614*	0.533*	0.356	0.578*	0.778	0.444	0.550*
		He	0.776	0.746	0.344	0.880	0.782	0.383	0.651
10. Kvernvika	45	A	9	10	4	7	9	5	44
		Ar	7.4	8.1	4.0	6.3	7.6	4.2	6.3
		R	155–223	258–290	155–161	206–242	121–159	209–233	121–290
		Ho	0.400*	0.533*	0.689	0.289*	0.622	0.356*	0.481*
		He	0.702	0.617	0.713	0.555	0.677	0.512	0.629
11. Gløtfossen	45	A	8	6	3	10	8	7	42
		Ar	7.2	4.8	2.8	8.8	6.4	5.7	6.0
		R	155–223	266–286	155–159	206–250	121–161	209–233	121–286
		Ho	0.857	0.533	0.556	0.556*	0.600	0.333	0.569*
		He	0.714	0.590	0.521	0.809	0.569	0.365	0.593

Appendix 2 *Continued.*

Location-population	N tot		SsBgIIIM.26	Cocl-23	Bwf2	C1-g	C2-157	Bwf1	Overall
12. Isteren-dwarf	30	A	7	4	4	10	7	4	36
		Ar	6.7	6.8	3.9	16.3	8.6	3.8	7.7
		R	155–219	274–286	155–163	204–242	121–151	207–229	121–286
		Ho	0.655	0.633	0.500	0.733	0.700	0.233	0.575
		He	0.578	0.707	0.572	0.866	0.716	0.216	0.609
13. Isteren-normal	30	A	6	7	4	17	9	4	47
		Ar	6.0	3.9	3.8	9.7	6.7	3.9	5.7
		R	155–219	248–286	151–161	204–254	121–155	209–233	121–286
		Ho	0.630	0.333	0.433	0.933	0.667	0.733	0.621
		He	0.627	0.395	0.430	0.809	0.724	0.610	0.599
14. Engeren	45	A	7	7	3	9	9	4	39
		Ar	6.2	6.2	3.0	8.4	7.7	3.4	5.8
		R	155–223	268–288	155–161	202–248	121–155	209–229	121–288
		Ho	0.863*	0.533	0.578	0.523*	0.644	0.200	0.556*
		He	0.719	0.687	0.511	0.769	0.741	0.275	0.616
15. Drevsjø	45	A	17	11	8	26	13	9	84
		Ar	14.1	9.5	7.1	21.4	10.8	8.3	11.8
		R	155–219	260–314	147–173	198–264	121–159	209–229	121–314
		Ho	0.756	0.667	0.622	0.659*	0.867	0.733	0.717*
		He	0.887	0.824	0.634	0.941	0.845	0.803	0.822
16. Vurrusjø	45	A	13	16	8	26	12	10	85
		Ar	12.1	14.0	7.4	22.2	10.9	9.4	12.7
		R	177–219	258–336	147–173	184–260	121–161	209–233	121–336
		Ho	0.795	0.844	0.733	0.738*	0.778	0.867	0.793*
		He	0.841	0.859	0.771	0.952	0.876	0.847	0.856
17. Storsjøen	30	A	5	6	5	10	5	8	39
		Ar	5.0	5.9	5.0	9.7	4.8	7.8	6.4
		R	155–199	270–308	153–163	200–248	121–157	207–231	121–308
		Ho	0.643	0.414	0.533	0.310*	0.633*	0.633	0.528*
		He	0.626	0.417	0.628	0.565	0.551	0.790	0.597
18. Lossnen	29	A	10	9	6	12	7	8	52
		Ar	10.0	8.9	5.9	11.8	6.9	7.8	8.5
		R	155–219	272–306	147–161	204–250	121–153	209–229	121–306
		Ho	0.741*	0.862	0.724	0.500	0.517	0.759	0.684*
		He	0.856	0.850	0.647	0.757	0.509	0.765	0.729