

Sympatric diversification as influenced by ecological opportunity and historical contingency in a young species lineage of whitefish

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ABSTRACT

Background: In adaptive radiations, ecological opportunity (i.e. niche availability) is considered to be an important driver to increase phenotypic variation, but diversity may also be constrained by historical factors related to colonization events.

Question: How do ecological opportunity and post-glacial colonization history affect the phenotypic diversity in a young species lineage?

Data: We quantified phenotypic diversity by the number of co-existing morphs and a heritable morphological trait (gill raker number) in 39 European whitefish (*Coregonus lavaretus*) populations. Level of diversity was compared within and between three sub-arctic watercourses, and explored in relation to ecological opportunity (habitat availability and lake productivity) and colonization history (lake elevation and position).

Results: We found three main distribution patterns of gill raker number: unimodal (approximate range 20–30), bimodal (20–30 vs. 30–40), and trimodal (15–20 vs. 20–30 vs. 30–40), representing monomorphic, dimorphic, and trimorphic populations respectively. In addition, a pattern intermediate to the monomorphic and dimorphic populations was recorded in all watercourses. Polymorphism increased from west to east among watercourses, which can mainly be explained by post-glacial colonization history. Higher diversity was also observed in downstream sites within each watercourse, and increased with lake size and productivity.

Conclusion: Our findings confirm that both ecological opportunity and historical constraints related to post-glacial colonization influence phenotypic patterns in a diverging lineage.

Keywords: Fennoscandia, phenotypic divergence, post-glacial fish, salmonid, speciation continuum.

INTRODUCTION

When a species colonizes novel environments such as remote islands or post-glacial lakes, competition among conspecifics for limiting resources is predicted to promote divergence in resource use and increased intraspecific phenotypic variation (Van Valen, 1965; Roughgarden, 1972; Robinson and Wilson, 1994; Bolnick, 2004). The population divergence may continue to gradually fill and match the available niches in the new environment. The diversification process can be viewed as a continuum of increasingly discrete variation from individual variation within panmictic populations to polymorphisms and may finally lead to complete reproductive isolation between species (Smith and Skúlason, 1996; Hendry, 2009; Hendry *et al.*, 2009; Nosil *et al.*, 2009; Seehausen, 2009). The extent of phenotypic diversity may show substantial temporal and spatial variation among populations (Smith and Skúlason, 1996; Grant and Grant, 2002), and studying populations at different places or stages along this continuum may be useful to explore mechanisms and constraints related to phenotypic diversity, the evolution of polymorphism, and incipient speciation. The level of phenotypic divergence and the number of new types formed in an adaptive radiation process are thought to be determined by the extent of ecological opportunity, which refers to increased niche availability due to resource redundancy and heterogeneity and freedom from competition (Schluter, 2000). In addition to the properties of the environment affecting availability of niche space, historical events during the colonization process are likely to influence the extent of phenotypic diversity (Taylor and McPhail, 2000). Diversity within a population may be constrained by the time available for the diversification process (Coyne and Orr, 2004) and by loss of genetic variation through bottlenecks and founder effects during colonization (Barrett and Schluter, 2008; Caldera and Bolnick, 2008; Schluter and Conte, 2009). That is, even when ecological opportunity for diversification is present, limitations due to the colonization history may constrain further divergence; in contrast, with limited ecological opportunity, diversification may not occur even over an indefinite period of time.

Adaptive radiations in post-glacial fish are excellent model systems to explore mechanisms and constraints of diversifying phenotypic evolution. Northern temperate lakes are young (approximately 10,000–15,000 years) and represent discrete and partly isolated environments. Several fish species inhabiting these lakes have shown rapid and convergent phenotypic divergence and adaptive radiation into multiple ecotypes, morphs or species (Robinson and Wilson, 1994; Taylor, 1999; Schluter, 2000). Sympatric forms have commonly diverged along the limnetic–benthic resource axis and occur as morph-pairs with distinct segregation in habitat and food resource use (Schluter and McPhail, 1993; Robinson and Wilson, 1994; Skúlason and Smith, 1995; Robinson and Parsons, 2002). Although the adaptive divergence is generally constrained to pelagic and littoral morphs, some species have a tendency to diversify into more fine-scaled specialists (Svärdson, 1979; Malmquist *et al.*, 1992; Kahilainen *et al.*, 2003; Knudsen *et al.*, 2006, 2011).

European whitefish (*Coregonus lavaretus* (L.)) is a highly polymorphic species, commonly occurring as sympatric morphs, thus offering a good opportunity to explore factors behind adaptive divergence and incipient speciation. Whitefish morphs are traditionally named and identified by differences in morphology and number of gill rakers (Svärdson, 1979; Amundsen, 1988; Sandlund and Næsje, 1989), which also correlates with other traits in head and body morphology (Kahilainen and Østbye, 2006; Harrod *et al.*, 2010). The number of gill rakers is a highly stable and heritable morphological trait, and has a central role in adaptive radiation of coregonids in general (Svärdson, 1950, 1952, 1979; Bernatchez, 2004; Rogers and Bernatchez, 2007). The arrangement of gill rakers is believed to influence the retention efficiency of small-sized prey (Sanderson *et al.*,

2001), and more densely rakered morphs are better adapted to planktivorous feeding than sparsely rakered morphs (Kahilainen and Østbye, 2006; Kahilainen *et al.*, 2011). In northern Fennoscandian lakes there are three main patterns of whitefish morph constellations. Allopatric whitefish populations consist of a large growing, sparsely rakered morph (LSR) that is a generalist that utilizes a wide variety of habitat and diet resources (Amundsen *et al.*, 2004b; Harrod *et al.*, 2010). Dimorphic populations consist of a benthivore LSR morph in sympatry with a densely rakered morph (DR) that typically utilizes a pelagic niche, feeding on zooplankton (Svårdson, 1979; Amundsen, 1988; Amundsen *et al.*, 2004a, 2004b). In a third pattern, the LSR and DR morphs co-exist with a small, sparsely rakered morph (SSR) that resides in deep benthic (i.e. profundal) habitats foraging on benthic macroinvertebrates buried in soft sediment (Kahilainen *et al.*, 2003). Thus, whitefish has diverged into one resource specialized morph for each of the three principal lake habitats, the littoral (LSR), the pelagic (DR), and the profundal (SSR) zone respectively.

Adaptive divergence within each lake has been suggested to be the most likely origin of the different whitefish morphs in northern Fennoscandia (Østbye *et al.*, 2005, 2006). Phylogeographic studies using mitochondrial DNA indicate a single ancestral clade for the whole region, probably originating from an eastern refugium of glacial lakes (Bernatchez and Dodson, 1994; Østbye *et al.*, 2005). Furthermore, analyses at six genetic microsatellite loci of sympatric morph pairs has indicated that similar morphs from different lakes likely have a polyphyletic origin, which is compatible with the process of parallel divergence of similar morphs within separate lakes (Østbye *et al.*, 2006). This unique system with multiple whitefish populations with varying degrees of phenotypic divergence makes it possible to explore the roles of both historical events (post-glacial colonization) and contemporary environmental factors related to ecological opportunity on the spatial patterns of phenotypic diversity.

The first aim of the present study is to explore and describe patterns of phenotypic divergence in terms of number of co-existing morphs and modality of the gill raker number distributions of whitefish from three watercourses in northern Fennoscandia. Second, we explore how post-glacial colonization history and contemporary environmental factors related to ecological opportunity may have influenced phenotypic patterns among whitefish populations. Patterns of phenotypic divergence are contrasted both between and within watercourses, suggesting that lakes colonized early during the post-glacial immigration will have the highest whitefish diversity. The possible relationship between phenotypic divergence and ecological opportunity (i.e. the availability of potential trophic niches) is explored using a measure of habitat and prey resource availability (i.e. lake productivity). Because whitefish dominate the fish communities, differences in resource availability are expected to influence ecological opportunity more than interspecific competitors. Habitat availability and heterogeneity are described by lake area, depth, and perimeter; Secchi depth, total nitrogen, and total phosphorus provide a proxy for lake productivity. The largest, deepest, and most productive lakes are predicted to harbour the most diverse whitefish populations (i.e. the highest number of morphs).

MATERIALS AND METHODS

Study area and fish sampling

The study region – northern parts of Norway, Finland, and Russia – has a sub-arctic climate with low mean temperatures and a short growing season. We sampled 39 lakes, with

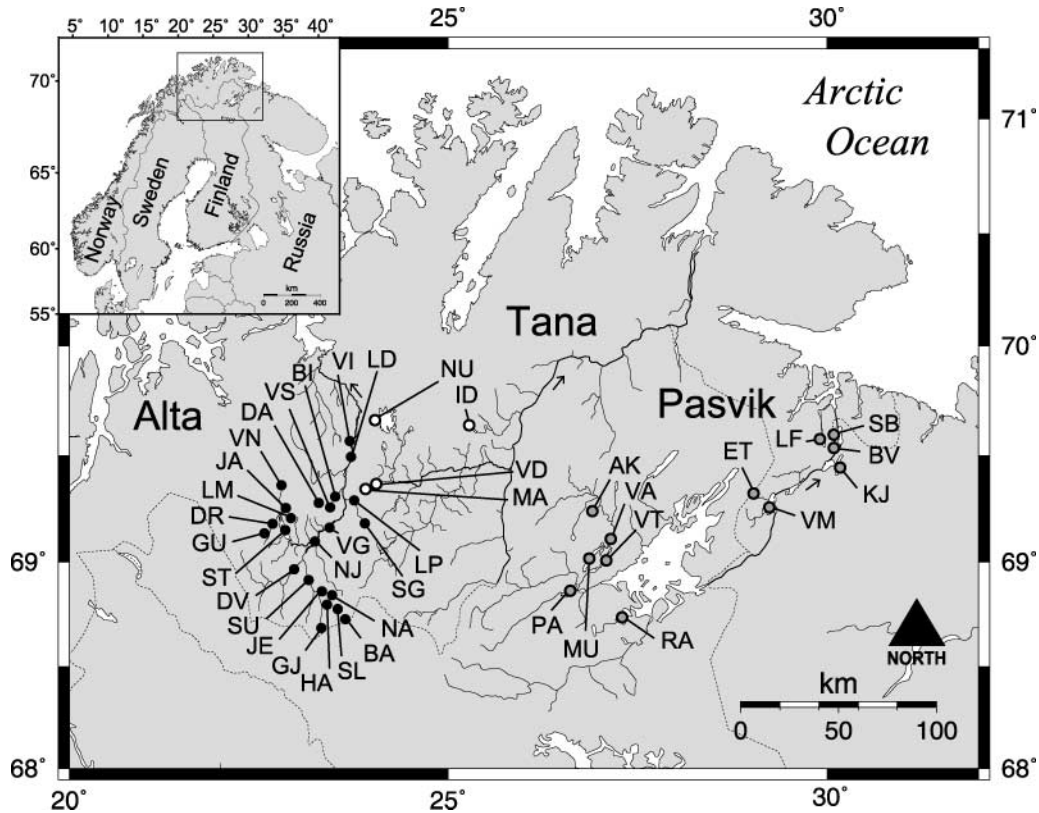


Fig. 1. Map of the study area in northern Fennoscandia where whitefish were sampled from the three major watercourses: Alta (black), Tana (white), and Pasvik (grey). Sampling sites are indicated with abbreviations of lake names: AK = Aksujärvi, BA = Bajasjärvi, BI = Biggijarvi, BV = Bjørnevatt, DA = Datkujarvi, DR = Durbunjarvi, DV = Dävajarvi, ET = Ellentjern, GJ = Gædgejarvi, GU = Guorbajarvi, HA = Havgajarvi, ID = Iddjarvi, JA = Jårgajarvi, JE = Jevdesjarvi, KJ = Kuetsjarvi, LD = Ladnetjarvi, LF = Langfjordvatn, LM = Læmbejarvi, LP = Lahpojarvi, MA = Måkkejarvi, MU = Muddsjärvi, NA = Naggitjarvi, NJ = Njallajarvi, NU = Nuorbejarvi, PA = Paadar, RA = Rahajarvi, SB = Skrukkebukta, SG = Stuora Galbajarvi, SL = Suolojarvi, ST = Stuurajarvi, SU = Suopatjarvi, VA = Vastusjärvi, VD = Vuoddasjarvi, VG = Vuolgamasjarvi, VI = Virdejarvi, VM = Vaggatem, VN = Vuolit Njivlujarvi, VS = Vuolit Spielgajarvi, VT = Vuontisjärvi.

surface areas ranging from 0.2 to 48 km², from the three largest watercourses in the region, which all drain to the Arctic Ocean (Fig. 1). The lakes in this area were formed when the ice-sheet retreated after the last ice age, about 10,000 years B.P. (Andersen and Borns, 1994; Berglund *et al.*, 1996; Kujansuu *et al.*, 1998). The lakes are oligotrophic to meso-oligotrophic (Table 1) and most are relatively pristine with little or no human impact. Most of the surveyed lakes are located within four different tributaries of the Alta-Kautokeino (hereafter Alta) watercourse, while the others are located in the Tana-Teno (Tana) and Pasvik-Paatsjoki (Pasvik) watercourses (Fig. 1). Lake surface area and perimeter were extracted from topographic maps (1:50,000) and maximum lake depth was obtained from echo-sounding. All sampling was performed during August and September in the years presented in the Online Appendix for each lake (evolutionary-ecology.com/data/2601appendix.pdf). For lakes sampled in multiple years,

Table 1. Summary statistics (median with minimum and maximum values in parentheses) for six environmental variables related to habitat availability and productivity, elevation, and the total number of fish species registered in each lake (n is the number of lakes with available information on the different variables in each watercourse)

Environmental variable	Alta		Tana		Pasvik	
	n	Med. (min.–max.)	n	Med. (min.–max.)	n	Med. (min.–max.)
Surface area (km ²)	23	2.1 (0.2–23.7)	4	2.6 (1.6–6.4)	12	8.7 (1.1–48.0)
Perimeter (km)	23	13.3 (1.9–86.4)	4	13.6 (12.3–30.5)	12	28.9 (6.6–160.1)
Maximum depth (m)	23	19 (6–52)	3	30 (30–32)	11	31 (7–73)
Total phosphorus ($\mu\text{g}\cdot\text{l}^{-1}$)	12	6 (3–18)	3	9 (8–11)	11	7 (3–13)
Total nitrogen ($\mu\text{g}\cdot\text{l}^{-1}$)	12	186 (98–296)	3	248 (150–258)	11	170 (100–246)
Secchi depth (m)	23	6.0 (3.5–9.0)	3	5.5 (4.5–8.0)	12	4.5 (2.0–8.0)
Elevation (m a.s.l.)	23	374 (265–474)	4	340 (275–435)	12	101 (7–206)
Number of fish species	23	5 (3–7)	4	5 (4–6)	12	9 (5–10)

mean values of Secchi depths were used. In a sub-set of lakes, water samples for chemical analyses were collected over the deepest area (for details on lakes and years, see evolutionary-ecology.com/data/2601appendix.pdf). Whitefish is the dominant fish species in all habitats in the majority of the surveyed lakes. Other recorded fish species in the study lakes were: perch (*Perca fluviatilis* L.), pike (*Esox lucius* L.), burbot (*Lota lota* (L.)), Arctic charr (*Salvelinus alpinus* (L.)), brown trout (*Salmo trutta* L.), grayling (*Thymallus thymallus* (L.)), vendace (*Coregonus albula* (L.)), three-spined stickleback (*Gasterosteus aculeatus* (L.)), nine-spined stickleback (*Pungitius pungitius* (L.)), and minnow (*Phoxinus phoxinus* (L.)).

Fish were sampled in benthic and pelagic habitats using standardized gill nets with bar mesh sizes from 10 to 60 mm (knot to knot). The morph of each individual whitefish was classified in the field according to appearance, head and body form, and a visual evaluation of the gill raker morphology (Amundsen, 1988; Amundsen *et al.*, 2004a; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010). The SSR whitefish have large eyes, a large head, pronounced subterminal mouth, reddish fins, and extremely short and widely spaced gill rakers. The LSR whitefish are larger in size with typical whitefish coloration with silvery sides, dark back and fins, and robust gill rakers of intermediate length and spacing. The DR whitefish are usually of smaller size, silvery and have long, thin, and densely packed gill rakers. In some lakes, a substantial number of individuals were difficult to assign to either the DR or the LSR morph by appearance and gill raker morphology, typically having relatively thin, long, and widely spaced gill rakers. These individuals were classified as ambiguous but not excluded from further analyses. In the laboratory, the number of gill rakers of the first left branchial arch was counted under a dissecting microscope.

Analyses

The level of phenotypic diversity within each whitefish population was based on a combination of (1) the number of morphs identified by gill raker morphology, head and body form and appearance (Amundsen *et al.*, 2004a; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010), and

(2) the modality of the gill raker number distribution. Based on previous knowledge, we expected distributions to be unimodal, bimodal or trimodal (Amundsen *et al.*, 2004b; Kahilainen and Østbye, 2006; Harrod *et al.*, 2010). We used three different methods to infer modality in the distributions of gill raker number. First, we explored the frequency histograms of gill raker number of 36 to 1203 fish per lake. When available, data from several years were used to increase sample sizes (see evolutionary-ecology.com/data/2601appendix.pdf for more details), since gill raker number has been shown to be a stable morphological trait in whitefish populations from the study region (Siwertsson *et al.*, in press). Second, we used normal probability plots to assess the fit of each gill raker distribution to a single normal distribution (Zar, 2010). If the data are from a single normal distribution, the points will approximately lie on a straight line. Distributions with heavy tails, such as bimodal or trimodal distributions, will result in sigmoid shape curves. Third, we determined whether the gill raker number distribution in a population was best described by a single normal distribution or a mixture of two or three normal distributions. We used a model-based clustering approach implemented in the package MCLUST version 3 for R (Fraley and Raftery, 2006) to determine the number of groups in each population based on gill raker number. The observed data were fitted to models with one or a mixture of up to three Gaussian distributions. With univariate data there are only three possible models: unimodal normal distribution, and mixtures of normal distributions with either equal or varying variances. The best model was selected based on the Bayesian information criterion [BIC, analogous to Akaike's information criterion (Fraley and Raftery, 2002)]. For each population, we compared the best model with the next best model (resulting in a different number of groups) by calculating ΔBIC as the difference between BIC for the best model minus BIC for the next best model. Following Kass and Raftery (1995), we interpreted $\Delta\text{BIC} > 10$ as very strong support for the best model, $6 < \Delta\text{BIC} < 10$ as strong support, $2 < \Delta\text{BIC} < 6$ as moderate support, and $\Delta\text{BIC} < 2$ as equivalent support for the best and the next best model.

Based on the above criteria, four patterns of whitefish diversity in the different lakes were identified (see Results section). These patterns, which represented increasing levels of whitefish phenotypic diversity, were examined in relation to the geographical distribution between and within watercourses as well as to the potential niche availability in the different lakes. Differences in phenotypic diversity between watercourses were tested with Fisher's exact test using the number of lakes per level of diversity in each watercourse. To compare the distribution of different divergence patterns within watercourses, we first calculated within-watercourse elevation for each lake. This was measured as the difference in lake elevation from the lowest known whitefish population in each watercourse. This standardization allowed us to include lakes from all watercourses in one analysis of variance (ANOVA) in order to look for general differences in the spatial distribution of the diversity in whitefish populations within watercourses. The effect of lake size (area, perimeter, maximum depth) and productivity (total phosphorus, total nitrogen, Secchi depth) (Table 1) on whitefish phenotypic diversity was explored using a principal components analysis (PCA) including 25 lakes where all six variables were available. Principal components analysis was used to account for correlations between variables, and differences between lakes with different whitefish diversity in the resulting first two PC axes were tested using ANOVA. Prior to the analysis, all variables were log-transformed to obtain normality. The number of co-occurring fish species were compared between lakes with different whitefish diversity using two-way ANOVA to account for differences between watercourses. Significant differences in ANOVAs were tested *post-hoc* using Tukey's HSD

test as implemented in the R *STATS* package (version 2.9.0). To assess the explanatory ability of the variables relating to ecological opportunity and colonization history, we estimated a cumulative logit model with the four levels of gradually increasing whitefish diversity as the categorical, ordered response variable, and lake area, Secchi depth, elevation, and watercourse as explanatory variables (Agresti, 2002). The choice of explanatory variables was made to maximize sample size. Lake area and Secchi depth were used as surrogates for habitat availability and productivity, and elevation and watercourse were used to describe colonization effects. To eliminate collinearity between elevation, lake area, and Secchi depth, we used the residuals of Secchi depth and lake area regressed against elevation as variables in the model. The fitted model was used to classify whitefish populations by level of diversity, and the success rate of the classification was recorded to obtain a measure of predictive potential. All statistical analyses were performed with the help of the statistical software R (version 2.9.0), using the package *VEGAN* (version 1.15-2 by Jari Oksanen) for the multivariate analyses, and the package *MASS* (version 7.3-7) for estimation of the cumulative logit model.

RESULTS

General patterns of phenotypic diversity

Gill raker number from the 39 lakes varied from 13 to 46 with a mean value of 28. Based on (1) the number of morphs identified by gill raker morphology, head shape, and body form, and (2) the modality of the gill raker number distribution (see evolutionary-ecology.com/data/2601appendix.pdf), we classified the level of diversity within each whitefish population into three major groups, representing increasing levels of diversification (Fig. 2).

First, the pattern representing the least diverse whitefish populations was characterized by the presence of only the LSR morph and a unimodal and relatively narrow gill raker distribution. Unimodality was concluded for 13 of 14 lakes in this group, based on weak deviations from normality in probability plots and strong to moderate support for only a single normal distribution (Δ BIC: 2.6–11.1; see Fig. A1 at evolutionary-ecology.com/data/2601appendix.pdf). The gill raker numbers in these LSR whitefish populations typically ranged from around 20 to 30 (Fig. 3a). The same range and average number of gill rakers was also found in LSR whitefish living in sympatry with other whitefish morphs (Fig. 2).

Second, 13 lakes were found to harbour sympatric LSR and DR whitefish morphs. In 12 of these lakes, bimodality in gill raker distributions was evident based on strong deviations from normality in probability plots and strong to moderate support for a mixture of two normal distributions as the best fit (Δ BIC: 2.8–27.2; see Fig. A3 at evolutionary-ecology.com/data/2601appendix.pdf). The two modes in the gill raker distributions in these populations corresponded with the LSR morph (*c.* 20–30 gill rakers) and the DR morph (*c.* 30–40 gill rakers) (Fig. 3c).

Third, trimorphic whitefish populations, with all three whitefish morphs present, represented the highest level of divergence observed in this study and were recorded from six lakes. The trimorphic whitefish populations had the widest total range of gill raker number and for three of the lakes we found strong or very strong support for trimodal distributions (Δ BIC: 8.1–63.2; see Fig. A4 at evolutionary-ecology.com/data/2601appendix.pdf), which corresponded with the three morphs: SSR (*c.* 15–20 gill rakers), LSR (*c.* 20–30), and DR (*c.* 30–40) whitefish (Fig. 3d). In some lakes, the density of the SSR morph was relatively

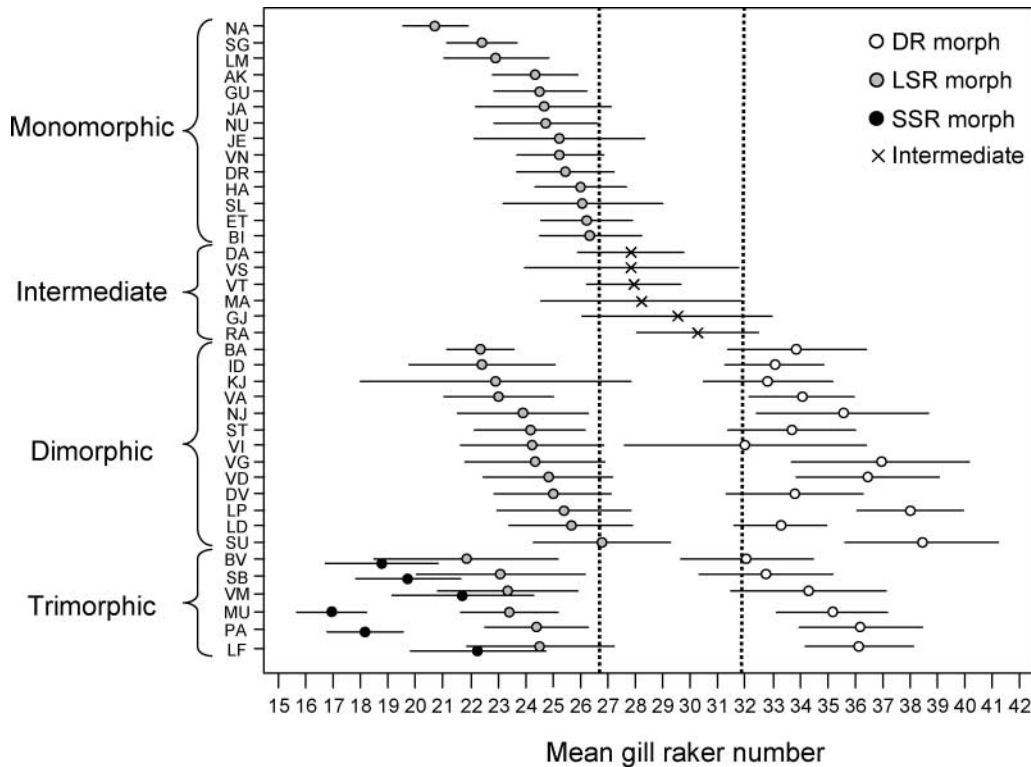


Fig. 2. Gill raker number distributions in the studied whitefish populations (mean \pm s.d.). Population diversity increases from top (monomorphic populations) to bottom (trimorphic populations) and the distribution is shown for each morph. Each row represents a lake inhabited by 1–3 whitefish morphs. Dotted lines indicate the highest mean gill raker number in LSR, and lowest in DR, from polymorphic populations. This illustrates the position of intermediate populations between the monomorphic and dimorphic populations. For lake name abbreviations, see legend to Fig. 1.

low, which may explain why some populations classified as trimorphic were not characterized by trimodal gill raker distributions [equal support for a mixture of two and three normal distributions in one population (Δ BIC: 1.8), and moderate to strong support for a mixture of two rather than three normal distributions in two populations (Δ BIC: 2.5 and 11.5); see Fig. A4 at evolutionary-ecology.com/data/2601appendix.pdf]. The SSR and DR whitefish morphs always co-existed with LSR whitefish, and these two morphs were thus never found in allopatry.

In addition to these three main patterns representing different numbers of co-occurring morphs, an intermediate group was identified based on six lakes that contained a substantial number of individuals with an ambiguous appearance relative to the criteria used to separate the different morphs. This group was quite heterogeneous in gill raker modality, containing three populations with unimodal distributions but mean values higher than other LSR morphs, and three populations with bimodal distributions (see Fig. A2 at evolutionary-ecology.com/data/2601appendix.pdf). These populations were labelled 'intermediate' due to the large number of individuals with intermediate head, body, and gill raker morphology relative to the LSR and DR whitefish morphs, as well as their

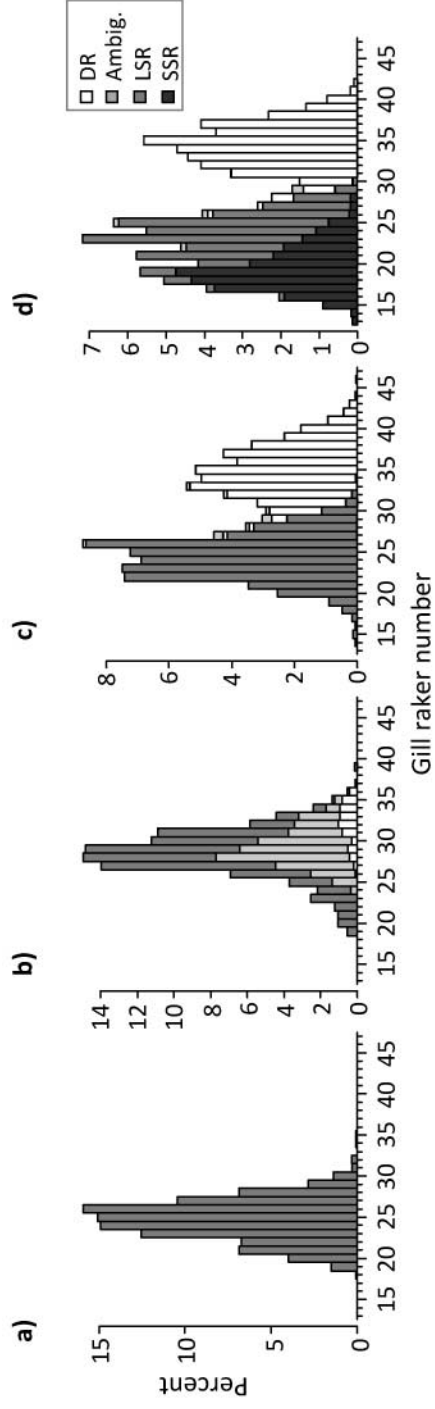


Fig. 3. Four patterns of gradually increasing population diversity described by gill raker number distributions of the studied whitefish populations: (a) monomorphic populations ($n = 14$), (b) intermediate populations with many individuals with ambiguous appearance ($n = 6$), (c) dimorphic populations ($n = 13$), and (d) trimorphic populations ($n = 6$).

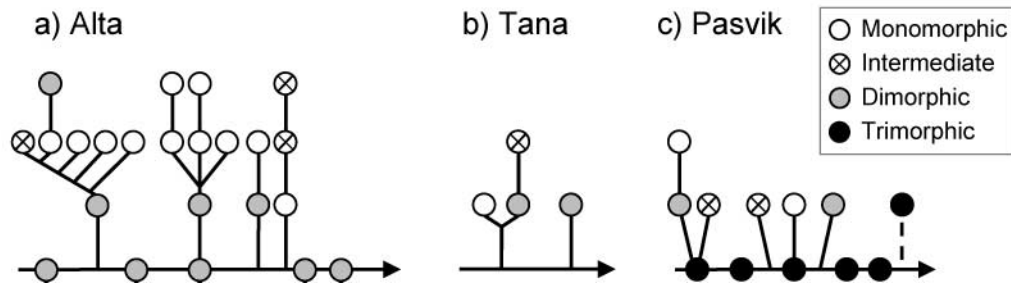


Fig. 4. Schematic illustration of the whitefish divergence patterns in the geographical context of the three studied watercourses. Circles represent different lakes, and the fill patterns indicate the level of phenotypic divergence in the whitefish population. The watercourse is presented from left (upstream) to right (downstream) and the outlet of each watercourse is marked with an arrow. The main stem is represented by the lowest horizontal line, with different tributaries reaching upwards. Connecting lines are for illustration only and do not represent geographical distances. Note that the lowest lake (hatched connection to the main river) in Pasvik represents the historical outlet of the watercourse.

intermediate position with regard to level of divergence between monomorphic and dimorphic whitefish populations (Fig. 3b). Hence, altogether we identified four patterns of gradually increasing whitefish diversity: monomorphic, intermediate, dimorphic, and trimorphic populations (Figs. 2 and 3).

Geographical distribution of different phenotypic patterns

The different levels of phenotypic diversity in whitefish populations were not equally represented in the three watercourses (Fisher's exact test: $P = 0.004$; Fig. 4). Pasvik, the easternmost watercourse, contained all the trimorphic populations and fewer of the monomorphic populations than the other watercourses (only significantly different from Alta; Fisher's exact test: $P = 0.001$). In contrast, the westernmost watercourse, Alta, was dominated by monomorphic populations and no trimorphic populations were observed.

The level of diversity was not equally distributed within watercourses (ANOVA: $F_{3,35} = 3.4$, $P = 0.028$). Polymorphic populations were generally found at lower elevations than monomorphic or intermediate populations (Table 2). This was evident in the Pasvik (trimorphic populations) and Alta (dimorphic populations) watercourses, where the majority of the lakes with most diverse whitefish populations were situated in or closely connected with the main stem (Fig. 4). In the Tana watercourse, a similar pattern could not be explored since lakes are generally absent from the main stem. Notably, all six lakes harbouring intermediate populations of whitefish were situated in the uppermost parts of tributaries in all three watercourses.

Resource availability and phenotypic patterns

Variation between lakes in variables related to habitat availability and productivity was well explained by the first two principal axes (PC1: 45%; PC2: 28%) in the principal components analysis (Fig. 5). PC1 had large positive loadings for area (0.58), perimeter (0.58), and maximum depth (0.46), indicating strong correlations between these three variables. Hence, PC1 could be interpreted as a proxy for habitat availability. Increasing values of PC1 are

Table 2. Mean values (\pm s.d.) of within-watercourse position (elevation), habitat availability (PC1), productivity (PC2), and number of co-occurring fish species in lakes (n) with increasingly diverse whitefish populations, from monomorphic to intermediate, dimorphic, and trimorphic populations

	n	Monomorphic	Intermediate	Dimorphic	Trimorphic
Elevation (m)	39	163 \pm 87	184 \pm 80	95 \pm 100	58 \pm 63
PC1 (habitat)	26	-0.18 \pm 0.15	-0.02 \pm 0.22	0.04 \pm 0.14	0.18 \pm 0.19
PC2 (productivity)	26	0.23 \pm 0.13	-0.02 \pm 0.22	-0.11 \pm 0.14	-0.08 \pm 0.14
Fish species	39	3.4 \pm 1.6	5.2 \pm 2.7	5.5 \pm 1.2	7.8 \pm 1.0

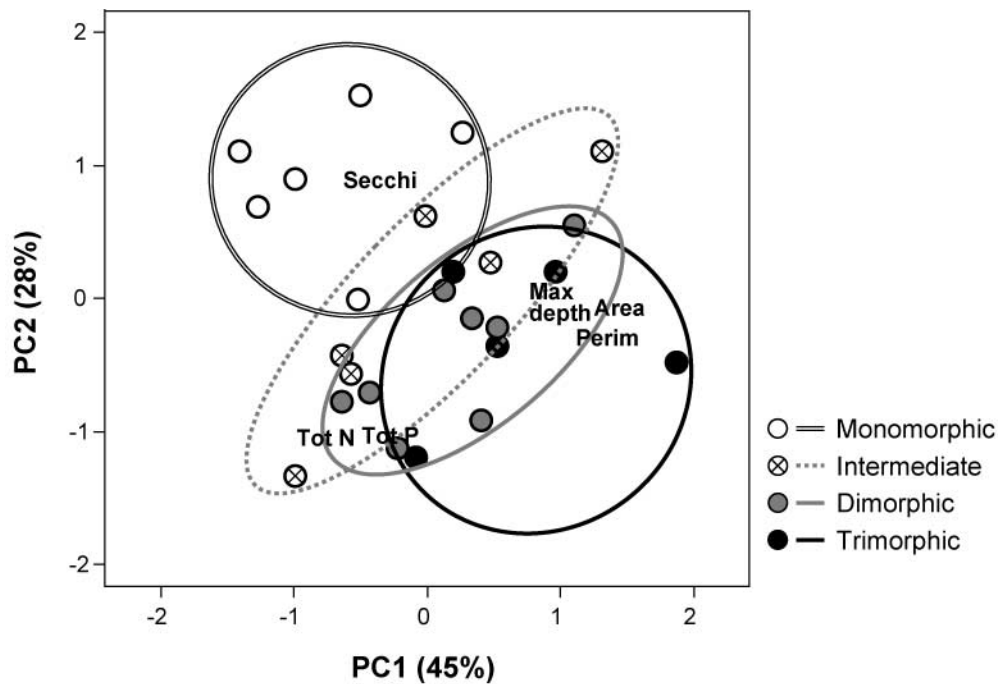


Fig. 5. The first two principal axes from a principal component analysis of environmental factors influencing whitefish divergence. Data from 25 lakes on six lake variables were explored: lake surface area (Area), perimeter (Perim), maximum depth (Max depth), Secchi depth (Secchi), total phosphorus (Tot P), and total nitrogen (Tot N). The variance explained by the different principal axes is shown in parentheses and the main dimensions of both axes are indicated. PC1 is mainly correlated with habitat availability (lake area, depth, perimeter) and PC2 with lake productivity (total phosphorus, total nitrogen, Secchi depth). Circles were fitted by eye to include all lakes with the same level of whitefish diversity, indicated by different fill patterns.

associated with larger sized, deeper lakes. PC2 was associated with positive values of Secchi depth (0.49) and negative values of total nitrogen (-0.61) and total phosphorus (-0.59) levels. PC2 thus illustrates the productivity of lakes, with increasing values of PC2 representing lower productivity, as described by lower nutrient availability and greater Secchi depths. Significant differences in both habitat availability (PC1) and productivity

(PC2) were observed between populations with differing levels of diversification (ANOVA: PC1: $F_{3,21} = 4.1$, $P = 0.020$; PC2: $F_{3,21} = 5.6$, $P = 0.006$). Structuring of the four different levels of whitefish diversification (as shown in Fig. 3) was observed, with more diverse whitefish populations associated with higher values of PC1 and lower values of PC2 (i.e. with larger, deeper, and more productive lakes; Fig. 5, Table 2). *Post-hoc* pairwise Tukey HSD tests showed significant differences between monomorphic and dimorphic populations in productivity (PC2: $P = 0.005$), and between monomorphic and trimorphic populations in both habitat availability and productivity (PC1: $P = 0.013$; PC2: $P = 0.021$). The three watercourses did not differ in either habitat availability or productivity (ANOVA: PC1: $F_{2,22} = 3.0$, $P = 0.07$; PC2: $F_{2,22} = 1.0$, $P = 0.39$). There were significant differences in number of co-occurring fish species both between lakes with different whitefish diversity (Table 2) and between watercourses (two-way ANOVA: $F_{5,33} = 19.3$, $P < 0.001$). Lakes in the Pasvik watercourse were more species rich (on average eight species) than those in Alta (four) and Tana (four) (*post-hoc* Tukey HSD tests: Pasvik–Alta: $P < 0.001$; Pasvik–Tana: $P = 0.010$). Lakes with three sympatric whitefish morphs also had the most diverse fish communities (*post-hoc* Tukey HSD tests: monomorphic–trimorphic: $P < 0.001$; intermediate–trimorphic: $P = 0.002$; dimorphic–trimorphic: $P = 0.001$), while lakes with monomorphic populations had the lowest numbers of co-occurring fish species (monomorphic–intermediate: $P = 0.023$; monomorphic–dimorphic: $P < 0.001$).

Modelling phenotypic patterns

The cumulative logit model confirmed an effect of ecological opportunity and colonization history on the level of phenotypic diversity of whitefish populations. The degree of polymorphism increased significantly with decreasing elevation, increasing lake area, and decreasing Secchi depth (Table 3). There was also a significant difference in whitefish diversity between the most western (Alta) and eastern (Pasvik) watercourse (Table 3). The estimated model classified correctly 68% of the lakes with regard to level of phenotypic diversity of the whitefish population.

DISCUSSION

Whitefish populations from northern Fennoscandia displayed profound variation in the level of phenotypic divergence that could be explained by niche availability and historical

Table 3. Cumulative logit model parameter estimates with standard errors (S.E.) and 95% confidence limits (95% CL)

Parameter	Estimate	S.E.	95% CL
Elevation	-0.0296	0.0081	-0.0477 to -0.0149
Lake area	0.9188	0.3823	0.2180 to 1.7494
Secchi depth	-0.4621	0.2309	-0.9497 to -0.0294
Pasvik watercourse	-4.9429	2.0769	-9.2949 to -0.9823
Tana watercourse	0.4972	1.1554	-1.7751 to 2.8525

Note: The model was run with the western watercourse (Alta) as reference, and the results show the differences to the middle (Tana) and eastern (Pasvik) watercourses.

contingency. The most diverse and morph-rich populations were found in the easternmost watercourse, and a general pattern of increasing polymorphism towards the main stem was repeated among the studied watercourses. Altogether, four patterns of gradually increasing phenotypic diversity were demonstrated. Three of these have been documented in previous studies in the region: a unimodal (approximate range in number of gill rakers: LSR: 20–30), a bimodal (LSR: 20–30; DR: 30–40), and a trimodal (SSR: 15–20; LSR: 20–30; DR: 30–40) distribution, reflecting monomorphic, dimorphic, and trimorphic whitefish populations respectively (Amundsen *et al.*, 2004a; Kahilainen and Østbye, 2006). In addition to these previously described patterns, lakes with a substantial number of individuals with an ambiguous appearance relative to the morph criteria used here were recorded in all three watercourses, apparently representing a pattern intermediate between the monomorphic and dimorphic populations. Further investigation revealed an almost continuous variation in gill raker distributions between populations from narrow to unimodal to wide multimodal distribution patterns. This continuum of increasing divergence in a single trait resembles the more general concept of a diversification continuum, with gradual evolution of specialization, polymorphism, and eventually speciation (Smith and Skúlason, 1996; Hendry, 2009; Nosil *et al.*, 2009). Whitefish populations seem to have reached varying levels of divergence, which may represent different stages in a common evolutionary process, a scenario recently suggested for different cichlid assemblages (Seehausen, 2009). Gill raker characteristics have been identified as central adaptive traits in divergence of whitefish and other post-glacial fishes (Schluter and McPhail, 1993; Robinson and Wilson, 1994; Bernatchez, 2004; Kahilainen *et al.*, 2011), in the same way as beak morphology in the classical example of differentiation among Darwin's finches (Grant and Grant, 2002). In the present study, we have identified three specialist whitefish ecotypes related to the three principal lake habitats (i.e. the littoral, pelagic, and profundal). However, the diversification process has not always resulted in three co-existing morphs, which may be explained by a combination of historical contingency and differences in ecological opportunity for diversification.

The LSR whitefish is most likely the ancestral morph that colonized Fennoscandian lakes and later radiated into different morphs (Østbye *et al.*, 2006). The LSR whitefish is a generalist with respect to habitat and diet choice (Amundsen *et al.*, 2004b; Harrod *et al.*, 2010) and to our knowledge the only allopatric morph in northern Fennoscandia. Presumably, the DR and SSR morphs may have evolved from the LSR phenotype through competition-mediated disruptive selection and adaptive divergence (*sensu* Rueffler *et al.*, 2006; Doebeli *et al.*, 2007). This is likely the case in sympatric morph-pairs of lake whitefish (*Coregonus clupeaformis* (Mitchill)), where the planktivore dwarf ecotype was originally derived from an ancestral normal benthivore ecotype (Bernatchez, 2004; Bernatchez *et al.*, 2010). Similar divergence in gene expression of several adaptive traits has been found in sympatric pairs of both lake whitefish and European whitefish (Jeukens *et al.*, 2009), supporting a possible similar scenario with an ancestral LSR morph also in Fennoscandian lakes. Since there are no allopatric populations of DR or SSR whitefish, a divergence pre-dating the colonization is unlikely. Dimorphic and trimorphic populations may thus be the result of either double invasions of a LSR morph, with subsequent within-lake divergence upon secondary contact, or sympatric divergence of LSR populations from a single colonization event. A previous study of dimorphic populations using six microsatellite loci provides some support to the latter scenario (Østbye *et al.*, 2006). However, ecological opportunity is important for both local diversification and for the persistence of morphs following separate invasions. With resource depletion and increased competition (i.e. less ecological opportunity), closely

related morphs experiencing secondary contact are likely to hybridize and collapse to a hybrid swarm. This is a process of reverse speciation and has been described for pairs of sticklebacks and cichlids following changes in the environment (Seehausen, 2006b; Taylor *et al.*, 2006; Seehausen *et al.*, 2008; Behm *et al.*, 2010). Colonization history may also affect both the double invasion and the single colonization scenario through limiting the spread of morphs and genetic variability, and differences in the time available for diversification in different areas (Coyne and Orr, 2004; Barrett and Schluter, 2008; Caldera and Bolnick, 2008). To the extent that the different patterns of phenotypic divergence represent different stages in a common evolutionary process, the intermediate gill raker pattern suggests that a gradual increase in the range and mean of gill raker numbers precedes a potential split into two separate morphs. Whether these intermediate populations experience strong divergent selection and are evolving towards a dimorphic population, or alternatively represent either a stable situation or a hybrid swarm in a collapsing system, remains an important but so far unresolved question.

The most diverse whitefish populations with three sympatric morphs were found only in the easternmost Pasvik watercourse, and never in the Alta watercourse despite extensive sampling. Genetic variability of whitefish in these three watercourses gradually decreases from east to west (Østbye *et al.*, 2006), indicating repeated bottlenecks during the colonization. Bottlenecks may also reduce the standing genetic variation, which may restrict the potential for diversification of whitefish in the western watercourses (Barrett and Schluter, 2008; Schluter and Conte, 2009). The spatial trend in genetic diversity, together with the deglaciation history of the region [i.e. the ice-sheet retreating towards the south-west (Andersen and Borns, 1994; Berglund *et al.*, 1996; Kujansuu *et al.*, 1998)], suggest that the most eastern watercourse (Pasvik) was colonized by whitefish earlier than the western ones (Alta and Tana) and therefore holds the oldest whitefish populations in the studied area (Østbye *et al.*, 2006). Limited time for divergence may thus also explain the less diverse whitefish populations in the western watercourses, especially if not all morphs diverged simultaneously but rather in a sequential pattern as suggested by the lack of sympatric LSR and SSR in the absence of the DR morph. Age is also a common predictor of species richness in island biogeography, where older islands have had more time to accumulate species both through colonization and within-island speciation (MacArthur and Wilson, 1967; Losos and Schluter, 2000). However, time itself does not guarantee adaptive divergence, which may be illustrated by the profound differences in diversity between Darwin's finches and other taxa (e.g. mockingbirds) that colonized the Galápagos Islands at approximately the same time (Grant and Grant, 2008). High intrinsic diversification potential (i.e. generalized morphology, behavioural flexibility, and high levels of genetic variation) and ecological opportunity have been used to explain the radiation success of Darwin's finches (Grant and Grant, 2008).

From the theory of ecological opportunity, higher availability of ecological niches (i.e. habitat and food resources) is expected to facilitate population divergence (Losos and Schluter, 2000; Schluter, 2000; Seehausen, 2006a). Accordingly, we found the highest diversity of whitefish populations in larger, deeper, and more productive lakes. Lake size is generally a good predictor of inter- and intraspecific fish diversity (Barbour and Brown, 1974; Tonn and Magnuson, 1982; Nosil and Reimchen, 2005), as expected from the widely applicable species–area relationship (MacArthur and Wilson, 1967; Lawton, 1999; Losos and Schluter, 2000). Larger areas (e.g. of islands and lakes) are expected to have more heterogeneous resources and to be able to sustain a higher number of species (Ricklefs and Lovette, 1999), as a result of both colonization and within-island speciation processes (Losos and Schluter, 2000). More specifically, lakes with larger surface area and greater depth have more available pelagic and profundal habitats, important for the

existence of the DR and SSR whitefish morphs. Lakes with higher productivity are expected to have more prey resources available in all habitat types, thus offering enhanced opportunities for diversification and morphological specialization in foraging traits. Ecological opportunity (lake size, depth, and productivity) typically increases downstream in a watercourse, which may explain the higher diversity of whitefish populations we observed at lower elevations within each watercourse. The most diverse whitefish populations, with three sympatric morphs, were found in large, deep, and productive lakes in the Pasvik watercourse, which have relatively high sedimentation rates of organic matter that likely increase both the diversity and density of available prey in both the pelagic and profundal habitats (Kahilainen *et al.*, 2003, 2005). Whitefish populations classified as intermediate were all situated in the upper parts of each watercourse or tributary. These lakes were generally larger and more productive than lakes harbouring monomorphic LSR populations, but somewhat smaller and less productive than lakes with polymorphic populations. Thus, the ecological opportunity may not be sufficient to support separate morphs in these lakes. The shorter time available for diversification in these distant populations may in addition have limited their possibility for divergence. The large-scale trend in whitefish diversity between watercourses is explained mainly by the post-glacial colonization history, while the within-watercourse patterns of diversity more likely are the result of a combination of historical contingency and contemporary environmental conditions related to ecological opportunity.

Only some aspects of ecological opportunity have been considered in the present study. To fully understand disruptive selection processes, all factors influencing individuals' possibilities and constraints to utilize different resources should preferably be included. Besides habitat and food resource availability, constraints from ecological interactions are generally incorporated in the concept of ecological opportunity (Robinson and Wilson, 1994; Smith and Skúlason, 1996; Schluter, 2000). The presence of other fish species may, through competition or predation, constrain population divergence by restricting whitefish to only utilize a sub-set of the total available resource spectrum. The majority of lakes in this study were dominated by whitefish, and are relatively species-poor with usually less than ten fish species present. The spatio-temporal differences in the post-glacial colonization (i.e. westernmost watercourses were the last to be colonized) described for whitefish also restricted the immigration of other fish species. Accordingly, and contradicting the theory of ecological opportunity, the most species-rich lakes are found in the easternmost watercourse where we also find the most diverse whitefish populations. This suggests that interspecific interactions (competition and predation) probably have had minor constraining effects on, and possibly facilitated, the whitefish diversification (Vamosi, 2005), although potential interspecific consequences remain unexplored.

In conclusion, we have documented three main patterns of phenotypic divergence in whitefish from Fennoscandia based on the distribution of gill raker numbers, including unimodal, bimodal, and trimodal distributions representing monomorphic, dimorphic, and trimorphic systems respectively, as well as a group intermediate to the mono- and dimorphic populations. A spatial trend of increasing polymorphism from west to east as well as from upstream to downstream lake localities was revealed and is likely related to the post-glacial colonization history in concert with differences in contemporary environmental factors related to ecological opportunity. Phenotypic divergence was correlated with lake habitat availability and productivity, with the most diverse populations being found in lakes with highest niche availability (i.e. large, deep lakes with high productivity). This pattern

of gradually increasing whitefish diversity replicated across several watercourses and numerous lakes provides a unique opportunity to explore genetic and ecological processes of population divergence, polymorphism, and incipient speciation.

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