



# Experimental evidence for reduced hybrid viability between dwarf and normal ecotypes of lake whitefish (*Coregonus clupeaformis* Mitchill)

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Forces driving the evolution of reproductive isolation among natural populations, as well as the mechanisms involved to maintain it, are still poorly understood. Because sympatric fish ecotypes mainly differ in phenotypic traits associated with occupying distinct trophic niches, it is generally believed that reproductive isolation is mainly driven by ecological divergent selection, excluding genome incompatibility as a basis for postmating isolation. We performed cross experiments between dwarf and normal ecotypes of lake whitefish (*Coregonus clupeaformis* Mitchill) originating from distinct glacial refugia to test the hypothesis that their geographical isolation during the Pleistocene may have led to sufficient genetic divergence for the development of reproductive isolation between them before their secondary contact. Similar fertilization success in pure and hybrid crosses indicated the absence of gametic incompatibility between the two ecotypes. In contrast, daily embryonic mortality rates were 2.4–4.7 times higher in reciprocal hybrid crosses compared to pure crosses, which supports our working hypothesis. These results, along with previous morphological and population genetic studies, indicate that both genetic and ecological mechanisms may jointly act to promote speciation among northern freshwater fish ecotypes.

**Keywords:** speciation; hybrids; reproductive isolation; fish; ecotypes; *Coregonus*

## 1. INTRODUCTION

Understanding the process of species formation is a central goal in evolutionary biology. Because such a phenomenon is generally too slow to be directly observed, many speciation studies have more often relied on the comparison of closely related, yet fully isolated species (Otte & Endler 1989). A main drawback of such an approach is that it is more likely to provide information about the characteristics of species than about the processes that gave rise to them (McPhail 1993). The study of populations still capable of exchanging genes but showing the capacity to retain separate gene pools may potentially provide better insight into the early stages of species formation (Chouinard *et al.* 1996).

Several characteristics of sympatric fish ecotypes found in north temperate lakes make them of particular interest for such a purpose. These forms are specialized for occupying distinct niches, and are typically found in northern lakes that developed following the last glacial retreat, which places a maximum time of approximately 15 000 years for their radiation and/or their secondary contact. Genetic analyses generally provided evidence for restricted, yet variable, extent of gene flow between sympatric ecotypes within a given species (see, for example, Bernatchez & Dodson 1990; Taylor & Bentzen 1993; Bernatchez *et al.* 1996; Taylor *et al.* 1996). This indicates that such ecotypes are in the process of speciation,

but that reproductive isolation has not completely been achieved in most cases.

Evolutionary forces driving the reproductive isolation of sympatric fish ecotypes, as well as the mechanisms involved to maintain it, are still poorly understood. Because sympatric fish ecotypes mainly differ in phenotypic traits associated with the occupation of distinct trophic niches, it is generally believed that reproductive isolation between them is mainly driven by ecological divergent selection. This hypothesis has been supported by experimental evidence for decreased hybrid fitness, microhabitat choice, and assortative mating by size in sympatric species of sticklebacks (*Gasterosteus* sp.), in the apparent absence of other mechanisms such as reduced hybrid fertility with gametic incompatibility or higher hybrid mortality owing to developmental problems (McPhail 1992, 1993; Schluter 1993; Hatfield 1995).

Sympatric pairs of lake whitefish (*Coregonus clupeaformis* Mitchill) are mainly found in several lakes of the St John River basin in northern Maine (USA) and south-eastern Quebec (Canada) (Fenderson 1964; Chouinard *et al.* 1996). Adults of these ecotypes, referred to as dwarf and normal, display differences associated with distinct trophic niches, namely one being associated with a more pelagic and the other with a more benthic mode of life (Bernatchez & Dodson 1990). Phylogeographical studies based on mitochondrial DNA analysis revealed that the existence of these ecotypes resulted primarily from the secondary intergradation between different glacial races that have been isolated from each other since the Illinoian glacial period (150 000 years ago) (Bernatchez & Dodson

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1990, 1991; Pigeon *et al.* 1997). Typically, the dwarf ecotype is associated with the Acadian race, whereas the normal ecotype is associated with either the Atlantic or Mississippian race, depending on lakes (Bernatchez & Dodson 1991; Pigeon *et al.* 1997).

The exact mechanisms for reproductive isolation in lake whitefish have not been documented. The recent observations that levels of gene flow between dwarf and normal ecotypes from different lakes are variable, and maintained independently of reproductive habitat segregation, suggest that mechanisms of reproductive isolation of these ecotypes may be complex and involve both ecological and genetic processes (Chouinard *et al.* 1996; Pigeon *et al.* 1997). The role of ecological processes in driving reproductive isolation of whitefish ecotypes has been suggested by a positive correlation between the extent of reproductive isolation and that of trophic specialization (Bernatchez *et al.* 1996). On the other hand, it is also plausible that geographical isolation in separate refugia may have been sufficient for the development of genetic incompatibility between whitefish ecotypes belonging to distinct races prior to their secondary contact in postglacial times.

In this study, we tested the latter hypothesis by quantifying fertilization success and embryonic mortality among pure and hybrid crosses of dwarf and normal ecotypes, respectively, originating from the Acadian and Atlantic–Mississippian races of lake whitefish that came into secondary contact in several lakes of north-eastern North America.

## 2. MATERIALS AND METHODS

### (a) *Experiment*

Spawning fish were collected from two allopatric whitefish populations belonging to distinct glacial races. Acadian lake whitefish of the dwarf ecotype were sampled in Témiscouata L. (47°36' N, 68°45' W), whereas Atlantic–Mississippian fish of the normal ecotype were sampled in Aylmer L. (45°50' N, 71°26' W). Artificial fertilization was conducted in the field on 23–24 October 1996. Eggs and sperm of Témiscouata L. fish were kept on ice and transported to Aylmer L. within less than 24 h to do hybrid crosses.

Pure dwarf crosses ( $D_F D_M$ ) consisted of 20 females and 20 males, whereas pure normal crosses ( $N_F N_M$ ) involved three females and three males. One hybrid cross ( $D_F N_M$ ) consisted of 20 dwarf females and three normal males, whereas the other hybrid cross ( $N_F D_M$ ) involved three normal females and 20 dwarf males. The use of different numbers of fish for dwarf and normal ecotypes was necessary to equilibrate the number of gametes. Thus, crosses in each group resulted in the fertilization of approximately 10 000 eggs, representing a randomized family admixture. Because different families could not be raised individually owing to limited incubating facilities, all further comparisons were based on the two pure groups against the two hybrid groups.

Fertilized eggs of the four groups were disinfected and separately put into four incubators (10 l) in which flow rate was adjusted to 30–50 ml s<sup>-1</sup> to make eggs roll gently, and water temperature was maintained between 3 and 4 °C. Photoperiod followed natural conditions for the same time of the year. Dead eggs were counted daily and removed. We determined developmental stages following Brooke (1975) and using the criterion of 50% of surviving embryos reaching distinct developmental stages to express embryonic survival as a function of time.

### (b) *Data analyses*

Fertilization success was estimated by counting a subsample of 100 ml of eggs under a dissecting microscope. The numbers of eggs per millilitre were then estimated, and the total number of eggs extrapolated from the total egg volume in each incubator. Fertilization success corresponded to the proportion of fertilized eggs, which can be differentiated readily from non-fertilized ones by external coloration.

Daily mortality rates were estimated from day 1 following fertilization as the proportion of dead embryos per 24 h. Overall difference in daily mortality rates among groups was tested by the Kolmogorov–Smirnov test. As daily mortality rates were close to zero before day 15 and after day 44 (see §3), statistical comparisons were based on observations between days 15 and 44.

We also quantified the number of surviving embryos as a function of time to further assess differences in mortality rate among developmental stages. As different mortality rates occurred between developmental stages of days 15–28 and days 28–44 (see §3), comparisons among groups were made separately for these stages using an analysis of covariance (Zar 1984).

## 3. RESULTS

### (a) *Fertilization success and embryonic mortality*

High fertilization success was observed in all cross groups, being 94.2% in  $D_F D_M$ , 96.3% in  $N_F N_M$ , 93.1% in  $D_F N_M$ , and 90.7% in  $N_F D_M$ . In contrast, a higher embryonic mortality was observed in hybrid compared with pure groups (see figure 1). Daily mortality rates were  $2.8 \pm 1.9\%$  for  $D_F N_M$  and  $1.9 \pm 1.6\%$  for  $N_F D_M$  compared with  $0.6 \pm 0.5\%$  for  $D_F D_M$  and  $0.8 \pm 0.9\%$  for  $N_F N_M$ , i.e. 2.4–4.7 times higher for hybrid than for pure crosses, depending on comparisons. Daily mortality rates were not significantly different, either between hybrid crosses ( $D_F N_M$  and  $N_F D_M$ ) ( $p=0.06$ ), or between pure crosses ( $D_F D_M$  and  $N_F N_M$ ) ( $p=0.48$ ), whereas differences in any hybrid against pure crosses ( $D_F N_M$  against  $D_F D_M$ ,  $D_F N_M$  against  $N_F N_M$ ,  $N_F D_M$  against  $D_F D_M$ , and  $N_F D_M$  against  $N_F N_M$ ) and pooled hybrid against pure crosses ( $D_F N_M + N_F D_M$  against  $N_F N_M + D_F D_M$ ) were all statistically significant ( $p < 0.01$ ) following correction for simultaneous tests.

The relations of the proportion of surviving embryos as a function of time showed that mortality rate within groups, as well as among groups, varied with developmental stages (figure 2). As determined by the average date at which 50% of surviving embryos reached a given developmental stage for each group, day 15 following fertilization corresponded to the optic primordia appearance stage, day 28 to the stage of tail curved and body movement, and day 44 to the stage of appearance of circulation in the branch of the vitelline vein lateral to the heart or yolk (Brooke 1975). Mortality rates were essentially null before day 15 and after day 44 in all groups (figure 2). During days 15–28, significant difference in mortality rate was observed among all groups, except between the two pure crosses (table 1), for which the mortality rate was 5.2 times lower than in  $D_F N_M$ , and 1.9 times lower than in  $N_F D_M$  (table 2). Mortality rate was more pronounced for all groups between days 28 and 44, where no significant difference was observed in pure–pure or hybrid–hybrid comparisons (table 1). However, highly significant differences were observed in all comparisons of

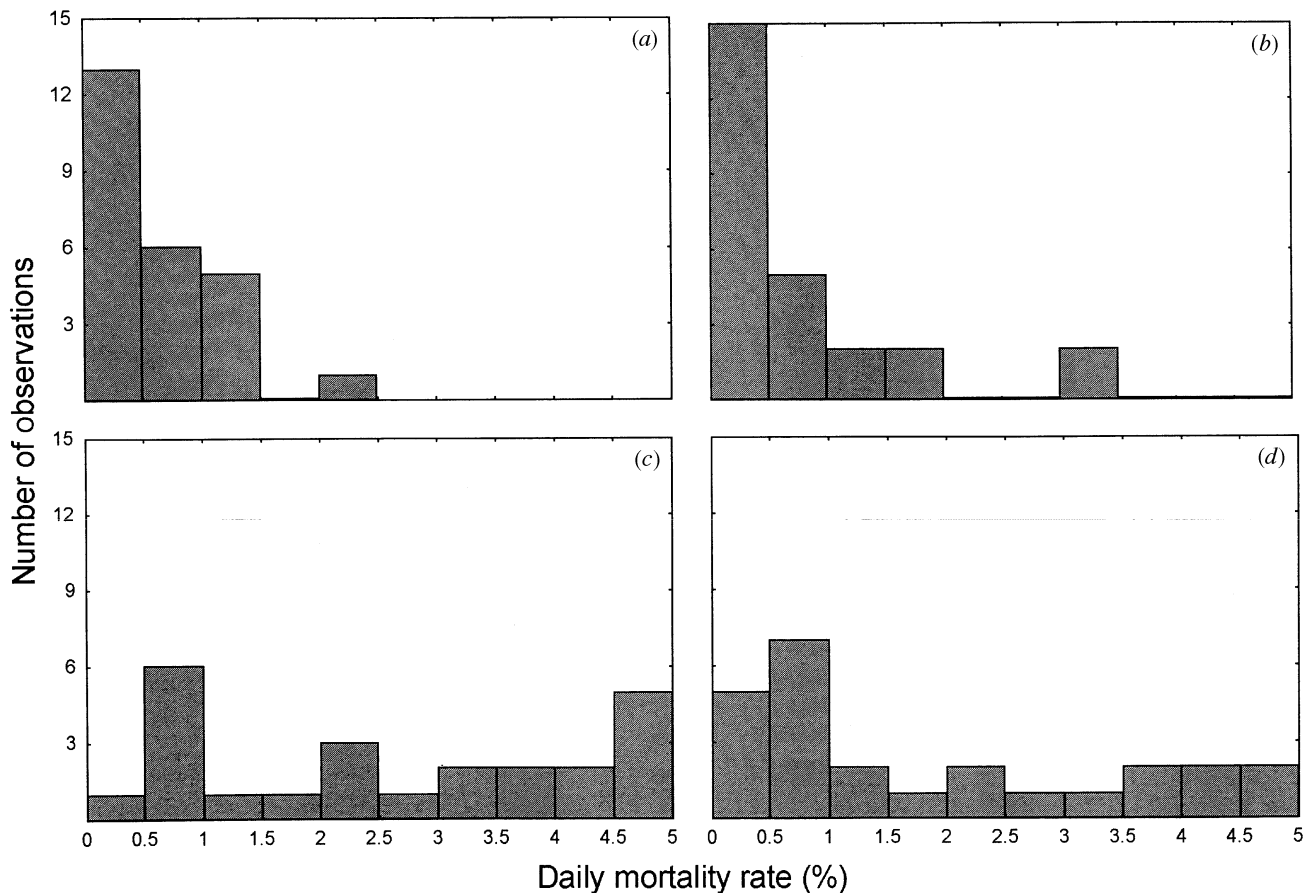


Figure 1. Distribution of mortality rates observed in pure and hybrid crosses of dwarf (Acadian race) and normal (Atlantic–Mississippian race) lake whitefish during developmental days 15–44, during which apparent mortality was observed: (a) pure dwarf crosses, (b) pure normal crosses, (c) hybrid crosses of dwarf females and normal males, (d) hybrid crosses of normal females and dwarf males. Note that an observation of 8.19% daily mortality rate is not illustrated in (c).

hybrid against pure groups, resulting in a mortality rate being 3.1–4.1 times higher in hybrid compared with pure crosses during this period (tables 1 and 2).

#### 4. DISCUSSION

The main objective of this study was to test the hypothesis that geographical isolation in separate glacial refugia led to sufficient genetic divergence to develop postmating reproductive isolation between dwarf and normal ecotypes of lake whitefish found in eastern North America. This was achieved by quantifying fertilization success and embryonic mortality among pure and hybrid crosses of these forms.

The salient features of the study were that (i) there was no evidence for reduced fertilization success in hybrid compared with pure matings; and (ii) overall embryonic daily mortality rates in reciprocal groups of hybrid progeny were 2.7–4.7 times higher than those observed in pure progeny. Embryonic mortality was also found to vary with developmental stages, with higher values observed between the stage of tail and body movement (day 28) and circulation in branches of vitelline veins (day 44) in both pure and hybrid cross groups. The much higher embryonic mortality in hybrid progeny therefore supports our working hypothesis, indicating that partial postmating

reproductive isolation may have already existed between different glacial races prior to their secondary contact in the St John River basin, approximately 12 000 years ago. Our results, however, do not support the traditional view that geographical isolation led to complete speciation (null gene flow induced by spatial isolation, *sensu* Mayr 1963). Indeed, the large percentage of hybrids surviving after 45 days, as well as the previous population genetic studies of sympatric pairs occurring in the zone of secondary intergradation, reveal that gene flow (although restricted) has occurred or is still occurring between sympatric dwarf and normal populations belonging to distinct races (Bernatchez & Dodson 1990; Pigeon *et al.* 1997). A similar scenario, whereby geographical isolation in separate glacial refugia during the Pleistocene resulted in sufficient genetic divergence to develop postmating isolation mechanisms and restrict gene flow in zones of secondary intergradation has recently been documented in waterstriders, genus *Aquarius*, from eastern North America (Gallant & Fairbairn 1997). Similar observations in highly divergent animal groups support the view of the important role of geographical isolation during Pleistocene glaciation events in promoting speciation processes in the northern aquatic fauna (Bernatchez & Wilson 1998).

Our results, however, are in contrast with previous studies of ecotypes found in other north temperate fish

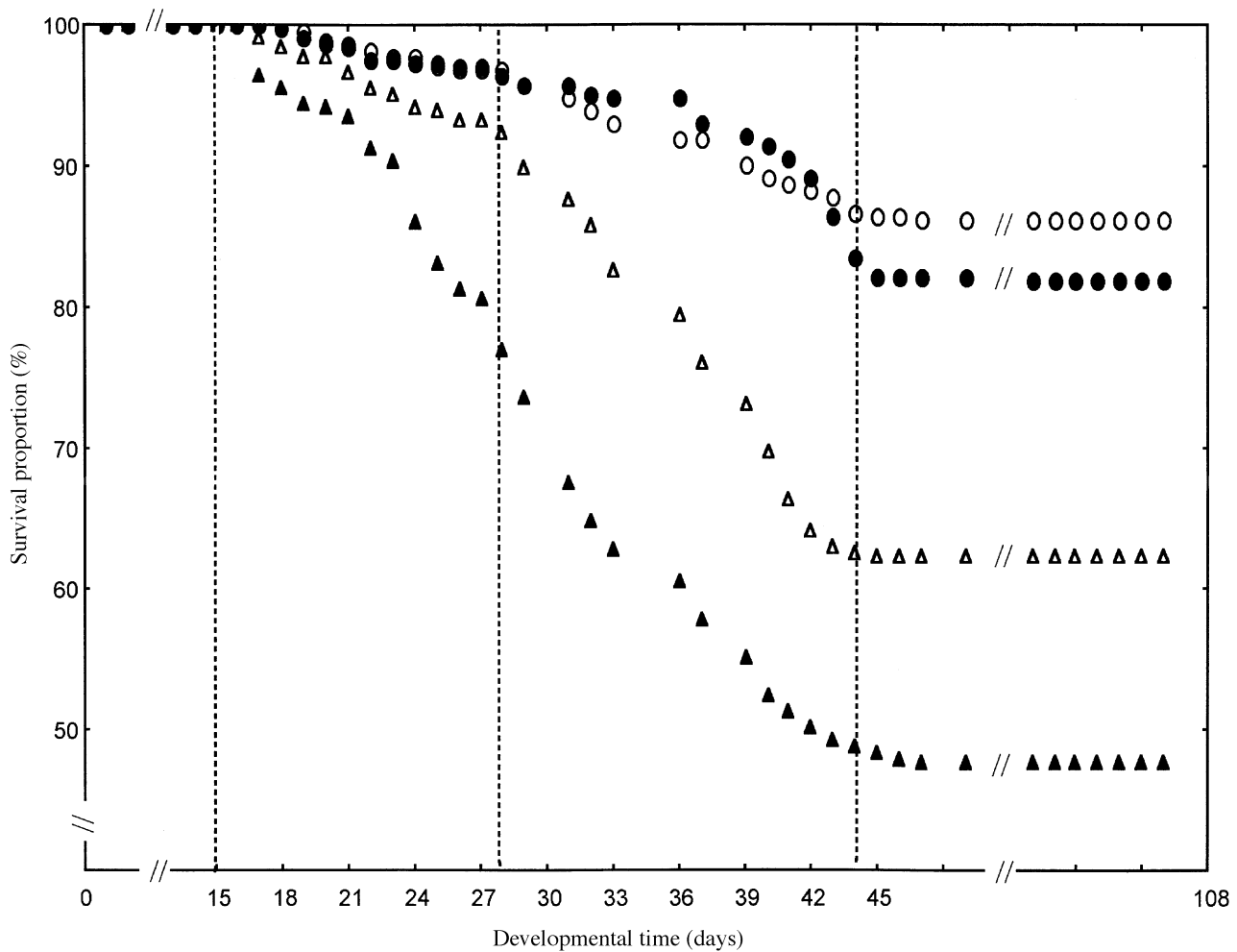


Figure 2. Surviving proportion of embryos (in per cent) as a function of developmental time for four cross groups of dwarf and normal ecotypes of lake whitefish. Open circles represent pure dwarf crosses; solid circles represent pure normal crosses; solid triangles represent hybrid crosses of dwarf females and normal males; and open triangles represent hybrid crosses of normal females and dwarf males. Vertical broken lines delineate dates of 50% developmental stages: day 15; optic primordia appearance stage, day 28; stage of tail curved and body movement, day 44; stage of appearance of circulation in branch of vitelline vein lateral to heart or yolk.

species complexes, namely sticklebacks (*Gasterosteus* sp.), and kokanee–sockeye salmon (*Oncorhynchus* sp.), for which no evidence of postmating isolation has been found (Wood & Foote 1990, 1996; McPhail 1984, 1993; Hatfield 1995). A likely explanation for the existence of an apparently unique potential genetic postmating isolation mechanism in whitefish may be the longer temporal scale involved in the divergence of ecotypes associated with distinct glacial races in this species complex. Indeed, dwarf and normal ecotypes of lake whitefish in eastern North America offer the clearest case of a species pair that resulted from separate invasion by previously allopatric populations that evolved in geographical isolation, most probably for at least 150 000 years (Bernatchez & Dodson 1990, 1991). In contrast, population genetic studies in stickleback and salmon did not support the hypothesis of secondary intergradation between ancestrally allopatric populations but, rather, a scenario of sympatric divergence that occurred in post-glacial times, that is within a time-frame of approximately 15 000 years (Taylor *et al.* 1996, 1997b). It is thus plausible

that this temporal period has not been sufficient for part reproductive incompatibility to develop through genetic processes between ecotypes of these species complexes.

The lack of evidence for reduced intrinsic viability and fertility of inter-ecotype hybrids observed in laboratory studies thus far has led to the conclusion that hybrid breakdown arising from genome incompatibility is not the basis of postmating isolation between northern fish ecotypes (Schluter 1996). Instead, the similar patterns of morphological, behavioural, and life history polymorphisms associated with specializations for exploiting distinct trophic niches (Schluter & McPhail 1993), along with experimental evidence for reduced feeding efficiency in hybrids compared with pure populations (Schluter 1993), supported the hypothesis that reproductive isolation in freshwater fish ecotypes is driven by divergent selection causing hybrid inferiority (Schluter 1996). The generality of ecological processes in driving reproductive isolation in fish ecotypes has, however, recently been questioned by the observations of sympatric occurrence of reproductively isolated populations of a same species complex despite the

Table 1. Analysis of covariance to test for difference in survival rate as a function of developmental stage for the four cross groups of dwarf (D) and normal (N) ecotypes of lake whitefish during two developmental periods delimited by distinct embryonic mortality rates

( $F_{0.05(1, 21)} = 4.32$ ;  $F_{0.01(1, 21)} = 8.02$ .)

	days 15–28		days 28–44	
	F	p	F	p
D <sub>F</sub> N <sub>M</sub> against N <sub>F</sub> D <sub>M</sub>	16.21	<0.01	0.004	>0.05
D <sub>F</sub> N <sub>M</sub> against D <sub>F</sub> D <sub>M</sub>	17.56	<0.01	18.94	<0.01
D <sub>F</sub> N <sub>M</sub> against N <sub>F</sub> N <sub>M</sub>	17.58	<0.01	17.77	<0.01
N <sub>F</sub> D <sub>M</sub> against D <sub>F</sub> D <sub>M</sub>	7.17	<0.05	20.11	<0.01
N <sub>F</sub> D <sub>M</sub> against N <sub>F</sub> N <sub>M</sub>	16.69	<0.01	19.00	<0.01
D <sub>F</sub> D <sub>M</sub> against N <sub>F</sub> N <sub>M</sub>	0.15	>0.05	3.30	>0.05
d.f.	(1, 21)	—	(1, 21)	—

Table 2. Slopes and correlation coefficients of the regression of surviving proportion of embryos as a function of time during days 15–28 and days 28–44 following fertilization in the four cross groups of dwarf (D) and normal (N) ecotypes of lake whitefish

cross	days 15–28		days 28–44	
	slope	R <sup>2</sup>	slope	R <sup>2</sup>
D <sub>F</sub> D <sub>M</sub>	−0.34	0.98	−0.87	0.98
N <sub>F</sub> N <sub>M</sub>	−0.32	0.92	−0.71	0.94
D <sub>F</sub> N <sub>M</sub>	−1.72	0.94	−2.67	0.98
N <sub>F</sub> D <sub>M</sub>	−0.63	0.98	−2.91	0.99

apparent absence of trophic polymorphisms between them. Thus, Taylor and co-workers (1997a) proposed that the development of reproductive isolation between morphologically similar sympatric kokanee salmon populations is most probably driven by competition for spawning habitats, whereas Bernatchez (1997) suggested that reproductive isolation between sympatric estuarine populations of smelt (*Osmerus* sp.) is most likely imputable to their long-term geographical isolation in distinct glacial refugia, as in the present case.

The present results, along with previous morphological and population genetics studies, suggest that both non-ecological and ecological mechanisms may be acting jointly to promote speciation processes between whitefish ecotypes. Thus, assuming that they represent conservative estimates of those realized in nature, which seems plausible given the fact that we reproduced natural incubation conditions in the absence of predation and/or natural diseases potentially encountered in nature, embryonic mortality rates obtained in this study would result in a selection coefficient ( $s$ ) against hybrids (estimated from the relative proportions of surviving embryos in each group at the end of the experiment) ranging between 0.24 and 0.44 compared with pure progeny, depending on comparisons (see figure 2). On a comparative basis, such values are considered high (Hartl & Clark 1989), and therefore may be compatible with the view that post-mating isolation as a product of genetic changes (either through mutations, drift or selection) in physically isolated

populations may enhance speciation by reinforcement when such a mechanism is very strong (Hostert 1997). On the other hand, several lines of evidence suggest that resource-based divergent selection and/or resource competition have played an important role in the genetic divergence of dwarf and normal whitefish ecotypes. These differ in behavioural and morphological traits associated with the alternate use of limnetic (dwarf) and benthic (normal) trophic niches (Fenderson 1964; G. Lu, A. Chouinard and L. Bernatchez, unpublished data). Phylogeographical studies revealed that the parallel evolution of a dwarf phenotype derived from an ancestral normal form occurred in at least three independent evolutionary lineages (Pigeon *et al.* 1997). Finally, the observation of a correlation in the amount of gene flow between whitefish ecotypes within different lakes, and the extent of their morphological differentiation associated with trophic ecology, led Bernatchez and co-workers (1996) to hypothesize that the extent of reproductive isolation achieved between ecotypes may be driven by the intensity of natural selection that generated, and is maintaining, ecotypic adaptive divergence in each lake.

As such, the present results and previous studies indicate that the roles of ecological and non-ecological reproductive isolation mechanisms are not mutually exclusive, but may act jointly to promote the speciation process in northern fish ecotypes. The fact that the level of gene flow occurring between dwarf and normal whitefish ecotypes in sympatry is generally more reduced than that reported between any other sympatric fish ecotypes (but see Chouinard *et al.* 1996) also suggests that the combined effects of different forces may accelerate the speciation process.

We dedicate this study to the late A. Chouinard, who died during his research on whitefish. We thank R. Tardif (Ministère de l'Environnement et de la Faune Québec) and M. Carette for their help in field collection, P. Gagné, S. Martin and S. Higgins for laboratory assistance. T. J. Schamerhorn (Ontario Ministry of Natural Resources) kindly provided protocols for the culture of lake whitefish as well as numerous valuable suggestions. We also thank, E. B. Taylor, J. Turgeon, S. Palumbi, T. N. Todd and two anonymous reviewers for constructive comments on the manuscript. This work was supported by a NSERC (Canada) research grant to L.B. for the GIROQ (Groupe Inter-universitaire de Recherches Océanographiques du Québec) programme.

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