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The genetic basis of intrinsic and extrinsic post-zygotic reproductive isolation jointly promoting speciation in the lake whitefish species complex (*Coregonus clupeaformis*)

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Abstract

Understanding the genetic architecture of reproductive barriers and the evolutionary forces that drove their divergence represents a considerable challenge towards understanding speciation. The objective of this study was to determine the genetic basis of intrinsic and extrinsic post-zygotic isolation in diverging populations of dwarf and normal lake whitefish with allopatric glacial origins. We found that the rate of embryonic mortality was 5.3–6.5 times higher in dwarf-normal hybrid backcrosses during development than in F1 dwarf and normal crosses. When comparing embryos that died during development against larvae that successfully hatched, patterns of Mendelian segregation at 101 loci whose linkage is known identified 13 loci distributed over seven linkage groups that exhibited significant shifts in segregation ratios leading to significant segregation distortion at these loci in the surviving progeny. Controlled crosses and quantitative trait loci analysis revealed a significant genetic basis for developmental time until emergence, a trait critical to fish larval survival in nature. Hatching backcross progeny exhibited asynchronous emergence and transgressive segregation, suggesting that extrinsic post-zygotic isolation may select against hybridization in specific environmental contexts. Evidence of a genetic basis for increased embryonic mortality followed by asynchronous emergence indicated that intrinsic and extrinsic mechanisms are not mutually exclusive in the formation and maintenance of reproductive isolation, but may be jointly promoting population divergence and ultimately speciation.

Introduction

Surprisingly little is known about the genetic basis of reproductive isolation (RI) despite its key role in speciation (Orr, 2005). Under a speciation model as envisioned by Mayr (1963), the evolution of RI mechanisms was seen as a by-product of genetic divergence between geographically isolated populations accumulating distinct alleles (Welch, 2004). Within this context, divergent alleles implicated in RI elicit a benign effect on fitness within their own genetic background but are deleterious otherwise (Johnson, 2000; Turelli & Orr, 2000). Understanding these discordant gene patterns that cause hybrid inviability represents an integral component towards understanding the origin of species (Harrison & Rand, 1998; Howard, 1998; Orr & Presgraves, 2000; Burke & Arnold, 2001, Coyne & Orr, 2004).

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Under the ecological theory of adaptive radiation, conditions of high ecological opportunity are also conducive to population divergence (Schluter, 2000; Rundle & Nosil, 2005). In these cases, RI is considered to evolve as a by-product of divergent natural selection sharing a common genetic basis with adaptation through pleiotropic interactions or physical linkage (Coyne, 1992; Rice & Hostert, 1993; Hawthorne & Via, 2001; Turelli *et al.*, 2001; Presgraves *et al.*, 2003). However, the underlying genetic basis of hybrid inviability in cases of adaptive divergence remains elusive for most species. Although hybrid fitness problems may be explained by

genetic incompatibilities, almost nothing is known about the genetic architecture of hybrid incompatibilities (Rieseberg *et al.*, 1996; Edmands *et al.*, 2005) or the evolutionary forces that may have driven their divergence (Orr & Turelli, 2001; Barbash & Ashburner, 2003; Barbash *et al.*, 2004; Presgraves *et al.*, 2003).

By examining the genetic basis of post-zygotic isolation during population divergence it may be possible to determine the factors that account for unfit hybrids and, as such, potentially offer insight into the evolutionary forces that led to formation of these barriers. This includes concurrently investigating intrinsic and extrinsic factors influencing RI (Rice & Hostert, 1993). Intrinsic post-zygotic isolation is considered to reflect developmental problems in hybrids independent of the environment and arises from divergent developmental systems that do not cooperate within a single genome (Coyne, 1992). Conversely, extrinsic post-zygotic isolation is environmentally dependent and occurs whenever hybrids inherit phenotypes that experience lower fitness in specific environments, due to the action of divergent natural selection (Schluter, 2000).

There are many reasons why understanding the role of post-zygotic isolation during population divergence is essential towards understanding speciation. Compared with prezygotic isolation, it is thought that it is difficult to 'undo' hybrid inviability based on genetic incompatibilities between diverging taxa while the evolution of multiple genetic incompatibilities reduces the probability of reversing intrinsic post-zygotic isolation (Coyne & Orr, 2004). Although post-zygotic isolation typically results in a reduction of fitness for hybrids between distinct taxa, very little is known about the genetic basis of the processes that may lead to the development of these reproductive barriers (Barton & Hewitt, 1985; Presgraves, 2003). This is partly complicated by the fact that it is difficult to establish if existing reproductive barriers arose during population divergence or following speciation. Another issue is that studies on the genetic basis of post-zygotic RI have almost exclusively focused on plant and invertebrate species (Rieseberg *et al.*, 1999, Coyne & Orr, 2004, Edmands *et al.*, 2005). Finally, in vertebrates, it has been difficult to study the genetic basis of post-zygotic RI because of considerable difficulties generating crosses past the F1 (Burke & Arnold, 2001; Price & Bouvier, 2002; Russell, 2003, but see Payseur *et al.*, 2004). Thus, investigating the genetic basis of RI in more recently diverged animal populations may increase our understanding the evolution of reproductive barriers. Overall, discovering the architecture of genetic regions contributing to hybrid inviability may be an important step towards identifying the processes that have led to population divergence and ultimately speciation. In particular, studies of post-zygotic RI have typically focused only on intrinisic barriers while the joint role of intrinsic and extrinsic forces influencing post-zygotic RI remains largely unknown (Johnson, 2000; Orr & Presgraves, 2000; Welch, 2004, Coyne & Orr, 2004).

The lake whitefish (*Coregonus clupeaformis*) species complex exhibits many ideal characteristics with which to address these issues. Allopatric divergence between populations took place during isolation within Pleistocene glacial refugia (18 000–500 000 ya, Bernatchez & Dodson, 1990, 1991). Secondary contact of glacial races subsequently occurred in several northern temperate post-glacial lakes (Bernatchez *et al.*, 1999; Lu *et al.*, 2001). Ecological opportunity in these lakes is hypothesized to have contributed to the very recent parallel phenotypic evolution of dwarf and normal ecotypes diverging in sympatry to exploit limnetic and benthic niches of the lakes, respectively (Fenderson, 1964; Pigeon *et al.*, 1997, Bernatchez *et al.*, 1999). Geographical isolation in these glacial refugia may have been sufficient for the development of genetic incompatibilities between populations prior to secondary contact in post-glacial times. Lu & Bernatchez (1998) tested this hypothesis by quantifying fertilization success and embryonic mortality among dwarf, normal 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. They found that embryonic mortality was higher in hybrid forms when compared with their dwarf and normal counterparts, but the genetic mechanisms contributing to the observed post-zygotic isolation remain unclear.

As the level of gene flow between dwarf and normal ecotypes from different lakes is negatively correlated to the degree of morphological specialization, mechanisms leading to RI may involve both ecological and genetic processes (Chouinard *et al.* 1996; Pigeon *et al.*, 1997; Lu *et al.*, 1999a; Rogers *et al.*, 2001). Overall, adaptive trait differentiation with respect to life-history (Bernatchez *et al.*, 1999), behavioural (Rogers *et al.*, 2002), physiological (Trudel *et al.*, 2001, Rogers & Bernatchez, 2005), levels of gene transcription (Derome *et al.*, 2006), and morphological traits (Bodaly, 1979; Lu & Bernatchez, 1999a; Rogers *et al.*, 2002; Bernatchez, 2004) suggests that both intrinsic and extrinsic processes may be involved in the formation of prezygotic and post-zygotic RI. However, the exact mechanisms for RI in lake whitefish have not been documented.

The objective of this study was to test the existence and elucidate the genetic basis of both intrinsic and extrinsic mechanisms of post-zygotic RI in the lake whitefish. Because fertile hybrids can be generated in the laboratory, we used a linkage mapping approach employing experimental hybrid dwarf and normal backcrosses originating from Acadian and Atlantic/Mississippian glacial races, respectively, in order to determine the genomic regions associated with RI. The utilization of hybrid backcrosses in which RI is incomplete between lineages is more likely to be informative towards representing the variation present

in natural populations as recombination between lineages liberates genetic variation normally 'hidden' by natural selection (Rieseberg *et al.* 1999, Rogers *et al.*, 2001). Consequently, reproductive barriers are more likely to represent those that have actually developed during population divergence. We focused on embryonic development because it is during this stage that post-zygotic isolation may have the most significant influence on development and viability (Lu & Bernatchez, 1998, 1999b, Chatti *et al.*, 1999). We tested the general hypothesis that both intrinsic and extrinsic forces have jointly contributed to the formation of reproductive barriers during the evolution of this species complex.

First, we predicted that elevated embryonic mortality in hybrid crosses would be associated with the development of intrinsic incompatibilities arising from either interactions between glacial races or those that have evolved during ecological divergence. During embryonic development, locus-specific deviations from Mendelian segregation, i.e. segregation distortion, may reflect such genomic incompatibilities associated with intrinsic hybrid inviability (Rice & Hostert, 1993; Whitkus, 1998; Vogl & Xu, 2000). We therefore tested patterns of Mendelian segregation at 101 AFLP loci with known linkage associations among whitefish hybrid backcrosses sampled at critical periods of embryonic development against larvae within the same family that successfully survived to emergence.

Second, the timing and synchronicity of larval emergence is critical to the survival of fishes in nature (Cushing, 1990; Powers *et al.*, 1991; Donaghy & Verspoor, 1997; Wood & Foote, 1996; Hawkins & Foote, 1998). We predicted that an additive genetic basis for time to emergence lending to an asynchronous emergence in hybrid crosses would be an underlying basis for extrinsic post-zygotic isolation if hybrids inherited phenotypes considered to be selectively inferior in specific environmental contexts (Rice & Hostert, 1993; Schluter, 2000; Coyne & Orr, 2004). We therefore tested for quantitative trait loci (QTL) associated with time to emergence to determine if extrinsic post-zygotic isolation barriers may contribute to hybrid inviability.

Materials and methods

Experimental hybrid crosses

Hybrids were produced between parents representing two allopatric whitefish populations belonging to different glacial races. The parental generation of the Acadian glacial origin (dwarf) and Atlantic-Mississippian glacial origin (normal) were sampled from Témiscouata Lake (47°36′N, 68°45′W) and Aylmer Lake (45°50′N, 71°26′W) respectively. The F1 consisted of dwarf, normal, and hybrid dwarf/normal crosses generated in 1996 (detailed in Lu & Bernatchez, 1998). The use of fish originating from different lakes was necessary because of logistic constraints (e.g. ice cover, accessibility, partial asynchrony in spawning schedule) of catching spawning dwarf and normal populations within the same lake and making successful crosses. On 25 October 1999, two divergent backcross lines were generated by crossing: (i) an F1 hybrid female (normal ♀ × dwarf ♂) with an F1 dwarf male hereafter referred to as hybrid x dwarf and (ii) an F1 hybrid female (dwarf ♀ × normal ♂) with an F1 normal male hereafter referred to as hybrid × normal. These backcross families were both used to study embryonic hybrid inviability and developmental time until emergence.

During development, fertilized eggs were maintained in cylindrical 10 L incubators with an upwelling flow rate of 30–50 mL s^{-1} to keep the eggs oxygenated. Photoperiod was kept at minimal lux while the temperature was maintained at 4 °C to approximate natural lake conditions. Dead eggs were removed twice daily and stored in 95% EtOH daily for genetic analyses. Upon hatching, whitefish larvae were automatically carried by an upwelling flow whereupon they fell into separate holding tanks and could be individually counted to measure daily hatching success. These procedures permitted accurate counts of daily embryonic mortality and hatching success within each cross.

Experimental test of intrinsic inviability

The first phase of mortality following fertilization during whitefish development begins at approximately 15 days (at 4 °C) corresponding to the initial appearance of the optic primordia (Brooke, 1975). Mortality rates are most pronounced between 28 (tail curvature stage) and 44 days (circulation appears). The mortality rate subsequently diminishes from this phase until emergence begins at approximately 75 days of development. Embryonic mortality was therefore compared for three different stages of development (days 15–28, 29–44, and 45–75 at 4 °C) in order to ascertain the degree of hybrid inviability in the backcross families relative to the F1 (see Lu & Bernatchez, 1998).

To assess whether hybrid embryonic mortality was a function of the development of intrinsic incompatibilities inferred from the distortion of Mendelian segregation ratios, AFLP loci with known linkage associations in the hybrid × dwarf backcross (Rogers *et al.*, 2001) were genotyped for embryos that died during these three stages of development (days 15–28; $n = 30$, days 29–44; $n = 25$, days 45–75; $n = 20$) and for those that survived development (days 76–136; $n = 60$, hatched larvae). For comparative purposes, we also report results of embryonic mortality rate obtained by Lu & Bernatchez (1998) for the F1 hybrids.

The sex-specific linkage associations and marker orders for 101 informative AFLP loci (covering 25 of 40 linkage groups) were determined using MAPMAKER/EXP 3.0 (Lander *et al.*, 1987). All loci were informative

(heterozygote in one parent, −/+ homozygote in the other, −/−, see details in Rogers *et al.*, 2001). Thus, in a backcross these loci were expected to segregate in a 1 : 1 ratio of heterozygotes (−/+) over homozygotes (−/−) with an observed heterozygote genotypic frequency in the segregating progeny of 0.5. Heterozygote proportions that were either under-represented in the segregating progeny (in which case the genotypic frequency was lower than 0.5) or over-represented (in which case the genotypic frequency was over 0.5) were considered distorted or deviating from expected Mendelian segregation ratios.

Loci were genotyped as follows. DNA from all samples was extracted using DNeasy microcolumns (Qiagen Inc., Crawley, UK). The final elution stages were reduced according to the manufacturer's directions in order to maximize total genomic DNA extraction from embryos when limited quantities of nuclear DNA were expected in the tissue. The AFLP plant mapping kit (Applied Biosystems Inc., Foster City, CA, USA) was used according to the protocol of (Vos *et al.*, 1995). Following the preselective amplification step, 12 selective primer combinations were used to amplify loci. Analysis of loci employed a fluorescent signal detection threshold set to 50 fluorescent units implemented in the software GENESCAN (Applied Biosystems, Inc.). Loci were automatically placed within 1 bp size-specific bins (from 50.5 to 499.5 bp) allowing an objective scoring of presence/absence scored using the software BINTHERE (Garnhart and Kocher, University of New Hampshire, http://hcgs.unh.edu/). An additional manual verification of all bins was performed in order to identify co-migrating loci within the same bin as well as loci that were inadvertently of similar size to the 1 bp binning threshold. The average size and standard deviation of all loci were calculated to identify potential genotyping errors (denoted by elevated standard deviations greater than 0.3 bp). AFLP locus notation consisted of the dinucleotide extensions representative of their selective primer combination (*Eco*RI Axx : *Mse*I Cxx) followed by the locus size in base pairs.

Dead embryos and live hatched samples were first assessed for 1 : 1 Mendelian segregation ratio using a χ^2 test implemented in MAPDISTO V1.0 (Lorieux, http://mapdisto.free.fr/). Mendelian segregation ratios were then tested for all samples pooled together under the null hypothesis that when all samples within a family are considered, dead and alive, there should be no allele frequency distortion. The significance of distorted segregation ratios was corrected for multiple comparisons using sequential Bonferonni correction (Rice, 1989).

We then determined if there was a significant shift, i.e. genotypic response, in the observed Mendelian segregation ratios during the three sampled periods of embryonic development compared with the segregation ratios of those that survived development. Possible locus-specific shifts of segregation ratios were assessed with logistic regression using the categorical data modelling (CATMOD) procedure implemented in SAS V8.0 (SAS Inc., Cary, NC, USA). CATMOD uses maximum likelihood estimation to test the categorical data (segregation ratios) over independent variables (developmental periods) (Stokes *et al.*, 2000). Because logistic regression implements maximum likelihood towards testing categorical responses, independent variables are treated within one statistical model, which increases the power of analysis. Comparatively, analyses of segregation ratios using repeated χ^2 tests are difficult to interpret, may induce type-I error, and would be limited with respect to comparative analyses between genotypic classes. If a significant genotypic response in Mendelian segregation ratios was detected among developmental periods, this would support the hypothesis that elevated embryonic mortality was associated with locus-specific incompatibilities.

We also performed a permutation procedure in order to determine the probability of observing shifts of segregation ratios by chance alone. Permutations employed the segregation ratio differential between dead and live samples (the differential between a 1 : 1 segregation ratio in both the dead and live samples would equal zero). As the whitefish hatch spanned approximately 60 days (see Results), we calculated the Mendelian segregation ratio for all dead embryo samples together to represent a similar temporal scale (day 15–75 = 60 days). The permuted distribution of differential values used a randomization procedure (employing 1000 permutations and 50 iterations) to randomly sample the absolute segregation differential values from all loci towards the construction of a permuted distribution. Our observed locus-specific segregation differential values were then compared with this distribution to obtain the corresponding probability (*P*-value) of observing such a value by chance alone when comparing dead and hatched samples. While logistic regression also detected such shifts in segregation ratios over development periods, the permutation procedure offered the additional advantage of assessing the significance given permutations of empirical values observed within the overall data set, thereby representing outcomes, which were as likely to have been observed as the original data. The source code for the permutation was written in MAPLE V.7 and is available upon request.

Segregation distortion as a function of hybrid inviability is likely the result of between locus incompatibilities (Fishman & Willis, 2001; Orr & Turelli, 2001). Thus, observing linked loci exhibiting similar shifts in Mendelian segregation ratios over embryonic development would be stronger evidence of association between elevated mortality and intrinsic incompatibilities. Parallel deviations at linked loci from Mendelian segregation over development also reduce the likelihood that these observations could be the result of scoring errors alone (Vogl & Xu, 2000). Given the observed frequency of loci exhibiting a significant shift with logistic regression, a χ^2

test was subsequently used to assess the probability of observing more locus-specific shifts over development per linkage group than expected by chance alone. The expected probability of significant loci was calculated using the frequency calculated over all loci (the total number of significant observations in logistic regression divided by the total number of loci). Expected numbers of loci per linkage group were obtained by multiplying these probabilities by the absolute number of loci observed per linkage group.

Experimental test of extrinsic inviability

Developmental time until emergence was quantified as the percentage of larvae hatching daily for the F1 and backcross families. Time to emergence in the laboratory was compared with a sympatric dwarf and normal natural population by standardizing larval hatching dates to developmental days at 4 °C using daily water temperature data available from the area (Chouinard & Bernatchez, 1998). This did not reflect the exact local temperature conditions but nevertheless provided a reasonable way by which overall patterns in developmental time to emergence could be compared between an experimental and a natural population.

A QTL analysis was performed employing each of the surviving backcross larvae genotyped. A maximum-likelihood interval analysis, performed in QTL CARTOGRAPHER (Wang *et al.*, 2006), estimated the initial likelihood of QTL from linked marker intervals spanning 2 cM along each linkage group. Experiment-wise significance thresholds for QTL identification were then determined by permutation analysis (Churchill & Doerge, 1994), which randomly permutes the phenotypic data over samples and recalculates the log of odds (LOD) test statistic across all mapping intervals. The most extreme LOD value from each permutation is saved and used to generate a distribution of LOD values to which empirical data could then be compared (Doerge & Churchill, 1996). One thousand permutations were performed for the trait with a threshold set to maintain a 5% experiment wise error rate.

Results

Intrinsic post-zygotic isolation

The success of fertilization in the hybrid backcross families was over 99% for both families, which is consistent with observations in the F1 hybrids (Lu & Bernatchez, 1998). However, both backcross families experienced severe embryonic mortality during development (Fig. 1). Mortality was highest overall in the hybrid × normal backcross with 78.9% (4384 of 5559) of embryos dying during development, compared with 55.5% (3569 of 6427) in the hybrid × dwarf backcross. A significant mortality rate (greater than zero) was observed from days 15 to 28 for both BC families (hybrid × dwarf slope = −0.68, $r^2 = 0.64$; hybrid × normal slope = −1.84, $r^2 = 0.85$). Additionally, the rate of embryonic mortality increased during days 29–44 for both backcross families (hybrid × dwarf slope = −4.59, $r^2 = 0.81$; hybrid × normal slope = −2.20, $r^2 = 0.60$), consistent with observations of the highest rate of mortality of F1 hybrids during this period (average slope = −2.79, $r^2 = 0.99$) (Fig. 1, but see details in Lu & Bernatchez, 1998).

When incorporating all embryonic samples (dead embryos and hatched surviving, average N per locus = 92), segregation ratios over all loci largely approached the 1 : 1 expected Mendelian ratio with an average genotypic frequency of 0.46 (compared with expected heterozygosity of 0.50). Overall, 97% of all loci conformed to Mendelian segregation ratios when all samples were

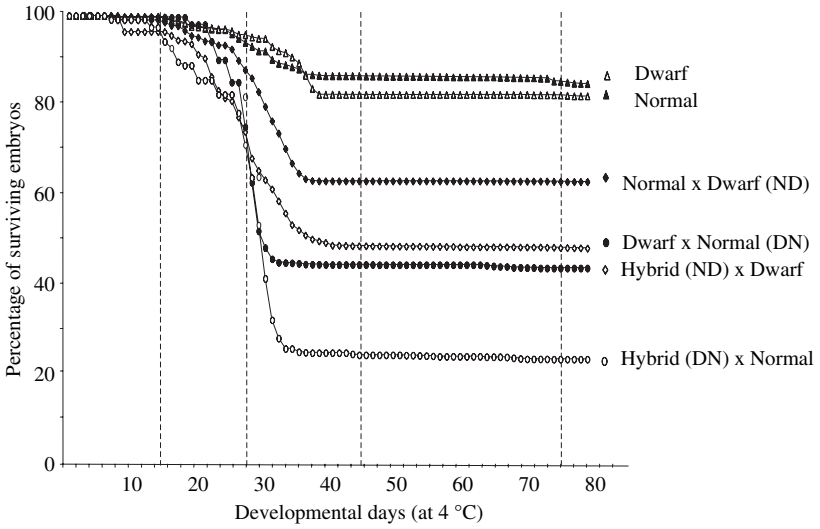

Fig. 1 Intrinsic hybrid inviability as illustrated by the percentage of surviving embryos as a function of developmental time for dwarf, normal, F1 hybrids (ND and DN, from Lu & Bernatchez, 1998), and backcrosses; hybrid × dwarf and hybrid × normal. Vertical lines delineate distinct developmental stages.

combined, with the exception of three loci from three different primer combinations (CATA064, AGTT061 and CTAG065) that exhibited significant segregation distortion at the table-wide 0.05 threshold ($P < 0.001$). In these three cases, heterozygote genotypes were significantly over-represented in the backcross progeny leading to significant genotypic frequency distortions (CATA064 = 0.72, AGTT061 = 0.67, and CTAG065 = 0.66, $P < 0.0005$).

In contrast, in embryos that died for all periods combined (average N per locus = 43), 21 loci (20.8%) exhibited significant segregation distortion ($P < 0.001$) with 19 loci under-represented and two loci over-represented in the backcross progeny. Consequently, the overall average genotypic frequency for loci in the dead embryo samples was 0.34, significantly different when compared with all samples combined (arcsine transformed ANOVA, F = 45.29, $P < 0.0001$). Conversely, in the hatched larvae (average N per locus = 60), 16 loci (16.8%) exhibited significant segregation distortion ($P < 0.001$) in the opposite direction, with two loci under-represented and 14 loci over-represented in the segregating progeny. An average genotypic frequency in the hatched backcross larvae of 0.55 was significantly different from the segregation ratios of all samples combined (arcsine transformed ANOVA, F = 24.93, $P < 0.0001$). This contrast between segregation ratios for dead embryos and those that survived was the first line of evidence indicating differential viability for embryos that inherited incompatible genotypes.

Logistic regression of segregation ratios over developmental periods indicated that 13 of 101 loci (13%) distributed over seven linkage groups illustrated a highly significant shift in segregation ratios over developmental periods ($P < 0.0001$) (Table 1). With the exception of locus CAAG069, the inheritance of homozygous genotypes was mainly associated with elevated mortality resulting in an excess of the heterozygote genotypes in the surviving progeny. Permutations of the segregation differentials between dead and live embryos supported these observations with the exception of two loci (CAAG069 and CAAG081) on Lg14 (Table 1). Two linkage groups (Lg3 and Lg18) were composed of more linked loci exhibiting a significant shift in segregation ratios among developmental periods per linkage group than expected by chance alone and therefore represented the best candidates to be implicated in intrinsic incompatibilities associated with increased hybrid mortality (Table 2). On Lg3, a positive relationship between loci CAAG143, CAAG174, ACTA104, and CAAG145 (average distance between loci = 13.25 cM) suggested that on this segment of the whitefish genome, observations of increased embryonic mortality were a function of genomic incompatibilities between dwarf and normal genomes during development (Fig. 2). During the first two developmental periods, when embryos experienced the highest mortality, significant allele frequency distortion (under the 1 : 1 expected) was observed for the genotypes of these linked loci in the dead embryos sampled. As a result, the surviving embryos exhibited genotypes with allele frequency distortion over the expected 1 : 1 segregation ratio. A similar, but weaker genotypic response during development, was also observed for Lg18 (Fig. 2).

Extrinsic post-zygotic isolation

The developmental time to emergence for both dwarf and normal families was consistent with the hypothesis that hatching times among individuals are synchronous.

Table 1 Loci exhibiting a significant ($P < 0.0001$) genotypic response to embryonic mortality as revealed by a shift in Mendelian segregation ratios over developmental periods under logistic regression.

					Day 15–28			Day 29–44			Day 45–75			Day 76–110			
Locus	LG	N	χ^2	d.f.	Aa	Aa	H_o	aa	Aa	H_o	aa	Aa	H_o	Aa	Aa	H_o	*P*-value
GGTG212	1	89	20.46	2		na		21	2	0.09	14	2	0.13	18	32	0.64	<0.01
GGTG115	1	89	20.36	2		na		17	6	0.26	13	3	0.19	13	37	0.66	<0.01
GGTG126	1	89	17.93	2		na		18	5	0.22	14	2	0.13	17	33	0.60	<0.01
CAAG143	3	114	20.53	3	20	6	0.23	19	2	0.10	9	8	0.47	17	33	0.62	<0.05
CAAG174	3	114	22.66	3	21	5	0.19	18	3	0.14	10	7	0.41	16	34	0.66	<0.01
ACTA104	3	102	21.51	2	20	2	0.09	14	0	0.00	13	3	0.19	18	32	0.58	<0.01
ACTA135	6	93	22.14	2	24	2	0.08		na		14	3	0.18	17	33	0.66	<0.01
AGAC152	12	89	20.46	2	17	10	0.37	14	7	0.33	15	2	0.12	11	39	0.74	<0.01
CAAG069	14	89	20.36	2	10	16	0.62	12	9	0.43	8	9	0.53	32	18	0.63	0.76
CAAG081	14	89	17.93	2		na		10	4	0.29	5	6	0.55	30	20	0.40	0.62
CTTC130	18	114	20.53	3		na		13	5	0.28	13	4	0.24	8	42	0.54	<0.001
CTTC216	18	114	22.66	3		na			na		13	4	0.24	20	33	0.84	<0.05
GGTG149	22	93	22.14	2		na		17	5	0.23		na		17	33	0.72	<0.01

AFLP were scored as aa, homozygote; Aa, heterozygote; H_o, heterozygosity; LG, linkage group; na, not available, indicating insufficient sampling from that period for analysis. Final column indicates probability of observing locus-specific shifts when estimating significance via 1000 permutations of the observed shifts in segregation ratios between dead and live embryos over all loci.

Table 2 Probability of observing the number of locus-specific incompatibilities per linkage group by chance alone under a χ^2 test.

		No. of loci per Lg		
Linkage group	Logistic regression	Observed	Expected	*P*-value
Lg1	Significant shift	3	2.06	0.4849
	Nonsignificant	13	13.94	
Lg3	Significant shift	3	0.77	0.0067*
	Nonsignificant	3	5.23	
Lg6	Significant shift	1	0.77	0.7826
	Nonsignificant	5	5.23	
Lg12	Significant shift	1	0.52	0.4708
	Nonsignificant	3	3.48	
Lg14	Significant shift	2	1.03	0.3059
	Nonsignificant	6	6.97	
Lg18	Significant shift	2	0.39	0.0055*
	Nonsignificant	1	2.61	
Lg22	Significant shift	1	0.39	0.3000
	Nonsignificant	3	3.61	

*denotes significance at $P <$ tablewide α of 0.05.

The overall variance was low (total $\sigma^2 = 12.5$) with a total hatching span between 9 and 17 days for these families (Table 3). The timing of development to hatching was similar between dwarf (mean = 96.9 days) and normal (mean = 97.6 days) natural populations (ANOVA, F = 1.20, $P = 0.27$). Under controlled environmental conditions, the dwarf and normal experimental families exhibited a synchronicity similar to what was observed in the natural populations (Fig. 3). However, the dwarf group hatched first causing mean hatching time to differ between dwarf (mean = 97.4 days) and normal (mean = 101.1 days) families (ANOVA, F = 2057.84, $P < 0.0001$). The variance of hatching time among these families ($\sigma^2 = 23.4$) was 1.8 times higher than observed in the natural populations. This may have been due to absence of extrinsic selective factors in the laboratory and/or the smaller number of families in a controlled environment.

In the F1 hybrids, the mean hatching time for both hybrid crosses (dwarf × normal = 99.9 and normal × dwarf = 98.8 days) was intermediate to the dwarf and normal crosses offering evidence that developmental time to emergence was under an additive, genetic control. The total variance observed in these F1 hybrids ($\sigma^2 = 22.7$) was comparable than what was observed in the dwarf and normal crosses (Table 3 and Fig. 3). This suggested that in the absence of recombination between dwarf and normal ecotypes, the timing to emergence in the F1 hybrids remained synchronous.

In contrast to the dwarf and normal and F1 hybrid families, the time to emergence in the hybrid × dwarf backcross was highly asynchronous. Time to emergence (mean = 110 days) was retarded approximately 11 days while the total hatching span was 30 days longer than what was observed in the dwarf and normal crosses (ANOVA, F = 6303.95, $P < 0.0001$) and the F1 hybrids (F = 3705.26, $P < 0.0001$) (Table 3 and Fig. 3). The

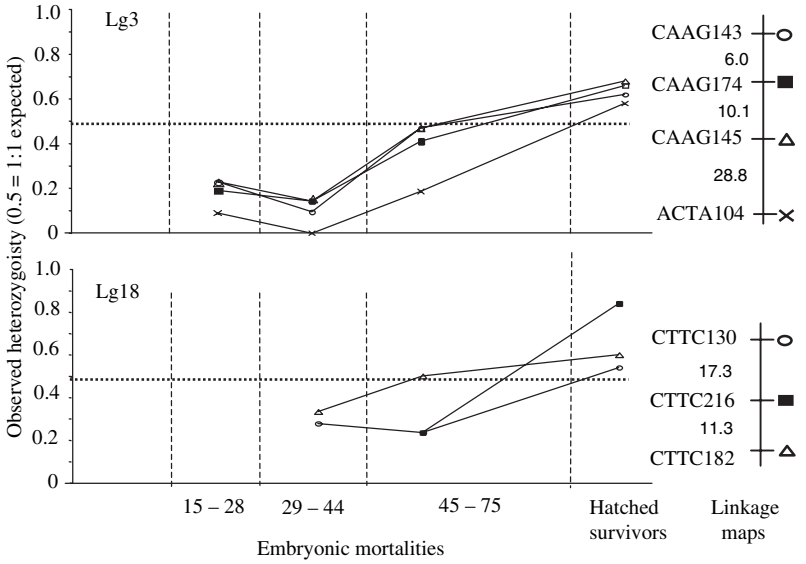

Fig. 2 Genotypic response of embryonic mortality as a function of linked intrinsic genic incompatibilities during development on linkage groups Lg3 and Lg18. Developmental time on the *x*-axis is proportional to Fig. 1 for comparison with embryonic mortality rates. On Lg3 three loci (CAAG143, CAAG174 and ACTA104) exhibited a significant shift in segregation ratios during development. A fourth locus, CAAG145, is included as it exhibited a similar genotypic response across development as the other loci but the significance level was slightly below the table wide level ($P = 0.0025$). Distances (cM) between loci are included in the legend. The dashed lines among periods schematically illustrate how these linked loci elicited similar genotypic responses throughout development when sampling dead embryos and emerging survivors upon hatching.

Source	Group	N	Mean	SD	Range	Variance	CV
Natural	Dwarf	111	96.9	3.7	93–104	14.9	3.8
	Normal	40	97.6	2.4	88–105	5.6	2.3
	All groups	151	97.1	3.5	88–105	12.5	3.1
Experimental	Dwarf	5375	97.4	3.7	82–107	13.7	3.8
	Normal	6774	101.1	5.0	93–113	25.0	4.9
	All groups	12 149	99.5	4.8	82–113	23.4	4.4
F1 hybrid	Dwarf × normal	2117	98.8	5.8	76–114	31.9	5.9
	Normal × dwarf	3372	99.9	4.3	81–114	16.5	4.3
	All groups	5489	99.5	4.8	76–114	22.7	5.1
Backcross	Hybrid × dwarf	2586	110.8	6.8	75–129	47.2	6.2
	Hybrid × normal	1041	93.5	10.9	80–119	127.8	11.6
	All groups	3627	105.8	11.4	75–129	131.1	8.9

Table 3 Summary data for developmental time until emergence measured in days at 4 °C.

Source indicates whether groups were sampled from the natural population (Chouinard & Bernatchez, 1998) or from crosses generated in the lab. CV indicates the coefficient of variation observed. See methods for complete description of cross design. SD, standard deviation; CV, coefficient of variation observed.

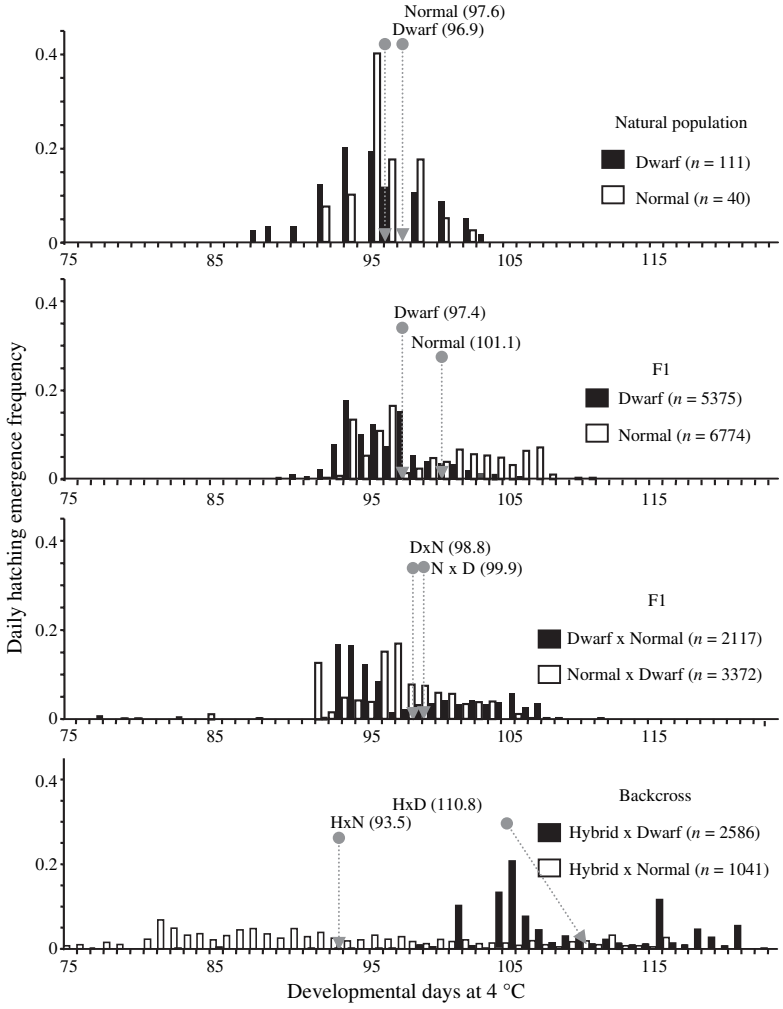

Fig. 3 Extrinsic hybrid inviability as illustrated by the distributions of the developmental time until emergence (at 4 °C) observed for (a) dwarf and normal sampled from a natural population (Chouinard & Bernatchez, 1998), (b) F1 dwarf and normal experimental crosses, (c) F1 hybrid crosses and (d) hybrid × dwarf and hybrid × normal backcrosses.

hybrid × normal backcross also exhibited an asynchronous developmental time to emergence. However, in the hybrid × normal backcross, the time to emergence (mean = 93.5 days) was significantly premature than what was observed in the F1 dwarf and normal crosses (ANOVA, $F = 1208.91$, $P < 0.0001$) and the F1 hybrid

Table 4 Summary of loci with a significant ($P < 0.05$) or suggestive ($P < 0.10$) association to developmental time to emergence in a quantitative trait loci (QTL) interval analysis of the hybrid × dwarf family.

Lg	Position (cM)	Marker interval		LOD	*P*-value	Additive effect	PVE (%)
9	8.01	CTAG118	CTAG072	2.8	0.074	+24.38	27.34
14	83.98	CAAG062	CAAG108	3.1	0.043	+25.84	29.26

The position (in cM) denotes the location within the linkage group showing the highest likelihood of association while marker interval specifies the loci within this interval. Significance of log of odds (LOD) values (LOD = 0.217 × LR, Wang *et al.*, 2006) is denoted by the *P*-value and was determined by 1000 permutations. The genetic effect of the QTL shows the mean phenotypic difference (days to hatch) between QTL genotypes as well as the direction of the informative allele in QTL analysis. The percentage of the phenotypic variance explained by the QTL is denoted by the PVE. Full information of linkage groups is available in Rogers *et al.* (2001). See Fig. 4 for the LR plots of QTL along linkage groups.

crosses ($F = 327.27$, $P < 0.0001$) (Table 3 and Fig. 3). Both hybrid backcrosses exhibited increased variance with respect to time to emergence, which was 5.6 times higher than what was observed in the dwarf and normal crosses and 10.5 times higher when compared with the natural dwarf and normal population (Table 3).

Linkage analysis showed an average distance of 18.15 cM between these 101 loci. Interval analyses revealed one significant and one suggestive QTL associated with time to emergence after experiment-wise permutations (Table 4 and Fig. 4). The first, on Lg14 (CAAG069-CAAG062) explained 29.26% of the phenotypic variance ($P = 0.043$) while a second, suggestive QTL on Lg9 (CTAG118-CTAG072) explained 27.34% of the phenotypic variance ($P = 0.074$). Notably, one of the QTL loci on Lg14, CAAG069, was also implicated in a shift in segregation ratios associated with embryonic mortality during development (Table 4).

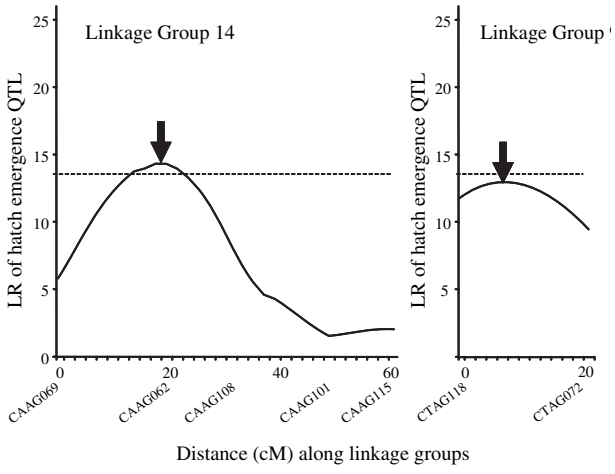

Fig. 4 Likelihood ratio (LR) plots of QTL associated with emergence using an interval analysis of the hybrid × dwarf family (See Rogers *et al.*, 2001 for full linkage map information). Black arrows indicate the most likely position of the QTL detected in linkage groups 9 and 14 while the dotted line shows the 5% experiment-wise significance threshold after 1000 permutations (LR = 13.95). A detailed description of these QTL can be found in Table 4.

Evidence for transgressive segregation

Additionally, the patterns of hatching in the backcross families were indicative of transgressive segregation, meaning the mean trait values of the hybrid crosses exceeded the phenotypic values of the parental crosses, either in a positive (hybrid × dwarf) or negative (hybrid × normal) direction. This pattern was significant, with variation in time to emergence in backcrosses exceeding the combined variation of both parental populations (Tukey *post hoc* multiple comparisons test for unequal N, Spjotvoll/Stoline; hybrid × dwarf, $P < 0.0001$; hybrid × normal, $P < 0.0001$).

Discussion

The objective of this study was to determine the genetic basis of intrinsic and extrinsic post-zygotic isolation that had evolved during the population divergence of lake whitefish. By concurrently investigating embryonic mortality and developmental time to emergence, this study demonstrated that population divergence in the lake whitefish has led to the evolution of distinct reproductive barriers whereby both intrinsic and extrinsic post-zygotic RI jointly contribute to hybrid inviability.

Intrinsic post-zygotic isolation

We predicted that the development of intrinsic incompatibilities would be associated with elevated embryonic mortality in the hybrid crosses. Our data provided strong evidence for the role of embryonic mortality as an intrinsic RI barrier. During the peak mortality (days 28–44 for all families), the mortality rate was 2.5 to 5.3 times greater that what was previously observed for dwarf crosses and 3.7 to 6.5 times greater than normal crosses confirming previous findings that divergence has resulted in genomes that are no longer fully complementary within lake whitefish hybrids (Lu & Bernatchez, 1998).

The sharp contrast between segregation ratios observed for dead and surviving embryos indicated that the inheritance of incompatible genotypes at certain linkage groups resulted in a higher likelihood of death. Thus,

more hybrids died than offspring of dwarf and normal crosses and the death rate of hybrids was not independent of the genotype. This was consistent with previous studies in which hybrid crosses between species resulted in unfavourable heterospecific interactions or discordant genomes at a locus-specific level (Wang & Eckmann, 1994; Whitkus, 1998; Livingstone *et al.*, 1999; Fishman *et al.*, 2001; Yin *et al.*, 2004; Myburg *et al.*, 2004). Comparisons of embryos that died during development with those that survived indicated a shift in segregation ratios for at least 13% of all loci over seven linkage groups in the hybrid × dwarf backcross (although this may be a conservative estimate as up to 20% of loci exhibited significant, opposite segregation distortion in the surviving progeny). Two of these linkage groups (Lg3 and Lg18) were associated with more genetic incompatibilities than expected by chance alone and elicited similar genotypic responses among linked loci with respect to patterns of segregation ratios over critical developmental periods. However, it remains unknown if these patterns have been influenced by epistasis or if these patterns are due to selection on one locus and linkage in the other loci (Rieseberg *et al.*, 1996, Orr & Presgraves, 2000; Burke & Arnold, 2001). It is noteworthy that several loci linked to Lg3 (e.g. CAAG174, CAAG140 and CAAG145) had previously been found to be significantly resistant to introgression between glacial races based on hybrid index estimates when compared with 995 other loci in natural populations (Rogers *et al.*, 2001). Together, these observations suggest that intrinsic inviability associated with this linkage group has evolved during divergence in allopatry rather than sympatry, lending to the hypothesis that historical contingency may have primed subsequent ecological determinism (Taylor & McPhail, 2000, Fraser & Bernatchez, 2005).

Segregation distortion has been frequently hypothesized to reflect incompatibilities associated with hybrid inviability during development because the genomes are no longer complementary (Rieseberg, 1993; Rice & Hostert, 1993; Kim & Rieseberg, 1999; Luo & Xu, 2003). However, the influence of segregation distortion as a form of post-zygotic isolation or hybrid breakdown has almost exclusively been demonstrated in plants (e.g. Li *et al.*, 1997; Burke & Arnold, 2001). In these cases, up to 49% of mapped loci have exhibited segregation distortion suggesting this was the result of substantial divergence between species (e.g. Whitkus, 1998; Livingstone *et al.*, 1999; Fishman *et al.*, 2001; Myburg *et al.*, 2004). These examples have cumulatively suggested that elevated levels of segregation distortion are indicative of linked sequences or loci under selection that reduce fitness upon hybridization between divergent lineages (Lyttle, 1991; Rieseberg *et al.*, 1993; Van Boven & Weissing, 1998).

However, selection for or against alleles during the life history of an organism is difficult to elucidate without evidence for the genotypic response of segregation ratios before zygotes have fully developed, i.e. events that may have occurred after fertilization (Sorensen, 1967; Bradshaw & Stettler, 1994; Vogl & Xu, 2000). Few examples have associated the deviation from Mendelian segregation with differential mortality during development. Christie & Macnair (1984, 1987) demonstrated that two natural populations of the *Mimulus guttatus* produce hybrids that die as embryos in response to population specific divergence of a copper resistance allele. McGoldrick & Hedgecock (1997) discovered that inbred crosses of oysters (*Crassostrea gigas*) exhibited significant segregation distortion when tested as adults, yet 6 h after fertilization the same loci conformed to Mendelian segregation ratios suggesting that selection against recessive deleterious mutations at closely linked genes during development was responsible for nonMendelian inheritance of markers (Launey & Hedgecock, 2001). We found that over 97% of loci conformed to Mendelian expectations when all individuals (dead and hatched) from our outbred crosses were combined, implicating a role for selection against loci during development. Altogether, these results showed that understanding the genetic basis of segregation distortion may contribute towards the understanding of post-zygotic reproductive barriers by identifying locus-specific regions of the genome susceptible to inviability upon hybridization between diverging genomes.

Extrinsic post-zygotic isolation

We predicted that if time to emergence has a genetic basis, recombination between diverging dwarf and normal lines should break up potentially co-adapted gene complexes thereby disrupting the time to emergence in the backcross families. Such a disruption would potentially lead to a reproductive barrier in specific environmental contexts. Our data indicated that developmental time to emergence has a genetic basis, which is consistent with previous experimental studies on the hatching times of fishes (Robison *et al.*, 2001; Granath *et al.*, 2004). In addition, the genetic basis for time to emergence also resulted in the asynchronous hatch of backcross hybrid individuals when compared with F1 hybrids, dwarf and normal experimental crosses, and dwarf and normal whitefish from natural populations.

Whitefish that hatched in both backcross families survived development and were healthy under the controlled experimental conditions. However, extrinsic RI barriers are hypothesized to occur in several forms of ecological inviability or behavioral sterility (see Coyne & Orr, 2004). For post-zygotic isolation to be considered extrinsic, hybrids must suffer inviability because of poor ecological fit as a consequence of divergent natural selection as opposed to intrinsic developmental defects (Schluter, 2000). For example, the role of differential growth in limnetic and benthic sticklebacks (*Gasterosteus aculeatus*) is one of the few examples demonstrating

extrinsic post-zygotic isolation as hybrids grow more slowly than either parental form as a consequence of inheriting intermediate phenotypes and therefore suffering lower fitness (Hatfield & Schluter, 1999; Rundle, 2002). It should be noted, however, that a direct link between differential growth and fitness *per se* has not been determined in *Gasterosteus*.

Although it is not logisitically possible to document the fitness of backcross progeny in wild whitefish populations, an asynchronous hatch is highly likely to correspond to a poor ecological fit for the backcross hybrids (Cushing, 1990). Indeed, asynchronous emergence in some fish species has been commonly correlated to an increased risk of starvation (Cushing, 1975, 1990), decreased opportunities for optimal growth (Morse, 1989), as well as an increased risk of predation (Morse, 1989; Frank & Leggett, 1982; Elliott & Leggett, 1996). In fact, these observations have led to one of the most important paradigms in fisheries science, the 'match/mismatch hypothesis, which predicts that the timing of emergence in fishes is highly correlated with local environmental conditions, particularly prey production (Hjort, 1914; Morse, 1989; Cushing, 1990; Mertz & Myers, 1994). This hypothesis has been central to explaining temporal variation in recruitment (Cushing, 1990), however this has not been examined in lake whitefish. Lake whitefish do have limited reserves of endogenous energy available from the yolk sac, a trait known to influence survival with respect to food availability at the time of 'first feeding' (Brooke, 1975; Miller *et al.*, 1988). Since the frequency of early life-history mortality in fishes may commonly exceed 99.5%, selection is strong and the greatest contribution to fitness may be reached by surviving this stage (Cushing, 1975; Houde, 1987; Chouinard & Bernatchez, 1998; Einum & Fleming, 2000). For example, within the family Salmonidae, Atlantic salmon (*Salmo salar*) mortality is increased by 70% within 17 days following median emergence and this selection may result in phenotypic shifts in the timing of emergence in subsequent generations (Einum & Fleming, 2000). Therefore, recombination in the backcrosses at the hatching span QTL detected contributed to a hatching span where the timing was significantly different and resulted in asynchronicity of emergence. We propose that both of these dysfunctions observed under controlled conditions are likely to result in a poor ecological fit and ultimately a lower fitness in whitefish.

Evidence for transgressive segregation

An unanticipated result of this study was the observation that transgressive segregation in the emergence times of the backcross families may have contributed to the extreme phenotypes outside the parental distributions. While the hybrid × dwarf backcross exhibited a significantly retarded mean time to emergence, the hybrid × normal backcross revealed a significantly shorter mean time to emergence when compared with the combined variation of the parental phenotypes. The direction of the transgressive segregation was therefore dependent on the direction of the backcross, suggestive of complementary gene action as opposed to developmental instability alone (Lewontin & Birch, 1966; Rieseberg *et al.*, 1999). If transgressive phenotypes are because of fixed sets of alleles with opposing effects within lines, then the expression of extreme phenotypes is predicted due to recombination in segregating hybrid generations (Rieseberg *et al.*, 1999). As genetic differences accumulate between lineages, the maintenance of similar phenotypes (e.g., by stabilizing selection) may require the accumulation of allelic differences with differential effects in sign from those predicted by the parental phenotypes (DeVincente & Tanksley, 1993; Rieseberg *et al.*, 1999; Kim & Rieseberg, 1999). Transgressive segregation is possible given the genetic architecture of differentiated populations (Rieseberg *et al.*, 2003; Albertson & Kocher, 2005), yet the consequences of trangressive segregation in cases of adaptive divergence between naturally hybridizing species remain unclear (Seehausen, 2004). Namely, transgressive segregation may be a rapid way to generate the genetic-phenotypic diversity needed to occupy novel and unexploited adaptive zones (Rieseberg & Wendel, 1993; Lexer *et al.*, 2003; Seehausen, 2004, Bell & Travis, 2005). However, if selection acts on a trait strongly correlated to fitness (such as a narrow temporal window for time to emergence), and the parental forms are present, transgressive segregation may not be advantageous and may instead provide a post-zygotic reproductive barrier to divergence. This is consistent with the notion that the synchronicity of emergence and the timing of breeding have a profound impact on fitness in most animals, including fishes (Schultz, 1993; Einum & Fleming, 2000), amphibians (Tejedo, 1992), mammals (Majluf, 1992), reptiles (Sinervo & Doughty, 1996), birds (Svensson, 1997), and insects (Landa, 1992). Although not firmly demonstrated by our experimental approach, our data indicate that transgressive segregation may contribute to the formation of extrinsic post-zygotic RI between hybridizing animal populations.

Limitations to the current study

Certain limits currently impede further interpretations of the genetic basis of post-zygotic isolation in lake whitefish. First, other factors may contribute to patterns of locus segregation. Because AFLP loci are dominant and the alleles are anonymous, heterozygosity can only be deduced in backcross progeny if one parent is homozygous and the other is heterozygous. Thus, there may be occurrences when two or more segregating loci are of similar molecular weight, i.e. co-migrating loci. If co-migrating loci are informative in the opposite sex of each

parent, such fragments would not be informative for linkage mapping and would therefore not be included in analyses. However, if the informative parents were heterozygous in the same sex for co-migrating loci, this could generate the appearance of segregation distortion. Indeed, this may explain why we detected three candidate loci of 101 with an excess of heterozygotes when segregation ratios for all samples combined were calculated (and a 1 : 1 ratio should have been respected). However, both co-migrating loci would have to be associated with elevated embryonic mortality to elicit the same genotypic response among sampled developmental periods to be implicated in intrinsic incompatibilities, otherwise they would exhibit deviations among all samples such as we observed with these three loci. For this reason, this was unlikely a problem given the methodology employed.

There can also be additional underlying biological explanations for loci not following the laws of Mendelian inheritance. This is particularly evident in cases of segregation distortion where gametic proportions can become distorted, e.g. meiotic drive (Pennisi, 2003). Sex chromosome meiotic drive, the unequal transmission of sex chromosomes from the heterogametic sex, is hypothesized to be caused by intragenomic conflict and results in biased sex ratios or a reduction of mean fitness (Jaenike, 2001; Orr, 2005). However, individuals sampled in this family at a later age suggest that the sex ratios are equal (data not shown).

As we show here, segregation distortion because of hybrid inviability may be, in many ways, considered as locus-specific evidence of selection for or against genotypes (Rieseberg *et al.*, 1993, Launey & Hedgecock, 2001). However, because these loci may interfere with the construction of linkage maps, they are often discarded from analysis or ignored. Bradshaw *et al.* (1998) remarked that, because of this, the very genes we are targeting in many evolutionary studies may often be missed. These loci may therefore be very important to acknowledge when studying the genetic basis of RI (Rieseberg *et al.*, 1993, Orr, 2005), while recent studies have suggested that they do not necessarily impede the construction of linkage groups (Hackett & Broadfoot, 2003). However, it remains unclear how best to treat these loci within a QTL analysis, although it has been suggested that QTL detection may improve if permutation thresholds are calculated separately for the contiguously distorted regions (Doerge & Churchill, 1996, Fishman *et al.*, 2001). To this end, it is possible that segregation distortion at the locus CAAG069 (Lg14) reduced the resolution of QTL analysis when compared with the significant hatching span QTL detected at the adjacent loci (Fig. 4).

Of course, the generalization of our findings will require additional tests for the presence of RI across different genetic backgrounds (Orr & Presgraves, 2000; Payseur *et al.*, 2004; Welch, 2004). This will be particularly relevant to lake whitefish given evidence that the linkage group most likely involved in intrinsic reproductive barriers may also be contingent upon historical glacial origin (Rogers *et al.*, 2001). This exemplifies the importance of understanding the genetic basis and origin of extrinsic and intrinsic isolating mechanisms that may have developed both during phases of allopatric (prior to secondary contact) and sympatric (following secondary contact) population divergence. In this study, we had genotypic information for only one backcross family, and as such it will be useful to investigate patterns over other families, particularly families of similar glacial origins undergoing sympatric divergence within the same lake. This is especially important given that, in general, we still have very little understanding of how the variation generated in studies of genetic architecture reflects the variation present in natural populations (Pigliucci 2003).

Finally, live embryos were not sampled concurrently with dead ones limiting the resolution of a comparative analysis of segregation ratios. This will be especially important given the evidence that transgressive segregation was a significant factor in the developmental time to emergence. Transgressive segregation may have therefore influenced the variability over all periods of embryonic development, including peak mortality periods.

Summary

One of the central problems impeding our understanding of speciation is discovering the origin of reproductive barriers that 'actually or potentially' prevent gene flow in sympatry (Coyne & Orr, 2004). An understanding of this problem is only achieved by determining which barriers were involved in the initial reduction of gene flow between populations as well as an understanding of the evolutionary forces that produced these barriers. However, even when we understand the genetic architecture of RIs, they do not necessarily tell us about the speciation process. Because of the difficulty in determining the origin of these barriers, speciation is still considered the 'mystery of mysteries' (Coyne, 1992). Yet, it is still currently common practice to infer issues about the speciation process from the genetic basis of RI. In this study, the combination of a genotypic response to increased embryonic mortality as well as the asynchronous emergence of backcross larvae provided evidence that intrinsic (genetic architecture) and extrinsic (ecological) mechanisms are not mutually exclusive in the formation and maintenance of post-zygotic RI but are instead both implicated in the development of reproductive barriers during the population divergence of lake whitefish. Progeny from hybrid backcross families either died during development or hatched at a sub-optimal time, consistent with observations that hybrids between diverging populations are rare (1–3%) within their natural habitat (Bodaly, 1979). Overall, we found evidence for several genomic regions implicated in the

genetic architecture of these intrinsic and extrinsic reproductive barriers.

In addition to the role of both intrinsic and extrinsic mechanisms of post-zygotic isolation documented here, several traits may additionally contribute to pre-zygotic isolation mechanisms between dwarf and normal forms diverging in sympatry. For example, Rogers & Bernatchez (2005) found parallel signatures of selection among sympatric dwarf and normal population at adaptive growth QTL suggesting that divergent natural selection for differential growth between forms has maintained differentiation during ecological divergence. However, the possible associations between differential body size and assortative mating remain to be firmly established in whitefish.

Recent studies of the genetics of speciation have concluded that speciation genes correspond to ordinary loci having normal functions within species (Presgraves *et al.*, 2003; Barbash & Ashburner, 2003; Barbash *et al.*, 2004; Orr, 2005). Because the elevated mortality experienced by whitefish hybrids corresponds to the same developmental stages whereupon dwarf and normal forms also experienced a greater risk of mortality in nature, it is possible that hybridization between diverging dwarf and normal whitefish lends to atypical or aberrant gene expression causing increased inviability (Ranz *et al.*, 2004). As the 'normal' expression of as many as 1400 genes is likely required during the early development of fishes (Amsterdam *et al.*, 2004), discovering the genes linked to segregation distortion and time to emergence is the next logical step towards the understanding of the genomic basis of RI in the lake whitefish.

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