Landscape influences on genetic differentiation among bull trout populations in a stream-lake network

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Abstract

This study examined the influence of landscape heterogeneity on genetic differentiation between migratory bull trout (*Salvelinus confluentus*) populations in Glacier National Park, Montana. An information-theoretic approach was used to compare different conceptual models of dispersal associated with barriers, different models of isolation by distance, and the combined effects of barriers, waterway distance, patch size, and intra- and inter-drainage distribution of populations on genetic differentiation between bull trout populations. The effect of distance between populations on genetic differentiation was best explained by partitioning the effects of mainstem and tributary stream sections. Models that categorized barriers as having a one-way effect (i.e. allowed downstream dispersal) or a two-way effect were best supported. Additionally, patch size and the distribution of populations among drainages influenced genetic differentiation. Genetic differentiation between bull trout populations in Glacier National Park is linked to landscape features that restrict dispersal. However, this analysis illustrates that modelling variability within landscape features, such as dispersal corridors, will benefit landscape genetic analyses. Additionally, the framework used for evaluating the effects of barriers must consider not just barrier presence, but also potential asymmetries in barrier effects with respect to the organism under investigation.

Introduction

The influences of habitat connectivity and spatial distribution of populations on ecological processes are topics that have been of interest to ecologists for more than six decades (e.g. Wright 1943; MacArthur & Wilson 1967; Levins 1969; Pulliam 1988; Hanski & Simberloff 1997). Landscape ecology has advanced our understanding of how landscape heterogeneity affects ecological processes (Turner *et al.* 2001). Similarly, the emerging field of landscape genetics (Manel *et al.* 2003) has provided a framework for examining how the physical landscape affects genetic characteristics of populations. Landscape genetics aims to identify and understand movement corridors and barriers to gene flow and addresses questions related to the influence of landscape heterogeneity on genetic variation (Storfer *et al.* 2007).

A landscape genetics approach has been used to address questions related to the genetic characteristics of freshwater fish populations. Aquatic habitat available to fishes may be easily delineated, migratory and dispersal corridors are well constrained by surrounding terrestrial habitat, and some barriers to gene flow are readily identifiable (e.g. waterfalls, dams, and dewatered stream sections).

Patterns of isolation by distance (Wright 1943) have been evaluated for a variety of fishes as well as other taxa. Isolation by distance is a population genetic structure characterized by increasing genetic differentiation

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with increasing geographic separation (see Slatkin 1993); however, the relationship between genetic differentiation on geographic distance is often variable among species and study systems. For example, genetic differentiation between populations of brook trout (*Salvelinus fontinalis*) was positively related to the stream distance separating them in Penobscot River Drainage, but not in the St. John River Drainage, Maine (Castric *et al.* 2001). Additionally, genetic differentiation of coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) populations was positively related to the stream distance separating them in western Oregon, but a subset of the observations from the Coast Range ecoregion did not exhibit isolation by distance (Guy *et al.* 2008). Variability in the strength of isolation by distance has also been observed for bull trout (*S. confluentus*) in the Boise River, Idaho (Whiteley *et al.* 2006), and the upper Kootenay River and Pine River, British Columbia (Costello *et al.* 2003).

Isolation by distance is commonly examined based on the waterway distance between population pairs for freshwater fishes; however, landscapes may be heterogeneous over the distances examined. Streams generally exhibit longitudinal changes in characteristics such as gradient and discharge, which likely influence the ability of individuals to disperse through a stream network. Therefore, incorporating landscape heterogeneity, or partitioning distance based on landscape characteristics along stream sections connecting populations, may be useful when evaluating patterns of isolation by distance.

Dispersal barriers can fragment a landscape resulting in isolated and subdivided populations. Fragmentation can increase genetic differentiation among subdivided populations (Frankham *et al.* 2002). However, there is little consistency with respect to the framework used to examine patterns of genetic differentiation between fish populations in stream networks. Elevation differences between sample sites have been used as a surrogate for the presence of barriers (e.g. Castric *et al.* 2001). However, the presence of localized, discrete barriers in low-gradient streams may confound the use of elevation difference as a surrogate for the presence of barriers. Alternatively, barriers may be treated as discrete structures that fragment the landscape between populations regardless of potential asymmetries in their affect on dispersal and gene flow. For example, genetic differentiation has been related to the presence or absence of barriers between populations (e.g. Wofford *et al.* 2005) or the sum of barriers between populations (e.g. Crispo *et al.* 2006; Leclerc *et al.* 2008). However, many barriers restrict fish dispersal in one direction only. Barriers such as waterfalls, dams, and culverts can allow dispersal of fishes in a downstream direction, but limit upstream dispersal depending on the characteristics of the barrier (e.g. height, pool depth) and the fish being examined (e.g. maximum jumping height).

The potential for one-way dispersal past barriers can result in complex patterns of genetic differentiation among populations in landscapes with multiple barriers (e.g. Neville *et al.* 2006). The influence of barriers on gene flow may be more realistically evaluated in terms of barrier presence, spatial configuration between populations, and biological constraints of the organism being examined. For example, genetic differentiation was lowest when comparing between populations of rainbow trout (*O. mykiss*) that were not isolated by barriers, moderate when comparing between populations isolated by downstream barriers and populations not isolated by barriers, and highest when comparing between populations isolated by downstream barriers and in different drainages in the Russian River, California (Deiner *et al.* 2007). This type of analysis allows for comparisons between populations in which gene flow is not restricted by barriers (Fig. 1a), gene flow is restricted in one direction by barriers (Fig. 1b), and gene flow is restricted in both directions by barriers (Fig. 1c). Analyses that evaluate the effects of barriers on genetic differentiation between populations in terms of the biological constraints of the organisms being examined are warranted, and comparisons should be made to determine the most appropriate framework for examining the influence of barriers on genetic differentiation between populations.

In addition to evaluating patterns of isolation by distance and the influence of barriers on genetic differentiation among fish, the contributions of other landscape characteristics to genetic differentiation among fish populations have been examined. Habitat patch size (Neville *et al.* 2006; Whiteley *et al.* 2006) and stream drainage pattern (Angers *et al.* 1999; Costello *et al.* 2003) have been shown to affect genetic differentiation among fish populations to varying degrees. Additionally, studies have illustrated the utility of simultaneously examining multiple landscape characteristics in order to elucidate those characteristics that have the greatest affect on genetic differentiation between populations (e.g. Angers *et al.* 1999; Costello *et al.* 2003).

Migratory bull trout populations (hereafter bull trout) in Glacier National Park (GNP), Montana, provide an ideal system to examine patterns of genetic differentiation associated with landscape heterogeneity. Bull trout in GNP west of the Continental Divide occupy an interconnected stream-lake network (Fig. 2). It is likely that bull trout naturally colonized this region from upper Columbia refugia following the Wisconsinan glaciations (Haas & McPhail 2001). Lake habitat is necessary for expression of the lacustrine-adfluvial life-history strategy exhibited by bull trout in GNP; however, bull trout
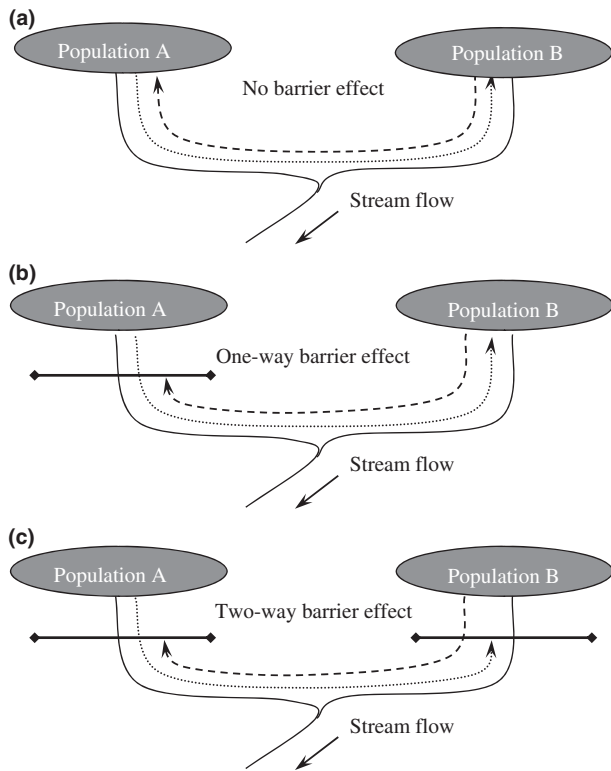
Fig. 1 Schematic representation of dispersal scenarios associated with the presence and configuration of barriers between two populations occupying different drainages in a stream network. Populations are represented by filled ovals, the stream network is represented by a solid line, the direction of dispersal is represented by a dotted line (Population A to B) and a dashed line (Population B to A), and barriers are represented by a solid line bound by diamonds. There is 'no barrier effect' on dispersal when barriers are absent (Fig. 1a). There is a 'one-way barrier effect' on dispersal when a barrier is located downstream of one population, but not the other (Fig. 1b). There is a 'two-way barrier effect' on dispersal when barriers are downstream of both populations (Fig. 1c).

are capable of long-distance migration and dispersal within streams (see Bjornn & Mallet 1964; Fraley & Shepard 1989). Therefore, bull trout are physiologically capable of dispersing among lakes in GNP, which could have ecologically important consequences (e.g. gene flow; Rieman & Allendorf 2001). The stream-lake network in GNP is variable in waterway distance between lakes, presence and spatial configuration of dispersal barriers (i.e. waterfalls), elevation differences between lakes, lake morphometry, and inter- and intra-drainage distribution of lakes. Consequently, a landscape-genetics approach may be useful for elucidating the effect of landscape heterogeneity on genetic differentiation of bull trout populations in GNP.

This study examined patterns of genetic differentiation between bull trout populations in GNP and consisted of three objectives. First, competing models for

examining the influence of waterway distance on genetic differentiation between bull trout populations were evaluated; incorporated landscape heterogeneity along dispersal corridors (i.e. differences between mainstem and tributary streams within the drainage network) was predicted to better represent patterns of genetic differentiation than a standard isolation by distance model. Second, competing models for examining the influence of barriers on genetic differentiation between bull trout populations were evaluated; partitioning the effects of one-way and two-way barriers was predicted to better represent patterns of genetic differentiation than treating all barriers the same or using elevation differences between populations as a surrogate for the presence of barriers. Third, competing models that included the combined effects of landscape and spatial characteristics between bull trout populations were compared to evaluate what characteristics where most useful for explaining patterns of genetic differentiation of bull trout populations in GNP.

Materials and methods

Sample collection

Bull trout were sampled from 16 lakes in GNP (Fig. 2) during the summers of 2004, 2005, and 2006. The sampled lakes represent the known distribution of bull trout in the Columbia Basin of GNP with the exception of Rogers Lake (Meeuwig *et al.* 2008), which was excluded from analyses due to low sample size ($N = 1$ bull trout). Each lake was assumed to represent a 'population' for the purpose of analyses performed hereafter; however, the term 'population' is used to represent a sample population as opposed to a biological population. Bull trout were sampled using gill nets, electrofishing, and hook and line (see Meeuwig *et al.* 2008). A small tissue sample (25 mm^2) was removed from the anal fin of all bull trout sampled and stored in 95% ethanol. Archived bull trout tissue samples were obtained to increase sample sizes for some lakes (Lake McDonald, A.M. Dux, unpublished data, collected 2004; Kintla Lake, Bowman Lake, Lower Quartz Lake, Logging Lake, and Harrison Lake, W.A. Fredenberg, unpublished data, collected 2000–2001). Allele frequencies did not differ ($\alpha = 0.05$) between archived bull trout tissue samples and bull trout tissue samples collected during 2004–2006 based on a Fisher exact test for genic differentiation (GENEPOP; Raymond & Rousset 1995).

Landscape variables

Landscape variables were measured either onsite during the summers of 2004, 2005, and 2006 or obtained
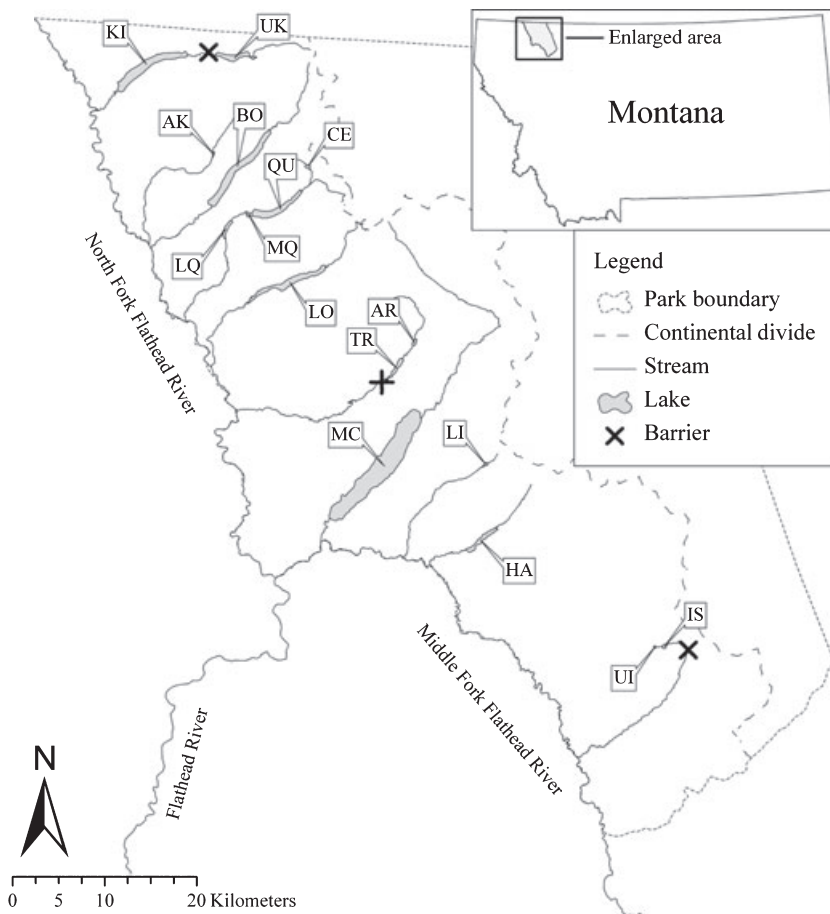
Fig. 2 Map of the study system (Glacier National Park) located in northwestern Montana. From north to south; UK, Upper Kintla Lake; KI, Kintla Lake; AK, Akokala Lake; BO, Bowman Lake; CE, Cerulean Lake; QU, Quartz Lake; MQ, Middle Quartz Lake; LQ, Lower Quartz Lake; LO, Logging Lake; AR, Arrow Lake; TR, Trout Lake; MC, Lake McDonald; LI, Lincoln Lake; HA, Harrison Lake; IS, Lake Isabel; UI, Upper Lake Isabel.

from available data [e.g. published data, map data, geographic information system (GIS) data]. The total waterway distance (hereafter total distance) between bull trout populations was measured in km from a GIS stream layer (simple polyline). Total distance was partitioned into mainstem distance and tributary distance between bull trout populations based on the trellised drainage pattern in the study system. Trellised drainage patterns have many small tributary streams running in parallel that do not join each other, but join a larger mainstem stream; therefore, the mainstem stream does not increase in stream order, but increases in discharge (Matthews 1998). Mainstem distance included portions of either the North Fork Flathead River or the Middle Fork Flathead River that were located between a population pair, and tributary distance included portions of the stream network between a population pair that were not the North Fork Flathead River or the Middle Fork Flathead River (see Fig. 2).

Within the study system, mainstem streams are fifth order streams and tributary streams are first through fourth order streams. The gradient of the mainstem streams varies from 2 to 4 m/km (lower and upper quartile) and the gradient of the tributary streams var-

ies from 14 to 26 m/km. Gradient estimates were calculated from the difference in elevation between branching points in the stream network (i.e. the confluence of mainstem and tributary streams and lake inlet and outlet elevations) and the distance along the stream network between those branching points. Elevation data were obtained from a GIS digital elevation model and distance data were obtained from a GIS stream layer.

Putative barriers to upstream dispersal by bull trout (hereafter barriers) were located by walking stream sections between each lake and either the North Fork Flathead River or the Middle Fork Flathead River; no barriers occur in the North Fork Flathead River or the Middle Fork Flathead River within the study system. Barriers were defined as waterfalls with a vertical drop of at least 1.8 m (Evans & Johnston 1980). At least one barrier was located in Kintla Creek downstream of Upper Kintla Lake (vertical drop = 6.7 m), Camas Creek downstream of Trout Lake (7.2 m), and Park Creek downstream of Lake Isabel (2.7 m; Table 1; Fig. 2). The structures identified as barriers for this analysis have been shown to limit the distribution of nonnative fishes within the study system and influence patterns of native species richness (Meeuwig *et al.* 2008).

Table 1 The presence of a downstream barrier, the number of individual bull trout sampled (N), expected heterozygosity (H_e), observed heterozygosity (H_o), and allelic richness (A_R) for bull trout sample populations from 16 lakes in Glacier National Park, Montana. Data sorted by the presence of barriers (yes to no) and expected heterozygosity (low to high) (see Fig. 2 for lake abbreviations)

Lake	Downstream barrier	N	H_e	H_o	A_R
AR	Yes	20	0.215	0.232	1.691
TR	Yes	20	0.251	0.255	1.891
UI	Yes	7	0.289	0.325	1.943
UK	Yes	20	0.291	0.305	2.005
IS	Yes	20	0.448	0.423	2.843
HA	No	20	0.342	0.264	2.382
MQ	No	11	0.487	0.529	2.934
CE	No	19	0.531	0.522	2.983
QU	No	20	0.573	0.555	3.283
LO	No	14	0.582	0.558	3.744
LQ	No	20	0.609	0.541	3.179
LI	No	12	0.616	0.583	3.597
AK	No	19	0.630	0.589	3.048
BO	No	20	0.658	0.595	3.575
MC	No	20	0.676	0.595	4.269
KI	No	17	0.686	0.642	4.147

Lake elevation (m) was obtained from a GIS lake layer (simple polygon). Elevation differences between lakes were calculated following the method of Castric *et al.* (2001), which quantifies the sum of elevation variation along the stream network between populations. For populations inhabiting lake a and lake b, this measurement was calculated as:

$$\text{Elevation difference} = (e_a - e_N) + (e_b - e_N),$$

where e_a is the elevation of lake a, e_b is the elevation of lake b, and e_N is the elevation of the lowest elevation at a common branching point in the stream network. For lakes in the same drainage with no branching point in the stream network, the lowest elevation lake of the pair was treated as e_N.

Lake surface area (km^2) was measured from a GIS lake layer (simple polygon). Lake surface area was used as a measurement of local patch size for each bull trout population. Total patch size was calculated for all bull trout population pairs. For populations inhabiting lake a and lake b, this measurement was calculated as the sum of local patch sizes for lake a and b.

An indicator variable was used to represent whether populations were located in the same drainage (drainage difference). The indicator variable was coded 0 to represent two populations located in the same drainage and 1 to represent two populations located in different drainages.

Laboratory methods

Genomic DNA was extracted from 279 bull trout tissue samples using a QIAGEN DNeasy Tissue Extraction Kit (QIAGEN Inc., Valencia, CA, USA). Sample sizes varied among populations (Table 1) with a median sample size of 20 individuals (lower quartile = 16; upper quartile = 20). Polymerase chain reaction (PCR) was used to amplify template DNA at 11 polymorphic microsatellite loci: *Omm*1128 (Rexroad *et al.* 2001), *Sco*102, *Sco*105 (Washington Department of Fish and Wildlife, unpublished), *Sco*200, *Sco*202, *Sco*212, *Sco*215, *Sco*216, *Sco*220 (DeHaan & Ardren 2005), *Sfo*18 (Angers & Bernatchez 1996), and *Smm*22 (Crane *et al.* 2004). PCR was performed in a DNA Engine DYAD thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). For each sample, one single and three multiplex PCR reactions were carried out. The number of loci examined per multiplex reaction and reaction conditions varied to optimize PCR products (Appendix). Percent amplification was high among samples for all populations and loci (97 ± 7%; mean ± standard deviation). Allele lengths were determined using an ABI 3100-*Avant* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and allele calls were made using GeneMapper software (GeneMapper version 3.7, Applied Biosystems).

Population genetic analyses

A Fisher exact test (GENEPOP; Raymond & Rousset 1995) was used to test for linkage disequilibrium between all pairs of loci within populations. Markov chain parameters were set at a dememorization of 1000 iterations, a batch size of 600, and 1000 iterations per batch. The number of loci pairs exhibiting linkage disequilibrium were determined for each population following a sequential Bonferroni adjustment ($\alpha = 0.05$; Holm 1979).

A Hardy–Weinberg exact test (GENEPOP; Raymond & Rousset 1995) was used to test for deviations from Hardy–Weinberg equilibrium among populations and loci. Markov chain parameters were set at a dememorization of 1000 iterations, a batch size of 1000, and 1000 iterations per batch. A sequential Bonferroni technique (Holm 1979) was used to control for group-wide type-I error rate when interpreting the Hardy–Weinberg exact test among loci within populations and among populations within loci.

A Fisher exact test (GENEPOP; Raymond & Rousset 1995) was used to estimate the probability of genic differentiation between all bull trout population pairs (i.e. differences in allelic distribution). Markov chain parameters were set at a dememorization of 1000 iterations, a batch size of 200, and 1000 iterations per batch.

The overall difference among loci was determined for each population pair following a sequential Bonferroni adjustment ($\alpha = 0.05$; Holm 1979).

Expected and observed heterozygosity for bull trout from each population were calculated using GENEPOP software (Raymond & Rousset 1995) and allelic richness (adjusted for sample size; see Kalinowski 2004) for bull trout from each population was calculated using HP-Rare software (Kalinowski 2005). Expected heterozygosity, observed heterozygosity, and allelic richness were averaged among loci by population. Pairwise F_{st} estimates (θ, Weir & Cockerham 1984) were calculated between all bull trout population pairs using GENEPOP software (Raymond & Rousset 1995). Gene flow between populations and F_{st} are negatively related (Frankham *et al.* 2002); therefore, F_{st} is often used as a measurement of genetic differentiation between populations (Frankham *et al.* 2002).

Genetic differentiation (F_{st}) among bull trout populations was partitioned among the stream sections connecting them using StreamTree software (Kalinowski *et al.* 2008) to determine if genetic differentiation among populations reflects contemporary patterns of stream connectivity. The fit of the StreamTree model was assessed using a coefficient of determination (R^2).

Modelling approach

The effects of landscape heterogeneity on genetic differentiation between bull trout populations were evaluated using linear statistical models following the method described by Yang (2004). Unlike traditional techniques for examining dissimilarity matrices (e.g. partial Mantel test), this method incorporates a likelihood-based approach that directly models non-independence of residuals (Yang 2004). Additionally, this method allows different covariance structures to be specified, provides estimates and significance tests for the model parameters, and provides likelihood statistics that allow comparisons of competing models following an information-theoretic approach (Burnham & Anderson 2002; Yang 2004). The PROC MIXED procedure in SAS software (SAS Institute 1989) was used and a program provided by Yang (2004) was modified to include multiple predictor variables when necessary; a modification suggested by Yang (2004).

Statistical models were examined for the presence of outlier populations following the method described by Koizumi *et al.* (2006). This procedure involves fitting a statistical model to pairwise genetic data (e.g. pairwise F_{st}) and examining the model residuals by population. If the mean residual for a population has a 95% confidence interval that does not overlap zero the population is considered to be an outlier, the population is removed from the analysis, and the model is refit. This procedure is repeated until the 95% confidence intervals of all the remaining populations overlap zero. For this study, a population was considered an outlier if two times the standard deviation of the mean residual (approximately 95% of the normal distribution) did not overlap zero. This modification was made because the distribution of the residuals was of interest, and not the confidence associated with the estimated mean residual.

The response variable for all models was genetic differentiation between population pairs (F_{st}). Predictor variables included combinations of landscape variables (see above). Indicator variables were used to define the classes of qualitative predictor variables (e.g. barrier presence, drainage difference); for c classes of a qualitative predictor variable, $c - 1$ indicator variables were used (Neter *et al.* 1996). Models were fit without an intercept term because it was assumed that in the absence of landscape heterogeneity between populations there would be no geographic separation between populations and therefore genetic differentiation would also be zero.

A restricted maximum likelihood method was used to evaluate four residual covariance structures (independent observations, compound symmetry, first-order autoregressive, and first-order autoregressive moving-average) for each model. Residual covariance structure was selected based on a likelihood ratio test of -2 times the log-likelihood (Yang 2004). A maximum likelihood method and a likelihood ratio test (Yang 2004) were used to evaluate significance of model parameters (i.e. fixed effects). Parameters were considered significant if they differed from zero ($\alpha = 0.05$). Models were ranked using Akaike's Information Criterion (AIC; Akaike 1973) with a small sample size adjustment (AIC$_c$; Hurvich & Tsai 1989). Akaike differences (Δ_i; where i is the model rank) were calculated and models with Δ_i greater than 10 were considered to be poorly supported and were not examined further (Burnham & Anderson 2002). Evidence ratios (w_1/w_i; where w_1 is the Akaike weight for the highest ranked model and w_i is the Akaike weight for the ith highest ranked model) were calculated and interpreted as the likelihood that the highest ranked model was the best model relative to the ith highest ranked model given that one of the models must be the Kullback–Leibler best model (Kullback & Leibler 1951; Burnham & Anderson 2002). An adjusted coefficient of multiple determination (adjusted R^2) was calculated to measure the proportionate reduction of total variation in genetic differentiation associated with the independent landscape characteristics (Neter *et al.* 1996).

Genetic differentiation models

Three groups of models were used to evaluate the influence of landscape heterogeneity on genetic differentiation between bull trout populations. Group one was used to evaluate the influence of waterway distance on genetic differentiation, group two was used to evaluate the influence of barriers on genetic differentiation, and group three was used to evaluate the combined effects of landscape heterogeneity on genetic differentiation. Model ranking was performed separately for each group of models.

Two competing models were used to evaluate the influence of waterway distance on genetic differentiation between bull trout populations. Total distance between bull trout populations was the predictor variable for the first model (distance model 1). The second model (distance model 2) included two predictor variables, mainstem distance and tributary distance. Populations that were located upstream of barriers were omitted from these models because it was assumed that barriers would have an effect on genetic differentiation that would not be accounted for by only examining waterway distance.

Three conceptual models were used to evaluate the effects of barriers on genetic differentiation. The first conceptual model (barrier model 1) used elevation difference between populations (predictor variable) as a surrogate for the presence of barriers (e.g. Castric *et al.* 2001). The second conceptual model (barrier model 2) classified barrier effects in one of two ways. First, the effect was classified as 'no barrier' when no barriers were located between a population pair. Second, the effect was classified as 'barrier' when at least one barrier was located between the population pair, regardless of the potential for one-way dispersal. One indicator variable was used to define the two classes of the qualitative predictor variable (i.e. no barrier, barrier). The third conceptual model (barrier model 3) classified barrier effects in one of three ways. First, the effect was classified as 'no barrier' when no barriers were located between a population pair (Fig. 1a). Second, the effect was classified as 'one-way barrier' when at least one barrier was located between a population pair, but the spatial configuration of the barrier or barriers was such that one-way dispersal could occur between the population pair (Fig. 1b). Third, the effect was classified as 'two-way barrier' if at least two barriers were located between a population pair and the spatial configuration of those barriers constrained dispersal in both directions (Fig. 1c). Two indicator variables were used to define the three classes of the qualitative predictor variable (i.e. no barrier, one-way barrier, two-way barrier). Barrier models were evaluated while accounting for water-way distance between populations as either total distance or the combined effects of mainstem distance and tributary distance; therefore, six competing models were evaluated (three barrier models × two distance models).

The combined effects of landscape heterogeneity on genetic differentiation between bull trout populations were examined by comparing 18 competing models. All models included a waterway distance effect (distance model 1 or distance model 2; defined above), a barrier effect (either barrier model 1, or barrier model 2, or barrier model 3; defined above), and either total patch size, or drainage difference, or both.

Results

Population genetic analyses

No consistent deviations from Hardy-Weinberg expectations were observed among loci within populations or among populations within loci. Bull trout in Harrison Lake deviated from Hardy-Weinberg equilibrium at *Sco*220 ($P < 0.001$; heterozygote deficiency) and bull trout in Lower Quartz Lake deviated from Hardy–Weinberg equilibrium at *Sco*216 ($P = 0.001$; heterozygote deficiency). Linkage disequilibrium was detected in 8 of 880 population and locus-pair combinations, but no patterns of disequilibrium were observed.

Allelic distributions differed significantly for 115 of 120 pairwise population comparisons (Table 2). Allelic distributions did not differ between bull trout in Kintla Lake and Lake McDonald, Cerulean Lake and Quartz Lake, Cerulean Lake and Middle Quartz Lake, Quartz Lake and Middle Quartz Lake, and Arrow Lake and Trout Lake.

Among bull trout populations and loci, expected heterozygosity varied from 0.215 to 0.686, observed heterozygosity varied from 0.232 to 0.642, and allelic richness varied from 1.691 to 4.269 (Table 1). Genetic differentiation (F_{st}) between bull trout populations varied from <0.001 to 0.658 (Table 2).

Genetic differentiation between bull trout populations was well explained by contemporary patterns of stream connectivity. The StreamTree model resulted in a $R^2 = 0.909$ when all bull trout populations were included. The model was re-evaluated without the Harrison Lake bull trout population (see below) resulting in a $R^2 = 0.969$.

Genetic differentiation models

A first-order aoutoregressive moving-average residual covariance structure was used for all models. The Harrison Lake bull trout population had a positive mean

Table 2 Pairwise genetic differentiation estimates (F_{st}; upper diagonal) for bull trout sample populations from 16 lakes in Glacier National Park, Montana. Superscript 'NS' denotes populations that did not differ in allelic distributions based on a Fisher exact test for geneic differentiation. Lower diagonal is the total waterway distance (km) between populations (see Fig. 2 for lake abbreviations)

Lake	UK	KI	AK	BO	CE	QU	MQ	LQ	LO	AR	TR	MC	LI	HA	IS	UI
UK		0.241	0.385	0.377	0.485	0.45	0.514	0.411	0.396	0.615	0.561	0.297	0.349	0.55	0.421	0.561
KI	3.8		0.081	0.068	0.175	0.145	0.175	0.104	0.088	0.357	0.333	0.006[NS]	0.059	0.301	0.205	0.275
AK	50.4	46.6		0.138	0.244	0.202	0.243	0.167	0.165	0.368	0.352	0.106	0.132	0.345	0.265	0.333
BO	42.7	38.9	27.9		0.212	0.163	0.21	0.126	0.148	0.335	0.313	0.073	0.162	0.322	0.319	0.363
CE	63.9	60.1	49.1	40.5		0.005[NS]	<0.001[NS]	0.058	0.154	0.524	0.503	0.192	0.227	0.396	0.343	0.375
QU	60.8	57.1	46.1	37.5	3.0		0.012[NS]	0.048	0.121	0.482	0.458	0.159	0.198	0.351	0.307	0.338
MQ	60.4	56.6	45.6	37.0	3.5	0.4		0.063	0.168	0.568	0.541	0.202	0.245	0.43	0.371	0.412
LQ	58.4	54.7	43.7	35.1	5.4	2.4	1.9		0.124	0.413	0.392	0.118	0.172	0.34	0.284	0.322
LO	55.9	52.2	41.2	32.6	31.6	28.5	28.1	26.2		0.454	0.43	0.116	0.159	0.326	0.283	0.333
AR	82.6	78.8	67.9	59.2	58.3	55.2	54.8	52.8	42.9		0.015[NS]	0.385	0.454	0.641	0.562	0.658
TR	80.2	76.5	65.5	56.8	55.9	52.8	52.4	50.4	40.5	2.4		0.364	0.429	0.621	0.542	0.625
MC	98.2	94.5	83.5	74.9	73.9	70.8	70.4	68.5	58.5	66.7	64.3		0.078	0.318	0.219	0.297
LI	121.9	118.2	107.2	98.6	97.6	94.5	94.1	92.1	82.2	90.4	88.0	31.2		0.311	0.226	0.306
HA	115.3	111.6	100.6	91.9	91.0	87.9	87.5	85.5	75.6	83.8	81.4	24.6	25.3		0.431	0.52
IS	169.1	165.3	154.3	145.7	144.7	141.7	141.3	139.3	129.4	137.5	135.1	78.4	79.1	66.7		0.216
UI	169.8	166.1	155.1	146.5	145.5	142.4	142	140.1	130.1	138.3	135.9	79.1	79.8	67.4	0.8	

residual with a distribution (two times the standard deviation) that did not overlap zero in all models. Therefore, the Harrison Lake bull trout population was considered an outlier due to greater genetic differentiation relative to other populations and was removed from the analyses (Koizumi *et al.* 2006).

The effect of waterway distance on genetic differentiation between bull trout populations was best explained by the model that incorporated mainstem distance and tributary distance. Distance model 2 was ranked higher than distance model 1 and the Akaike difference between distance model 2 and distance model 1 was 42.4; therefore, distance model 1, which was analogous to a traditional method for evaluating isolation by distance, was considered to be poorly supported and was not examined further. Mainstem distance did not have a significant effect on genetic differentiation between bull trout populations ($P = 0.299$), but F_{st} between bull trout populations was predicted to increase by 0.006 ($P < 0.0001$) for each 1 km increase in tributary distance; adjusted R^2 for this model was 0.677.

The effect of barriers on genetic differentiation between bull trout populations was best explained by the model that incorporated the effects of one-way barriers and two-way barriers, and which accounted for waterway distance between populations using mainstem and tributary distance (adjust $R^2 = 0.666$). Akaike differences between the highest ranked model and the five other models used to evaluate the influence of barriers were greater than 41.3. Therefore, models that used elevation difference between populations as a sur-

rogate for the presence of barriers, that did not consider the potential for one-way dispersal past barriers, and that used the total distance between populations to account for waterway distance were considered to be poorly supported and were not examined further. In the highest ranked model, a one-way barrier was predicted to increase F_{st} between populations by 0.200 ($P < 0.0001$) and a two-way barrier was predicted to increase F_{st} between populations by 0.378 ($P < 0.0001$).

The combined effects of landscape heterogeneity on genetic differentiation between bull trout populations were best explained by three models that had Akaike differences less than or equal to 10 (Table 3). The highest ranked model (Fig. 3) was 2.123 times more likely to be the best model than the second highest ranked model and 60.499 times more likely to be the best model than the third highest ranked model (Table 3). Adjusted R^2 of the three highest ranked models varied from 0.789 to 0.852 (Table 3).

Only models that included both one-way barrier and two-way barrier effects had Akaike differences less than or equal to 10. The estimated effect of a one-way barrier on F_{st} varied from 0.209 to 0.225 and the estimated effect of a two-way barrier varied from 0.399 to 0.423 among the top ranked models (Table 3). Drainage difference was included in the top ranked models, and its effect on F_{st} varied from 0.101 to 0.206 (Table 3) indicating that bull trout populations in different drainages were genetically more different than populations located in the same drainage. Total patch sizes had significant negative effect on genetic differentiation

Table 3 Model rank, Akaike Differences (Δ_i), evidence ratios (w_1/w_i), adjusted coefficient of multiple determination (Adj. R^2), effects in model, model effect estimates, likelihood ratio statistics, and probability (P) that the effect was different from zero for the three top-ranked models used to examine the effect of landscape heterogeneity on genetic differentiation (F_{st}) between bull trout populations in Glacier National Park, Montana

Rank	Δ_i	w_1/w_i	Adj. R^2	Effect	Effect estimate	Likelihood ratio	P
1	0.0		0.844	Mainstem distance	−0.001	26.486	< 0.001
				Tributary distance	0.002	5.896	0.015
				One-way barrier	0.209	105.005	< 0.001
				Two-way barrier	0.399	109.901	< 0.001
				Total patch size	−0.002	3.854	0.050
				Drainage difference	0.138	13.639	< 0.001
2	1.5	2.1	0.852	Mainstem distance	−0.001	26.382	< 0.001
				Tributary distance	0.003	15.867	< 0.001
				One-way barrier	0.209	103.178	< 0.001
				Two-way barrier	0.401	108.420	< 0.001
				Drainage difference	0.101	9.787	0.002
3	8.2	60.5	0.789	Total distance	−0.001	19.387	< 0.001
				One-way barrier	0.225	113.261	< 0.001
				Two-way barrier	0.423	113.880	< 0.001
				Total patch size	−0.003	16.973	< 0.001
				Drainage difference	0.206	48.652	< 0.001
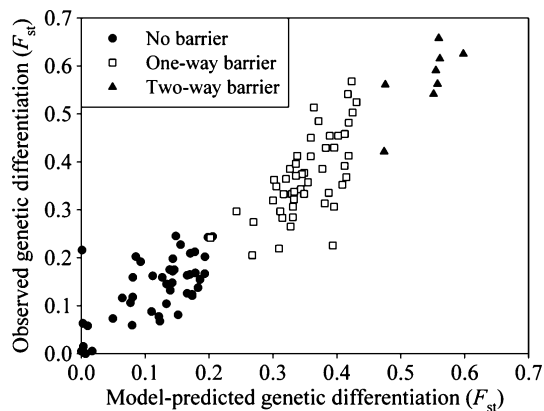
Fig. 3 Scatterplot of observed genetic differentiation (F_{st}) versus predicted genetic differentiation (F_{st}) from the landscape model which included the effects of mainstem distance, tributary distance, one-way barrier, two-way barrier, total patch size, and drainage difference. Filled circles represent populations not separated by a barrier, open squares represent populations separated by a one-way barrier, and filled triangles represent populations separated by a two-way barrier.

between bull trout populations in the first and third ranked models. Each 1 km² increase in total patch size of two populations was predicted to decrease F_{st} by 0.002–0.003 (Table 3).

The first and second ranked models included the effects of mainstem distance and tributary distance. Each 1 km increase in tributary distance was estimated to increase F_{st} between populations from 0.002 to 0.003 (Table 3). Mainstem distance had a negative effect on genetic differentiation between bull trout populations,

with an effect size of −0.001 (Table 3). Total distance was included in the third ranked model with an effect size of −0.001 (Table 3).

Discussion

Barriers have an effect on genetic differentiation among bull trout populations in a trellised drainage network. Genetic differentiation between bull trout populations was greater when barriers were present than when absent, and the magnitude of the effect of these barriers was larger than other landscape characteristics examined. Additionally, patterns of genetic differentiation were best described by partitioning barrier effects into one-way and two-way barriers.

Identifying barriers to gene flow and evaluating their influence on population genetic structure are common themes in the field of landscape genetics (Storfer *et al.* 2007). For aquatic organisms barriers may be readily-identifiable, discrete structures such as dams and waterfalls, or may be continuous landscape variables, such as elevation gradient (e.g. Castric *et al.* 2001). Modelling barriers as discrete structures has often been useful for explaining patterns of genetic differentiation. For example, the presence of dams had a greater effect on population genetic structure than geographic distance between samples for yellow perch (*Perca flavescens*) in the Saint Lawrence River, Quebec, Canada (Leclerc *et al.* 2008), waterfalls decrease dispersal and gene flow for Trinidadian guppies (*Poecilia reticulata*) in the Marianne River, Trinidad (Crispo *et al.* 2006), and barriers have been shown to increase genetic differentiation

between samples for a variety of salmonid species [e.g. bull trout (Whiteley *et al.* 2006), cutthroat trout (Wofford *et al.* 2005; Neville *et al.* 2006), and rainbow trout (Deiner *et al.* 2007)].

Various conceptual and analytical frameworks have been used to evaluate the influence of barriers on genetic differentiation between populations or samples; many of which have not distinguished between barriers that restrict dispersal between populations in one direction only from barriers that restrict dispersal in two directions. The analytical framework used in this study allowed this distinction to be made and provided greater biological insight into how barriers are influencing genetic differentiation between bull trout populations in GNP. Models that incorporated the effects of both one-way and two-way barriers were better supported than models that treated all barriers and barrier configurations the same or that used elevation gradients as a surrogate for the presence of barriers. The trends observed for bull trout in GNP are similar to those observed for rainbow trout in the Russian River (Deiner *et al.* 2007), where F_{st} was lowest between below barrier samples (i.e. no barrier effect), moderate between above barrier and below barrier sites (i.e. one-way barrier effect), and greatest between above barrier sites in different drainages (i.e. two-way barrier effect). This trend may be common among fishes and other taxa and should be evaluated further in landscapes with multiple barriers and barrier configurations.

Patterns of isolation by distance for bull trout in GNP were best described by partitioning the effects of mainstem and tributary distance. When evaluating the effects of waterway distance, the model that included only the total distance between populations was considered to be poorly supported relative to the model that included both mainstem distance and tributary distance effects. Additionally, models that incorporated multiple landscape characteristics generally favoured models that included both mainstem and tributary distance between populations. Variability in stream characteristics (e.g. width, depth, gradient, discharge) can influence the dispersal ability of aquatic organisms. Tributary distance between bull trout populations in GNP had a greater effect on genetic differentiation between populations than mainstem distance. Therefore, the effect of waterway distance on genetic differentiation between bull trout populations in GNP is principally influenced by the amount of tributary distance between populations as opposed to mainstem distance or total distance.

Similarity in allelic distributions and the small observed F_{st} between bull trout populations in Kintla Lake and Lake McDonald provides an example of the tributary distance effect. Of 105 possible comparisons

between population pairs (excluding Harrison Lake), Kintla Lake and Lake McDonald had the 76th greatest waterway distance between them, but genetic differentiation between these populations was the third smallest. Only 8.90 km of the waterway distance between these populations consisted of tributary distance; the 10th shortest tributary distance. Therefore, partitioning waterway distance into mainstem and tributary streams helps explain the genetic similarity between these populations.

Landscape heterogeneity along dispersal corridors between populations may influence dispersal capabilities and gene flow for a variety of species. For example, mountain crests, open deserts, and grasslands have been shown to partially restrict gene flow for puma (*Puma concolor*) (Ernest *et al.* 2003; McRae *et al.* 2005). These types of habitat features do not completely block dispersal of puma (as do metropolitan areas; Ernest *et al.* 2003), but they impede dispersal to a greater extent than other habitat types (e.g. undeveloped mountain foothills; Ernest *et al.* 2003). Similarly, areas of high relief, ecotone boundaries, and catchment boundaries act as partial barriers to dispersal of the sand frog (*Heleioporus psammophilus*) (Berry 2001). Alternatively, selection for preferred habitats may result in variability in dispersal and gene flow within a heterogeneous landscape. For example, dispersal of natterjack toad (*Bufo calamita*) was shown to be a function of juvenile habitat preference (Stevens *et al.* 2006). Although distance between populations or samples influences population genetic structure, incorporating landscape heterogeneity along dispersal corridors and consideration of species-specific biological constraints will aid interpretation of patterns in complex landscapes.

The presence and spatial configuration of barriers between populations and the tributary distance separating populations were important in explaining genetic differentiation between bull trout populations. Additionally, other variables had an influence on genetic differentiation between populations. Bull trout occupying lakes in the same drainage were predicted to be more similar than bull trout occupying different drainages. This type of drainage effect has been shown for bull trout in the upper Kootenay River and Pine River in British Columbia (Costello *et al.* 2003) and for brook trout in La Mauricie National Park, Québec (Angers *et al.* 1999).

Within drainage similarities are exemplified by the Middle Quartz Lake, Quartz Lake, and Cerulean Lake complex. These lakes are located within the same drainage, are separated by a relatively short geographic distance, and are not separated by barriers. There was little genetic differentiation among bull trout sampled from these lakes and allelic distributions did not differ.

The observed genetic similarity may be linked to frequent movement of bull trout among these lakes to fulfil life-history needs. Anecdotal information and available spawning-survey data (Meeuwig & Guy 2007; L. B. Tennant, Montana Cooperative Fishery Research Unit, personal communication) indicate that the stream section located between Quartz Lake and Cerulean Lake is a high-use spawning area for bull trout in the Middle Quartz Lake, Quartz Lake, and Cerulean Lake complex and that the majority of spawning occurs in close proximity to Quartz Lake. Consequently, genetic similarities could be a result of frequent gene flow among bull trout sampled from these three lakes. These data indicate that treating bull trout from Middle Quartz Lake, Quartz Lake, and Cerulean Lake as one demographic unit for management purposes is appropriate. A similar situation was observed in the Arrow Lake and Trout Lake complex. These lakes are also within the same drainage, are geographically in close proximity, and are not separated by a barrier. Therefore, frequent gene flow between bull trout in Arrow Lake and Trout Lake is plausible. However, additional information related to the location of spawning habitat used by bull trout in Arrow Lake and Trout Lake would aid this interpretation.

The total patch size of population pairs was negatively related to genetic differentiation between bull trout populations in GNP; therefore, genetic differentiation decreased as overall patch size increased. Similarly, differences in habitat size and quality were related to genetic differentiation between populations of Lahontan cutthroat trout *O. c. henshawi* in the Marys River Basin, NV (Neville *et al.* 2006). Conversely, patch size was a poor predictor of genetic variation among bull trout samples from the Boise River (Whiteley *et al.* 2006). Patch size is often used as a surrogate for habitat carrying capacity or population size, which is related to genetic drift and differentiation (Frankham *et al.* 2002). Patch size is often easily quantified; however, other factors may influence local population size (e.g. habitat quantity and quality, interactions with native and nonnative species, and environmental perturbations). Therefore, patch size alone may not adequately represent the influence of habitat carrying capacity or population size on genetic differentiation. Additional information on the census or effective population size of populations under examination may aid in interpretation of patterns of genetic differentiation when these parameters are known.

The modelling procedure used in this study was generally useful for explaining patterns of genetic differentiation between bull trout populations in GNP. However, the Harrison Lake bull trout population was considered an outlier in all models examined. Analysis of model residuals by population indicated that Harrison Lake had greater genetic differentiation than expected based on the model parameters; suggesting increased genetic drift in this population relative to other populations examined. Harrison Lake may have been historically isolated by a downstream barrier. Genetic diversity (i.e. H_e and A_R) for the Harrison Lake population was similar to that of populations isolated by barriers; however, there are no data to indicate the presence of a historical barrier downstream of Harrison Lake. Alternatively, this may be the result of a recent reduction in the size of the Harrison Lake bull trout population. Numerous nonnative species are present in Harrison Lake, including lake trout (*S. namaycush*; Meeuwig *et al.* 2008), which have been implicated in the decline of bull trout populations in other portions of their native range (Donald & Alger 1993).

Bull trout in GNP occupy a heterogeneous landscape. Genetic differentiation between bull trout populations is clearly linked to features that restrict dispersal, but more subtle influences can be observed by considering the influence of potential one-way dispersal as well as by partitioning dispersal corridors based on characteristics of the stream drainage pattern. Analytical models that aim to explain the influence of landscape heterogeneity on ecological processes will benefit from considering not only distinct landscape features (e.g. barriers, dispersal corridors, or other environmental gradients), but variability within or associated with those features.

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Michael Meeuwig's research focuses on various aspects of landscape ecology, conservation genetics, native species conservation, and aquatic ecology as they relate to fishery science. Christopher Guy's research falls within the broad mission of ecology of fishery and aquatic resources and includes research on life history, movements, habitat use, population ecology and dynamics, exploitation, hybridization, and nonnative species eradication. Steven Kalinowski's research focuses on developing new ways to describe genetic variation within species. Wade Fredenberg's research focuses on protection and recovery of native populations of bull trout in the presence of invasion by nonnative lake trout.

Appendix

Reaction (single or multiplex), reagents and quantity, and thermal profile for single and multiplex PCRs.

Reaction	Reagent	Quantity (µL)	Thermal profile
Single*	HPLC water	5.7	95 °C for 10 min; 45 cycles
	Gold buffer 10X	1.0	of 95 °C for 30 s, 55 °C for
	MgCl (24 mM)	0.6	30 s, 72 °C for 30 s; 72 °C
	BSA (2 µg/µL)	1.0	for 10 min; 20 °C for 1 min
	dNTPs (10 mM)	0.2	
	*Sco*105-F (10 µm)	0.2	
	*Sco*105-R (10 µm)	0.2	
	Amplitaq Gold	0.1	
	Template DNA	1.0	
Multiplex*	RNase free water	3.2	95 °C for 15 min; 15 cycles
	QIAGEN Multiplex Master Mix	5.0	of 94 °C for 30 s, 67 °C for
	*Sco*102-F (10 µm)	0.2	1.5 min (stepped down by
	*Sco*102-R (10 µm)	0.2	0.5 °C each cycle), 72 °C for
	*Sco*220-F (10 µm)	0.2	1 min; 25 cycles of 94 °C for
	*Sco*220-R (10 µm)	0.2	30 s, 60 °C for 1.5 min, 72 °C for 1 min; 60 °C for 30 min; 20 °C for 1 min
	Template DNA	1.0	
Multiplex*	RNase free water	2.4	95 °C for 15 min; 15 cycles
	QIAGEN Multiplex Master Mix	5.0	of 94 °C for 30 s, 67 °C for
	*Sco*200-F (10 µm)	0.2	1.5 min (stepped down by
	*Sco*200-R (10 µm)	0.2	0.5 °C each cycle), 72 °C for
	*Sco*212-F (10 µm)	0.2	1 min; 25 cycles of 94 °C for
	*Sco*212-R (10 µm)	0.2	30 s, 60 °C for 1.5 min, 72 °
	*Sco*215-F (10 µm)	0.2	C for 1 min; 60 °C for 30
	*Sco*215-R (10 µm)	0.2	min; 20 °C for 1 min
	*Smm*22-F (10 µm)	0.2	
	*Smm*22-R (10 µm)	0.2	
	Template DNA	1.0	

Appendix (Continued)

Reaction	Reagent	Quantity (µL)	Thermal profile
Multiplex	RNase free water	2.4	95 °C for 15 min; 15 cycles
	QIAGEN Multiplex Master Mix	5.0	of 94 °C for 30 s, 67 °C for
	*Omm*1128-F (10 µm)	0.2	1.5 min (stepped down by
	*Omm*1128-R (10 µm)	0.2	0.5° C each cycle), 72 °C for
	*Sco*202-F (10 µm)	0.2	1 min; 25 cycles of 94 °C for
	*Sco*202-R (10 µm)	0.2	30 s, 60 °C for 1.5 min, 72 °
	*Sco*216-F (10 µm)	0.2	C for 1 min; 60 °C for 30
	*Sco*216-R (10 µm)	0.2	min; 20 °C for 1 min
	*Sfo*18-F (10 µm)	0.2	
	*Sfo*18-R (10 µm)	0.2	
	Template DNA	1.0	

*Diluted to 1 part PCR product to 9 parts HPLC grade water post PCR.