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Parasite phylogeographical congruence with salmon host evolutionarily significant units: implications for salmon conservation

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Abstract

Comparative phylogeographical studies between parasites and their hosts or with biogeographical regions are useful to predict parasite dispersal potential over a broad geographical range. We used both microsatellite markers and mtDNA sequence data from a trematode parasite, *Plagioporus shawi*, to test for congruence across two evolutionarily significant unit (ESU) boundaries of its salmonid hosts (*Oncorhynchus* spp.). We find congruent patterns with the nuclear loci of *P. shawi* and the ESU boundaries of its salmonid hosts. This pattern indicates that broad-scale phylogeographical patterns of a parasite can be predicted by the biogeographical history of their hosts. Furthermore, this pattern provides independent support for these ESU boundaries as biologically relevant barriers. The mtDNA shows some discordance with nuclear loci and a level of genetic differentiation greater than can be explained by genetic drift. Thus, the mtDNA cannot be used in isolation to infer the population history of *P. shawi*. The genetic differentiation at both the nuclear and mtDNA markers will be useful for salmon fisheries management by providing a tool to assign ocean-migrating salmonids back to their freshwater population of origin.

Keywords: comparative phylogeography, conservation, gene flow barriers, *Oncorhynchus* spp., *Plagioporus shawi*, Trematoda

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Introduction

Comparative phylogeographical studies between parasites and their hosts or with biogeographical regions can be useful in several regards. (i) As with any comparative study among species, congruent phylogeographical patterns can identify historically and evolutionarily independent regions, and suggest shared biogeographical factors in shaping intraspecific population histories (Bermingham & Moritz 1998; Avise 2000). Furthermore, when species are of conservation concern, the identification of distinct evolutionarily units is informative in management strategies (Bermingham & Moritz 1998). (ii) Comparative studies between hosts and their parasites can expand our understanding of parasite evolution and population history. For example, Nieberding *et al*. (2004) used cophylogeographical patterns between a parasite and its host to calibrate a molecular clock for the parasite. Because parasites are tightly bound to their hosts for survival, host movement or other behaviours may influence parasite genetic structure on local or regional scales (McCoy *et al*. 2003; Criscione & Blouin 2004). Thus, comparative host-parasite studies are useful to predict parasite dispersal potential and scale of gene flow over a broad geographical range. (iii) Recent studies indicate that parasite molecular data can potentially elucidate host population history or demography (Nieberding *et al*. 2004; McCoy *et al*. 2005; Whiteman & Parker 2005; Wirth *et al*. 2005; Biek *et al*. 2006), and can serve as useful markers to indicate host-source populations (Criscione *et al*. 2006). Despite the potential usefulness of parasite genetic data, phylogeographical studies on nonhuman parasites are relatively scarce (reviewed in Criscione *et al*. 2005; recent papers by McCoy *et al*. 2005; Nieberding *et al*. 2005).

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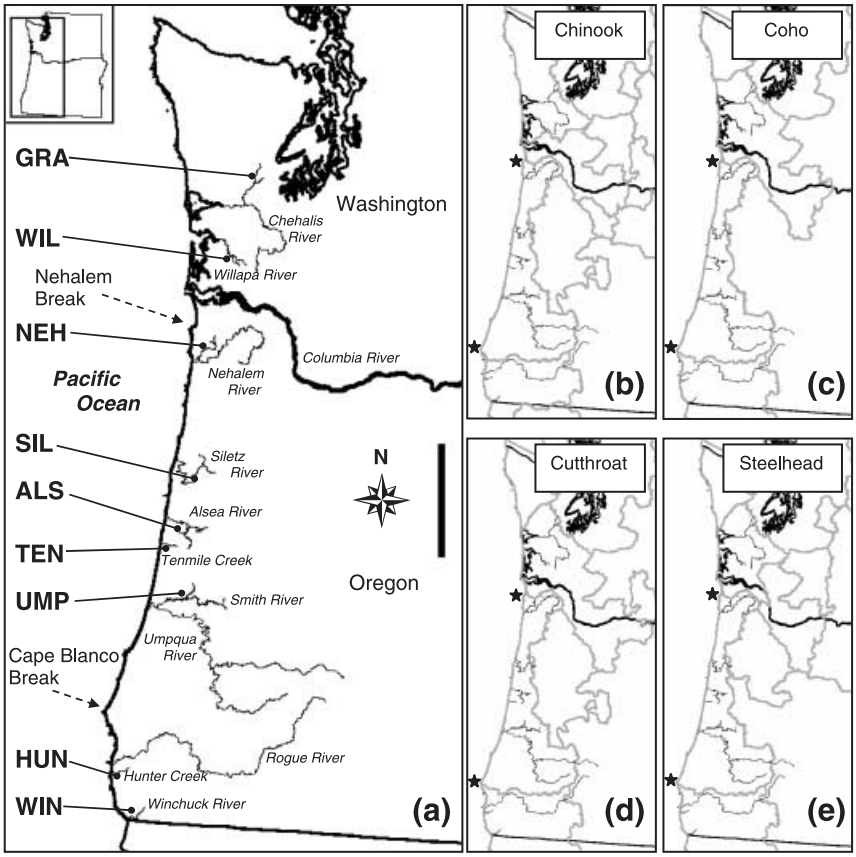

Fig. 1 (a) Map of sampling locations and ESU boundaries (grey lines) for (b) *Oncorhynchus tshawytscha* (chinook salmon) (c) *Oncorhynchus kisutch* (coho salmon) (d) *Oncorhynchus clarki* (cutthroat trout), and (e) *Oncorhynchus mykiss* (steelhead trout). Dashed arrows in (a) indicate the Nehalem and Cape Blanco breaks. In (b–e), the breaks are indicated by a star. GRA and WIL are part of coastal drainages of southwestern Washington. NEH, SIL, ALS, TEN, and UMP are within the Oregon Coast ESU. HUN and WIN are within the Klamath Mountains Province. Scale bar in (A) is 100 km.

Here our primary aim is to test for congruence between the coastal phylogeographical patterns of a digenean trematode parasite (*Plagioporus shawi*) and the evolutionarily significant unit (ESU) boundaries of its salmonid hosts (*Oncorhynchus* spp.). The Oregon Coast ESU (Fig. 1) on the Pacific coastline of the USA is a common ESU designation among four salmonid species (see Fig. 1) known to be infected with *P. shawi*. Thus, these salmonids share two ESU boundaries: in the north, the Nehalem break and in the south, the Cape Blanco break (Fig. 1). A test of congruence across these breaks would elucidate if broad-scale phylogeographical patterns of parasites can be predicted by host phylogeographical structure. *Plagioporus shawi* has a strict freshwater life cycle (snail to aquatic arthropod to salmonid fish) (Schell 1975) and thus, is dependent on nonnatal migrations of its anadramous hosts for dispersal among freshwater drainages. Therefore, we expect that the Nehalem and Cape Blanco breaks will demarcate phylogeographical boundaries for *P. shawi*. In a previous study, we compared the accuracy of genetic assignment tests between *P. shawi* and one of its salmonid hosts (Criscione *et al*. 2006). Individuals of *P. shawi* were shown to assign back to regions of origin on either side of the Cape Blanco break with high accuracy. These microsatellite data, although from a small number of populations, suggested that the Cape Blanco break was a barrier. However, it was unknown if this pattern would remain if populations from other ESUs were included. Here we provide a more robust analysis by adding more populations (including populations across the Nehalem break), provide explicit tests of population differentiation, and use both mtDNA and microsatellite markers from every parasite individual to test for congruence across the Nehalem and Cape Blanco breaks.

The data we present is also of conservation relevance for two reasons. First, testing for congruence will also determine if the parasite genetic data support the ESUs as historically unique regions. This is of significance because neutral markers from some salmonids show weak genetic differentiation (F_{ST} ~ 0.02) across ESU boundaries (Teel *et al*. 2003; Criscione *et al*. 2006). Thus, independent tests of these phylogeographical regions with other organisms are warranted. *Plagioporus shawi* has its own evolutionary history and therefore, provides an independent test of these phylogeographical boundaries. Second, we want to extend previous work (Criscione *et al*. 2006) to establish a baseline on which the parasite genetic data could be used as a conservation management tool to indicate freshwater origins (across multiple ESUs) of ocean-migrating salmonids.

Materials and methods

Description of salmonid ESUs

The salmonid ESUs are based on a combination of biogeographical data, geological history, salmonid neutral genetic markers, and salmonid life-history traits (reviewed in Waples *et al*. 2001). Figure 1 shows the ESU boundaries of the four salmonids. Briefly, the Cape Blanco break demarks different ecological regions (Waples *et al*. 2001). The region south of Cape Blanco (Klamath Mountains Province) has a unique geological history with an east to west mountain range that bridges the Cascade and Coastal mountain ranges, which extend north to south from Washington into California (Minckley *et al*. 1986). This region is also characterized by distinct ichthyofaunal assemblages compared to coastal streams north of Cape Blanco (Minckley *et al*. 1986; Hughes *et al*. 1987). Furthermore, steelhead, coho, and chinook that originate from streams north of Cape Blanco tend to migrate northward in the ocean, whereas those from streams south of Cape Blanco tend to be south migrating (Busby *et al*. 1996; see Waples *et al*. 2001 for other life-history trait differences across both boundaries). The Oregon Coast ESU and the region extending north to the Chehalis River system belong to the same ecological region (Waples *et al*. 2001). The ichthyofauna on either side of the Nehalem break, which is essentially demarcated by the mouth of the Columbia River, originated from the Columbia River system. However, above the Nehalem break, the ichthyofauna of Washington coastal streams (the Chehalis and Willapa river systems; Fig. 1) are morphologically differentiated from their Columbia River counterparts, thus suggesting a period of isolation (McPhail & Lindsey 1986). Over our sampled range, neutral genetic markers of both steelhead and chinook delineate three population clusters: the Klamath Mountains Province, the Oregon Coast ESU, and southwest Washington coastal streams. The Oregon Coast ESU and Washington clades are more closely related than to the clade of populations south of Cape Blanco (Busby *et al*. 1996; Myers *et al*. 1998). Cutthroat shows moderate structure across the Cape Blanco break, but high structure across the Nehalem break indicates that the Columbia River is a barrier to cutthroat gene flow (Johnson *et al*. 1999). Coho shows moderate genetic structure across both ESU boundaries (Weitkamp *et al*. 1995). Given the above descriptions of the ESU breaks, we expect that the Nehalem break represents a weaker phylogeographical boundary when compared to the Cape Blanco break. Full details on the descriptions of the ecological regions and ESU boundaries are given in Waples *et al*. (2001) and in several technical memoranda (Weitkamp *et al*. 1995; Busby *et al*. 1996; Myers *et al*. 1998; Johnson *et al*. 1999) on the four salmonid species (available at www.nwfsc.noaa.gov/publications/index.cfm).

Parasite background

Plagioporus shawi completes its life cycle within a freshwater stream. Sexually mature adults infect the intestines of salmonids and pass eggs into the freshwater via host faeces. A miracidium hatches and penetrates a freshwater snail where a period of asexual reproduction occurs prior to cercarial development. Cercariae leave the snail and penetrate aquatic arthropods (e.g. amphipods, caddis larvae) where they encyst as metacercariae. The life cycle is completed when a fish ingests an infected arthropod (Schell 1975). The geographical range of *P. shawi* extends west of the Cascade Mountains from northern California to northern Washington, with reports in eastern Washington and western Idaho, USA (Hoffman 1999). Adults of *P. shawi* are most often found in juvenile or resident (i.e. nonanadramous) adult salmonids in freshwater streams, but there are also reports of adult *P. shawi* infecting salmonids that were collected from the Pacific Ocean (Olson 1978; Margolis 1984, 1985). These reports indicate that adult *P. shawi* can survive in ocean-migrating salmonids. Host range appears to be limited to salmonid fishes with reports only from *Oncorhynchus* spp. (across the geographical range) and mountain whitefish, *Prosopium williamsoni* (in Idaho only) (Hoffman 1999). *Plagioporus shawi* is hermaphroditic, but a previous study indicates that random mating predominates within populations of *P. shawi* (Criscione & Blouin 2006).

Sample collection

We sampled 23 individuals of *P. shawi* from each of nine rivers located in the coastal mountain ranges of Washington and Oregon, USA (Fig. 1). Most trematodes were mature; however, some were still developing. All sampled rivers ultimately drain into the Pacific Ocean: Bingham Creek (GRA), Fork Creek (WIL), North Fork Nehalem River (NEH), Mill Creek (SIL), Cascade Creek (ALS), West Fork Smith River (UMP), Ten Mile Creek (TEN), Hunter Creek (HUN), and Winchuck River (WIN). Two rivers are above the Nehalem break (GRA and WIL) and two are below the Cape Blanco break (HUN and WIN). To ensure that parasites and fish originated in the stream from which they were sampled, we collected out-migrating smolts (juvenile salmonids leaving the drainage for the ocean) rather than returning adults. All samples were collected from March to June of 2002 in conjunction with Oregon and Washington salmonid-monitoring projects under the permits OR2002-019 and 02–033, respectively. To obtain a random sample of each parasite population, each parasite was collected from a different individual salmonid host. Prevalence (percent infected), mean intensity of infected fish, and the number of genotyped individuals of *P. shawi* from the nine rivers and four host species is given in Table S1, Supplementary material.

Genotyping and sequencing

Protocols for DNA extraction, polymerase chain reaction (PCR), and genotyping of the eight microsatellite loci (m26, m41, m48, d04, d09, d36, d43, and d47) were previously described in Criscione & Blouin (2005). We sequenced a 636-bp region of the NADH-dehydrogenase subunit 1 (ND1) mitochondrial gene (see Criscione & Blouin 2004 for primers and protocols). Microsatellite genotypes and mtDNA sequences were obtained from the same individual parasites. Sequences were deposited in GenBank (AY269691–AY269782 and DQ868547–DQ868656).

Microsatellite analyses

Within populations, we tested for Hardy–Weinberg equilibrium at each locus and genotypic disequilibrium for pairs of loci using GENEPOP version 3.4 (Raymond & Rousset 1995). Significance was determined using the Markov chain method (5000 dememorizations, 5000 batches, 5000 iterations per batch). A sequential Bonferroni correction was applied for multiple comparisons within each population sample. Observed heterozygosities and gene diversities (H_E) were also calculated in GENEPOP. Multilocus F_{IS} for each population as estimated by Weir & Cockerham (1984) was tested in FSTAT version 2.9.3 (Goudet 1995) with 1000 permutations of alleles among individuals. The number of alleles (A) and allelic richness (R_s, number of alleles rarefied to smallest population sample size) per locus were calculated in FSTAT version 2.9.3 (Goudet 1995). Based on finding a large reduction in genetic diversity in terms of both H_E and R_s in HUN and WIN (see results below), we performed a post hoc analysis to determine if these populations had undergone bottlenecks. The Wilcoxon sign rank test as implemented in BOTTLENECK (Piry *et al.* 1999) was used to test all populations for bottlenecks (based on 10 000 replications in each population). We used the infinite allele model because most of our loci showed allele-size variation that is inconsistent with a stepwise mutation model (e.g. alleles that are not multiples of the basic repeat number).

We used several methods to test for structure among populations and ESU boundaries. FSTAT was used to calculate pairwise F_{ST} (Weir & Cockerham 1984) among populations and to test (1000 permutations of individuals) for differentiation between pairs of populations. Sequential Bonferroni correction was applied. We did a hierarchical analysis with HIERFSTAT (Goudet 2005) to test genetic structure for individuals within rivers (F_{IndRiv}, which is the average F_{IS} among populations), among rivers within ESUs (F_{RivEsu}), and among ESUs ($F_{EsuTotal}$). The northern ESU consisted of GRA and WIL, the middle had NEH, SIL, ALS, TEN, and UMP, and the southern had HUN and WIN. Significance of F_{IndRiv} was tested in FSTAT with 15 000 permutations of alleles among individuals within populations. Significance of F_{RivEsu} and $F_{EsuTotal}$ was tested in HIERFSTAT with 15 000 permutations of individuals among rivers within ESUs and of rivers among ESUs, respectively. If the two breaks did not represent phylogeographical boundaries for *P. shawi*, we might expect to find isolation by distance assuming that non-natal salmonid migrations occur among nearby streams. We tested $F_{ST}/(1 - F_{ST})$ against geographical distance using partial Mantel tests (20 000 permutations) implemented in FSTAT. Two indicator variables were included in the analysis. One was a matrix where a 1 was entered for pairs of populations across the Cape Blanco break and a 0 for the other pairs. The second indicator variable was the same, but across the Nehalem break. Geographical coordinates used to calculate surface distance were GRA (46.9178°N, 124. 1807°W), WIL (46.7250°N, 124.0978°W), NEH (45.6562°N, 123.9443°W), SIL (44.9263°N, 124.0253°W), ALS (44.4223°N, 124.0817°W), TEN (44.2260°N, 124.1122°W), UMP (43.6692°N, 124.2205°W), HUN (42.3882°N, 124.4263°W), and WIN (42.0037°N, 124.2158°W). These coordinates correspond to the point at which each river system meets the Pacific Ocean rather than the actual sampling location. Distances between populations were added between pairs of populations (e.g. the distance between GRA and SIL = GRA-WIL + WIL-NEH + NEH-SIL).

Within-population heterozygosity can be high with variable loci such as microsatellites, thus the maximum F_{ST} among populations may be much less than 1 (Hedrick 2005). In order to facilitate comparisons among different hierarchical levels and between microsatellites and mtDNA (see below), we standardized the Weir & Cockerham (1984) estimates of F-statistics among populations by dividing $F_{estimate}$ by F_{max}. The formula for standardization in Hedrick (2005) is not for hierarchical structure nor may it be appropriate for the Weir & Cockerham (1984) estimators of F-statistics. Therefore, in order to calculate F_{max}, we recoded our data to obtain maximum divergence among populations (i.e. no shared alleles among populations) (Meirmans 2006). For the hierarchical analyses, we created two recoded data sets. One was used to get the max F_{RivEsu}. Here we coded each river as having unique alleles. This data set cannot be used to get the max $F_{EsuTotal}$ as the within ESU heterozygosity will be wrong. Thus, a second data set where alleles were unique among ESUs but not unique to rivers within ESUs was used to calculate the max $F_{EsuTotal}$. We only report standardized F-statistics for all between or among population comparisons. Tables S2 and S3, Supplementary materials provide all $F_{estimate}$ values. Significance testing was always carried out with the original data sets as this is not affected by within population heterozygosity. There is no need to standardize for F_{IS}.

Lastly, we examined genetic structure by constructing population dendrograms. A neighbour-joining tree based on the Cavalli-Sforza & Edwards (1967) chord distance,

which is the distance measure that provides the best estimation of tree topology (Takezaki & Nei 1996), was made in PHYLIP version 3.65 (Felsenstein 2005). Stability of topology was assessed by 1000 bootstraps over loci. We also used a Bayesian method of clustering groups of individuals (i.e. each river) into populations that do not rely on a priori expectations of population delineation (BAPS version 4.13; Corander *et al*. 2003, 2004). For input as the maximum number of populations (*K*), we used 10 replicates for all values between two and nine as the latter was the number of rivers we sampled. We then constructed a neighbour-joining tree with the Kullback-Leibler divergence matrix provided as output with BAPS. This matrix can be used as a measure of relative genetic distance between the BAPS-identified clusters.

In order to evaluate the potential use of parasite genetic data to indicate host-source populations across multiple ESU boundaries, we assessed the accuracy of assignment back to the population of origin. The Bayesian method of Rannala & Mountain (1997) as implemented in the program GENECLASS2 (Piry *et al*. 2004) was used for assignment. Each individual was assigned to the population in which that individual had the highest assignment score as calculated in GENECLASS2. We did the analysis two ways: (i) by treating all nine rivers as separate populations and (ii) by using the BAPS-identified clusters as the populations of origin.

Mitochondrial analyses

To provide a basis of comparison with the microsatellites, we did a hierarchical analysis with HIERFSTAT (Goudet 2005) on the haplotype frequencies to test for genetic structure among rivers within ESUs (F_{RivEsu}), and among ESUs (F_{EsuTotal}). Significance was determined as mentioned for the microsatellites. Analyses using the sequence information (i.e. analysis of molecular variance) yielded similar results and are not reported. FSTAT was used to calculate and test pairwise F_{ST} among populations as reported for the microsatellite analyses. A statistical parsimony network was computed with the program TCS 1.21 (Clement *et al*. 2000) to provide a visual representation of the sequence data.

Recent studies have emphasized that mtDNA may not be suitable for inference on population history or phylogeography because of direct or indirect selection on the mtDNA (Ballard & Whitlock 2004; Hurst & Jiggins 2005; Bazin *et al*. 2006). To determine if the mtDNA was behaving in a possibly non-neutral fashion, we compared the level of genetic divergence (F_{ST}) across all seven of the populations north of Cape Blanco for each microsatellite locus and for the mtDNA. HUN and WIN were omitted as the mtDNA had an obvious discordant pattern across the Cape Blanco break (see Results below). To account for the potential difference in genetic divergence resulting from different effective sizes between the mtDNA and nuclear loci, we transformed the F_{ST} values to N_m according to an island model (Wright 1931) using the formula $F_{\mathrm{ST}} = 1/(4N_m + 1)$ for each microsatellite locus and $F_{\mathrm{ST}} = 1/(2N_m + 1)$ for the mtDNA. $2N_m$ is used for the mtDNA conversion because *P. shawi* is hermaphroditic. We then used a one-sample *t*-test to see if the N_m of mtDNA fell outside the range of the nuclear loci.

Results

Of the 207 individuals genotyped and sequenced, we removed 17 from our analyses for the following reasons. Two individuals, one from TEN and one from UMP, showed three or four alleles at one or more loci suggesting possible gene duplications, an increase in ploidy, or contamination. We concluded that the other 15 possibly represented cryptic species. This conclusion was based on the perfect concordance between distinct microsatellite genotypes (e.g. some loci had close to 100 bp allele size differences) and mtDNA haplotypes that fell out in separate clades (data not shown). Some of these individuals included previously published ND1 sequences in Criscione & Blouin (2004). It should be noted that removal of these individuals does not alter the conclusions of our previous or current study. The finding of potential cryptic species will be reported elsewhere as our focus here is on intraspecific population history. Final sample sizes for all populations are shown in Table 1.

Microsatellite results

After sequential Bonferroni, there were no pairs of loci within populations that showed significant genotypic disequilibrium. Only m41 in HUN and d09 in TEN showed a significant deficit of heterozygotes after sequential Bonferroni within populations (Table 1). Only WIN had a multilocus estimate of F_{IS} that was significantly different from zero (Table 1, $F_{\mathrm{IS}} = 0.147$, $P = 0.002$). HUN was marginally nonsignificant ($F_{\mathrm{IS}} = 0.088$, $P = 0.054$). Mean R_s ranged from 11.6 to 12.6 in all rivers above the Cape Blanco break, whereas mean R_s in HUN and WIN was 4.5 and 4.9, respectively. Likewise, mean H_{E} was much lower in HUN and WIN (0.46 and 0.52), when compared to populations north of Cape Blanco (range 0.8–0.87). Furthermore, HUN and WIN were fixed for the same alleles at m26 and d43 (Table 1). A bottleneck was detected in WIN ($P = 0.039$), whereas HUN was marginally nonsignificant ($P = 0.078$). *P* values for bottleneck tests in the rivers north of Cape Blanco were all nonsignificant, ranging from 0.13 to 0.68.

Table 2 shows the estimates of F_{ST} between pairs of populations. All pairwise comparisons were highly significant ($P < 0.0001$) except for the pairwise comparisons among SIL, ALS, TEN, and UMP ($P > 0.1$ or $0.01 < P \leq 0.05$ after sequential Bonferroni). Using the ad hoc method of

Table 1 Summary statistics by locus and population, and averaged over loci within populations: number of alleles (A), allelic richness (R_s), observed heterozygosity (H_O), gene diversity (H_E)

		GRA (15)*	WIL (23)	NEH (22)	SIL (23)	ALS (23)	TEN (18)	UMP (21)	HUN (23)	WIN (22)
Locus										
m41	A (38)†	6	5	16	10	9	6	8	7	7
	R_s	6.00	4.65	13.23	7.64	7.03	5.64	6.49	5.80	6.46
	H_O	0.4	0.739	0.818	0.478	0.522	0.444	0.476	0.304¶	0.591
	H_E	0.363	0.735	0.838	0.485	0.534	0.508	0.452	0.604	0.667
m26	A (21)	8	11	7	14	14	11	13	1	1
	R_s	8.00	9.21	6.63	11.52	11.71	10.25	10.75	1.00	1.00
	H_O	0.933	0.783	0.727	0.696	0.826	0.667	0.714	—	—
	H_E	0.844	0.799	0.826	0.762	0.827	0.765	0.718	—	—
m48	A (35)	17	22	21	24	22	20	16	7	10
	R_s	17.00	17.61	17.31	18.77	17.32	18.20	14.44	6.26	9.02
	H_O	1.000	0.913	0.955	1.000	1.000	0.944	1.000	0.739	0.682
	H_E	0.959	0.949	0.955	0.960	0.951	0.963	0.938	0.759	0.853
d36	A (43)	15	19	22	19	16	12	17	3	7
	R_s	15.00	15.70	17.28	14.75	13.21	10.95	14.51	2.88	6.48
	H_O	0.933	1.000	1.000	0.957	0.913	1.000	1.000	0.348	0.682
	H_E	0.943	0.940	0.943	0.913	0.910	0.870	0.936	0.305	0.751
d47	A (20)	13	11	8	12	11	13	10	3	4
	R_s	13.00	9.51	7.62	10.11	9.68	11.74	8.46	2.88	3.36
	H_O	0.933	0.739	0.727	0.783	0.783	0.889	0.571	0.435	0.318
	H_E	0.874	0.735	0.809	0.809	0.841	0.816	0.681	0.456	0.488
d04	A (16)	7	9	5	11	7	9	10	3	3
	R_s	7.00	8.27	5.00	9.37	6.30	8.63	9.18	3.00	3.00
	H_O	0.867	0.652	0.818	0.826	0.783	0.833	0.905	0.652	0.500
	H_E	0.805	0.831	0.799	0.862	0.758	0.862	0.839	0.658	0.591
d09	A (31)	16	19	20	19	18	20	20	14	10
	R_s	16.00	15.95	16.55	16.16	15.51	18.04	17.28	12.75	8.87
	H_O	1.000	1.000	0.909	0.913	0.913	0.833¶	0.952	0.913	0.818
	H_E	0.949	0.951	0.944	0.953	0.949	0.957	0.961	0.929	0.844
d43	A (26)	14	14	11	15	14	11	13	1	1
	R_s	14.00	12.42	9.50	12.56	11.63	10.31	11.64	1.00	1.00
	H_O	0.800	0.957	0.682	0.913	0.826	0.833	1.000	—	—
	H_E	0.924	0.920	0.823	0.908	0.892	0.898	0.905	—	—
Mean										
	A	12	13.75	13.75	15.5	13.88	12.75	13.38	4.88	5.38
	(SD)‡	(4.34)	(5.82)	(6.84)	(4.81)	(4.88)	(4.07)	(4.07)	(4.36)	(3.66)
	R_s	12	11.67	11.63	12.6	11.55	11.72	11.59	4.45	4.90
	(SD)	(4.34)	(4.5)	(5.08)	(3.73)	(3.84)	(4.36)	(3.61)	(3.86)	(3.25)
	H_E	0.832	0.857	0.867	0.832	0.833	0.830	0.804	0.464	0.524
	(SD)	(0.20)	(0.09)	(0.07)	(0.16)	(0.14)	(0.15)	(0.18)	(0.34)	(0.35)
	F_{IS}§	−0.032	0.012	0.044	0.013	0.015	0.030	−0.030	0.088	0.147¶

*Sample sizes; †total number of alleles across populations; ‡standard deviation; §multilocus estimates of F_{IS}; ¶$P \leq 0.05$ after sequential Bonferroni.

delimiting populations from Waples & Gaggiotti (2006), SIL, ALS, TEN, and UMP would be considered a single population. These four populations are adjacent and within the Oregon Coast ESU (Fig. 1). The hierarchical analysis showed significant structure among ESUs ($F_{EsuTotal} = 0.611$, $P = 0.0013$) and lower, but significant structure among rivers within ESUs ($F_{RivEsu} = 0.196$, $P < 0.001$). The average F_{IS} among populations ($F_{IndRiv} = 0.026$, $P = 0.006$) was also significantly different from zero (Table 3). However, it is obvious from the pairwise F_{ST} analyses (Table 2) and from the within population F_{IS} values (Table 1) that both HUN and WIN strongly influence $F_{EsuTotal}$ and F_{IndRiv}. Therefore, we repeated the hierarchical analysis after removing HUN and WIN. F_{IndRiv} decreases to 0.01 and is not significant. However, there remains significant genetic differentiation across the Nehalem break ($F_{EsuTotal} = 0.102$, $P = 0.046$) (Table 3).

There was no correlation between the geographical distance and genetic distance when the indicator variable for

Table 2 Standardized F_{ST} between pairs of populations. Multilocus values from the microsatellites are below the diagonal and the mtDNA values are above the diagonal. All values are highly significant $P < 0.0001$ after sequential Bonferroni unless noted otherwise

	GRA	WIL	NEH	SIL	ALS	TEN	UMP	HUN	WIN
GRA	—	1	1	1	1	1	1	1	1
WIL	0.328	—	1	1	1	1	1	1	1
NEH	0.316	0.341	—	1	1	1	1	1	1
SIL	0.167	0.297	0.344	—	0.204†	0.135^{NS}	0.955	1	0.998
ALS	0.217	0.353	0.352	0.020†	—	0^{NS*}	0.858	0.975	0.952
TEN	0.248	0.331	0.371	0^{NS*}	0.027†	—	0.846	0.969	0.935
UMP	0.188	0.325	0.345	0.020^{NS}	0.029^{NS}	0.007^{NS}	—	0.584	0.015^{NS}
HUN	0.867	0.795	0.835	0.799	0.826	0.785	0.797	—	0.612
WIN	0.865	0.766	0.815	0.751	0.787	0.735	0.770	0.279	—

*These values are 0 as the F_{ST} estimate was negative; †$0.05 \geq P > 0.01$ after sequential Bonferroni; NS, not significant.

Table 3 Standardized hierarchical *F*-statistics for the full ESU hierarchy or Nehalem break hierarchy (excludes HUN and WIN)

	ESU hierarchy (microsatellites)	Nehalem break hierarchy (microsatellites)	ESU hierarchy (mtDNA)
$F_{EsuTotal}$	0.611**	0.102*	0.829**
F_{RivEsu}	0.196***	0.179***	0.794***
F_{IndRiv}	0.026**	0.01^{NS}	N/A

$^{NS}P > 0.05$; *$0.05 \geq P > 0.01$; **$0.01 \geq P > 0.001$; ***$0.001 \geq P$.

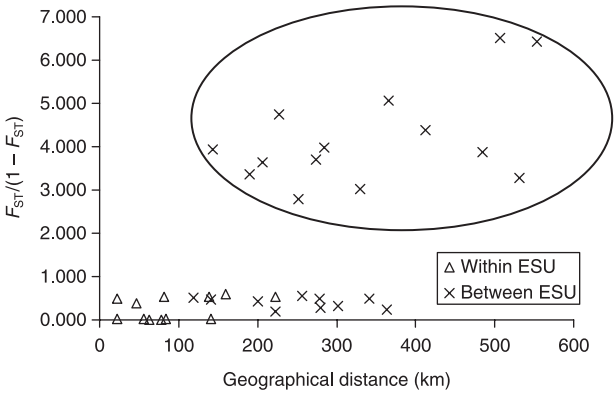

Fig. 2 Plot of genetic distance ($F_{ST}/1 - F_{ST}$) against geographical distance showing no significant relationship with distance after accounting for the ESU breaks. However, the values enclosed in the circle (all pairwise comparisons between HUN and WIN to populations north of the Cape Blanco break) illustrate that the Cape Blanco break is a significant barrier to gene flow. Standardized F_{ST} values were used in the analysis.

the Cape Blanco break was included (Fig. 2). However, the variable for the Cape Blanco break was highly significant even after accounting for geographical distance and the variable for the Nehalem Break (model $r^2 = 0.897$, $P < 0.01$). In fact, the Cape Blanco break alone had an $r^2 = 0.876$, indicating that the addition of geographical distance had little effect in explaining the variance in the data. The variable for the Nehalem break was not significant.

The population dendrograms are shown in Fig. 3. The BAPS analysis clustered the nine rivers into five groups. The SIL, ALS, TEN, and UMP were a single cluster, a result consistent with the pairwise differentiation among populations. GRA and WIL, the two populations north of the Nehalem Break were also found to be a single cluster. Both population dendrograms show that the longest branch is the one separating the two populations south of the Cape Blanco break, HUN and WIN, from the rest of the populations. This provides strong support for the Cape Blanco break as a barrier to *Plagioporus shawi*. Furthermore, both analyses show that the SIL, ALS, TEN, and UMP populations form a single clade and that WIL and GRA cluster together. These data also suggest that the Nehalem break is barrier for *P. shawi*. Unexpectedly, the NEH population always falls out by itself. Its relationship to the northern populations is unresolved.

When all nine rivers are considered separate source populations, there was 63.2% correct assignment. However, 80% of the misassignments (56/70) were among the adjacent SIL, ALS, TEN, and UMP rivers (Table S4, Supplementary material). Furthermore, there was no misassignment across the Cape Blanco break and only 3.7% (7/190) misassignment across the Nehalem break. When the BAPS-identified clusters are treated as source populations, there was 94.7% correct assignment (Table 4). There was over 90% correct assignment back to all of the BAPS-identified clusters with no misassignment across the Cape Blanco break and only 3.2% (6/190) misassignment across the Nehalem break.

Mitochondrial results

Table 2 shows the estimates of F_{ST} between pairs of populations for the mtDNA. Most of the estimates are at or

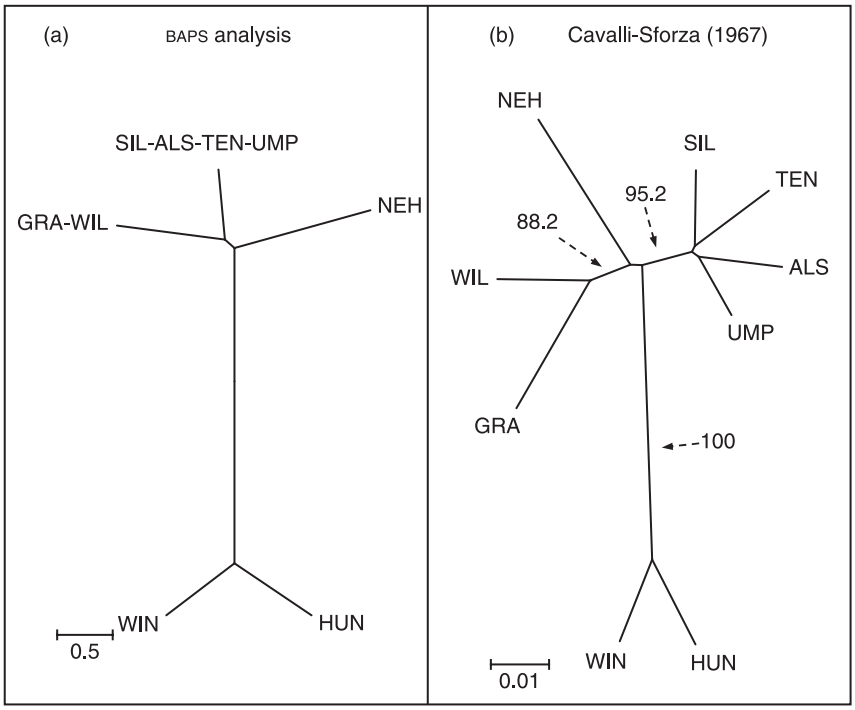

Fig. 3 Population dendrograms based on the (a) BAPS analysis and (b) Cavalli-Sforza & Edwards (1967) chord distance. Branches with bootstrap support greater than 70% are illustrated in (b) with dashed arrows. Both trees support the Cape Blanco break as a barrier as the longest branch in both trees separates HUN and WIN from the other populations. Likewise, the Nehalem break is supported in that GRA and WIL cluster together in both analyses. SIL, ALS, TEN, and UMP seem to form a single population, whereas the relationship of NEH to other populations is unresolved when comparing the two analyses.

	GRA-WIL (38)*	NEH (22)	SIL-ALS-TEN-UMP (85)	HUN (23)	WIN (22)
GRA-WIL	**35**†	1	2		
NEH		**20**	1		
SIL-ALS-TEN-UMP	3	1	**82**		
HUN				**21**	
WIN				2	**22**
% correct assignment‡	92	91	96	91	100

*Sample size for each population; †numbers in bold indicate assignment back to the sampled population; ‡percentage of correct assignment back to the sampled population.

Table 4 Results of the assignment tests based on the BAPS identified clusters. Columns show the populations from which individuals were sampled and rows show the number of individuals that were assigned to that respective population

near 1 because there were no alleles shared between most of the populations (i.e. all the alleles in most populations were found only in those populations). The exceptions were between SIL, ALS, and TEN comparisons and between UMP, WIN, and HUN. This latter pattern is due to a common shared mtDNA allele between UMP, WIN, and HUN (starred haplotype in Fig. 4) and represents a discordant pattern with respect to the microsatellite data. There is also a single copy of this haplotype in ALS and TEN. The hierarchical analysis showed highly significant structure among ESUs ($F_{EsuTotal} = 0.829$, $P = 0.004$) and among rivers within ESUs ($F_{RivEsu} = 0.794$, $P < 0.001$) (Table 3).

When comparing the N_m values of the microsatellite loci to that of the mtDNA, we did not include HUN and WIN because of the discordant pattern observed between the markers. F_{ST} across the other seven populations for the microsatellites ranged from 0.07 to 0.43, which gives a range of N_m values from 0.33 to 3.22. The F_{ST} and N_m for the mtDNA was 0.9 and 0.05, respectively. The N_m for the mtDNA was significantly smaller ($P = 0.015$) than the average of the microsatellites ($N_m = 1.11$), thus indicating that that the mtDNA had greater differentiation across populations than can be explained by genetic drift alone.

Discussion

Summary of main results

1 All microsatellite analyses support the Cape Blanco break as a barrier to gene flow.

2 All microsatellite analyses except the isolation-by-distance analysis support the Nehalem break as barrier to gene flow.

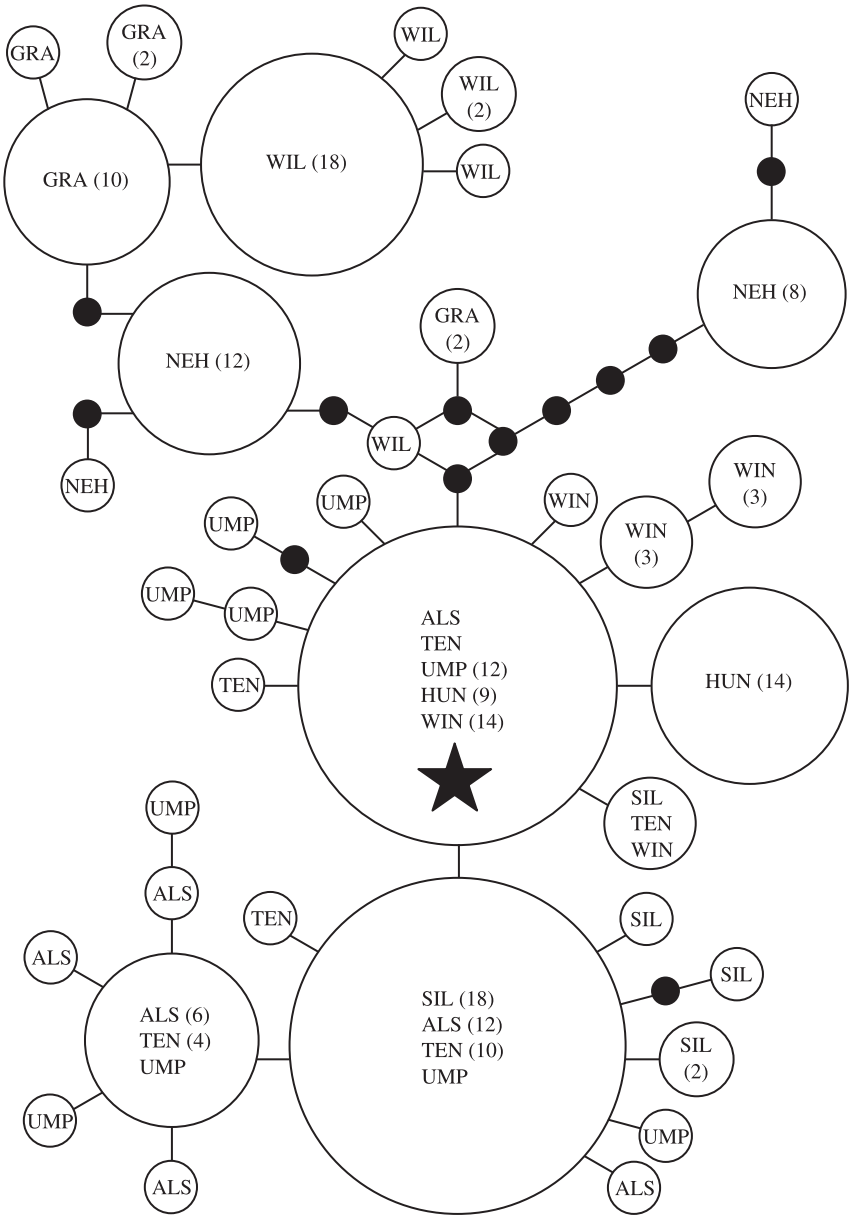

Fig. 4 Statistical parsimony network of the ND1 locus of the mtDNA. Each connection is a single mutational step with black circles representing inferred haplotypes. Observed haplotypes are shown as open circles with the geographical location(s) indicated inside the circle. Numbers in parentheses indicate the frequency of the haplotype in that location. No number indicates a single copy of the haplotype. Sizes of circles are proportional to the number of individuals with that haplotype. Notice that GRA and WIL have only unique alleles indicating that no alleles are shared across the Nehalem break. NEH also has only unique alleles, but also has two somewhat divergent clades. The starred haplotype indicates a common haplotype that is shared across the Cape Blanco break. This is a pattern that is discordant with the microsatellite data.

3 Microsatellite genetic differentiation across the Cape Blanco break is two times greater than differentiation across the Nehalem break (Table 2).

4 HUN and WIN have reduced microsatellite genetic diversity relative to populations north of Cape Blanco.

5 No mtDNA haplotypes are shared across the Nehalem break. However, there is a discordant pattern with the microsatellites in that a common haplotype is shared among UMP, WIN, and HUN.

6 The mtDNA shows greater differentiation among populations than can be explained by genetic drift alone.

7 Assignment tests show that accurate assignment back to population of origin for parasites (> 90%), and thus fish host individuals that they infect, can be obtained across and sometimes within ESUs.

Parasite population history inferred from nuclear markers

We find strong support that the salmonid ESU boundaries at the Nehalem and Cape Blanco breaks also represent phylogeographical boundaries for *Plagioporus shawi*. Previous studies have shown that host movement can determine the degree of genetic differentiation among parasite populations (Blouin *et al*. 1995; McCoy *et al*. 2003; Criscione & Blouin 2004). Here we see that the broad-scale phylogeography of a host predicts the broad-scale

phylogeographical patterns of the parasite. All microsatellite analyses show strong divergence across the Cape Blanco break (Figs 2 and 3, Tables 2 and 3). Although the genetic differentiation across the Nehalem break is not as strong, we still find that all but the isolation-by-distance analyses support this boundary as a gene flow barrier for *P. shawi*. Overall, the pattern of greater divergence across the Cape Blanco break and lesser, but significant structure across the Nehalem break is consistent with what is seen with salmonid genetic data (e.g. steelhead and chinook allozyme data; Busby *et al*. 1996; Myers *et al*. 1998) and biogeographical data on fish distributions. For example, we see a clustering of the southwest Washington populations (GRA and WIL), Oregon Coast ESU populations (SIL, ALS, TEN, and UMP, but excluding NEH), and Klamath Mountains Province populations (HUN and WIN) (Fig. 3). In particular, we see a greater affinity between the populations in the Oregon Coast ESU (NEH, SIL, ALS, TEN, and UMP) and southwest Washington (GRA and WIL) relative to the populations in the Klamath Mountains Province (HUN and WIN) (Fig. 3). Given that we did not detect isolation by distance even within ESU units, it appears that populations of *P. shawi* exist as discrete units as opposed to a gradation of genetic divergence among neighbouring populations. Interestingly, the NEH population within the Oregon Coast ESU tends to fall out by itself in the clustering analyses (Fig. 3) and has equal divergence to other populations within this ESU that is similar to the level of divergence across the Nehalem break (Table 2). A possible explanation for why NEH falls out by itself is discussed in light of the mtDNA data below.

The within-population levels of genetic diversity also present an obvious pattern. The two populations south of Cape Blanco (HUN and WIN) have reduced R_s and H_E across the majority of loci (Table 1). This pattern and the high degree of divergence between HUN and WIN are consistent with reduced effective population sizes (N_e), in these populations. The reduced N_e's could be caused by several factors: (1) the populations have smaller census sizes; (2) a higher selfing rate, or (3) experienced a bottleneck. We currently do not have the data to address 1. All populations except WIN did not deviate from Hardy–Weinberg equilibrium (HUN was marginally nonsignificant). This result is consistent with a more in-depth analysis of within-population structure in two populations of *P. shawi* (Criscione & Blouin 2006). The higher F_{IS} values in HUN and WIN (Table 1) are therefore consistent with explanation 2 (higher selfing rates). However, we believe that the higher F_{IS} values in HUN and WIN may in part be due to null alleles. We observed unique indel allelic variation at several loci (data not shown) in HUN and WIN that was not seen in populations north of Cape Blanco. This pattern raises the possibility that there may be low frequency nulls in these populations. Two aspects of the data suggest that these populations have experienced a bottleneck via a founder effect. First, the bottleneck test in WIN was significant and marginally nonsignificant in HUN even though power was reduced due to the fact that only six loci were used (two loci were fixed) (Piry *et al*. 1999). Perhaps more convincing of a founder event is that both HUN and WIN are fixed for the same alleles at d43 and m26. The allele fixed at d43 is found only two other times, once in UMP and once in ALS. The allele fixed at m26 is found in all other populations, where it ranges in frequency from 0.21 to 0.52. Furthermore, three alleles at d47 (Table 1) have nearly identical frequencies between HUN and WIN. If HUN and WIN had experienced separate standing bottlenecks or had reduced diversity due to small population sizes or high selfing rates, it seems unlikely that the same allele would get fixed at two separate loci. A bottleneck via a founder event, on the other hand, is more parsimonious in that the founders may have already been fixed for those alleles. This founder event may be somewhat recent as the signature to detect a bottleneck with the method used in BOTTLENECK is within $2N_e$ to $4N_e$ generations (Piry *et al*. 1999). In part, the high genetic differentiation across the Cape Blanco break may be a reflection of the bottlenecking effects of a founder event. This would indicate that the ability of *P. shawi* to cross the Cape Blanco break is very limited and that dispersal across this barrier is a rare chance occurrence.

Mitochondrial DNA phylogeographical patterns

Three patterns stand out with the mtDNA. (i) First, there is discordance with the microsatellite data in that there is a shared common haplotype among UMP, HUN, and WIN. Although there are several explanations for this pattern (e.g. selection, incomplete lineage sorting), this occurrence is also consistent with a recent founder event possibly originating from the SIL-ALS-TEN-UMP region. (ii) Second, within the NEH population, there are two separate lineages (Fig. 4). There was no obvious association with these lineages and the microsatellite data. An interesting hypothesis that warrants investigation is that there has been an introgression of mtDNA from a population of Columbia River ancestry. This hypothesis stems from a biogeographical study of fish assemblages that suggests the Nehalem River once flowed into the Columbia River and then switched course to the Pacific Ocean (Reimers & Bond 1967). Such an introgression event may explain why the nuclear loci do not cluster NEH with other rivers in the Oregon Coast ESU (SIL, ALS, TEN, and UMP). We have three mtDNA sequences for *P. shawi* caught in the Columbia River estuary (data not shown) that indicate that introgression via stream capture is plausible. We do not know the exact freshwater origin of those parasites and more data are needed for a more conclusive test. Thus, we cannot rule out other possible causes such as the emergence of deep phylogenetic lineages within populations of a continuously low-dispersal

species (Irwin 2002; Kuo & Avise 2005). (iii) The third striking pattern from the mtDNA is the high level of divergence observed among most of the populations relative to that seen in nuclear markers (Table 2). Because *P. shawi* is hermaphroditic, sex-biased dispersal can be ruled out as an explanation. We accounted for differences in N_e between nuclear and mtDNA loci, assuming that *P. shawi* is hermaphroditic with random mating. Unequal male or female function by some individuals could also reduce mtDNA N_e below the one-half N_e expected in a completely hermaphroditic population. However, an eight-fold difference in nuclear to mtDNA N_e is the maximum expected due to a highly male-biased sex ratio (Hoelzer 1997). If the N_m of the mtDNA was 0.326 (i.e. the value needed to fall within the 95% CI of the microsatellite estimates), then there would have to be a 12-fold difference in the nuclear to mtDNA N_e to obtain the F_{ST} we observed for the mtDNA. Thus, even a highly male-biased sex ratio would not cause the excess mitochondrial differentiation we observed (Hoelzer 1997). Because genetic drift alone cannot explain the pattern, either higher mutation rates (and more homoplasy) in the microsatellites compared to the mtDNA (reviewed in Prugnolle & de Meeus 2002) or selection on the mtDNA (Hurst & Jiggins 2005; Bazin *et al*. 2006) could be possible explanations for the excess mtDNA differentiation that we observed.

Given the extreme differentiation and discordant patterns observed for the mtDNA, it is obvious that inferences on the phylogeographical history of *P. shawi* using the mtDNA alone would be misleading. However, the observed patterns have generated some interesting avenues for future study, and the extreme differentiation may prove useful in certain applications (see below).

Salmon conservation implications

There are two conservation implications stemming from our results. First, the congruence of parasite genetic differentiation and host ESU boundaries provides independent support that these boundaries are of biological significance. Second, the high levels of genetic differentiation at both the nuclear and mtDNA of *P. shawi* offer a unique tool for the management of salmon fisheries. The individual genotypes of *P. shawi* can be used to identify the freshwater source populations of several salmonid species. Salmon fisheries cross international borders and there are several endangered and protected ESUs (Waples *et al*. 2001). Thus, there is a need for information on the freshwater origins of salmonids harvested in the ocean and for data on juvenile near shore migratory patterns. We have previously shown that the genotypes of *P. shawi* are more accurate than steelhead genotypes in assignment back to population of origin (Criscione *et al*. 2006). Here, we have extended the analysis to include populations in ESUs north of the Nehalem break. Using the BAPS identified clusters as indicators of distinct parasite populations, we could assign fish back to any one of these five populations with over 90% accuracy. There is over 96% accuracy across the Nehalem break and 100% across the Cape Blanco break. Furthermore, assignment could potentially be narrowed to specific rivers within a population cluster if one used mtDNA in addition to microsatellites. For example, the three northernmost populations GRA, WIL, and NEH, share no mitochondrial haplotypes. Even within the SIL-ALS-TEN-UMP cluster, UMP showed high mtDNA divergence (F_{ST} = 0.85–0.95; Table 2) when compared to the other three rivers.

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Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3220/MEC3220sm.htm

Table S1 Prevalence (percent infected), mean intensity, and number of genotyped individuals of *Plagioporus shawi* collected from the nine rivers and four host species.[a]

Table S2 Nonstandardized F_{ST} between pairs of populations. Multilocus values from the microsatellites are below the diagonal and the mtDNA values are above the diagonal. All values are highly significant $P < 0.0001$ after sequential Bonferroni unless noted otherwise.

Table S3 Nonstandardized hierarchical *F*-statistics for the full ESU hierarchy or Nehalem break hierarchy (excludes HUN and WIN).

Table S4 Results of the assignment tests. Columns show the populations from which individuals were sampled and rows show the number of individuals that were assigned to that respective population.

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