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PARASITE GENOTYPES IDENTIFY SOURCE POPULATIONS OF MIGRATORY FISH MORE ACCURATELY THAN FISH GENOTYPES

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Abstract. DNA-based assignment of individuals to their population of origin has many applications such as mixed-stock analysis, identifying individuals from protected populations, and elucidating migration patterns. However, low genetic differentiation among populations will cause misassignments. Thus, an alternative means of determining an individual's population of origin is needed in cases where there is little or no neutral differentiation among source populations. Here, we test the hypothesis that parasite genotypes can be used to identify the origins of hosts more accurately than host genotypes. Using microsatellite markers from steelhead trout and their trematode parasites, we show that the odds of correct assignment are four times greater with the parasite's genotypes than with the host's genotypes. Our analyses show that this result is simply explained by the greater genetic structure among populations of the trematode parasite. Recent studies on the comparative genetic structure of other host and parasite species suggest that our results are not unusual or unique to the host–parasite system we studied. Thus, our work indicates that parasites will be useful for a wide range of applied and basic research that requires the assignment of individuals to source populations.

Key words: assignment test; genetic structure; Oncorhynchus mykiss; *parasite;* Plagioporus shawi; *salmon; source population; Trematoda.*

Introduction

DNA-based methods of assigning individuals to populations of origin have become increasingly important in addressing questions about dispersal patterns, population delineation, and conservation (Manel et al. 2005). For example, assignment methods are used in fisheries management to identify source populations for individuals that have migratory life histories and occur in mixed stocks (Beacham et al. 2004, Wirgin and Waldman 2005). Unfortunately, assignment is very inaccurate when neutral genetic differentiation among source populations is low (e.g., $F_{st} < 0.1$; Cornuet et al. 1999, Manel et al. 2002). Thus, alternative methods of assignment are needed in cases where there is little or no neutral differentiation.

The use of parasites as biological tags for assignment has a long history, and has proven useful in fisheries stock discrimination (MacKenzie and Abaunza 1998, MacKenzie 2002). Here the broad geographical origins of fish are identified based on the presence or absence of parasite species. It has been suggested that genotypes of individual parasites of a single parasite species could be used to assign hosts to source populations even more accurately (Beverley-Burton 1978, Manel et al. 2002). Indeed, if a parasite species is more finely subdivided among populations than its host, then one could potentially use the genotypes of individual parasites to assign hosts to their population of origin with higher probabilities than by using the host's own genotypes (Criscione et al. 2005). At first glance, it may not be apparent how a parasite could be more genetically structured than its dispersing host. However, host gene flow does not necessarily equate to parasite gene flow because not all hosts are infected, parasites may be locally adapted in ways that hosts are not, and effective sizes of host and parasite may be very different. In fact, several studies that compared genetic structure of parasites with that of their dispersing host all found equal or higher structure in the parasite (Nadler et al. 1990, Mulvey et al. 1991, Jobet et al. 2000, Nieberding et al. 2004, McCoy et al. 2005, Prugnolle et al. 2005). Thus, parasites may be useful for a wide range of applied and basic research that requires the assignment of individuals to source populations. Nevertheless, the hypothesis that parasite genotypes will be more accurate in assigning hosts to source populations than the host genotypes themselves has never been tested.

Here we compare the accuracy of assignment back to known source populations between *Oncorhynchus mykiss* (steelhead trout) and its trematode parasite *Plagioporus shawi*. This system is particularly interesting for testing the use of parasite genotypes to assign hosts to source populations for several reasons. First, the identification of stock composition is important in the management of depleted fish stocks (Banks 2005, Hammer and Zimmerman 2005). Thus, assignment methods are becoming more common in fisheries management (Cadrin et al. 2005). Second, there is competing international economic

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interest in steelhead fisheries (Banks 2005), and there are several endangered and protected evolutionarily significant units (ESUs) in the Pacific Northwest of the U.S. (Waples et al. 2001). Thus, there is a need for information on the freshwater origins of steelhead harvested in the ocean and for data on juvenile near shore migratory patterns. Finally, the strict freshwater life cycle of *P. shawi* (snail to aquatic arthropod to salmonid fish) (Schell 1975) and recent mitochondrial DNA data suggest that there is limited dispersal of the parasite among freshwater drainages (Criscione and Blouin 2004).

Methods

Sampling

We sampled between 21 and 24 individuals of both *P. shawi* and *O. mykiss* from each of five rivers located in the coastal mountain range of Oregon, USA (Fig. 1). All sampled rivers ultimately drain into the Pacific Ocean: the North Fork Nehalem River (NEH; 45°48′32″ N, 123°45′15″ W) flows into the Nehalem Bay; Mill Creek (SIL; 44°44′57″ N, 123°47′36″ W) flows into the Siletz River; the West Fork Smith River (UMP; 43°48′26″ N, 123°46′17″ W) feeds the Smith River, which connects to the Umpqua River; Hunter Creek (HUN; 42°22′18″ N, 124°24′24″ W) and Winchuck River (WIN; 42°00′19″ N, 124°12′05″ W) connect directly to the Pacific Ocean. These five rivers span the boundary between two major ESUs of steelhead (Waples et al. 2001). Three rivers (NEH, SIL, UMP) are located in the Oregon Coast ESU and two rivers (HUN, WIN) are in the Klamath Mountains Province ESU (Fig. 1). 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 of *P. shawi* were collected from March to May of 2002. The same was true for samples of *O. mykiss* except for three individuals from NEH and 12 from SIL that were sampled in April 2003 and 2004, respectively. To obtain a random sample of each parasite population, each parasite was collected from a different individual salmonid host including many of the steelhead used in the analyses. Some individuals of *P. shawi* were also obtained from cutthroat trout (*O. clarki*) and Chinook salmon (*O. tshawytscha*). Previous mitochondrial and microsatellite data indicate that there is no host specificity among the different salmonid species (Criscione and Blouin 2004; C. Criscione, *unpublished data*). Collections were made in conjunction with Oregon salmonid-monitoring projects under the permits OR2002-019 and OR2004-1431. Parasitological techniques for recovering parasites from hosts were previously described in Criscione and Blouin (2004).

Genotyping

Protocols for DNA extraction, polymerase chain reaction (PCR), and genotyping of *P. shawi* and *O. mykiss* were previously described in Criscione and Blouin (2005) and Araki and Blouin (2005), respectively. Both trematodes and steelhead were genotyped at eight microsatellite loci. The loci for *P. shawi* were m26, m41, m48, d04, d09, d36, d43, and d47 (Criscione and Blouin 2005) and the loci for *O. mykiss* were Omy77, One2, Ssa407, Omy1011, Str2, Rt191, Omy1001, and One108 (Araki and Blouin 2005).

Data analyses

The Bayesian method of Rannala and Mountain (1997) as implemented in the program GENECLASS2 (Piry et al. 2004) was used for assignment. GENECLASS2 gives an assignment score (the likelihood value of an individual belonging to a given population divided by the sum of likelihood values for that individual in all sampled populations) for each individual (Piry et al. 2004). Thus, the assignment score is a percentage. Each individual was assigned to the population in which that individual had the highest score. For the calculation of the likelihood of an individual belonging to the population from which it was sampled, GENECLASS2 excludes that individual prior to estimating that population's allele frequencies (i.e., the leave-one-out procedure; Piry et al. 2004).

The accuracy of assignment back to the population of origin was compared between *P. shawi* and *O. mykiss* in two ways. First, a Fisher's exact test and odds ratio were used to compare the percentage of correct assignments between *P. shawi* and *O. mykiss*. The second method compares the quality index calculated in GENECLASS2. The quality index is the mean value of the assignment scores of each individual in the population from which it was sampled (Piry et al. 2004). A Mann-Whitney test was used to compare the quality index between species because the assignment scores were not normally distributed.

To assess whether any individual locus had a large influence on the results, we redid the assignment tests using a single locus at a time and via a jackknife procedure where two of the eight loci were excluded at a time. The mean percentage of correct assignments and mean quality index were calculated from the results of eight individual loci or from the 28 possible combinations of excluding two loci. Two-sample t tests with unequal variances on arcsine-square-root transformed data were used to test for differences between *P. shawi* and *O. mykiss*.

Gene diversity and number of alleles per locus were calculated over all individuals grouped together. Nei's (1987) genetic diversity (H_s) was calculated with FSTAT v2.9.3 (Goudet 1995). Mean H_s of eight loci was compared with a two-sample t test with unequal variances on arcsine-square-root transformed gene diversities. We compared the number of alleles per locus (A) with and without rarefying. We rarefied to a sample size of 100 individuals using HP-RARE (Kalinowski 2005). A two-

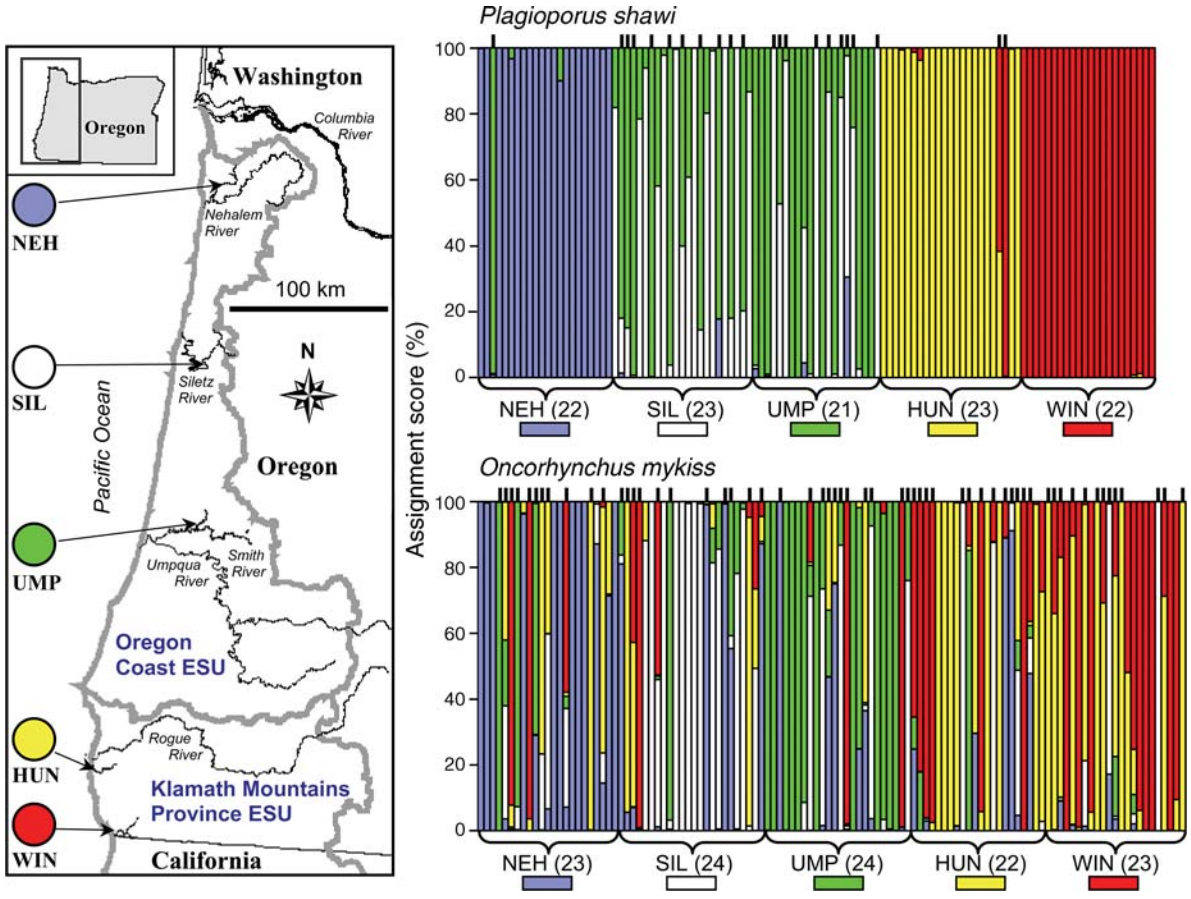

FIG. 1. Map of sampled locations and individual assignment scores of *Plagioporus shawi* (top graph) and *Oncorhynchus mykiss* (bottom graph). The thick gray lines in the map demarcate the boundaries for the Oregon Coast ESU (evolutionarily significant unit) and Klamath Mountains Province ESU of steelhead (Waples et al. 2001). Sampled locations in the map are color coded to match the graphs of individual assignments (NEH, North Fork Nehalem River; SIL, Mill Creek; UMP, West Fork Smith River; HUN, Hunter Creek; WIN, Winchuck River). The assignment graphs show the color-coded assignment scores (expressed as a percentage) for each individual (separated by black lines). Brackets on the bottom of each graph indicate from which population an individual was sampled. Numbers in parentheses are the number of individuals sampled from each population. Black bars on the top of each graph indicate an incorrect assignment. For example, the third individual from the left of *P. shawi* that was sampled from NEH was incorrectly assigned (assignment score = 98.7%) to UMP. The assignment graphs clearly illustrate the greater assignment accuracy in *P. shawi*. Notice that most incorrect assignments for *P. shawi* occur between SIL and UMP and that there was 100% correct assignment across the ESU boundary.

sample *t* test was used to test for a difference in mean *A* or rarefied mean *A*. Genetic structure within and among populations was analyzed with Weir and Cockerham's (1984) unbiased estimators of F_{is} (average within populations) and F_{st} (among populations) as calculated by FSTAT v2.9.3.

Population dendrograms were inferred from Nei's (1978) unbiased genetic distance. Distance matrices among populations and bootstrap distance matrices (an exhaustive bootstrap of 6435 replicates over eight loci) were calculated with MICROSAT (Human Population Genetics Laboratory, Stanford University, *unpublished program*). Unrooted neighbor-joining trees were created using PHYLIP v3.6 (J. Felsenstein, *unpublished program*) and drawn with TREEVIEW v1.6.6 (Page 1996).

RESULTS AND DISCUSSION

Our results clearly show that the genotypes of the parasites are more accurate than the genotypes of the fish in assigning individuals to their population of origin. Tests for both percentage of correct assignment and quality index indicate that *P. shawi* has significantly higher assignment accuracy than *O. mykiss* (Fig.1, Table 1; $P < 0.00001$). The odds of correct assignment were four times greater (odds ratio, 4.2; 95% CI, 2.3–7.6) with the parasite's genotypes than with the steelhead's genotypes. Furthermore, among populations of *P. shawi*, all but three incorrect assignments were confined to assignments between the adjacent SIL and UMP rivers (Fig. 1). There were many more misassignments for *O. mykiss,* and these misassignments were spread widely among different rivers. If we only try to assign

TABLE 1. Measures of assignment accuracy, genetic diversity, and population genetic structure

Species	Correct assignment (%)	Quality index (%)	Correct with one locus (%)	Correct with jackknife (%)†	H_s	A	F_{is}	F_{st}
Plagioporus shawi	80.2	80.5	51.9 (42.8–60.9)	78.7 (77.4–79.9)	0.84 (0.74–0.92)	23.9 (16.6–31.2)	0.042 (0.008–0.082)	0.19 (0.11–0.28)
Oncorhynchus mykiss	49.1	49.6	35.4 (31.6–39.3)	47.4 (46.1–48.8)	0.93 (0.9–0.95)	26.6 (19.8–33.4)	0.048 (0.008–0.093)	0.019 (0.014–0.024)

Notes: Values reported are means (with 95% CI in parentheses). Confidence intervals for F_{is} and F_{st} were calculated in FSTAT by bootstrapping over loci. Parameters are: H_s, Nei's (1987) gene diversity as calculated in FSTAT; F_{is}, proportion of the genetic variance in an individual relative to the variance in the subpopulation (inbreeding coefficient); F_{st}, proportion of genetic variance in a subpopulation relative to the total variance of the population; and A, the number of alleles per locus. Rarefied mean A (95% confidence intervals) was 23.3 (16.3–30.4) for *P. shawi* and 25.8 (19.2–32.4) for *O. mykiss*.

† The mean percentage correct assignment of all 28 combinations of excluding two loci.

steelhead back to their correct ESU, rather than to particular rivers, there is still only 80.2% correct assignment (Fig. 1). In stark contrast, there was 100% correct assignment for individuals of *P. shawi* across the ESU boundary (Fig. 1).

Our choice of loci did not bias the assignment results as assessed by the reanalysis of the assignment tests using a single locus at a time or via a jackknife procedure where two of the eight loci were excluded at a time. The mean percentage of correct assignment was significantly higher in *P. shawi* than *O. mykiss* for both the single locus ($P < 0.003$) and jackknife tests ($P < 0.0001$; Table 1). Similar results were obtained for the mean quality index (data not shown). The results of the single locus tests indicate that on average, a single locus from *P. shawi* will have higher assignment accuracy than a locus from *O. mykiss*. Furthermore, the jackknife results indicate that assignments were not substantially biased by any set of two loci as the mean percentage of correct assignment was only lowered by 2% for both *P. shawi* and *O. mykiss* (Table 1).

Assignment to source populations can be affected by sample size, number of loci, number of alleles, genetic diversity, and the amount of genetic structure among populations (Cornuet et al. 1999, Manel et al. 2002, Kalinowski 2004). We used an equal number of loci and similar population sample sizes for *P. shawi* and *O. mykiss*. There was no statistical difference between species in the mean A, rarefied mean A, (Table 1) or in F_{is}. Mean gene diversity (H_s) was slightly higher ($P = 0.04$) in *O. mykiss* than *P. shawi* (Table 1). However, this difference should bias the results toward more correct assignments in *O. mykiss* (Kalinowski 2004). These results indicate that the difference in assignment accuracy between *O. mykiss* and *P. shawi* was not the result of differences in genetic polymorphism. F_{st}, on the other hand, was 10 times greater among the populations of *P. shawi* (Table 1). Thus, the higher assignment accuracy for *P. shawi* relative to *O. mykiss* is most

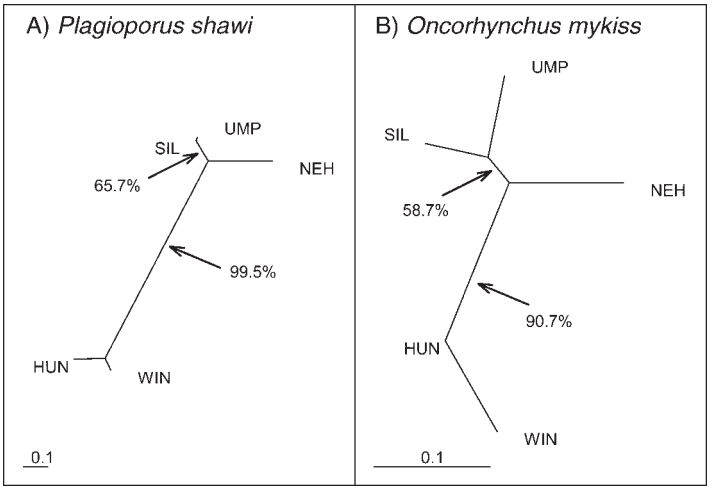

FIG. 2. Unrooted population dendrograms (inferred from Nei's [1978] unbiased genetic distances among populations) for (A) *Plagioporus shawi* and (B) *Oncorhynchus mykiss*. Numbers and arrows indicate the percentage of bootstraps that support the branches. Notice the congruence of the topologies and that the longest branch in both species separates the populations on either side of the ESU boundary shown in Fig. 1. There is very little genetic distance between the SIL and UMP populations of *P. shawi*. In general, however, genetic distances are larger among populations of *P. shawi* than *O. mykiss* (notice the difference in the scale bars in the bottom left of each panel).

simply explained by the greater genetic structure among populations of *P. shawi*. We can also ask if the higher genetic structure in *P. shawi* results from current differences between species in gene flow and/or effective size, or if it results from different biogeographic histories. The population dendrograms of *O. mykiss* and *P. shawi* show perfect concordance in their topologies (Fig. 2). In addition, the longest branch in the population dendrograms of both species separates the rivers on either side of the ESU boundary (Fig. 2), so the distributions of both the steelhead and parasite genotypes are consistent with the previous ESU designations shown in Fig. 1 (Waples et al. 2001). Thus, different biogeographic histories do not obviously account for the difference in assignment accuracy.

Our data show that assignment accuracy is greater for the trematode parasite than for its host, and that this difference probably results from differences in equilibrium levels of gene flow and/or genetic drift. In this case, the assignment of ocean-caught steelhead to freshwater source populations should be more exact when using the genotypes of *P. shawi* than the genotypes of the steelhead themselves (Fig. 1). It is interesting to note that coho salmon (*O. kisutch*) also have low levels of genetic subdivision ($F_{st} \approx 0.02$) among streams in Oregon and Washington (Teel et al. 2003, Ford et al. 2004). Thus, *P. shawi* may be useful in assigning other species of salmonids to their freshwater source populations.

Here, we have a clear demonstration of principle that parasite genotypes can be used to accurately assign hosts to their population of origin. Thus, a few comments on the general applicability of this method are in order. An obvious drawback is that it cannot be used on uninfected hosts. Parasites tend to have an aggregated distribution among hosts, and not all hosts may be infected (Shaw et al. 1998). For example, the mean prevalence (percentage of hosts infected) for *P. shawi* in our sampled steelhead populations was 64.9% (range among sampled populations; 37.5 to 100%). On the other hand, a host that carries two or more individual parasites can be assigned with extremely high accuracy. In our system, the average mean intensity of infection was 6.9 (range among sampled populations; 2.9–15.9) parasites per infected steelhead. So here we have the ability to assign some fish with almost complete certainty, while other, uninfected, fish cannot be assigned at all. The utility of this method may also depend on when and where infections are acquired. For example, in the *P. shawi*–steelhead system, older fish at sea may have lost some of the parasite infections acquired in freshwater. So for steelhead this method may be more useful for questions such as studying the near-shore movements of recently out-migrated juveniles than say, testing the origins of returning spawners. The opposite will be true in species where hosts accumulate parasites over time so that older hosts are more likely to be infected than younger hosts.

Here we have shown that, on average, any given parasite locus should be of much more assignment value than any given fish locus. However, we are not arguing that one should use only parasite genotypes for assigning hosts. Rather, we view parasites as a valuable additional tool. In practice, one would presumably use all available information to assign hosts, including host genotypes. Indeed, for an economically important host like salmon, there may be many more marker loci available for the host than for the parasite. A preselected subset of the most discriminating fish loci may work better than an equal number of randomly chosen parasite loci. One could also include any phenotypic traits of the host that differ among populations (e.g. fish otolith chemical composition). Note that variation among populations in parasite prevalence or intensities could provide one such additional source of information (as in the classical "biotag" methods for assigning fish stocks [MacKenzie 2002]), thus making the parasites useful in two ways.

This is the first study to show that parasite genotypes can assign a host back to its source population more accurately than the host's own genotype. More importantly, our trematode–steelhead system does not appear to represent a unique case. Arthropod, nematode, and other trematode parasites typically have equal or higher genetic structure than their dispersing hosts (Nadler et al. 1990, Mulvey et al. 1991, Jobet et al. 2000, Nieberding et al. 2004, McCoy et al. 2005, Prugnolle et al. 2005). Thus, the use of parasites to assign hosts back to source populations may have broad application. For example, it may be possible to use the assignment of parasites in epidemiological studies to indicate foci of transmission for infected hosts. Other potential applications include identifying (1) source populations of poached animals in wildlife forensics (Manel et al. 2002), (2) individuals from protected populations in conservation management, and (3) dispersal patterns or feeding grounds for other migratory species.

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Literature Cited

Araki, H., and M. S. Blouin. 2005. Unbiased estimation of relative reproductive success of different groups: evaluation and correction of bias by parentage assignment errors. Molecular Ecology **13**:4097–4109.

Banks, M. A. 2005. Stock identification for conservation of threatened or endangered species. Pages 609–630 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock identification methods: applications in fishery science. Elsevier, Burlington, Massachusetts, USA.

Beacham, T. D., K. D. Le, and J. R. Candy. 2004. Population structure and stock identification of steelhead trout (*Onco-*

rhynchus mykiss) in British Columbia and the Columbia River based on microsatellite variation. Environmental Biology of Fishes **69**:95–109.

Beverley-Burton, M. 1978. Population genetics of *Anisakis simplex* (Nematoda: Ascaridoidea) in Atlantic salmon (*Salmo salar*) and their use as biological indicators of host stocks. Environmental Biology of Fishes **3**:369–377.

Cadrin, S. X., K. D. Friedland, and J. R. Waldman. 2005. Stock identification methods: applications in fishery science. Elsevier, Burlington, Massachusetts, USA.

Cornuet, J.-M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics **153**:1989–2000.

Criscione, C. D., and M. S. Blouin. 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. Evolution **58**:198–202.

Criscione, C. D., and M. S. Blouin. 2005. Eleven polymorphic microsatellite loci for the salmonid trematode *Plagioporus shawi*. Molecular Ecology Notes **5**:562–564.

Criscione, C. D., R. Poulin, and M. S. Blouin. 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Molecular Ecology **14**:2247–2257.

Ford, M. J., D. Teel, D. M. Van Doornik, D. Kuligowski, and P. W. Lawson. 2004. Genetic population structure of central Oregon Coast coho salmon (*Oncorhynchus kisutch*). Conservation Genetics **5**:797–812.

Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate *F*-statistics. Journal of Heredity **86**:485–486.

Hammer, C., and C. Zimmerman. 2005. The role of stock identification in formulating fishery management advice. Pages 631–658 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock identification methods: applications in fishery science. Elsevier, Burlington, Massachusetts, USA.

Jobet, E., P. Durand, J. Langand, C. D. M. Muller-Graf, J.-P. Hugot, M.-E. Bougnoux, C. Rivault, A. Cloarec, and S. Morand. 2000. Comparative genetic diversity of parasites and their hosts: population structure of an urban cockroach and its haploid-diploid parasite (oxyuroid nematode). Molecular Ecology **9**:481–486.

Kalinowski, S. T. 2004. Genetic polymorphism and mixed-stock fisheries analysis. Canadian Journal of Fisheries and Aquatic Sciences **61**:1075–1082.

Kalinowski, S. T. 2005. HP-RARE: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes **5**:187–189.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology **124**: S153–S163.

MacKenzie, K., and P. Abaunza. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fisheries Research **38**:45–56.

Manel, S., P. Berthier, and G. Luikart. 2002. Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. Conservation Biology **16**:650–659.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology and Evolution **20**:136–142.

McCoy, K. D., T. Boulinier, and C. Tirard. 2005. Comparative host-parasite population structures: disentangling prospecting and dispersal in the Black-legged Kittiwake *Rissa tridactyla*. Molecular Ecology **14**:2825–2838.

Mulvey, M., J. M. Aho, C. Lydeard, P. L. Leberg, and M. H. Smith. 1991. Comparative population genetic structure of a parasite (*Fascioloides magna*) and its definitive host. Evolution **45**:1628–1640.

Nadler, S. A., M. S. Hafner, J. C. Hafner, and D. J. Hafner. 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomydiae). Evolution **44**:942–951.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583–590.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York, USA.

Nieberding, C., S. Morand, R. Libois, and J. R. Michaux. 2004. A parasite reveals cryptic phylogeographic history of its host. Proceedings of the Royal Society of London. Series B **271**: 2259–2568.

Page, R. D. M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences **12**:357–358.

Piry, S., A. Alapetite, J.-M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity **95**:536–539.

Prugnolle, F., A. Theron, J. Pierre Pointier, R. Jabbour-Zahab, P. Jarne, P. Durand, and T. de Meeus. 2005. Dispersal in a parasitic worm and its two hosts: consequence for local adaptation. Evolution **59**:296–303.

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences (USA) **94**:9197–9201.

Schell, S. C. 1975. The life history of *Plagioporus shawi* (McIntosh 1939) (Trematoda: Opecoelidae), an intestinal parasite of salmonid fishes. Journal of Parasitology **61**:899–905.

Shaw, D. J., B. T. Grenfell, and A. P. Dobson. 1998. Patterns of macroparasite aggregation in wildlife host populations. Parasitology **117**:597–610.

Teel, D. J., D. M. Van Doornik, and D. R. Kuligowski. 2003. Genetic analysis of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington reveals few Columbia River wild fish. Fishery Bulletin **101**:640–652.

Waples, R. S., et al. 2001. Characterizing diversity in salmon from the Pacific Northwest. Journal of Fish Biology **59**:1–41.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution **38**:1358–1370.

Wirgin, I., and J. R. Waldman. 2005. Use of nuclear DNA in stock identification: single-copy and repetitive sequence markers. Pages 331–370 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock identification methods: applications in fishery science. Elsevier, Burlington, Massachusetts, USA.