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Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic differences with distance

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Abstract

In many species genes move over limited distances, such that genetic differences among populations or individuals are expected to increase as a function of geographical distance. In other species, however, genes may move any distance over a single generation time, such that no increase of genetic differences is expected to occur with distance. Patterns of gene dispersal have been assessed typically using this theoretical property. In this study, this classical approach based on a Mantel test was compared to a new method using individual assignment to reveal contrasts in dispersal patterns between 15 populations of brook charr *Salvelinus fontinalis* and 10 populations of Atlantic salmon *Salmo salar* sampled in eastern Canada, where both species co-occur naturally. Based on the Mantel test, we found evidence for neither an increase of genetic differences with distance in either species nor a significant contrast between them. The individual-based method, in contrast, revealed that individual assignment in both species was non random, being significantly biased toward geographically proximate locations. Furthermore, brook charr were on average assigned to a closer river than were salmon, according to a priori expectations based on the dispersal behaviour of the two species. We thus propose that individual assignment methods might be a promising and more powerful alternative to Mantel tests when isolation by distance cannot be postulated a priori.

Keywords: dispersal, individual assignment, isolation by distance, Mantel test, microsatellite, salmonids

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Introduction

Dispersal can be considered as one of the most ubiquitous processes in the living world (Dingle 1996; Clobert *et al*. 2001), and as a consequence, genes of virtually any species move in space from one generation to the next; yet, in many species, the location of a gene is not independent from the location of its parental copy. Rather, genes generally move limited distances, as modelled by the process of isolation by distance (Wright 1943). In other species, however, individuals may move very large distances during their lifetime, such that the genes they carry virtually have the potential to cover any distance in the species' range over a single generation time (Dingle 1996). The way genes of a species disperse over its range has major consequences on many ecological and evolutionary features, including the species' demographic dynamics, the potential for local adaptations to evolve or the development of spatial patterns of genetic diversity (Clobert *et al*. 2001). As such, an accurate assessment of dispersal is of the utmost importance, both from a fundamental and conservation perspective.

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Typically, a direct method for measuring dispersal in animals is achieved by physical tagging (Dingle 1996). However, several logistical and technical drawbacks may limit the usefulness of physical tagging, especially in small and highly vagile organisms living in open habitats. Alternatively, the availability of highly variable genetic markers [e.g. microsatellite loci, amplified fragment length polymorphisms (AFLP)] has allowed the definition of individual multilocus genotypes and made it possible to identify an individual as a disperser to the population where it has been sampled (e.g. Paetkau *et al*. 1995; Palsbøll *et al*. 1997; Waser & Strobeck 1998; Roques *et al*. 1999; Primmer *et al*. 2000; Potvin & Bernatchez 2001; Campbell *et al*. 2003) or to estimate migration rates among populations (Wilson & Rannala 2003; Paetkau *et al*. 2004). However, the usefulness of such individual-based genetics methods for assessing the correlation between patterns of dispersal and geographical distances has not been tested rigorously (but see Paetkau *et al*. 1997 in a different context). More typically, this has been achieved using an 'indirect' genetic approach at the scale of populations rather than individuals, based on the expected increase of genetic differences with distance when the variance of parental position relative to offspring position (σ^2) is finite (Wright 1943; Slatkin 1993; Rousset 1997). This indirect approach assumes that dispersal patterns translate unequivocally into spatial patterns of genetic diversity. However, there are a number of reasons why there may be no detectable increase of genetic differences with distance in a species even though dispersal may be geographically restricted, including departure from equilibrium expectation (Leblois *et al*. 2000), insufficient statistical power (Peterson & Denno 1998) and temporal variance in dispersal (Li 1976; Whitlock 1992).

These limitations of classical tests of isolation by distance can be circumvented partly by considering a comparative approach that contrasts spatial patterns of genetic diversity in species using similar habitats but that differ in dispersal potential. Such an approach has, for instance, been used to reveal the impact of dietary behaviour on isolation by distance patterns in phytophagous insects (Peterson & Denno 1998). A major asset of this approach is that one species may serve as a baseline to ensure that the sampling scheme had sufficient statistical power to reveal a pattern if it existed. Ideally, such comparisons should involve sets of species differing only by their dispersal behaviour.

Anadromous salmonid fishes are notorious for their homing behaviour by which adults return to their home river following the oceanic phase of their life cycle. However, knowledge on patterns of dispersal of fish that do not reproduce in their home river (a phenomenon known as straying) is still limited for many species. Anadromous Atlantic salmon (*Salmo salar*) and brook charr *Salvelinus fontinalis* Mitchill are two salmonids naturally co-occurring on the East coast of North America that are believed to differ in their potential for dispersal. Young salmon spend 1–3 years in freshwater, migrate to their oceanic feeding grounds where they spend one (grilse) to several (multisea-winter fish) years before returning as adults to spawn (Klemetsen *et al*. 2003). Oceanic feeding grounds may be up to several thousands of kilometres away from their home river, such that salmon can potentially stray to virtually any river in its range. Mark–recapture studies generally indicate that salmon tend to stray in an inverse proportion to the distance from their home river (e.g. Hvidsten *et al*. 1994; Mills 1994; Potter & Russell 1994). In contrast, the bulk of studies on the geographical structure of genetic diversity in Atlantic salmon (reviewed in Youngson *et al*. 2003) failed to detect an increase of genetic differences with distance, as would be predicted from restricted dispersal (but see Nielsen *et al*. 1999; King *et al*. 2001). More recently, a meta-analysis revealed that studies at large geographical scales tended to show higher levels of genetic differences among populations (F_{ST}) than studies at smaller scales (Hendry *et al*. 2004). This suggested that isolation by distance in previous empirical studies may have remained undetected because of limited statistical power. Altogether, empirical evidence for an increase of genetic differences with distance has remained equivocal in Atlantic salmon. In contrast to salmon, brook charr have low salinity tolerance (Besner & Pelletier 1991) such that the species tends to remain in the close vicinity of its home river (White 1941, 1942; Curry *et al*. 2002), where it also overwinters every year. Thus, the probability of long-distance migration in brook charr appears lower than in Atlantic salmon and is more likely to result in an increase of genetic differences with geographical distance (Castric & Bernatchez 2003).

In the present study, we performed a comparative analysis of spatial patterns of genetic diversity in Atlantic salmon and brook charr sampled over a single stretch of a linear coast in their native range to test for restricted dispersal using two different methods. We first tested whether samples from geographically remote rivers showed higher genetic differences than samples collected at smaller distances using a Mantel test. We then used individual-based assignment methods (Cornuet *et al*. 1999) to test whether fish identified as potential migrants had moved randomly over any distance or preferentially over limited distance from their home river. Based on the contrasted potential for dispersal of both species, we predicted that (1) genetic differences should correlate more strongly with geographical distances in brook charr than in Atlantic salmon (*Salmo salar*), (2) straying individuals should be found more frequently in neighbouring than in remote rivers if dispersal is geographically restricted and (3) straying salmon should be found further away from their home river than straying charr.

Contrary to the results obtained from the classical test for isolation by distance, the assignment method showed clearly that dispersal of both species was restricted geographically.

Table 1 Sampling locations of Atlantic salmon and brook charr; *n* refers to sample size and the coastal distance from km 0 is the distance to the westernmost population (Ste-Marguerite River)

	Location		*n*	Coastal distance from km 0	Latitude	Longitude
S. salar	S1	Rivière Ste-Marguerite	76	0	48°15′49″	69°56′47″
	S2	Rivières des Escoumins	43	32	48°20′50″	69°27′00″
	S3	Rivière Trinité	50	230	49°25′05″	67°18′16″
	S4	Rivière Moisie	32	386	50°16′00″	65°56′00″
	S5	Rivière St-Jean	40	514	50°17′00″	64°20′00″
	S6	Rivière Mingan	29	574	50°18′00″	63°59′00″
	S7	Rivière Piashti	21	634	50°17′00″	62°48′00″
	S8	Rivière Natashquan	50	709	50°07′00″	61°48′00″
	S9	Rivière du Gros Mécatina	50	899	50°46′06″	59°05′40″
	S10	Rivière St-Paul	42	1034	51°27′00″	57°42′00″
S. fontinalis	F1	Rivière Ste-Marguerite	50	0	48°15′49″	69°56′47″
	F2	Rivière des Escoumins	50	32	48°20′50″	69°27′00″
	F3	Rivière Laval	68	72	48°46′00″	69°03′00″
	F4	Rivière Godbout	22	200	49°19′00″	67°35′00″
	F5	Rivière Trinité	50	230	49°25′05″	67°18′16″
	F6	Rivière du Calumet	48	256	49°37′00″	67°13′00″
	F7	Rivière Ile de Mai	50	306	49°55′38″	66°57′50″
	F8	Rivière Moisie	49	386	50°16′00″	65°56′00″
	F9	Rivière St-Jean	50	514	50°17′00″	64°20′00″
	F10	Baie-Johann-Beetz	13	634	50°17′00″	62°48′00″
	F11	Rivière Washicoutai	48	778	50°13′00″	60°52′00″
	F12	Rivière Watasheistic	31	888	50°24′00″	59°50′00″
	F13	La Tabatière	48	938	50°50′00″	58°59′00″
	F14	Rivière St-Augustin	46	986	51°12′00″	58°35′00″
	F15	Rivière St-Paul	41	1034	51°27′00″	57°42′00″

It also revealed contrasts in the dispersal pattern of both species, which were not apparent from classical tests for isolation by distance. We therefore conclude that the use of individual-based genetic assignment methods may provide an efficient alternative to traditional tests of isolation by distance for inferring patterns of dispersal.

Materials and methods

Sample collection

Samples of both Atlantic salmon and brook charr were collected along a 1034 km-long linear stretch of coastline in the Gulf of St Lawrence River (Québec, Canada) where both species co-occur. A total of 433 (mean $n = 43$) adult anadromous Atlantic salmon (including both grilses and multisea-winter fish) were collected during their upstream migration from 10 rivers along the coast (Table 1, Fig. 1). Also, the data for 666 brook charr (mean $n = 44$) analysed in Castric & Bernatchez (2003) and that were collected in 15 rivers in the same region were used in this study (Table 1, Fig. 1). Adipose fins were nonlethally removed and total DNA was isolated using a standard phenol–chloroform protocol (Sambrook *et al*. 1989). Individual genotypes of Atlantic salmon were obtained using an ABI 377 semiautomated sequencer (PerkinElmer) at six microsatellite loci (SSA-85, SSA-171, SSA-197, SSA-202, O'Reilly *et al*. 1996; SSOSL-85, Slettan *et al*. 1995; and MST3, Presa & Guyomard 1996) as described in Garant *et al*. (2000). Genetic analyses for brook charr samples were performed at six microsatellite loci (SFO-12, SFO-18, SFO-23, SFO-8, Angers *et al*. 1995; SSA-197, O'Reilly *et al*. 1996; MST-85, Presa & Guyomard 1996) as detailed in Castric *et al*. (2001).

Genetic diversity within and among populations

Intrapopulation genetic diversity was estimated as the number of different alleles per locus, expected and observed heterozygosity (A, H_E and H_O, respectively). Conformance to Hardy–Weinberg (HW) expected genotypic proportions was tested using the permutation test (5000 iterations) implemented in GENETIX 4.02 (Belkhir *et al*. 2000). Genetic differentiation among samples from different rivers was quantified using F_{ST} as estimated by θ (Weir & Cockerham 1984) and the 95% credible intervals were obtained by performing 1000 bootstrap iterations over loci using

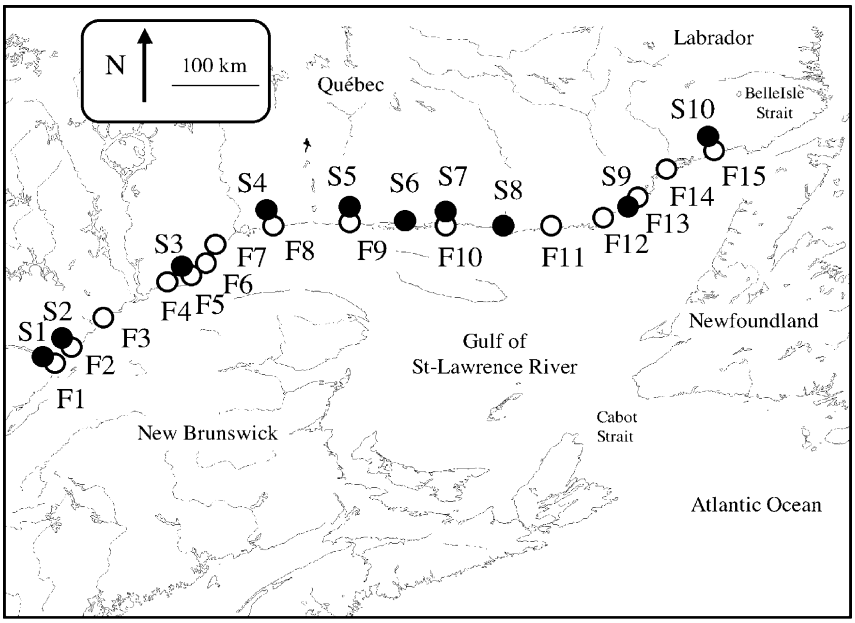

Fig. 1 Location of Atlantic salmon (filled circles) and brook charr (open circles) samples. River names refer to locations in Table 1.

GENETIX. Significance for the overall F_{ST} value and pairwise comparisons was assessed using 30 000 permutations. For the latter, the critical significance threshold was maintained at 5% using Bonferroni sequential adjustment for multiple tests (Rice 1989).

Testing for isolation by distance

Regression method. Because reproduction in both anadromous salmon and brook charr is restricted to freshwater, populations are discrete and linearly organized along a single dimension. In such habitat, isolation by distance (finite variance of parental position relative to offspring position σ^2) results in a linear increase of genetic differences with distance (Rousset 1997). Note that this expected pattern of increase has been referred to commonly as the 'isolation by distance pattern', but should not be confused with the underlying isolation by distance *process* (= geographically restricted dispersal), as originally defined by Wright (1943). We used GENETIX to compute the Mantel correlation coefficient (Mantel 1967) between the pairwise matrices of geographical and genetic $[F_{ST}/(1 - F_{ST})]$ distances between populations and to assess the significance of the correlation using 5000 random permutations of matrices. Slopes of the relationship between these two estimates obtained for both species were also compared in the mathematics computer language MAPLE 6 (Waterloo Inc. 1999) by bootstrapping slopes over loci (1000 iterations).

Scaling effect over the isolation by distance signal. Because spatial patterns are also sensitive to scaling effects and may fade out at larger geographical scales (Castric & Bernatchez 2003), we then tested whether the slope of isolation by distance remained constant over increasing geographical scales by computing the regression slope of $F_{ST}/(1 - F_{ST})$ as a function of coastal distance, successively including pairwise comparisons of populations separated by increasingly large distances (from 32 to 1034 km for salmon, from 26 to 1034 km for charr). The variation pattern was depicted using a log–log graph in which the steepness of the regression illustrates the rate of decrease of the isolation by distance slope with geographical scale (see details of this method in Castric & Bernatchez 2003). The rate of decrease in salmon and brook charr was compared using a bootstrap procedure implemented in MAPLE (1000 iterations).

Individual assignment methods. Rannala & Mountain's (1997) Bayesian individual assignment method implemented in GENECLASS (www.montpellier.inra.fr/URLB/geneclass/geneclass.html) was used to estimate the likelihood that a fish originated from each of the rivers of our sampling scheme. An individual fish whose river of sampling (thereafter 'sampling river') differed from the river where its multilocus genotype was most likely to originate from (thereafter 'assignment river') was referred to as being misassigned and the distance between sampling and assignment rivers as the misassignment distance. Under the null hypothesis of random dispersal, misassignment should theoretically occur randomly with geographical distance as well (H_O). In contrast, isolation by distance should result in misassigned individuals being found preferentially in geographically proximate rivers, such that short-distance classes should be over-represented compared to expectation under random dispersal. Sources of misassignment can be

classified into three types. First, misassigned fish can be immigrants sampled outside from but assigned to the river from which they emigrated. Second, a proportion of fish may be assigned by chance to another river when the likelihood functions of two rivers overlap, which may occur when differentiation is low (Waser & Strobeck 1998). Because restricted dispersal will result in lower genetic differences at smaller distances, misassigned individuals should also tend to be assigned to nearby rivers. Thus, restricted dispersal should affect these first two sources of misassignment in the same direction, i.e. fish assigned preferentially at short distances. Third, misassigned individuals may originate from a river outside our sampling scheme. In such a case misassignement distance is unknown, such that these individuals have to be discarded from further analyses. The simulation method of Cornuet *et al*. (1999) was used to generate the rejection zone for each river (using the 'leave one out' option in GENECLASS) and exclude rivers as a potential source for each individual whose multilocus genotype was outside the 95% likelihood region of the river. Thus, individuals found to be outside the 95% likelihood region of all rivers we sampled were considered as potential immigrants from an unknown source and were not considered for further analyses.

We then tested whether misassigned fish were equally likely to originate from any river whatever its distance from the sampling river (H_O) or whether individuals tended to be assigned to rivers geographically closer from the sampling river (H_1). The average distance of misassigned individuals was computed and compared to the expected distribution under random dispersal. Thus, the expected random distribution of geographical distance between source and misassigned rivers was generated by drawing without replacement 10 000 pairs of populations, and estimating the proportion of pairs obtained as a function of geographical distance using MAPLE. Variation in sample size among rivers was taken into account by weighting the probability of drawing a river by the number of individuals sampled in that river. Because sampling designs differed between both species, random distributions were generated separately for each of them.

The distribution of misassignment distances was then quantified as frequencies among 14 distance classes, each being 75 km wide, which allowed the identification of distance classes causing departure from random expectations. The expected census and 95% credible intervals of each distance class were produced by simulating random dispersal in MAPLE as presented above, and observed and expected frequency distributions of misassignment distances in each species were compared using a *G*-test with $\times$-2 degrees of freedom, where $\times$ is the number of distance classes with non-null expected census (Sokal & Rohlf 1995).

We then used a Mantel test to determine whether misassignment occurred randomly with geographical distance. The distance between each pair of rivers was measured, and the proportion of fish whose multilocus genotypes would have been more likely to occur in the other river was computed using GENECLASS and averaged over the two populations of the pair. Significance of the correlation of both matrixes was tested using 10 000 random matrix permutations in GENETIX. For comparison purposes, we also used a Mantel test to assess the correlation between geographical distances and Paetkau *et al*.'s (1997) genotype likelihood ratio distance (D_{LR}), which is also based on individual assignment among pairs of populations. Finally, the distribution of Atlantic salmon and brook charr misassignment distances was compared directly using a *G*-test.

Results

Genetic diversity within and among populations

High levels of microsatellite polymorphism were observed in both species for all populations (Appendix I). The mean expected heterozygosity (H_E) over the six microsatellite loci used for Atlantic salmon was higher [H_E = 0.8230, range (0.7781–0.8559)] than that for brook charr [H_E = 0.7088, range (0.6001–0.7798)]. Highly significant heterogeneity in allele frequencies was observed overall in both Atlantic salmon and brook charr ($P < 0.00003$ in both species). Each population was differentiated clearly from all others within both species, as all pairwise populations comparisons were significant in brook charr ($P_{\alpha=0.05} = 0.0014$) and all but one in Atlantic salmon ($P_{\alpha=0.05} = 0.0023$) following sequential Bonferroni corrections. However, brook charr populations were on average genetically more differentiated (F_{ST} = 0.1070, 95% CI = 0.0833–0.1300) than were Atlantic salmon populations (F_{ST} = 0.0445, 95% CI = 0.0310–0.0597), providing a first indication of a more restricted gene flow among brook charr than Atlantic salmon populations.

Isolation by distance

For Atlantic salmon, the Mantel test revealed no significant association between geographical and genetic distances (Fig. 2). Despite a more pronounced genetic differentiation on average, the Mantel test was also not significant in brook charr (Fig. 2). Furthermore, although the slopes of isolation by distance differed by an order of magnitude between both species (1.12×10^{-6} km^{-1} for Atlantic salmon vs. 1.34×10^{-5} for brook charr), the bootstrap procedure revealed that this difference was not statistically significant (P = 0.0847). Therefore, the classical tests of isolation by distance did not reject the null hypotheses that dispersal in salmon and brook charr followed an identical pattern in both species and occurred randomly with respect to geographical distance.

Table 2 Proportion of salmon sampled in a river that are classified in each of the rivers. Numbers in bold are the proportion of fish classified into their sampling river

Sampled in	Excluded from all rivers	Classified in									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
S1	0.11	**0.69**	0.13	0.01	0	0	0.08	0.01	0.05	0.01	0
S2	0.16	0.28	**0.44**	0.07	0.02	0.02	0	0.07	0.02	0.02	0.06
S3	0.06	0.08	0.04	**0.46**	0.08	0.06	0.14	0.04	0.02	0.04	0.04
S4	0.09	0.03	0.12	0.09	**0.37**	0	0.06	0	0.19	0.06	0.06
S5	0.08	0	0.02	0.10	0.05	**0.65**	0.10	0.02	0.05	0	0
S6	0.03	0.10	0	0.07	0.03	0.14	**0.38**	0	0.07	0.03	0.17
S7	0.14	0.05	0.05	0	0.09	0.09	0	**0.67**	0.05	0	0
S8	0.14	0.08	0.02	0.04	0.08	0.04	0.06	0	**0.58**	0.04	0.06
S9	0.04	0	0.02	0.02	0.06	0.02	0.04	0.02	0.02	**0.76**	0.04
S10	0.02	0.07	0	0.12	0	0.02	0.02	0.05	0.09	0.14	0.48

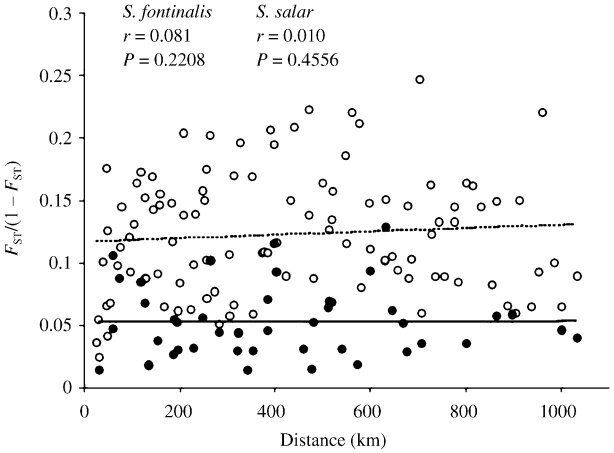

Fig. 2 Relationship between genetic differences $[F_{ST}/(1 - F_{ST})]$ and geographical distances in Atlantic salmon (black dots, solid line) and brook charr (open dots, dotted line) from Eastern Canada. *P*-values are the significances of the observed correlations as estimated by a Mantel test.

Individual assignment methods. A total of 395 salmon (91.33%, Table 2) and 591 brook charr (89.01%, Table 3) were within the 95% likelihood limits of at least one river of our sampling scheme for each species. Of those, 225 salmon (56.9%) were assigned to their sampling river and 170 (43.1%) were assigned into one of the nine other rivers ('misassignment rivers'). A greater proportion of brook charr (449 = 76.0%) were assigned to the river from which they were sampled, such that misassignment into one of the 14 other rivers in that species was observed in 142 fish (24.0%). Results of the assignment test thus further pointed toward higher gene flow in salmon than in brook charr.

In sharp contrast with the classical Mantel test, analysis of assignment distances revealed a strong pattern of restricted dispersal in both Atlantic salmon and brook charr. In both species, misassigned individuals were assigned significantly closer from their sampling river than expected if they had been assigned randomly to any river indiscriminately. The average assignment distance of 170 individual salmon randomly dispersing over the 10 sampled populations by simulations was 454 km [95%CI = (413–496)]. The observed distribution for the 170 misassigned salmon departed clearly from this simulated random distribution, as they were assigned at an average distance of 358 km from their sampling river ($P < 0.0001$). Accordingly, the frequency distribution of the 75-km wide distance classes (Fig. 3A) departed from its random expectation (*G*-test, d.f. = 11, $P = 0.0078$), and the Mantel test showed that misassignment significantly decreased with distance (Fig. 4A, Mantel's $r = -0.3266$, $P = 0.0134$). In brook charr, the 142 misassigned individuals were assigned at an average distance of 286 km from their sampling river, which also departed significantly from the random distribution generated by simulations [average distance 436 km, 95%CI = (383–486), $P < 0.0001$]. The distribution of dispersal distances (Fig. 3B) also departed significantly from its random expectation (*G*-test, d.f. = 12, $P = 1.31 \times 10^{-7}$), and misassignment significantly decreased with distance (Fig. 4B, Mantel test $r = -0.3179$, $P < 0.0001$). Interestingly, the variation pattern of D_{LR} distances did not parallel the observed decrease of misassignment with distance in both species, as no correlation was found between D_{LR} and geographical distances in salmon ($P = 0.5280$) and brook charr ($P = 0.0521$). Finally, the distribution of misassignment distances of the two species strongly departed from each other (Fig. 3A and B, *G*-test, d.f. = 10, $P = 6.12 \times 10^{-5}$). This showed that misassigned brook charr were found closer from their sampling rivers than misassigned salmon.

Table 3 Proportion of brook charr sampled in a river that are classified in each of the rivers. Numbers in bold are the proportion of fish classified into their sampling river

Sampled in	Excluded from all rivers	Classified in														
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
F1	0.08	**0.60**	0.16	0.04	0.06	0	0.02	0.02	0	0	0	0	0.04	0	0.02	0.04
F2	0.12	0.14	**0.58**	0.06	0.02	0.04	0	0.04	0	0.04	0.02	0	0	0	0.06	0
F3	0.07	0.09	0.01	**0.85**	0	0	0	0.01	0	0	0	0.01	0.01	0	0	0
F4	0.23	0	0	0.04	**0.73**	0.14	0	0	0	0.04	0	0	0	0.04	0	0
F5	0.14	0.04	0.02	0	0.04	**0.72**	0.10	0	0	0.02	0	0	0.02	0	0.04	0
F6	0.17	0	0.04	0	0.08	0.10	**0.69**	0.02	0.02	0	0	0.02	0	0.02	0	0
F7	0.04	0	0.04	0.02	0	0.04	0	**0.68**	0.06	0	0.02	0.04	0.08	0	0	0.02
F8	0.06	0	0	0	0	0.06	0.02	0	**0.82**	0.02	0	0	0	0.02	0.02	0.04
F9	0.16	0	0.02	0	0.10	0.06	0.02	0	0.04	**0.72**	0	0	0.02	0	0	0.02
F10	0	0	0	0	0	0	0	0.08	0.08	0	**0.85**	0	0	0	0	0
F11	0	0	0	0	0	0	0	0	0	0	0.02	**0.96**	0	0.02	0	0
F12	0.13	0.10	0	0	0.03	0.03	0	0.03	0	0	0	0.06	**0.64**	0.06	0.03	0
F13	0.15	0.02	0.02	0	0	0.02	0	0.02	0	0	0.02	0	0.04	**0.62**	0.19	0.04
F14	0.17	0	0.04	0	0	0.02	0	0	0.04	0	0	0	0.04	0.06	**0.74**	0.04
F15	0.15	0.02	0.05	0	0.02	0	0.05	0	0	0	0	0	0.05	0.02	0.05	0.73

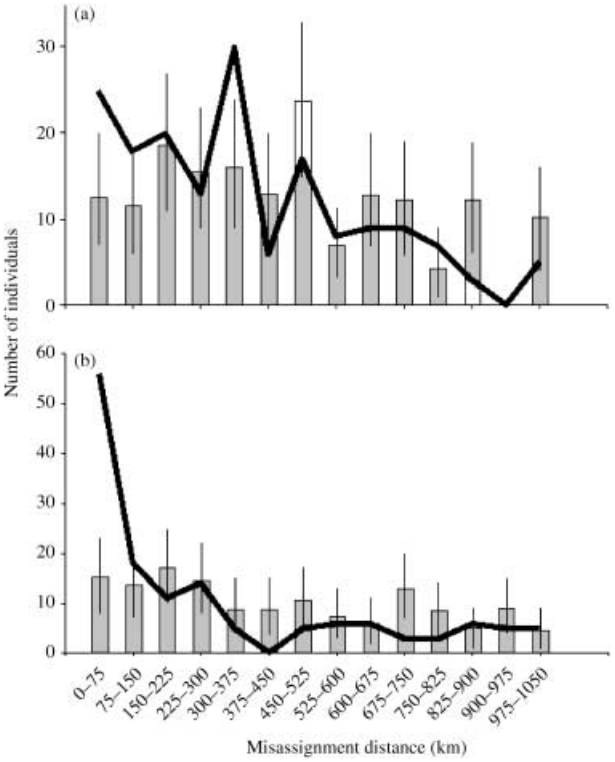

Fig. 3 Observed distribution of misassignment distances (heavy black line) compared with its expectation under random dispersal (grey bars) in (A) Atlantic salmon and (B) brook charr. The 95% CI for the expected distribution are obtained by simulating N_{ma} individuals randomly dispersing over any distance as observed in 10 000 iterations in the simulation procedure.

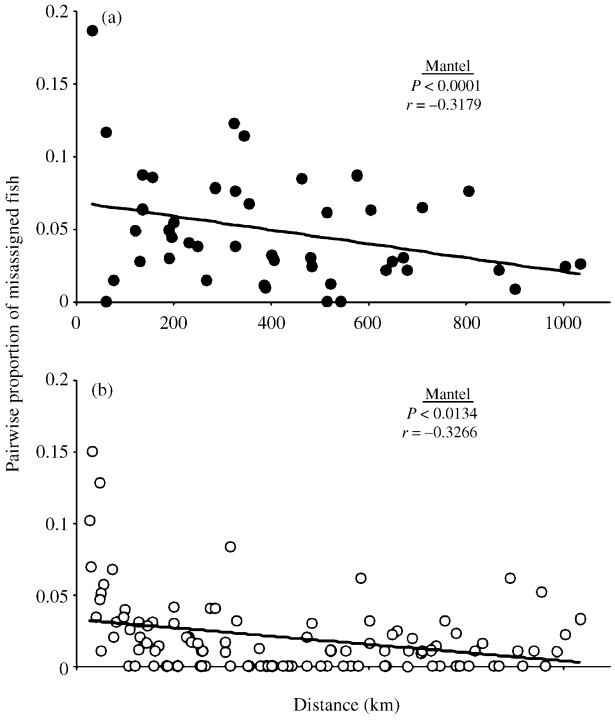

Fig. 4 Relationship between geographical distance (km) and pairwise proportion of misassigned fish in Atlantic salmon (A) and brook charr (B). Significance of the correlation was tested using a Mantel test (10 000 iterations).

Discussion

This study showed that dispersal in both Atlantic salmon and anadromous brook charr populations occurred preferentially into nearby rivers. Furthermore, as predicted from a priori knowledge on the dispersal behaviour of the two species, the methodology presented in this study also revealed that the two species had contrasting dispersal patterns among rivers. Because these signals remained undetected by the commonly used Mantel test to reveal an increase of genetic differences with distance, our results illustrate the promises of individual-based methods for detecting restricted dispersal by means of molecular markers. In addition, these results have important management consequences for the conservation and restoration of wild Atlantic salmon populations.

Limits of the regression-based method

Isolation by distance processes in natural populations have been investigated mainly through the increase of genetic differences they are expected to produce with distance. Several methods are available in the literature to test and quantify this increase, among which the regression-based method (Slatkin 1993; Rousset 1997) has been used extensively in empirical population genetic studies because it relies on a more explicit model than alternative methodologies (Sokal *et al*. 1989; Cassens *et al*. 2000; Epperson 2000; but see Hardy & Vekemans 1999 for the link with autocorrelation methods). However, the Mantel test we used to test for an increase of genetic differences with distance was unable to reject the null hypothesis that both salmon and brook charr can disperse randomly over the 1034 km of coast sampled. Although this could be plausible for Atlantic salmon, this is clearly at odds with basic knowledge on brook charr behaviour at sea (White 1941, 1942; Power 1980; Curry *et al*. 2002).

Two limits of the regression method may explain this lack of statistical power. First, the statistical power of the regression-based approach depends upon the number of populations analysed. For instance, Peterson & Denno (1998) showed in a review of allozyme variation in phytophagous insects that with fewer than 15 populations analysed, one might conclude erroneously that isolation by distance is lacking in a species in which gene flow does indeed decline with distance. Although the use of microsatellite loci in the present study may have increased statistical power (see Leblois *et al*. 2003), the number of populations included in this study (10 for salmon and 15 for brook charr) may have resulted in the reduced statistical power of the Mantel test to reveal an increase of genetic differences with distances. Accordingly, Castric & Bernatchez (2003) found a significant increase of $F_{ST}/(1 - F_{ST})$ with distance when using a more extensive sampling of

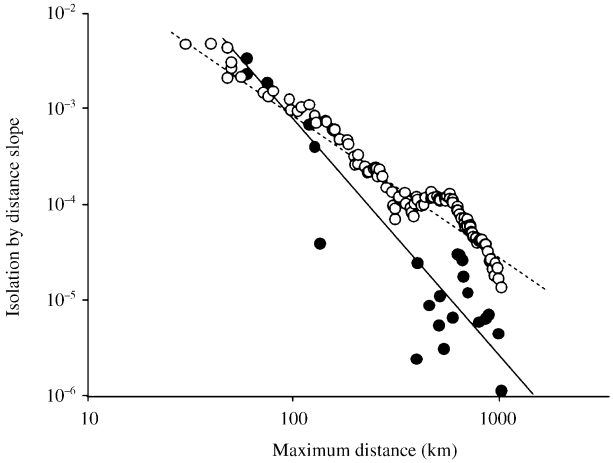

Fig. 5 Variation of the slope of the isolation by distance relationship in Atlantic salmon (black dots, solid line) and brook charr (open dots, dotted line) as a function of the geographical scale of observation. Note the steeper decline for Atlantic salmon than for brook charr.

59 brook charr populations, including the 15 samples from the present study. A second limitation may be that the slope of the isolation by distance relationship varied with the geographical scale at which it was observed in both Atlantic salmon and brook charr (Fig. 6). Thus, slopes computed from pairs of populations separated by the shortest distances only (e.g. 0.0033 km^{-1} for Atlantic salmon and 0.0021 km^{-1} for brook charr at distances < = 60 km) were over three orders of magnitude higher than those computed with all pairs of populations considered together (1.12×10^{-6} km^{-1} and 1.34×10^{-5}, respectively, at distances < = 1034 km Fig. 5). Expanding the geographical scale of investigation thus resulted in a dramatic decrease of the slope, ultimately reaching the very low slopes observed at the global scale. Scaling considerations have been a recurrent theme in empirical studies of isolation by distance (Hellberg 1995; Palumbi *et al*. 1997; Johnson & Black 1998; Ruckelshaus 1998; Pogson *et al*. 2001, and references therein). This phenomenon has received various explanations (reviewed in Castric & Bernatchez 2003) but has typically been considered as problematic when attempting to infer $N\sigma^2$ from isolation by distance patterns, such that Leblois *et al*. (2003) suggested that the slope estimation should be restricted to a given range of distances. Alternatively, Heuertz *et al*. (2003) have taken advantage of the change in the shape of the kinship-distance curve to infer the relative contribution of seeds and pollen to gene flow among individuals at the 'within population' geographical scale. In the present case, the slope of isolation by distance was similar for Atlantic salmon and brook charr when restricting slope estimation to distances < = 60 km (0.0033 vs. 0.0021, respectively, Fig. 5), but the decay was more rapid for salmon than brook charr (1000 bootstrap replicates over loci: $P < 0.001$), such that the slope estimated from all

distance classes was overall lower for salmon than brook charr. Altogether, the regression-based approach was unable to reveal an increase of genetic differences with distances because it inherently collapses all spatial scales together. This is problematic, since the slope of isolation by distance is not independent from the spatial scale of investigation.

Promises of the individual-based method. The scope of issues accessible to population genetic investigation has expanded considerably in the recent years by making use of individual multilocus genotypic information (Paetkau *et al*. 1995; Rannala & Mountain 1997; Cornuet *et al*. 1999; Pritchard *et al*. 2000; Wilson & Rannala 2003; Paetkau *et al*. 2004). Parentage analysis has been used recently by Telfer *et al*. (2003) to investigate the distribution of individual dispersal distances in water voles *Arvicola terrestris*. In many species, however, parentage assignment cannot be performed because populations are too large. Here, we presented a more general application of individual-based methods that had, to our knowledge, not been considered before and can be applied to any subdivided species. Although based on the same samples, the assignment method revealed a pattern of restricted dispersal that remained unapparent by the more classical regression-based method in both Atlantic salmon and brook charr. Two explanations can be put forward to account for the discrepancy between both approaches. First, the regression method is expected to be affected more by nonequilibrium conditions than the individual-based method. As shown theoretically by Slatkin (1993), genetic differences need time to build up after a set of populations undergoing isolation by distance was founded, and consequently genetic differences initially only increase weakly with geographical distance, resulting in low statistical power in recently founded populations. In contrast, dispersing individuals contribute instantaneously to increase the misassignment distance. The distribution of misassignment distances is thus skewed towards short distances as soon as isolation by distance occurs in a species. Consequently, the assignment method provides information on a shorter time scale, and is expected to be less affected by nonequilibrium conditions (Wilson & Rannala 2003). The brook charr populations included in the present study are the northernmost samples analysed in Castric & Bernatchez (2003), who reported that isolation by distance patterns were decreasing in intensity with increasing distance from the glacial refugium of the species located in the south of the current range. These populations are thus likely to be affected by nonequilibrium dynamics, which may explain why the two methods gave contrasting results.

Second, both methods are based on different kinds of information. On one hand, the regression method is based on pairwise estimates of $F_{ST}/(1 - F_{ST})$, summarizing information over several alleles, several loci and several individuals into a single estimate. On the other hand, the individual-based method uses each multilocus individual genotype as an independent unit of information and combines the effect of two distinct phenomena resulting from restricted dispersal: (1) the fact that dispersers move limited distances, and thus tend to be assigned at shorter distances than if they were moving randomly and (2) the fact that assignment errors are more likely to occur into geographically close populations because they are expected to be the most genetically similar populations under restricted dispersal. This second phenomenon is indeed the basis for the regression method, but is used here as a complementary asset to the assignment of migrant individuals. The issue of analytical power in detecting 'true' migrants from individuals misassigned 'by error' have been investigated only recently by simulations (Paetkau *et al*. 2004), but analytical procedures to achieve this have yet to be developed. Thus, because both processes result from the same underlying cause (restricted dispersal) and are acting in the same direction, we rather took advantage of the combined information contained in the distribution of misassignment distances. An investigation of the conditions under which both methods perform best will now be necessary, especially to assess whether scaling effects affect them equally. Obviously, the regression-based approach will remain the method of choice to infer demographic parameters from population genetics data. None the less, as illustrated in this study, alternative approaches now exist that will test more efficiently for restricted dispersal among natural populations.

Isolation by distance in Atlantic salmon

Restricted dispersal has important management consequences for the conservation and restoration of wild Atlantic salmon populations. The consideration of local adaptation is considered central in the design of management and conservation plans in salmonid species (Dodson *et al*. 1998). Whether populations can adapt to local conditions depends essentially on the relative rates of gene flow, selection and drift. If genes diffuse across a continuous habitat, then an allele can become established provided that it is favoured in a region larger than a critical distance set by the characteristic scale $1 = \frac{\sigma}{\sqrt{2s}}$ (Slatkin 1973; Nagylaki 1975), where s is the selective advantage of an allele. For a given intensity of selection (s), local adaptation is thus more likely to evolve when dispersal is infrequent and / or geographically restricted than under an island model of population structure. In Atlantic salmon, clear evidence for local adaptation is scarce. Perhaps the most compelling evidence stems from biochemical studies that evidenced an association between allele frequencies at the NADP-dependent malic enzyme-2 locus (mMEP-2*) and summer freshwater temperature at both a local (among sections

within a stream) and a regional scale (parallel north–south clines in North America and Europe, Verspoor & Jordan 1989). Furthermore, differences in growth between mMEP-2* homozygote genotypes have been demonstrated experimentally (Jordan & Youngson 1991), thus providing strong evidence that this cline has been shaped by natural selection. Cumulatively, such clines suggest that Atlantic salmon populations are adapted to their local environment, and our conclusion that dispersal is geographically restricted thus suggests that these patterns of adaptation have evolved clinally, following the latitudinal clines of environmental variables. Consequently, restoration of wild populations when the local stock has been extirpated should use fish from proximate rivers rather than from distant rivers. The same recommendations would apply to brook charr as well (Castric & Bernatchez 2003).

Conclusion

Geographically restricted dispersal in a species has important consequences for the evolution of a number of traits. As revealed for Atlantic salmon, restricted dispersal may be more frequent than generally thought, even in species with potential for long-distance genetic exchanges. One of the most astonishing examples may be the European eel, believed classically to form a single panmictic unit, but found recently to exhibit isolation by distance (Wirth & Bernatchez 2001). In such species, understanding the forces selecting for geographically restricted dispersal despite their potential for long-distance genetic exchanges is a stimulating issue. When the cost of dispersal increases with distance, selection should favour short-distance dispersal under stable and damped local dynamics (Murrell *et al*. 2002). Clinal local adaptation may cause the cost of dispersal to increase with geographical distance. We expect that more extensive research on species with a potential for long-range dispersal may shed more light on the selective factors determining dispersal distances.

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V.C. is interested in how genetic diversity evolves in natural populations of both plant and animal species. He is now a postdoc in University of Lille and investigates the molecular and population genetic consequences of natural selection acting on the self-incompatibility locus in Arabidopsis. This study was part of his PhD in L.B.'s team, whose major interests are in the understanding of patterns and processes of molecular and organismal evolution, as well as their significance to conservation.

Appendix I

Microsatellite diversity in Atlantic salmon sampled in rivers S1–S10 and brook charr sampled in rivers F1–F15 (see Table 1 for locations). H_E and H_O are expected and observed heterozygosity, respectively; f is Weir & Cockerham's F_{IS} estimate. P(HW) is the probability that f is null. 0 = P(HW) lower than 0.0002

		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Overall
SSA-171	H_E	0.9211	0.8848	0.9104	0.8724	0.9215	0.9080	0.9349	0.8981	0.9176	0.9179	0.9087
	H_O	0.9474	0.8182	0.6818	0.8966	0.8667	0.9091	0.7778	0.7250	0.8222	0.7826	0.8227
	f	−0.0290	0.0760	0.2530	−0.0280	0.0600	−0.0010	0.1720	0.1950	0.1050	0.1500	0.0953
	P(HW)	0.7096	0.0104	0.0009	0.7870	0.0200	0.4198	0.0166	0.0037	0.0333	0.0402	0
SSA-197	H_E	0.8855	0.8687	0.8974	0.8969	0.8693	0.8708	0.8048	0.8361	0.8402	0.8424	0.8612
	H_O	0.9474	0.8000	0.8125	0.9032	0.7750	0.9286	0.7778	0.7551	0.7400	0.7500	0.8190
	f	−0.0700	0.0800	0.0950	−0.0070	0.1100	−0.0680	0.0340	0.0980	0.1200	0.1110	0.0503
	P(HW)	0.9213	0.1351	0.0232	0.5013	0.1097	0.4168	0.3810	0.0020	0.1936	0.1355	0.0039
SSA-202	H_E	0.8380	0.8881	0.9054	0.8367	0.6870	0.8856	0.8943	0.8942	0.9194	0.8948	0.8644
	H_O	0.8421	0.8718	0.7556	0.6087	0.4615	0.6667	0.6190	0.5000	0.8000	0.7778	0.6903
	f	−0.0050	0.0190	0.1670	0.2770	0.3310	0.2510	0.3130	0.4440	0.1310	0.1320	0.2060
	P(HW)	0.6813	0	0.0014	0.0420	0.0562	0.0011	0.0125	0	0.0118	0.0043	0
SSA-85	H_E	0.6504	0.7547	0.8483	0.8414	0.9021	0.7669	0.7974	0.8731	0.7577	0.7479	0.7940
	H_O	0.6842	0.6667	0.8723	0.8065	0.8421	0.6786	0.6500	0.7600	0.7500	0.6410	0.7351
	f	−0.0520	0.1180	−0.0290	0.0420	0.0670	0.1170	0.1890	0.1310	0.0100	0.1450	0.0738
	P(HW)	0.7327	0.0033	0.6494	0.4358	0.0537	0.0201	0.0869	0.0209	0.4785	0.3394	0.0013
SSOSL-85	H_E	0.8696	0.8576	0.8322	0.8633	0.8632	0.8672	0.7897	0.8765	0.8279	0.8782	0.8525
	H_O	0.8947	0.7667	0.7500	0.6667	0.7632	0.8148	0.7500	0.6563	0.7381	1.0000	0.7801
	f	−0.0290	0.1080	0.1000	0.2310	0.1170	0.0620	0.0520	0.2540	0.1100	−0.1410	0.0864
	P(HW)	0.7874	0.0061	0.3639	0	0.0537	0.1539	0.3461	0.0018	0.1282	1	0
μ3	H_E	0.7482	0.7691	0.6604	0.6499	0.7349	0.7459	0.4476	0.7574	0.5067	0.5558	0.6576
	H_O	0.7895	0.6429	0.6600	0.6774	0.7353	0.6522	0.4375	0.7000	0.4800	0.6765	0.6451
	f	−0.0560	0.1660	0.0010	−0.0430	−0.0010	0.1280	0.0230	0.0760	0.0530	−0.2210	0.0126
	P(HW)	0.8308	0.0012	0.5803	0.5345	0.3921	0.0354	0.5649	0.3199	0.0936	0.9920	0.0347
Multilocus	H_E	0.8188	0.8372	0.8423	0.8267	0.8296	0.8407	0.7781	0.8559	0.7949	0.8061	0.8188
	H_O	0.8509	0.7610	0.7554	0.7598	0.7406	0.7750	0.6687	0.6827	0.7217	0.7713	0.7487
	f	−0.0500	0.1160	0.0912	0.0441	0.0689	0.0456	0.0916	0.1582	0.0820	0.0237	0.0671
	P(HW)	0.9830	0	0	0.0995	0.0175	0.1225	0.0410	0	0.0025	0.2280	0

Appendix I *Continued*

		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	Overall
SFO-12	H_E	0.7677	0.6992	0.6716	0.6597	0.4290	0.4895	0.3022	0.5236	0.3055	0.6522	0.5513	0.6098	0.6397	0.7827	0.7716	0.5904
	H_O	0.7200	0.6739	0.5692	0.6667	0.4286	0.4792	0.2000	0.3659	0.2200	0.8333	0.3542	0.6000	0.5532	0.7727	0.7179	0.5437
	f	0.0630	0.0370	0.1530	−0.0110	0.0010	0.0210	0.3410	0.3040	0.2820	−0.2940	0.3600	0.0160	0.1360	0.0130	0.0700	0.0995
	P(HW)	0.2583	0.4502	0.0474	0.6579	0.7170	0.2613	0.0003	0.0001	0.0014	0.9877	0	0.0118	0.1057	0.5616	0.1892	0
SFO-18	H_E	0.5234	0.7740	0.3797	0.5833	0.7201	0.7273	0.1861	0.6171	0.6834	0.7354	0.5439	0.3152	0.5901	0.4831	0.7879	0.5767
	H_O	0.5000	0.7391	0.3019	0.5500	0.6977	0.6000	0.1087	0.6250	0.6122	0.8462	0.5208	0.3226	0.4375	0.4222	0.6923	0.5317
	f	0.0450	0.0460	0.2060	0.0590	0.0320	0.1770	0.4190	−0.0130	0.1050	−0.1580	0.0430	−0.0240	0.2610	0.1270	0.1230	0.0965
	P(HW)	0.4395	0.4304	0.0020	0.3324	0.5638	0.0012	0.0004	0.5345	0.0558	0.9437	0.4534	0.6588	0.0180	0.0887	0.0089	0
SFO-23	H_E	0.8471	0.9080	0.7481	0.8872	0.9119	0.9001	0.8534	0.8664	0.8870	0.7385	0.7495	0.8571	0.8579	0.8955	0.8422	0.8500
	H_O	0.8200	0.7955	0.6552	0.8000	0.8667	0.8205	0.8571	0.7778	0.8837	0.5385	0.7727	0.7143	0.7447	0.9318	0.8049	0.7856
	f	0.0320	0.1250	0.1250	0.1010	0.0500	0.0890	−0.0040	0.1030	0.0040	0.2790	−0.0310	0.1690	0.1330	−0.0410	0.0450	0.0786
	P(HW)	0.0077	0	0.0509	0.0415	0	0	0.4026	0	0.3647	0.1423	0.3822	0.1048	0.1481	0.7014	0.2975	0
SFO-8	H_E	0.9270	0.8898	0.8561	0.8002	0.9215	0.8813	0.8588	0.9203	0.8966	0.8551	0.8274	0.8768	0.9402	0.9073	0.9367	0.8863
	H_O	0.9167	0.6939	0.7460	0.6667	0.8750	0.5435	0.6957	0.8889	0.7234	1.0000	0.7500	0.5667	0.8750	0.8095	0.7308	0.7655
	f	0.0110	0.2220	0.1290	0.1700	0.0510	0.3860	0.1920	0.0350	0.1950	−0.1790	0.0940	0.3580	0.0700	0.1090	0.2230	0.1377
	P(HW)	0.3595	0.0134	0.0328	0.0110	0	0	0.0059	0.2841	0	1	0.1294	0	0.0325	0	0.0019	0
SSA-197	H_E	0.5497	0.5773	0.6823	0.7705	0.7291	0.7465	0.5822	0.6804	0.6943	0.5815	0.3013	0.5019	0.6072	0.4948	0.2200	0.5813
	H_O	0.6000	0.4082	0.6034	0.7000	0.7209	0.5870	0.4783	0.4750	0.7143	0.6923	0.3125	0.4839	0.6458	0.4762	0.1579	0.5370
	f	−0.0930	0.2950	0.1160	0.0940	0.0110	0.2160	0.1800	0.3050	−0.0290	−0.2000	−0.0380	0.0360	−0.0640	0.0380	0.2850	0.0768
	P(HW)	0.7013	0.0615	0.1290	0	0.4076	0.0052	0.0386	0.0012	0.7141	1	0.7261	0.5047	0.2001	0.5114	0.0559	0
Mst-85	H_E	0.7840	0.8304	0.7843	0.7794	0.8531	0.8753	0.8181	0.7493	0.8197	0.3007	0.7408	0.8119	0.7673	0.8114	0.8042	0.7687
	H_O	0.7200	0.5510	0.7576	0.7778	0.8200	0.7609	0.7442	0.7447	0.6977	0.0833	0.7917	0.6897	0.5833	0.8500	0.6765	0.6832
	f	0.0820	0.3390	0.0340	0.0020	0.0390	0.1320	0.0910	0.0060	0.1500	0.7320	−0.0690	0.1530	0.2420	−0.0480	0.1610	0.1364
	P(HW)	0.0080	0.0000	0.3934	0.6336	0.0092	0.0281	0.0073	0.2208	0.0139	0.0062	0.7912	0.1529	0.0072	0.7052	0.0014	0
Multilocus	H_E	0.7331	0.7798	0.6870	0.7467	0.7608	0.7700	0.6001	0.7262	0.7144	0.6439	0.6190	0.6621	0.7337	0.7291	0.7271	0.7089
	H_O	0.7128	0.6436	0.6056	0.6935	0.7348	0.6318	0.5140	0.6462	0.6419	0.6656	0.5836	0.5628	0.6399	0.7104	0.6300	0.6411
	f	0.0309	0.1873	0.1136	0.0884	0.0270	0.1995	0.1148	0.0927	0.0578	0.0024	−0.0119	0.1597	0.1369	0.0073	0.1225	0.0886
	P(HW)	0.0075	0	0	0.0009	0	0	0	0	0	0.6866	0.0672	0	0	0.0850	0	0

