Population genetics of plaice (pleuronectes platessa L.) in Northern Europe

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Chapter 4

POPULATION STRUCTURE OF PLAICE (*Pleuronectes platessa* L.) IN NORTHERN EUROPE: A COMPARISON OF RESOLVING POWER BETWEEN MICROSATELLITES AND MITOCHONDRIAL DNA DATA

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Abstract
We used Single Strand Conformation Polymorphism (SSCP) of mtDNA control region to assess the population structure of the flatfish *Pleuronectes platessa* (plaice), to compare these data with a previous study based on microsatellite loci, and to test for possible sex-biased dispersal. From 461 individuals, 163 haplotypes were identified across 11 locations. Diversity was higher with mtDNA ($h = 0.776$ to 0.981; $\pi = 0.0178$ to 0.0298) as compared to microsatellite loci using the same samples ($H_e = 0.721$ to 0.77). Genetic diversity was lower in samples from Iceland and Faroe, as compared to the continental shelf samples. Although both classes of markers revealed a relatively strong differentiation between shelf and off-shelf populations ($\theta = 0.1015$ and $\theta = 0.0351$, respectively), only the mtDNA data was able to detect differentiation within the continental shelf, i.e., a North Sea-Irish Sea group which was weakly distinguishable from Norway ($\theta = 0.0046$), the Baltic ($\theta = 0.0136$) and the Bay of Biscay ($\theta = 0.0162$). No evidence was obtained for isolation by distance, nor for sex-biased dispersal. This study demonstrates the importance of using more than one class of markers, especially for species like plaice, with large populations, high dispersal and recent colonization histories.
Introduction

The European plaice (*Pleuronectes platessa* L.) is an important flatfish for Northern Europe fisheries. It is widely distributed in shallow European waters (<100m) from the Western Mediterranean to Iceland and the White Sea (Wimpenny 1953). Reproduction takes place offshore during winter and the eggs/larvae are pelagic for about three to four months (Harding *et al.* 1978) after which they settle in shallow coastal waters where they metamorphose (Zijlstra 1972). Tagging data have shown that adults exhibit seasonal migration patterns from spawning grounds to feeding grounds (de Veen 1978) and that regional scale population structure of plaice appears to be mainly shaped by oceanographic features such as deep water, the occurrence of retention areas and suitable nursery grounds (Rijnsdorp and Grift, in press). This has suggested the possibility of philopatry which could lead to strong population differentiation despite a high dispersal potential via the long pelagic phase of eggs or migration of adults.

A recent population genetic study of plaice based on six microsatellite loci revealed high genetic homogeneity among continental shelf populations from Norway to southern Brittany (Hoarau *et al.* 2002a) regardless of whether individuals were collected from feeding, spawning or nursery grounds. Only the samples from Iceland and Faroe, that are separated from the continental shelf by deep ocean channels, showed significant population differentiation. These results were unexpected and suggested significant mixing among spawning grounds on the continental shelf.

Microsatellite loci are considered to be the marker of choice for many population genetic studies, mainly because of their high level of polymorphism (Jarne and Lagoda 1996) and ability to detect subtle population differentiation (*e.g.* Shaw *et al.* 1999), although in a few cases they have been found to produce similar (*e.g.* Buonaccorsi *et al.* 2001) or even lower resolution than mitochondrial DNA (mtDNA) (*e.g.* Bérubé *et al.* 1998). Direct comparison between microsatellite and mtDNA loci can be very informative, as evolutionary forces will differentially affect each class of marker. In animals, nuclear DNA is usually diploid and biparentally inherited whereas mtDNA is haploid and, with a few exceptions (*e.g.* Gyllensten *et al.* 1991), maternally inherited. Because of these differences in ploidy and inheritance, the mitochondrial effective population size (*N*<sub>e(mt)</sub>) is four times smaller than for nuclear
Plaice population structure: msats vs. mtDNA

loci (Birky et al. 1989), and thus more susceptible to the effects of genetic drift. Consequently, estimates of population differentiation are expected to be higher for mitochondrial markers (see discussion in Crochet 2000, but also Birky et al. 1989 for possible exceptions). This is of interest for populations with presumptively large effective population sizes \( (N_e) \) (such as plaice) where drift might be too small to be detected with nuclear markers. Another advantage of mtDNA is that it will reach migration-drift equilibrium sooner than nuclear DNA (Birky et al. 1989). Migration-drift equilibrium is the equilibrium between migration, which homogenises populations, and genetic drift which increases inter-population differentiation. Attainment of this equilibrium is relevant for species that are known to be part of the recent recolonization of the North Atlantic following the last glacial maximum (last 18-10,000 years). The use of mtDNA will, therefore, increase the chance of detecting population differentiation.

The possibility of sex-biased dispersal has been raised for plaice as a result of the recent finding of inbreeding (Hoarau et al., in prep). This is an important issue because inbreeding is generally not considered in species with very large populations. Even where the effective population size has been shown to be several orders of magnitude smaller than the census size in marine fish (Hauser et al. 2002, Turner et al. 2002), the number of individuals is still very large. The presence/absence of inbreeding avoidance mechanisms in plaice, therefore, need to studied and sex-biased dispersal provides a potential mechanism for inbreeding avoidance (Pusey and Wolf 1996). The availability of comparative data from mitochondrial and nuclear genomes makes it potentially possible to test for sex-biased dispersal. This approach has been successfully used in several species, mainly mammals and birds (Pusey 1987). Very few studies have looked at sex-biased dispersal in fish. No sex-biased dispersal was found in blue marlin (Buonaccorsi et al. 2001) whereas male-biased dispersal has been shown in brook trout (Hutchings and Gerber 2002) and white sharks (Pardini et al. 2001). In both of these cases, males dispersed more frequently and over longer distances.

The objective of the present study was therefore to: 1) re-examine the genetic structure of plaice based on the mtDNA control region; 2) compare these results with a previous analysis of the same individuals based on six microsatellite loci; and 3) test for sex-biased dispersal.
Material and Methods

Sampling

We used the same 480 individuals as Hoarau et al. (2002a). The samples consisted of 240 adults and 240 offspring of the year (0-group), collected from 11 locations (Fig.1).

**Fig. 1.** Sampling locations and sample sizes of *Pleuronectes platessa*. The grey line delimits the 200-m contour of the continental shelf. Adults samples (▲): LO Lofoten, Norway (N=24); FA Faroe’s Plateau (26); TR Trondheim fjord, Norway (48); IS Irish Sea, England (44); TB Terschelling Bank, Netherlands (49); FE Femer Bælt, Denmark (49). 0-group samples (●): ic Alftanes, Iceland (48); ob Oban, Scotland (48); ba Balgzand, Netherlands (48); am Amrum, Germany (48); bv Bay of Vilaine, France (48). Continental shelf samples: all except Iceland and Faroe. (From Hoarau et al. 2002a)
DNA extraction and PCR-SSCP

DNA was extracted according to Hoarau et al. (2002a). A ≈150 bp fragment of the mtDNA control region was amplified by PCR using the primers: DLF 5’-CCA CCT CTA ACT CCC AAA GC-3’ (5’ fluorescently labelled with 6-FAM) and DLR (5’-TGA AGG GAT TTT GAG TCT TGG -3’) (5’ fluorescently labelled with NED). All PCR amplifications were carried out in 10-µl volumes containing 1 µl of 1/10 diluted DNA, 1X reaction buffer (Promega), 0.2 mM of each dNTP, 2 mM MgCl₂, 0.30 U Taq DNA polymerase (Promega) and 0.1 µM of each primer. PCR was performed in either a PTC100 (MJ Research) or a MasterCycler gradient cycler (Eppendorf). The reaction profile was: 90 °C for 1 m; 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 m; and 72°C for 10 m.

Single strand conformation polymorphism (SSCP) (Orita et al. 1989, Sunnucks et al. 2000) was used to detect sequence variation in the mtDNA control region. Mutations that affect the conformation of single strand DNA are revealed by migration in non-denaturing polyacrylamide gels. This technique is very sensitive and provides a quick and relative inexpensive way to screen a large number of samples for sequence variation. SSCP gels were run on an ABI Prism-377 automatic sequencer (Applied Biosystems) as described in Coyer et al. (2002), except that 5% glycerol was added to the gels. The use of an automatic sequencer together with different labelling of the primers allows quicker and more accurate scoring of the haplotypes.

Sequence

All unique haplotypes detected by SSCP were subsequently sequenced and frequent haplotypes were sequenced for at least two individuals each. PCR products were cleaned using Sigmaspin (Sigma) purification columns. Both strands were cycle-sequenced using the dGTP Big Dye Terminator Kit (Applied Biosystems) and run on an ABI Prism-377 automatic sequencer (Applied Biosystems). Sequences were edited and aligned manually using BIOEDIT v.5.0.9 (Hall 1999).

Microsatellites

All microsatellite data are from Hoarau et al. (2002a). Six microsatellite loci were used: PL09, PL92, PL115, PL142, LIST1001 and LIST1003.
Data analyses

Statistical parsimony was used to analyze the intraspecific phylogeny of mtDNA haplotypes using the software TCS v.1.13 (Clement et al. 2000). Haplotype (h, Nei 1987) and nucleotide (π, Nei 1987) diversities were estimated from the mtDNA data using DNASP v.3.53 (Rozas and Rozas 1999); gene diversity (He, expected multilocus heterozygosity, Nei 1987) was estimated for the six microsatellites loci using GENETIX v.4.03 (Belkhir et al. 2002).

Population differentiation was analyzed using Wright’s $F_{ST}$ (Wright 1969); global $F_{ST}$ and pairwise $F_{ST}$ were estimated using Weir and Cockerham’s $\theta$ (Weir and Cockerham 1984). Significance of all $\theta$ estimates was tested with GENETIX using 2000 permutations. Sequential Bonferroni corrections (Rice 1989) for multiple comparisons were applied where necessary.

Isolation by distance was examined by plotting the pairwise $\theta(1-\theta)$ estimates against the Log of the geographic distances (Rousset 1997) and tested using the Mantel test (Mantel 1967) with 2000 permutations as implemented in GENETIX.

Sex-biased dispersal was tested by comparing $\theta_{\text{mitochondrial}}$ ($\theta_{\text{mito}}$) with $\theta_{\text{microsatellites}}$ ($\theta_{\text{msat}}$). The null hypothesis of no sex-biased dispersal is based on the equation, $\theta_{\text{mito}} = 4 \frac{\theta_{\text{msat}}}{1 + 3 \theta_{\text{msat}}}$ (Crochet 2000) which assumes equilibrium conditions, random mating, a 1:1 sex ratio, a Poisson-distributed reproductive success, and an infinite-island model. Departures from this theoretical relationship, therefore, indicate sex-biased dispersal; male biased if the data points are above this plot and female biased if they are below. The correlation between the two pairwise $\theta$ matrices was tested using the Mantel test in GENETIX with 2000 permutations. The software PRISM v.3.02 (GraphPad Software, www.graphpad.com) was used to fit the theoretical curve to the data and to compute the non-linear $R^2$ (analogue to the correlation coefficient in linear regression) as well as the distribution of the residuals from the regression.

Results

Genetic diversity

The mtDNA diversity was extremely high, with 167 mtDNA haplotypes recovered (GenBank accession number AY442522-AY442688) from 461...
individuals. Of these, 104/167 (62%) were unique. The most common haplotype was present in 10% of the samples. An attempt to establish a haplotype network was unsuccessful owing to the number of reticulations in which no clear-cut pattern could be established (Fig 2).

Fig. 2. Statistical parsimony network of *Pleuronectes platessa* mtDNA haplotypes

Genetic diversity was lower in the Icelandic population, as compared to the continental populations (Table 1). Comparison of the two approaches, showed that mtDNA diversity was higher than microsatellite diversity (Table 1)

*Population differentiation*

Significant population differentiation was detected in both data sets between continental shelf populations and those from Iceland and the Faeroe Plateau area. MtDNA, however, provided up to 10-fold higher resolution among continental-shelf populations. In the centre of the European continental shelf, a large undifferentiated group was found (North Sea and Irish Sea). This North Sea-Irish Sea group showed significant differentiation from Norway, the Baltic and the Bay of Biscay. As for microsatellite data (Hoarau *et al.* 2002a), no pattern of isolation by distance was detected among continental-shelf locations based on the mitochondrial data (*Z*=5.975, *P*=0.10). No isolation by distance could be demonstrated either between on- and off-shelf populations, or among the on-shelf populations.
Table 1. Genetic diversity and population differentiation in *Pleuronectes platessa*. \( N = \) sample size; mitochondrial DNA (mtDNA) data: \( N_h = \) number of haplotype, \( h = \) haplotype diversity, \( \pi = \) nucleotide diversity; microsatellite (msat) data: \( N_A = \) number of allele \( H_e = \) expected heterozygosity. ns = not significant, * = \( P<0.05 \), ** = \( P<0.01 \), *** = \( P<0.001 \)

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Sex-biased dispersal

A strong correlation was found for the population differentiation (\( \theta \)) matrix (\( Z=0.1, P<0.01 \)) between the mitochondrial and microsatellite estimates. Fitting the theoretical curve of no sex-biased dispersal to the observed data gave a non-linear correlation coefficient \( R^2 = 0.7977 \) (Fig. 3) and the normal distribution of the residuals from the regression was not rejected (Normality Test, \( P>0.1 \)). Therefore, our data did not significantly deviate from the expected curve and suggests that there
is no sex-biased dispersal in plaice. Separate analysis of the “low” and “high” differentiation clouds (Fig. 3) showed similar results, i.e., no deviation from the null hypothesis of “no sex-biased dispersal”. Despite this clear-cut result, violations of the underlying assumptions of the model lead us to accept this result with caution (see Discussion).

**Discussion**

Significant population substructure was detected on the continental shelf using the mtDNA data which was not the case with the microsatellite data. The mtDNA results are more consistent with ecological and fisheries observations which include differences in demographic parameters such as fecundity, maturation, and growth (Rijnsdorp 1989, 1991). The inability to detect seemingly distinct stocks with microsatellite data is, in this case, most likely related to the extremely shallow population history in the Northern North Atlantic. Plaice have only repopulated the North Atlantic and the North Sea within approximately the past 10,000 years. MtDNA, with its smaller effective population size, faster genetic drift and haploid maternal inheritance can actually provide a better view through this extremely short timeframe.

The mtDNA diversity in plaice is high but comparable to what has been found in other flatfish (Jones and Quattro 1999). The higher diversity in mtDNA as compared to microsatellites observed in the present study, is most likely to be related to a
larger female effective population size. A larger female effective population size may be expected because males show both a higher natural mortality as well as a higher fishing mortality rate (Beverton 1964, Rijnsdorp 1993, Solmundsson et al. 2003), which may lead to the development of a skew in the sex ratio (Rijnsdorp 1994). Furthermore, variance in female reproductive success is expected to be lower than in males because even a few males can fertilise many females. Finally, longer female life span with overlapping generations acts as a buffer against fluctuating population size (Gaggiotti and Vetter 1999), thus also increasing \( N_e(f) \). Taken together, these factors will increase \( N_e(f) \) relative to \( N_e(m) \) which is what is being detected with the mtDNA data.

The lower diversities associated with the Icelandic population are qualitatively similar to those observed with the microsatellite data (only the number of alleles differed). There are two potential explanations for this, which are not mutually exclusive. First, the lower diversity of the Icelandic population may be due to a lower overall population size as well as a lower effective population size. The census population size of Icelandic plaice is estimated to be an order of magnitude smaller than that found in the North Sea (ICES-www.ices.dk). Second, the geographic position of Iceland at the edge of the distributional range of plaice (Nielssen 1986) may also account for the lower diversity, as lower genetic diversity is typical of edge populations in general (Hewitt 2000, Comps et al. 2001, Coyer et al. 2003).

The reticulations in the haplotype network (Fig. 2) are due to homoplasy, which is the result of multiple substitutions at a given site. Such a large amount of homoplasy can only be explained by the very high mutation rate in this region and/or to recombination. High mutation rates are a natural feature of the mtDNA control region. To date, recombination has only been shown in flounder (Hoarau et al. 2002b). The structure of the mtDNA control region in flatfish seems to promote heteroplasy and recombination (Hoarau G. unpublished data). At present, these observations need further investigation. Such a process has implications for genealogical comparisons but not for \( F_{ST} \)-based studies which rely on unordered characters.

Although population structure is governed by both demographic and genetic processes, oceanographic features are also crucial. Deep water is a major barrier as shown by: 1) the relatively strong differentiation between shelf and off-shelf
populations in both mtDNA and microsatellite data sets; and 2) between the North Sea and Norway which are separated by the tongue of the Norwegian Deep. Currents (and salinity fluctuations) also play a role in separating Baltic and North Sea populations in the Skagerrak-Kattegat region. A similar restriction of gene flow has been observed in cod (Nielsen et al. 2003). With respect to the Bay of Vilaine (Fr), it seems most likely that these populations represent the northern range of a more southern stock within the Bay of Biscay. There was no significant relationship detected between population differentiation ($\theta$) and geographic distance regardless of inclusion or exclusion of the off-shelf populations. This is again consistent with recent population history combined with high dispersal and migration of adult populations.

The ability to demonstrate sex-biased dispersal depends upon how well the underlying assumptions of the model are met. In the present case at least two (and possibly three) of these underlying assumption are not met. First equilibrium conditions almost certainly do not exist given the short history of the North Atlantic over the past 10,000 years; and second, there is at least some evidence for a sex skew in plaice. Finally, the apparent absence of sex-biased dispersal in plaice is further supported by independent tagging data (L Bolle unpublished data) in which no difference in male/female movement was detected. If our analysis is correct, then the absence this inbreeding avoidance mechanism is an important issue in our understanding of inbreeding in plaice.

Conclusion

The present study demonstrates the value mtDNA as an addition to nuclear microsatellite loci in detecting genetic stock structure and gene flow in a species with a large population size, high dispersal capacity and shallow population history. The pattern of genetic differentiation depicted by the combined nuclear-mtDNA data sets is in good agreement with other biological and tagging data.
Chapter 4

Acknowledgments

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References


