Conservation aspects of natural populations and captive-bred stocks of turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) using estimates of genetic diversity

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Population genetic analyses have been highly successful in predicting inter- and intraspecific evolutionary relationships, levels of gene flow, genetic divergence, and effective population sizes. Parameters estimated are evolutionary averages and are therefore relevant for addressing contemporary ecological or conservation issues. Changes in genetic variation within the range of a species may indicate patterns of population structure resulting from past ecological and demographic events that are otherwise difficult to infer, so may provide an insight into evolutionary development. Genetic data, drawn from 14 enzyme loci amplified from two populations of turbot (*Scophthalmus maximus*) and five populations of Dover sole (*Solea solea*) from the Irish Sea were used to examine population structure estimated from measures of genetic diversity. The aim was to provide an empirical assessment of whether artificial propagation poses a genetic threat to conservation of naturally spawning populations, and whether the fitness for natural spawning and rearing can be rapidly and substantially reduced or increased by artificial propagation. Because of prolonged overfishing, turbot and sole populations in the region are below natural levels, and survive in small local populations in fragmented habitats. Genetic data derived from allozymes have shown that populations are characterized by relatively low levels of genetic diversity. A hypothetical model supporting genetic population substructure, such as range expansion with founder-flush effects, and subsequent population decline with small effective population sizes was considered. Observations support our belief that conservation measures based on genetic diversity have to be developed to ensure the survival of this diverse gene pool.

Keywords: conservation genetics, flatfish, genetic diversity, hatchery stocks, natural populations.

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Introduction

Conservation and recovery strategies for threatened or endangered aquatic species are usually developed with demographic and/or habitat-based ecological data. Such data typically derive from surveys of a few populations over a short period, but concordance of results drawn from demographic and ecological data is not guaranteed. Extinction risk models used to analyse population trends at scales from local to global consider three types of stochastic change, environmental, demographic, and catastrophic (Mace and Lande, 1991; Boyce, 1992; Caughley, 1994; Taylor, 1995), but neglect potential information derived from genetic effects (Dunham *et al.*, 1999). Environmental or catastrophic changes influence every individual in a population and can have large negative results on genetic population structure. Demographic change, on the other hand, influences each individual differently and can result in multiple remissions of fitness among individuals within a population (Lande, 1988; Taylor and Dizon, 1996; Gibbs, 2001).

Population decline, semi-extinction, and low overall abundance may result from any of the above effects, but genetic structure within and between survivors will differ (Ehrlic, 1988; Gilpin, 1991; Witlock and Barton, 1997; Montgomery *et al.*, 2000). A catastrophic loss of most individuals from a population in a given area can lead to recolonization by neighbouring populations with similar ecological and reproductive requirements. Colonizers, however, can quickly alter most of the original gene pool (McCauley, 1991; Avise, 1994; Gibbs, 2001). Expansion of the range of a species range through environmental shifts in habitat conditions or invasions following catastrophic change can retain significant genetic structure with sufficient gene flow, and, theoretically, lead to unique patterns of increased genetic diversity attributable to founder events (Slatkin, 1996). In contrast, altered environmental conditions that accompany demographic expansion may shift fitness and survival patterns within a population and can have a variety of effects on the gene pool (Slatkin, 1996). Changes in effective population size (the number of

individuals actually contributing genes to the next generation), population bottlenecks, age structure, and movement (i.e. gene flow) between surviving groups must also be considered (Avise, 1994; Allendorf and Waples, 1996).

Under all the conditions above, the degree of genetic variation found within and between existing populations provides information on past patterns of demographic and ecological events in the history of each population (Weir, 1996). Patterns of genetic variation have a strong historical component (Slatkin, 1985, 1993), and trends in isolation, gene flow, and evolutionary history derived from these may provide insights into historical population dynamics (Avise, 1994). These changes are especially informative in fragmented populations, or those found near the edge of a species' range (Fahrig and Merriam, 1994). The suite of events reflected in the gene pool can be used in analyses of population viability or extinction risk (Lande, 1995; Hedrick *et al.*, 1996; Allendorf *et al.*, 1997). Therefore, genetic data can serve as indicators of conservation and recovery requirements that are otherwise difficult to infer.

Genetic diversity can be measured on multiple scales. Evolutionary divergence is a dynamic process, with markers acting across many different temporal and spatial patterns (Avise, 1994; Nielsen *et al.*, 1997a, b). Changes in the bimolecular markers used in population studies are generally thought to be selectively neutral throughout their evolutionary range and to have no direct effect on individual fitness (Kimura, 1983). Change is assumed to result from stochastic events involving differences in genetic drift and/or gene flow between populations (Nei, 1987). Neutrality is, therefore, the most ungenerous explanation for the patterns of genetic variation found in fish population studies (Kreitman, 1996).

DNA mutation rates have long been known to vary extensively among genes and across taxa (Nei, 1987; Weber and Wong, 1993; Wilson *et al.*, 1997). With highly variable mutation rates and no evidence of selection, the three most common molecular systems used to estimate genetic diversity—allozymes, mitochondrial DNA (mtDNA), and microsatellite repeat loci—can present different temporal or spatial scales in any population (Boyce *et al.*, 1996; Nielsen *et al.*, 1997b). Allozyme loci reflect protein-coding genetic structure and are thought to have the slowest mutation rate of the three types of marker; they remain the molecular markers of first choice for fisheries-related population studies and stock identification (Ward and Grewe, 1995; Thorpe *et al.*, 2000; Exadactylos *et al.*, 2003).

According to the ecological "dispersal" model, populations near the edge of a species' range are thought to arise as a consequence of colonization from the centre of the range by individuals that have adapted to new environmental conditions through active or passive dispersal across a pre-existing geographic or ecological barrier (Avise, 1994). The objectives of this study were to examine allozyme genetic diversity over 14 loci in natural populations and in hatchery stocks of two commercially important marine flatfish, turbot (*Scophthalmus maximus*) and Dover sole (*Solea solea*) in the Irish Sea. Genetic diversity analyses were then used to examine population structure and to attempt to gain an insight into past evolutionary history through consideration of different genetic dispersal models.

Management implications

Dover sole provide a commercially important demersal fishery in many areas of northern Europe and in the Mediterranean Sea (Gibson and Robb, 1996), but stock levels and mean size composition have declined markedly in recent decades, suggesting a significant effect of commercial overexploitation (Rice and Gislason, 1996; Imsland *et al.*, 2004; http://www.fao.org/figis/servlet/species?fid=3367). Dover sole are in greatest abundance in the shallower areas of the North Sea, English Channel, and Irish Sea, but are infrequent in or absent from deeper parts. Although some constraints to full development still exist, the species remains a potential candidate for aquaculture (Howell, 1997; Flos *et al.*, 1998; Dinis *et al.*, 1999). There does not appear to have been any attempt to enhance wild populations by releasing captive-bred material in any of these areas; indeed to date their artificial propagation has had very limited success (Howell, 1997).

Turbot are also commercially important and are found throughout the eastern North Atlantic and in the Mediterranean Sea, but are most abundant in coastal waters and rare or absent from offshore areas (Rogers *et al.*, 1998). Aquaculture production of market-sized turbot across Europe rose from 2 000 t in 1995 to >12 000 t in 2004 (Josepuit, 1996). Wild catches of turbot have also declined markedly over the past 10–20 years or so (FAO, 1994; http://www.mfa.gov.uk/pdf/UKSeaFish2005.pdf). To balance population depletion, experimental releases of cultured fry have been carried out in Spain (Iglesias and Rodriguez-Ojea, 1994), Denmark (Nicolajsen, 1993), and Norway (Bergstad and Folkvord, 1998). As a consequence of such activities and the rapid increase in production of hatchery-reared turbot, there is need to understand the genetic composition of natural turbot populations in order to evaluate the potential genetic effects induced by hatchery operations.

Genetic variation within and between populations is thought to be essential to ensuring the evolutionary adaptability of species in the long term, and to maintaining individual fitness in the short term (Hedrick and Miller, 1992; Thorpe and Smartt, 1995). As noted by Ryman (1981), Allendorf *et al.* (1987), and Oldfield (1989), the need to preserve natural populations as reservoirs of genetic variation within fish species is becoming increasingly apparent. This need is particularly prevalent where natural populations have suffered serious declines from multiple sources. The pristine habitats required have been drastically eroded in many areas over the past two centuries by the effects of industrialization and growing human populations. Migratory passages have been lost through blockages and water diversions, and escalating market value has resulted commonly in overfishing.

Introductions of non-native fish have also played a major role in the decline and loss of indigenous populations. Such introductions have often been accompanied by the spread of disease into new habitats, sometimes to the detriment of native fish (e.g. Allendorf and Leary, 1988). Following introductions of exogenous fish, native populations have also declined as a consequence of displacement and interbreeding (Hindar *et al.*, 1991). Consequently, we also describe likely case scenarios for genetic interactions between existing native populations and exogenous hatchery populations of turbot and sole. Genetic data from a number of protein-coding loci, including previously undescribed variants, are presented for collections of fish representative of native and hatchery produced populations. The dataset is considered from the perspective of the biological and management implications of differences observed between native and hatchery populations.

Material and methods

Juvenile sole (S/CONWY) were obtained from the Cefas laboratory in Conwy, North Wales, UK, and from the Flødevigen Marine Research Station (S/ARENDAL) in Arendal, Norway. The broodstock in Conwy consisted of adult fish trawled from the Irish Sea and the broodstock in Arendal was a mixture of fish trawled from the Kattegat and the Skagerrak. Both broodstocks had been acclimated to captivity and spawned naturally for many years. Three natural populations of sole were fished southwest of the Isle of Man (S/IOM), off Kish Bank (S/IRL), and off King William Bank (S/CUM). A natural population of turbot (T/IOM) was caught off the coast of the Isle of Man. Sample collection sites and dates did not include patterns that would bias the results (e.g. summer feeding migrations, spawning season, or winter offshore migration of adults or juveniles). There are no records indicating hatchery stocking of turbot in the Irish Sea. Juvenile turbot (T/MANNIN) were provided by Mannin Sea Farms, Castletown, Isle of Man, and were derived from a locally caught broodstock. The Irish Sea was the study area (Figure 1); in all, 648 specimens were analysed.

Earlier studies on turbot and Dover sole based on allozymes (Rigby, 1986; Exadactylos *et al.*, 1998, 1999, 2003; Exadactylos and Thorpe, 2001) provided protocols and methodologies. New genetic data presented here are based on screening 14 enzyme-coding loci (*AK*, *CK*, *GPI*-1, *GPI*-3, *G3PDH*, *G6PDH*, *IDH*, *LDH*-1, *LDH*-2, *MDH*-2, *MDHP*-1, *MDHP*-2, *PGDH*, *PGM*) drawn from the same sources.

Data analysis

Phenotypic distributions of all co-dominantly expressed loci were tested for conformation to Hardy–Weinberg (i.e. binomial) expectations using exact tests for small sample sizes (Lessios, 1992; Sokal and Rohlf, 1995), analogous to Fisher's exact tests for 2×2 contingency tables. For loci with more than two alleles the variant alleles were pooled. Allele frequency differences within hatchery and wild samples, and between the pooled hatchery and wild samples, were tested by contingency Chi-squared analysis (Sokal and Rohlf, 1995). Heterozygosities and the proportion of polymorphic loci (P_{99}) within samples were estimated from allele frequencies assuming Hardy–Weinberg equilibrium. Deficiencies of heterozygotes in each population were estimated using the inbreeding index F_{IS}, the significance of which was tested using Li and Horvitz's (1953) formula. Genetic diversity analysis (Nei, 1978, 1987; Chakraborty *et al.*, 1982) was used to partition total genetic variation into its components within and between samples, combining data within. Standard genetic distance values were calculated according to Modified Rogers D_T (Wright, 1978); a dendrogram of these was constructed using the unweighted pair-group method with arithmetic averages (UPGMA; Sneath and Sokal, 1973), based on the genetic distance matrix of these values. Corrections for multiple tests were performed following the sequential Bonferroni procedure (Rice, 1989).

A measure of the effect of population subdivision based on F_{ST} was calculated, and its significance was tested by the method of Workman and Niswander (1970). The data were also used to estimate the extent of gene flow (N_em) in an island model at equilibrium (Slatkin, 1993) among all possible pairs of Dover sole populations. Geographic distance between the populations was calculated as coastal kilometres between the centres of the geographic distribution of each collection. We tested for a pattern of isolation by distance between them by regressing $\log_{10}$ (N_em) on $\log_{10}$ (distance), following Slatkin (1993). Based on simulation and tests of empirical data, Slatkin (1993) showed that a negative slope resulting from this type of regression analysis would suggest some degree of isolation by distance among equilibrium populations. Statistical analyses were performed on JMP 5.0, Minitab 13.0, GenePop 1.2 (Reymond and Rousset, 1995), F-stat 2.9.3 (Goudet, 2001), Genetix 4.04 (Belkhir, 2003), TreeView 3.2 (Roderic, 1996), and Biosys-2 (Swofford and Selander, 1989).

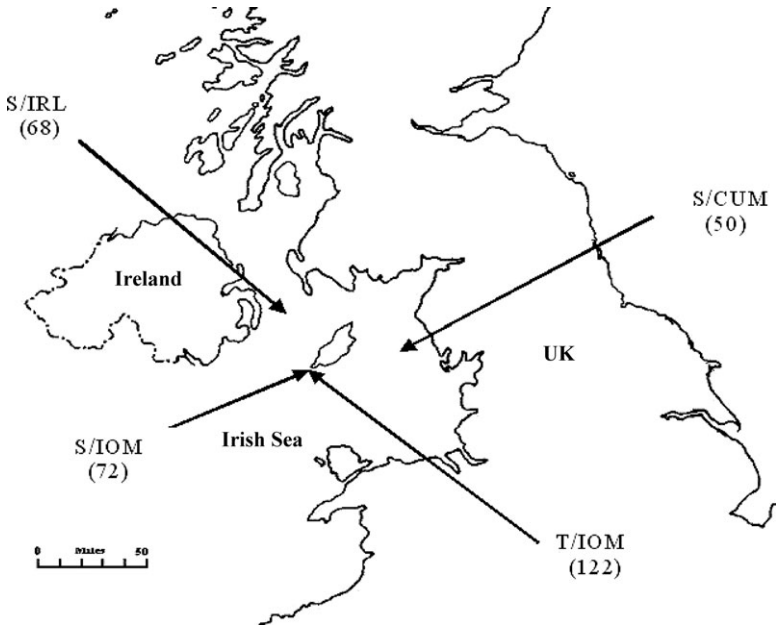

Figure 1. Map showing the sample locations of *Solea solea* and *Scophthalmus maximus* adults. The total numbers of individuals sampled are given in parentheses. The two Dover sole hatchery stocks were represented by 120 juveniles each, and the turbot hatchery stock by 96 juveniles.

Table 1. Significant coefficients for heterozygote deficiency (*D*) in our flatfish populations.

Population	Locus	Heterozygotes		Fixation index (*F*)	*D*	χ^2	*p-value*
		Observed	Expected				
T/IOM	MDH-2	1	2.795	0.642	−0.642	9.08	$<3 \times 10^{-4}$
S/IOM	*MDH-2*	8	11.188	0.285	−0.285	8.31	$<4 \times 10^{-4}$
S/CUM	*G3PDH*	2	3.840	0.479	−0.479	11.48	$<10^{-4}$
	MDHP-1	2	3.840	0.479	−0.479	11.48	$<10^{-4}$
	MDHP-2	0	1.960	1.000	−1.000	50.00	$<10^{-5}$
S/IRL	*GPI-3*	1	6.083	0.836	−0.836	27.08	$<10^{-5}$
	G6PDH	0	1.889	1.000	−1.000	18.00	$<10^{-5}$
	PGDH	0	1.889	1.000	−1.000	18.00	$<10^{-5}$
S/Arendal	*GPI-3*	8	11.400	0.298	−0.298	10.67	$<10^{-4}$

Results

All samples were within Hardy–Weinberg expected proportions, although there was a slight overall deficit of heterozygotes (Table 1). Mean heterozygosity ranged from 0.006 ± 0.003 in the farmed samples to 0.127 ± 0.03 in the wild samples. The proportion of polymorphic loci (P_{99}) ranged from 28.6% in hatchery-reared turbot (T/MANNIN) to 85.7% in wild-caught sole (S/IOM) (Table 2).

Contingency Chi-squared analyses and pairwise comparisons of allele frequencies (Table 3) revealed genetic heterogeneity between samples within species. Weir and Cockerham's (1984) F_{ST} were 0.024 ($p > 0.01$) for Dover sole and 0.029 for turbot ($p < 0.01$) (Table 4). However, this result should be interpreted with caution because of the founder effect and genetic drift associated with broodstock management. After correction of multiple tests by the sequential Bonferroni procedure, it was found that comparisons within wild and farmed samples revealed significant differentiation ($p < 9 \times 10^{-5}$; $p < 10^{-6}$, respectively) (Table 3).

The matrix of Nei's (1978) unbiased genetic distance coefficients within species and/or farmed/natural populations indicated relative homogeneity, with no evidence of population subdivision (Tables 5 and 6). The UPGMA dendrogram (Figure 2), using Modified Rogers' distance, indicated a clear separation at a species level and a pattern of distinctive clustering between two population groups, east and west of the Isle of Man. Our analyses indicated a limited pattern of isolation by distance, with a negative slope of 0.22 in the regression of Slatkin's (N_em) gene flow on geographic distance between our studied Dover sole populations. The analysis showed, however, that just 19% of the variation in the allozyme data could be explained by isolation by distance (Figure 3). Low regression slope and r^2 values found for comparisons could result from two factors: significant gene flow among the populations and very little actual isolation by distance, or one or more population not being in genetic equilibrium. The timescale of approach of F_{ST} and hence N_em, to equilibrium (where isolation by distance becomes apparent) is under the influence of local effective population size. A population is in genetic equilibrium when there are more or less constant allele frequencies in a gene pool through successive generations over a significant period.

Discussion

All seven populations we assessed showed slight, but individually not significant, heterozygote deficiencies when compared with Hardy–Weinberg expectations. These observed heterozygote deficiencies maybe attributable to inbreeding or to the existence of subpopulations. Other destabilizing forces could be the effect of selection on the loci in question, or mutation, or the presence of loci with rare alleles. Nevertheless, exact tests revealed no significant differentiation from Hardy–Weinberg equilibrium, so mating between populations was approximated as random (panmixia). Notwithstanding, the range of heterozygosity observed indicates significant heterogeneity between samples. For wild-caught fish, the heterozygosity values and the percentage of polymorphic loci are similar to those found in other studies (e.g. Blanquer *et al.*, 1992; Kotoulas *et al.*, 1995; Bouza *et al.*, 1997).

It is worth noting that for both species, levels of genetic variability are greatly reduced in hatchery-reared populations when compared with wild-caught fish. In the two hatchery samples of Dover sole, from Wales and Norway, the mean observed values of heterozygosity are 0.019 and 0.017, respectively, whereas for

Table 2. Genetic variability at all loci and populations. Standard errors in parentheses.

Population	Mean sample size per locus	Mean number of alleles per locus	Percentage of loci polymorphic[a]	Mean heterozygosity	
				Direct count	Hardy–Weinberg selected[b]
T/IOM	122.0 (0.0)	1.6 (0.2)	42.9	0.044 (0.019)	0.056 (0.023)
T/Mannin	96.0 (0.0)	1.3 (0.1)	28.6	0.006 (0.003)	0.006 (0.003)
S/IOM	72.0 (0.0)	2.6 (0.3)	85.7	0.110 (0.024)	0.127 (0.028)
S/CUM	50.0 (0.0)	2.1 (0.2)	78.6	0.053 (0.016)	0.073 (0.022)
S/IRL	68.0 (0.0)	2.1 (0.2)	78.6	0.048 (0.011)	0.105 (0.025)
S/Conwy	120.0 (0.0)	1.4 (0.2)	35.7	0.019 (0.009)	0.019 (0.008)
S/Arendal	120.0 (0.0)	1.4 (0.1)	42.9	0.017 (0.007)	0.020 (0.009)

[a]a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99; [b]unbiased estimate (Nei, 1978).

Table 3. Significances from contingency Chi-squared analysis at two levels (within species and origin) at all loci.

Species/source	Locus	Alleles	χ^2	d.f.	*p*-value
Turbot	*PGDH*	2	7.992	1	$<5\times10^{-4}$
	Total		26.958	8	$<7\times10^{-5}$
Dover sole	*GPI*-1	5	50.875	16	$<2\times10^{-6}$
	GPI-3	4	81.119	12	$<10^{-6}$
	G3PDH	3	59.013	8	$<10^{-6}$
	G6PDH	3	87.266	8	$<10^{-6}$
	IDH	2	16.099	4	$<3\times10^{-4}$
	LDH-1	2	30.921	4	$<10^{-6}$
	MDH-2	3	57.250	8	$<10^{-6}$
	MDHP-2	3	30.255	8	$<2\times10^{-5}$
	PGDH	3	112.976	8	$<10^{-6}$
	Total		564.402	96	$<10^{-6}$
Wild	*G6PDH*	3	17.424	4	$<2\times10^{-4}$
	PGDH	3	15.950	4	$<3\times10^{-4}$
	Total		79.198	44	$<9\times10^{-5}$
Captive bred	*GPI*-3	2	9.567	1	$<2\times10^{-4}$
	MDHP-1	2	9.172	1	$<2\times10^{-4}$
	MDHP-2	2	8.136	1	$<4\times10^{-4}$
	Total		45.905	8	$<10^{-6}$

wild-caught fish they ranged from 0.048 to 0.110. In turbot, the difference is even more marked: 0.006 in the hatchery sample when compared with 0.044 in wild fish (i.e. some 86% of genetic variation has been lost from the hatchery-produced fish, obviously as a result of the hatchery procedures or limited broodstock sampling).

Reduced genetic variability in farmed strains attributable to inbreeding effects associated with the use of too few parents to establish and maintain them has been described for other cultivated species (Cross and King, 1983; Verspoor, 1988), and low levels of genetic differentiation in wild turbot have been noted also in earlier studies (Blanquer *et al.*, 1992; Bouza *et al.*, 1997). Such low variability could result from persistent gene flow during the turbot pelagic phase or from post-glacial colonization from a single refuge without sufficient time having elapsed for differentiation.

Table 4. Summary of *F*-statistics at all polymorphic loci, within Dover sole and turbot natural and captive-bred populations (Weir and Cockerham, 1984).

Locus	Dover sole			Turbot		
	F_{IS}	F_{ST}	*p*-value	F_{IS}	F_{ST}	*p*-value
GPI-1	0.022	0.021	0.912			
GPI-3	0.255	0.028	0.163	−0.024	0.007	0.369
G3PDH				−0.065	0.031	0.060
IDH				−0.035	0.023	0.120
LDH-2	0.025	0.034	0.014			
MDH-2				−0.054	0.027	0.072
MDHP-1	0.341	0.024	0.158			
MDHP-2	0.130	0.020	0.140			
PGDH	0.141	0.025	0.182	−0.081	0.044	0.010
PGM	−0.054	0.024	0.062	−0.023	0.016	0.147
Mean	0.121	0.024	0.120	−0.056	0.029	0.004

Table 5. Matrix of genetic similarity and/or distance coefficients.

Population	I	II	III	IV	V	VI	VII
T/IOM		0.975	0.047	0.033	0.041	0.019	0.019
T/Mannin	0.047		0.034	0.020	0.028	0.006	0.007
S/IOM	0.954	0.966		0.956	0.968	0.938	0.945
S/CUM	0.968	0.980	0.057		0.956	0.961	0.968
S/IRL	0.960	0.973	0.048	0.056		0.949	0.956
S/Conwy	0.982	0.994	0.080	0.054	0.066		0.985
S/Arendal	0.981	0.993	0.076	0.053	0.056	0.023	

Below diagonal: modified Rogers distance (D_T) (Wright, 1978). Above diagonal: Rogers (1972) genetic similarity.

To maintain genetic variation within farmed strains at levels comparable with those of wild source populations, good broodstock management practices are required, especially the use of adequate numbers of effective parents and broodstock from different locations, or more locally if there are significant differences in the wild. Data from additional loci and more screened individuals may provide more precise estimates of genetic variation, and therefore perhaps demonstrate significant reductions in variability. It has been suggested that reduced genetic variation can result in reduced performance in aquaculture strains (Gjedrem, 1992; Smitherman *et al.*, 1997), and that loss of genetic diversity can be correlated with time elapsed since strains were founded. Moreover, year classes of the strains would need to be analysed to determine whether there is significant inter-cohort variation in allele frequency. Butler and Cross (1996) argued that allele frequency fluctuations between year classes may be a better indicator of founder effects than overall genetic variation.

The low allozyme heterozygosity, which we and others have detected in turbot, and the lack of population subdivision in sole could reflect genetic depletion in the whole genome and thus represent a load to the adaptive potential of the species, as studies have stated for endangered species (O'Brien, 1994). However, reduced allozyme heterozygosity would not necessarily indicate low global genomic variation because of the slow recovery rate of neutral variation (Lande and Barrowclough, 1987). Imsland *et al.* (2000) suggest genetic differentiation between Norwegian/Icelandic turbot and those from the Kattegat; many other flatfish species also show genetically differentiated population structures (e.g. Igland, 1994; Exadactylos *et al.*, 1998, 2003). Possibly, the variability exhibited by farmed strains is due to random genetic drift of allele frequencies in the hatcheries. However, some element of domestication or hatchery/husbandry (deliberate or inadvertent) selection may have led also to changes in the genetic composition of farmed strains compared with source populations. From the point of view of the natural biodiversity of the species, the current phase of a possible restocking process will lead to a considerable loss of alleles within populations. If similar stocking practices become widespread within Europe as aquaculture methods improve, various natural taxa will become genetically swamped and eventually replaced by uniform commercial domestic stocks; inevitably the average genetic distances between regions and therefore the genetic diversity within the species will be further reduced. Projects to replace domestic strains by strains created from wild populations would be of value, but great care would be needed to reduce the potentially deleterious genetic impacts of restocking.

The combination of a high effective population size and very low genetic diversity found in turbot and sole appears counter-intuitive

Table 6. Matrix of Nei's (1978) unbiased genetic distance coefficients averaged by species (first level) and origin (second level).

Species	**Populations**			
		I	**II**	
Turbot	2	0.001 (0.001 – 0.001)		
Dover sole	5		0.001 (0.001 – 0.001)	
Origin		**I**	**III**	**IV**
Wild turbot	1			
Captive-bred turbot	1	0.001 (0.001 – 0.001)		
Wild Dover sole	3		0.001 (0.000 – 0.002)	
Captive-bred Dover sole	2		0.003 (0.000 – 0.005)	0.000 (0.000 – 0.005)

(see Avise, 2000) and goes against most common molecular evolutionary models, in which the greatest diversity is expected in populations with the largest number of breeders. Several hypotheses have been suggested (Frankel and Soule, 1981; Soule, 1986; Avise, 1994; Risser, 1995; Charlesworth, 1997; Ruckelhaus *et al.*, 1997) to account for such patterns of genetic diversity.

Founder-flush effects represent one theoretical condition under which extensive genetic diversity can evolve rapidly from a single, panmictic source, although the theory remains controversial (Templeton, 1980; Slatkin, 1996; Charlesworth, 1997). Such models assume that populations founded by a small number of individuals, which then grow rapidly, are characterized by a distinct genetic evolution. If the founding population represents a random sample from the parent population, the model predicts that during the period of rapid growth, genetic drift will be much weaker than would normally be expected, particularly for low-frequency alleles. Therefore, the probability of loss of a neutral lineage is low, and the probability of fixation of advantageous alleles is much higher than in populations that remain at a constant size. For founder-flush effects to have contributed to the observed genetic diversity in turbot, we would need to find support for range expansion into other habitats, a period of rapid growth, and a period of habitat reduction leading to a significant and rapid population decline. Range expansion, following for example the last ice age, and habitat decline through anthropogenic influences, particularly overfishing, seem to be feasible circumstances in the recent evolutionary history of both turbot and sole.

Another hypothesis follows the "vicariance" model of genetic variation outlined by Avise (1994). From his hypothesis, it follows that the Irish Sea may play the role of an evolutionary ecotone between two Pleistocene source populations. Interfacing divergent conditions found in ecotones plays a significant role in generating genetic diversity (Mace *et al.*, 1996). Small, isolated populations found in such regions may exhibit greater inter-population diversity because individuals are more subject to drift and/or directional selection (Smith, 1993; Risser, 1995). Physical oceanographic conditions support the ecotone hypothesis. If the theory of two Pleistocene refugia and an ecotone in the Irish Sea, where the two evolutionary histories meet and combine, is true, it has important implications for management, because differing processes or pathways of evolution need to be considered in conservation programmes.

Most of the theory that underlies interpretation of population structure from observed allelic and genotypic frequencies assumes that the system is near equilibrium with respect to all the forces altering allele frequencies (Zhivotovsky *et al.*, 1994). Although most models that have been developed include appropriate caveats, in practice and because of lack of resource, equilibrium and neutrality are often assumed. The model most often used to describe population structure assumes that only random genetic drift and immigration are involved, i.e. that selection is negligible and the populations are in equilibrium (e.g. Chakraborty and Leimar, 1987). Because the models are all that are available for interpreting population structure, it is critical that their limitations

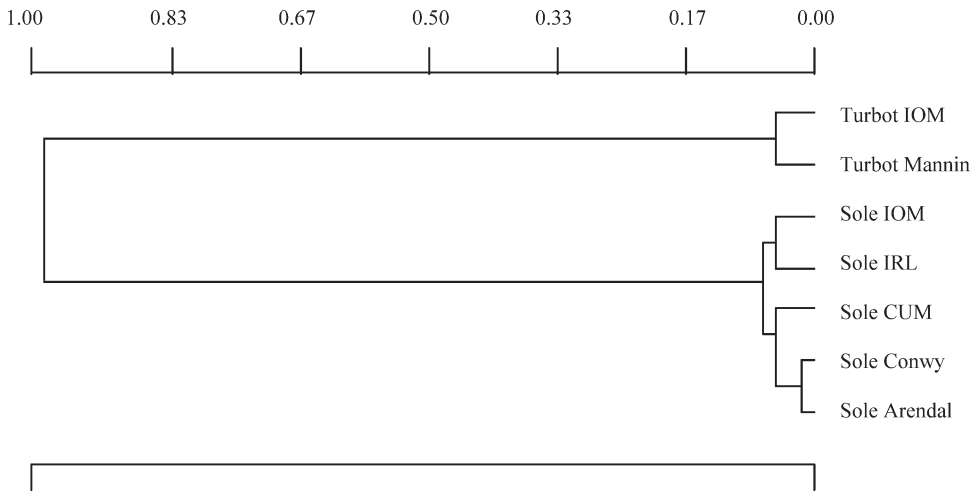

Figure 2. Cluster analysis using unweighted pair group method of modified Rogers (D_T) distance (Wright, 1978). Goodness of fit statistics: $f = 0.164$ (Farris, 1972); $F = 1.583$ (Prager and Wilson, 1976); % s.d. = 8.106 (Fitch and Margoliash, 1967); Cophenetic correlation = 1.000.

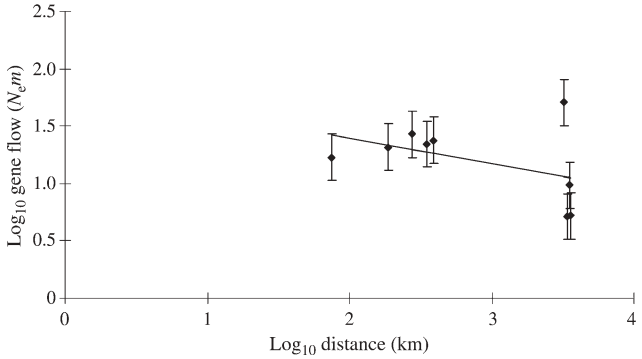

Figure 3. Gene flow plotted against distance for *S. solea* populations in the Irish Sea. Regression ($N_em = 1.837 - 0.221d$) was non-significant ($R = 0.44$; $F_{0.01,8} = 1.66$, $p = 0.239$).

be understood (see also Avise, 2000). Recent geological or climatological changes or human activities have affected many populations and make it unlikely that these populations are in equilibrium with respect to selection, immigration, and drift. We have no way of knowing how far from equilibrium they may be, or to what degree normal environmental fluctuations and local diversifying selection affect their genetic compositions. If the forces affecting allele frequencies are relatively weak, the approach to equilibrium may be very slow in a resource manager's timeframe.

Assessment of our results in the light of the above discussion indicates that departure from equilibrium and even relatively weak selection can severely bias parameter estimates and alter interpretations. Applications of such statistics from population systems or from loci that may not satisfy the assumptions must be carried out with caution. Parameters such as N_em estimated from allele frequency data may be affected substantially either by disequilibria or by loci influenced by relatively low levels of selection, especially diversifying selection. In addition, the magnitude of heterogeneity of allele frequencies among populations is sensitive to selection, but it indicates that statistically significant divergence takes place even with immigration numbers in excess of ten individuals per generation (see Allendorf and Phelps, 1981). At neutral loci, immigration eventually arrests further divergence, but not before there has been significant divergence.

Several of the hypotheses outlined above use results from ranges of migration and selection parameter values, which were intended to blanket actual values and only follow the fate of a single locus subjected to selection. It is unlikely that many of the traits which are important to average fitness of a population are polygenic or quantitative traits, such as those related to life-history characteristics (Gharrett and Smoker, 1993). Therefore, in order to model hatchery and wild stock interactions accurately in order to predict potential impacts, geneticists must have more biological information than is currently available (Pérez *et al.*, 2003). Such information includes normal gene exchange rates between populations for a variety of species, populations, and times, as well as on the magnitude of and variation in selection regimes that occur in different locations and that result from fluctuating environmental conditions. In addition, knowledge of the role and relationship of population structure to average fitness must be developed, which means that we need to learn much more about the genetics of life-history traits in populations. More sophisticated models must also be developed and include the ability to study quantitative loci. Such data are of critical value to managers and aquaculturists alike. The potential consequences of exposing wild stocks to immigration from hatchery stocks have serious economic implications for the entire fishing industry. These data are not easy to acquire but, considering the value of our fish resources, we must make the commitment to obtain them.

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