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# Spatio-temporal genetic variability in sea trout (*Salmo trutta*) populations from north-western Spain

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## SUMMARY

1. Genetic variation at five microsatellite loci was investigated in six sea trout (*Salmo trutta*) populations to describe their spatio-temporal genetic variation in north-western Spain. We observed significant genetic variation between river basins, and isolation by distance with restricted gene flow between neighbouring rivers, which suggests an important homing behaviour.
2. Despite these populations suffering a serious demographic decline during 1998, we did not detect any reduction in their genetic variation, suggesting a reasonably high effective population size and temporal stability.
3. Genetic differences among rivers should be taken into account in future management activities. Given the high genetic variability and the temporal stability observed, we believe that no supportive breeding programmes are presently needed in these populations.

*Keywords*: effective population size, gene flow, genetic differentiation, homing, microsatellite, sea trout

## Introduction

The brown trout (*Salmo trutta* L.) presents two alternative life strategies, a resident form that dwells in the river, and an anadromous form (sea trout) that migrates to the sea, where it stays from several months up to several years before returning to the river for spawning. Although both forms present many morphological, demographic and ecological differences (Bagliniere, 2000), they live in sympatry, can interbreed (Frost & Brown, 1967), can have progeny of the alternative life strategy, and the juvenile are indistinguishable. Moreover, no genetic differences have been reported so far between resident and anadromous individuals inhabiting the same rivers (Guyomard *et al*., 1984; Hindar *et al*., 1991; Cross, Mills & Williams, 1992; Petersson, Hansen & Bohlin, 2001; Charles *et al*., 2005).

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While there is complete reproductive isolation between resident populations (Ryman, 1983; Morán *et al*., 1995; Bouza *et al*., 1999), anadromous populations are also partially isolated from each other and show restricted gene flow. As a result of homing behaviour, appreciable genetic differentiation has been reported among neighbouring populations of the migratory form despite their access to the sea (Hansen *et al*., 2002; Hovgaard, Skaala & Naevdal, 2006). On the other hand, genetic similarity among populations indicates that straying among neighbouring basins also occurs (Morán *et al*., 1995; Bouza *et al*., 1999; Hansen *et al*., 2002; Ostergaard *et al*., 2003; Schreiber & Diefenbach, 2005; Ayllón, Morán & García-Vázquez, 2006). In addition, several tagging experiments suggest high levels of homing (Jensen, 1968; Le Cren, 1985; Caballero, Cobo & González, 2006), while in others straying reached values of at least 15.5% (Pratton & Shearer, 1983; Berg & Berg, 1987).

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Sea trout populations can suffer demographic changes either naturally or as a consequence of human activities such as fishing, contamination and dam construction. Under these circumstances it is very important to take into account this temporal variation to understand the genetic differentiation among populations (Waples, 1998). While some sea trout populations seem to be genetically stable through time (Hansen *et al.*, 2002; Charles *et al.*, 2005), others do show temporal variation (Laikre *et al.*, 2002; Ostergaard *et al.*, 2003). Temporal stability in the genetic composition of a population is intimately related to its effective size (i.e. the number of individuals who contribute offspring to the next generation), but also to gene flow among neighbouring populations. Indeed, temporal stability is of special concern in conservation and management activities, particularly in detecting sudden reductions in population size (bottlenecks) that might lead to loss of genetic variation and inbreeding.

In this study we examined genetic variation at five microsatellite loci in six sea trout populations sampled in NW Spain in 1997 and 2003. Our main objective was to assess gene flow among rivers in order to understand the homing behaviour of sea trout. From the records of captures (Fig. 1), five of these populations appeared to suffer a 30–70% demographic reduction in 1998. Therefore, we also measured genetic variation in these populations before and after 1998, in order to detect any potential loss of variability and/or reduction of the effective population size. Finally, all this information was used to evaluate the conservation status of these populations and to provide information for potential management activities in the future.

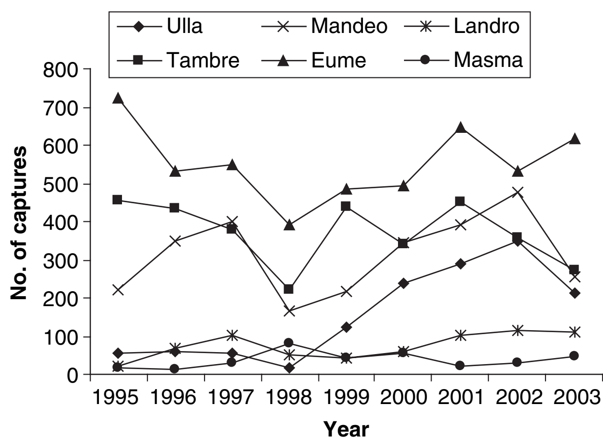


**Fig. 1** Reported number of angling captures per river and year (1995–2003).

## Methods

### *Populations sampled*

We screened genetic variation in sea trout populations from six rivers in three neighbouring regions in NW Spain: 'southern' (Ulla and Tambre, rivers that flow into the Atlantic through large estuaries known as 'rías'), 'central' (Mandeo and Eume, that flow further north into the Atlantic but have smaller rías) and 'northern' (Landro and Masma, that flow into the Cantabric Sea) (Fig. 2). All the rivers have dams that have considerably reduced the natural habitat distribution of these species (Table 1). We collected a total of 438 individuals in 1997 and 2003, all adults returning from the sea. The occurrence of a marine period was confirmed by scale reading.

These sea trout spend 1–4 years in freshwater (mean smolt age is 2.2), after which they migrate to the sea, where they stay from less than a year up to 2 years (Caballero *et al.*, 2006). Half of the individuals return for their first spawning in the same year as the smolt migration (finnocks), and the second largest spawning group is formed by one sea-winter individuals (44%). The proportion of multiple spawners

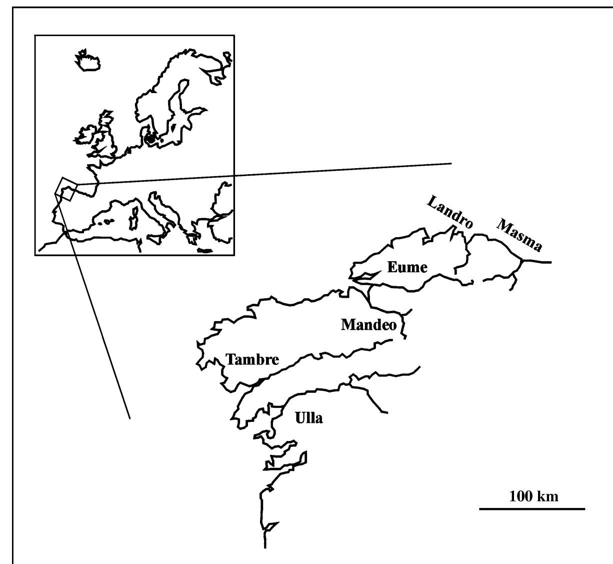


**Fig. 2** Geographical location of the rivers studied.

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**Table 1** Physical characteristics of the rivers studied showing the length of the main river and the area accessible to sea trout. We include also the total number of captures reported from 1995 to 2003 and the number of captures per ten fishing licences (CPUE).

| River | Length (km) | Passable stretch (km %) | No. captures | CPUE |
|---|---|---|---|---|
| Ulla | 132 | 74 (56) | 1407 | 2.63 |
| Tambre | 124 | 6 (5) | 3349 | 4.48 |
| Mandeo | 56 | 15 (27) | 2822 | 2.94 |
| Eume | 80 | 13 (16) | 4966 | 2.26 |
| Landro | 31 | 8 (26) | 675 | – |
| Masma | 46 | 22 (48) | 334 | – |

reported in this area is 14–28%. Introductions carried out in this area have not been successful (Martínez *et al.*, 1993; Arias, Sánchez & Martínez, 1995). Only recreational angling in rivers (from March to September) is permitted. Since 1995, all angled sea trout in this area have been monitored. Each individual is tagged and scale samples and morphological data (e.g. weight and size) are collected. Based on this information, most of these rivers apparently harbour stable populations, with an average number of captures of 37–552 individuals.

### Microsatellite genotyping

DNA was extracted from scales using the NucleoSpin® Tissue Kit BD Biosciences (MACHEREY-NAGEL, Düren, Germany). One tetranucleotide and four dinucleotide microsatellite loci were analysed: SSOSL311, SSOSL417 and SSOSL85 (Slettan, Olsaker & Lie, 1995), SS4 (Martínez, Morán & García-Vázquez, 1999) and Ssa197 (O'Reilly *et al.*, 1996). PCR reactions were carried out using 1 μL of extracted genomic DNA, 2 μL of reaction buffer (10X buffer), 2.5 mM $MgCl_2$, 0.35 μM of each primer (forward primer labelled with FAM, HEX or NED), 1 U of *Taq* DNA polymerase and deionised water up to a final volume of 20 μL. Cycling conditions consisted of a 5 min denaturation at 95 °C, followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C and 30 s extension at 72 °C, followed by a final 10 min extension at 72 °C. PCR products (1–3 μL) of separate reactions were combined into a final volume of 10 μL. Two microlitres of this mix were added to 12 μL of deionised formamide and 0.5 μL of the internal size standard GENESCAN 400HD [ROX] for electrophoresis. This mixture was denatured at 95 °C for 5 min and immediately chilled on ice and subjected to capillary electrophoresis on an ABI PRISM Genetic Analyser. Loci were identified according to their size range and attached fluorescent label. Genotype data was generated using the GENESCAN software (Applied Biosystems, Foster City, CA). Scoring errors, large allele dropout and null alleles were checked using Micro-Checker (Van Oosterhout *et al.*, 2004).

### Population genetics analysis

*Genetic variation.* We estimated population allele frequencies and heterozygosities, deviations from Hardy-Weinberg equilibrium and genotypic linkage equilibrium using GENEPOP v3.4 (Raymond & Rousset, 1995). Probabilities were adjusted for multiple testing with the sequential Bonferroni correction (Hochberg, 1988). Allelic richness and expected heterozygosities in 1997 and 2003 were compared through randomisation tests using FSTAT (Goudet, 1995).

*Demography.* We tested for the occurrence of genetic bottlenecks using three different methods. First, we used the BOTTLENECK program (Cornuet & Luikart, 1996), that compares the observed gene diversity ($H_E$) with that expected at the equilibrium (*Heq*) calculated from the observed number of alleles ($k$). The idea is that during a bottleneck the number of alleles decreases faster than gene diversity, and therefore after the bottleneck $H_E$ will be higher than *Heq*. We assumed a two-phase mutation model that consists mostly of one-step mutations and a small percentage (10%) of multi-step changes. We also used the graphical method of Luikart *et al.* (1998), which tests for a distortion of the allele frequency distribution. During a bottleneck, rare alleles are more likely to be lost than common alleles, shifting the histogram of the allele frequency distribution to the right. All the loci in a sample were pooled and alleles were binned in 10 frequency classes (i.e. 0–0.10, 0.11–0.20, 0.91–1.00). If no bottleneck occurred, we expect to see a high number of alleles in the 0–0.10 bin, and the distribution should be L-shaped. On the contrary, after a bottleneck there will be less number of alleles at the low frequency bins, and the distribution will appear shifted to the right. Finally, we used the coefficient of Garza (Garza & Williamson, 2001), $M = k/r$, where $k$

is the number of alleles and $r$ is the range in allele size. Note that when a population is reduced in size, the loss of any allele will decrease $k$, but only the loss of the smallest or largest allele will reduce $r$. Therefore $k$ is expected to be reduced more quickly than $r$, and $M$ will be smaller after a bottleneck. We computed the average $M$ across loci for each sample, and for each river by pooling the two temporal sets. The empirical value of $M$ was contrasted with the critical value $M_{\text{crit}}$ at the equilibrium. Because $M_{\text{crit}}$ depends on several parameters, such as the percentage of one-step mutations, the mean size of non-one-step mutations and $\Theta$ ($4N_e\mu$), with unknown values that are difficult to estimate, we considered three different scenarios. Basically, we assumed a two-phase mutation model with 90% of single stepwise mutations and a mean size of non-one-step mutations of 2.8 (see Garza & Williamson, 2001), but considered three possible values of $\Theta$. We estimated $\Theta$ using the long-term $N_e$ derived from the expected heterozygosity ($H_E$) and a mutation rate of $\mu = 10^{-4}$ (see Waldick *et al.*, 2002), and also used other realistic values often observed in natural populations ($\Theta = 10$ and $\Theta = 0.5$) (see Guinand & Scribner, 2003). Therefore, using the program Critical_M (Garza & Williamson, 2001), we obtained three different $M_{\text{crit}}$ for each data set. To be most conservative, we considered significant only those $M$ values that were below the highest $M_{\text{crit}}$ of the three obtained.

*Geographical differentiation and population structure.* In order to explain the distribution of the genetic variation among and within rivers between temporal samples, we carried out an analysis of molecular variance (AMOVA) (Excoffier, Smouse & Quattro, 1992) using ARLEQUIN version 2000 (Schneider, Roessli & Excoffier, 2000). We also performed an AMOVA to estimate the genetic variation distributed among the three geographical regions sampled. Pairwise $F_{\text{ST}}$ (Weir & Cockerham, 1984) were calculated using GENETIX (version 4.03) (Belkhir *et al.*, 1998), and their significance was calculated by randomisation (10 000 permutations). We calculated Nei's standard genetic distance, $D_A$ (Nei, Tajima & Tateno, 1983), between populations using the program MSA (Dieringer & Schlotterer, 2003). To detect any correlation between $D_A$'s and geographical distances we performed three different Mantel tests using GENEPOP v3.4 (Raymond & Rousset, 1995) for the 1997, 2003, and 1997 plus 2003 samples. From the $D_A$ matrix we constructed a neighbour joining tree using PHYLIP 3.6 (Felsenstein, 1993). Confidence in the observed relationships was assessed with the bootstrap technique (Felsenstein, 1985). Bootstrap values are the number of times a clade is present in the trees estimated from the bootstrap pseudosamples. Loci were bootstrapped 10 000 times. This procedure was implemented with the MSA software and the Neighbour and Consense programs of the PHYLIP package. We also performed a principal coordinates analysis (PCO) with the matrix of $D_A$ distances using the program PAST (Hammer, Harper & Ryan, 2001).

Assessment of genetic structure was performed with the program STRUCTURE v2.0 (Pritchard, Stephens & Donnelly, 2000), which estimates the number of populations represented by a sample of individuals by minimising Hardy–Weinberg and gametic phase disequilibria. The underlying model assumes that individual genomes consist of a mixture of genes originating from $K$ unknown populations. The program estimates the proportion of membership of each individual $j$ in each of the $K$ populations ($p_{jk}$). The analysis was run for 1 000 000 iterations, with a burn-in period of 100 000. Three independent runs were performed for each of the 12 values of $K$ assayed (1–12). We choose the value of $K$ that resulted in the maximum and most stable log-likelihood among runs. Evidence of genetic structure was supported by clusters presenting a higher $p_{jk}$ than $1/K$ (i.e. a membership proportion higher than expected by chance).

*Effective population size.* To estimate the effective size of these populations we used two methods that are based on the temporal change in allele frequencies. Berthier's *et al.* (2002) likelihood method relies on coalescence theory and uses Markov chain Monte Carlo simulations for generating a posterior distribution for $N_e$. It is implemented in the program TM3. We based our estimates on 100 000 MCMC generations, and assumed a maximum $N_e$ ($N_{e\max}$) of 1000 and a generation time of 3.8 years, based on demographic data from these populations (Caballero *et al.*, 2006). We also used the temporal method of Wang & Whitlock (2003) implemented in the program MLNE. This method allows for the estimation of $N_e$ by assuming isolated populations, implying that all genetical changes between the temporal samples are

due to genetic drift. Alternatively, it can also estimate $N_e$ considering migration as an additional source of variation in allele frequencies. In this case, it also estimates the percentage of immigrants ($m$) from the source population. We conducted the analyses for each river, considering as the source population the pooled temporal samples of the closest river, and assuming a $N_{emax}$ of 1000. As the intervals between temporal samples are not integers because of overlapping generations, all estimates were subsequently adjusted according to the equations provided by Wang & Whitlock (2003).

## Results

### *Genetic variation and temporal stability*

Allele frequencies and heterozygosities for each river and locus are given in the Appendix. The number of alleles per locus ranged between 16 and 44, being SSOSL417 the most variable. Size ranges and gene diversities (Table 2) were similar to those found in other sea trout populations in Spain (Ayllón *et al.*, 2006). Genetic diversity was similar for all samples. Allelic richness was comparable among samples, although samples from the River Masma showed smaller, albeit non-significant, values. Most samples were in Hardy–Weinberg equilibrium, except locus SSOSL85 in samples Tambre97, Eume97 and Eume03. Only seven out of 120 tests showed linkage disequilibrium, with different pairs of loci involved. Importantly, genetic variability based either on allelic richness or gene diversity was not significantly different between 1997 and 2003.

No evidence of a past bottleneck was found given the observed heterozygosities (BOTTLENECK $P$-value >0.05) or the allele frequency distribution, which presented a normal L-shaped distribution (Fig. 3). However, most Garza's $M$ values were significant under different hypothetical scenarios (Table 3).

**Table 2** Microsatellite loci studied

| Locus | Label | Size range | *NA* | $H_E$ |
|---|---|---|---|---|
| SSOSL311 | FAM | 118–172 | 23 | 0.85 |
| Ssa197 | HEX | 117–189 | 19 | 0.88 |
| SSOSL417 | FAM | 167–253 | 44 | 0.90 |
| SS4 | NED | 174–224 | 20 | 0.83 |
| SSOSL85 | HEX | 175–207 | 16 | 0.88 |

Label indicates the fluorescent dye used.
*NA*, number of alleles; $H_E$, expected heterozygosity under Hardy–Weinberg equilibrium.

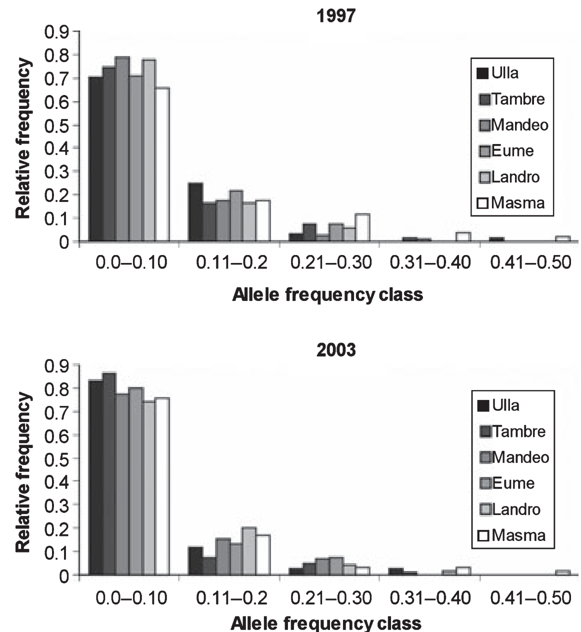


**Fig. 3** Distribution of allele frequencies across all microsatellites in each river and for each temporal data set.

**Table 3** Estimated values of $M$ (Garza & Williamson, 2001) for each temporal sample and river

| | | $M_{crit}$ | | |
|---|---|---|---|---|
| | $M$ | $\theta_{est}$ | $\theta = 10$ | $\theta = 0.5$ |
| Ulla97 | **0.567** | 0.648 | 0.698 | 0.800 |
| Ulla03 | **0.597** | 0.660 | 0.710 | 0.800 |
| Ulla | **0.702** | 0.717 | 0.742 | 0.800 |
| Tambre97 | **0.629** | 0.670 | 0.730 | 0.800 |
| Tambre03 | 0.728 | 0.700 | 0.728 | 0.800 |
| Tambre | 0.782 | 0.747 | 0.761 | 0.800 |
| Mandeo97 | **0.645** | 0.689 | 0.728 | 0.800 |
| Mandeo03 | **0.576** | 0.681 | 0.724 | 0.800 |
| Mandeo | **0.683** | 0.735 | 0.759 | 0.800 |
| Eume97 | **0.609** | 0.682 | 0.724 | 0.800 |
| Eume03 | **0.594** | 0.685 | 0.724 | 0.800 |
| Eume | **0.680** | 0.733 | 0.755 | 0.800 |
| Landro97 | **0.588** | 0.657 | 0.705 | 0.800 |
| Landro03 | **0.600** | 0.685 | 0.724 | 0.800 |
| Landro | **0.681** | 0.728 | 0.753 | 0.800 |
| Masma97 | **0.522** | 0.670 | 0.693 | 0.800 |
| Masma03 | **0.599** | 0.706 | 0.725 | 0.800 |
| Masma | **0.652** | 0.735 | 0.748 | 0.808 |

We include the expected $M$ value at equilibrium under different values of $\theta$ ($M_{crit}$). $\theta_{est}$ is the value estimated from the data. Significant values for the three population scenarios are shown in bold.

*Geographical variation and population structure*

The AMOVA indicated that there is significant geographical variation among rivers (2.22%; $F_{CT} = 0.048$, $P < 0.0001$) and between temporal samples within rivers (0.43%; $F_{SC} = 0.009$, $P < 0.001$). When we grouped the six rivers in three geographical regions, the AMOVA also showed significant geographical variation among regions (1.87%; $F_{CT} = 0.041$, $P < 0.0001$) and within regions (1.08%; $F_{SC} = 0.023$, $P < 0.0001$).

The global $F_{ST}$ was 0.024. In general, most pairwise $F_{ST}$ between contemporaneous samples were significant, although comparisons that involved the River Ulla and the neighbouring river Tambre were non-significant. Also, the $F_{ST}$ between the Mandeo and Eume in 1997 was not significant. On the other hand, all comparisons of temporal samples within rivers were non-significant, except for the River Eume (Table 4).

The neighbour joining tree grouped temporal samples belonging to the same river with very high bootstrap values, except for the River Ulla (Fig. 4). Rivers grouped according to the three geographical regions: southern (Tambre-Ulla), central (Mandeo-Eume) and northern (Masma-Landro). The PCO also showed the same three groups (Fig. 5). The Mantel test indicated that there was a significant correlation between geographical and genetic distances in 1997 ($r = 0.81$; $P = 0.017$) but not in 2003 ($r = 0.36$; $P = 0.1$). However, given that most temporal samples were not significantly different, we pooled all samples from the same river, obtaining a significant correlation ($r = 0.77$; $P = 0.008$) (Fig. 6).

The maximum likelihood estimate of the number of populations was $K = 3$. Stratification was supported because clusters presented samples assigned with a probability higher than expected by chance ($1/K = 1/3$). The three clusters observed corresponded to the southern, central and northern geographical regions (Table 5).

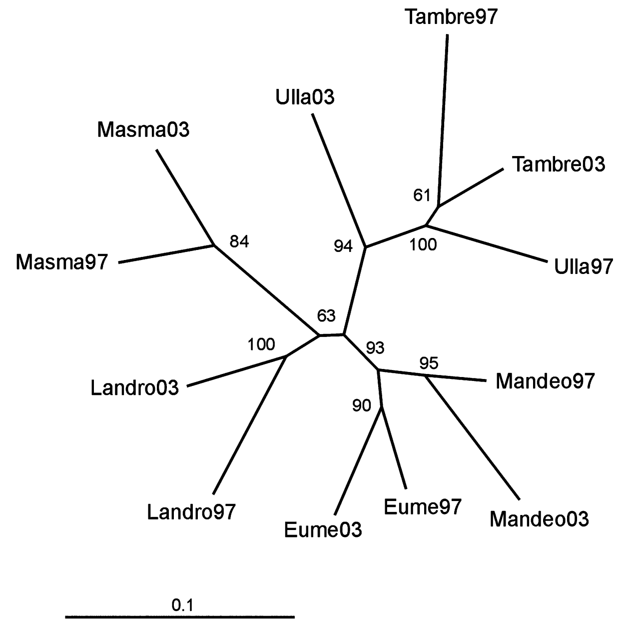


**Fig. 4** Neighbour joining population tree based on Nei's standard genetic distance ($D_A$). Bootstrap values are the number of times (%) a clade on the original tree is present in the trees estimated from the 10 000 pseudosamples.

*Effective population size*

The estimated 90% credible intervals of $N_e$ using TM3 ranged from 50 to 900. The median of the posterior distribution for $N_e$ was very similar ($N_e = 500$) for all rivers (Table 6). For MLNE, and assuming closed

**Table 4** Pairwise $F_{ST}$

| | Ulla97 | Tambre97 | Mandeo97 | Eume97 | Landro97 | Masma97 | Ulla03 | Tambre03 | Mandeo03 | Eume03 | Landro03 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tambre97 | 0.002 | | | | | | | | | | |
| Mandeo97 | **0.021** | **0.030** | | | | | | | | | |
| Eume97 | **0.023** | **0.031** | 0.003 | | | | | | | | |
| Landro97 | **0.022** | **0.030** | **0.020** | **0.018** | | | | | | | |
| Masma97 | **0.034** | **0.052** | **0.024** | **0.028** | **0.021** | | | | | | |
| Ulla03 | -0.001 | **0.011** | **0.014** | **0.017** | **0.020** | **0.035** | | | | | |
| Tambre03 | 0.002 | 0.002 | **0.030** | **0.028** | **0.026** | **0.047** | 0.007 | | | | |
| Mandeo03 | **0.031** | **0.041** | 0.006 | **0.014** | **0.022** | **0.024** | **0.020** | **0.035** | | | |
| Eume03 | **0.033** | **0.034** | **0.015** | **0.011** | **0.023** | **0.036** | **0.025** | **0.041** | **0.026** | | |
| Landro03 | **0.018** | **0.023** | **0.020** | **0.017** | -0.001 | **0.028** | **0.015** | **0.023** | **0.020** | **0.018** | |
| Masma03 | **0.045** | **0.053** | **0.028** | **0.033** | **0.016** | 0.009 | **0.042** | **0.049** | **0.035** | **0.046** | **0.023** |

Significant values ($P < 0.05$) after sequential Bonferroni correction are shown in bold.

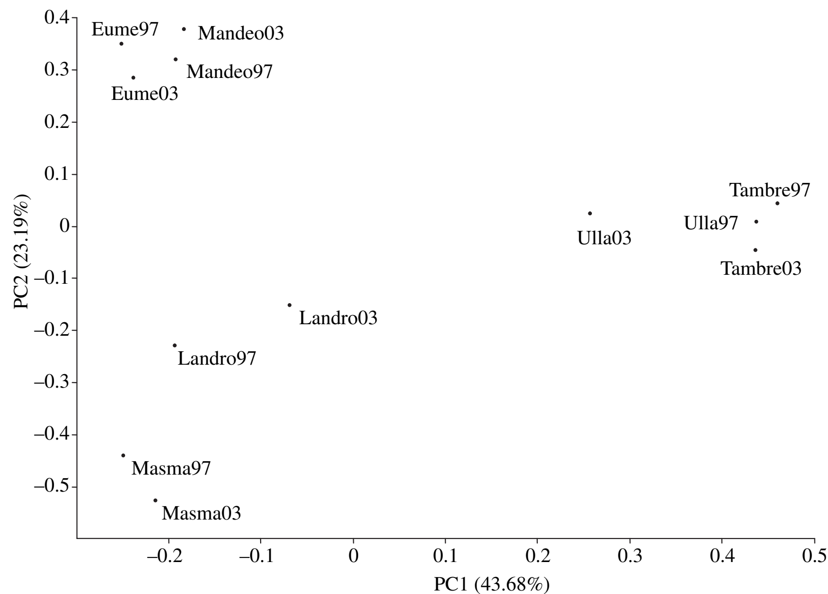


**Fig. 5** Principal coordinates analysis (PCO) based on Nei's standard genetic distance ($D_A$).

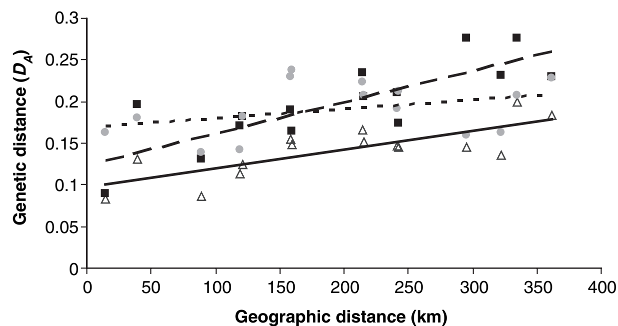


**Fig. 6** Correlation between genetic distance ($D_A$) and geographical distance (Km) for each temporal sample: 1997 (black squares) and 2003 (grey circles), and after pooling temporal samples of the same river, 1997 + 2003, (triangles). Lines represent the regression slope for each group: 1997 (dashed line; $r = 0.81$; $P = 0.017$), 2003 (dotted line; $r = 0.35$; $P = 0.1$) and 1997 + 2003 (solid line; $r = 0.77$; $P = 0.008$).

**Table 5** Proportion of membership of each of the samples in each of the three inferred clusters. Results in bold are higher than expected by chance (>1/3).

| | Cluster | | |
|---|---|---|---|
| Source sample | 1 | 2 | 3 |
| Ulla97 | **0.6500** | 0.1745 | 0.1753 |
| Ulla03 | **0.5327** | 0.2495 | 0.2177 |
| Tambre97 | **0.7290** | 0.1318 | 0.1392 |
| Tambre03 | **0.6873** | 0.1337 | 0.1788 |
| Mandeo97 | 0.2263 | **0.5378** | 0.2360 |
| Mandeo03 | 0.1795 | **0.5553** | 0.2653 |
| Eume97 | 0.1962 | **0.6008** | 0.2028 |
| Eume03 | 0.1750 | **0.5612** | 0.2640 |
| Landro97 | 0.2303 | 0.2907 | **0.4792** |
| Landro03 | 0.2818 | 0.3060 | **0.4120** |
| Masma97 | 0.1200 | 0.2537 | **0.6265** |
| Masma03 | 0.1115 | 0.1823 | **0.7063** |

populations, the lower 95% limit ranged from 50 to 100. However, the program could not estimate the 95% upper limit except for the River Eume (95% upper limit = 600). The estimates of $N_e$ assuming migration ranged from 15 to 80, while the migration rate estimates ranged from 0.09 to 0.36.

## Discussion

### *Genetic diversity and temporal stability*

The preservation of genetic diversity is fundamental if natural populations are to evolve and adapt to environmental changes (Frankel & Soulé, 1981). The high levels of genetic variation observed in these sea trout populations do not suggest a risk of extinction or inbreeding. Levels of genetic variation were also very similar among rivers, despite captures by angling suggesting that population sizes were different. Given the high similarity between temporal samples from the same river, and the lack of evidence of a bottleneck based on two different methods, these populations seem rather stable, regardless of important oscillations in the number of captures from 1995 to 2003. In particular, the 1998 demographic decline did not seem to reduce the genetic variation in these

**Table 6** Effective population sizes ($N_e$)

| Population | TM3 | MLNE | | |
|---|---|---|---|---|
| | | $N_e$ assuming no migration | $N_e$ assuming migration | $m$ |
| Ulla | 478.81 (52.53–905.09) | 784.31 (97.26 to 1000) | 23.53 (15.10–46.97) | 0.36 (0.20–1) |
| Tambre | 562.77 (170.67–954.86) | 337.50 (82.61 to 1000) | 26.85 (18.27–46.46) | 0.24 (0.13–0.41) |
| Mandeo | 497.90 (83.02–912.78) | 114.44 (50.17 to 1000) | 29.06 (19.09–53.28) | 0.13 (0.04–0.27) |
| Eume | 505.89 (85.96–925.82) | 96.79 (48.32–595.35) | 25.31 (17.43–41.06) | 0.15 (0.06–0.29) |
| Landro | 546.87 (145.48–948.27) | 224.73 (63.55 to 1000) | 33.90 (20.24–79.42) | 0.12 (0.03–0.23) |
| Masma | 589.35 (211.38–967.32) | 784.54 (83.81 to 1000) | 27.11 (17.25–51.50) | 0.09 (0.02–0.20) |

We used the programs TM3 (Berthier *et al.*, 2002) and MLNE of Wang & Whitlock (2003), either assuming migration or not. We show also the estimated migration rate ($m$) using MLNE. The 95% intervals are shown in brackets.

populations. Although, Garza's coefficient $M$ suggests the occurrence of a genetic bottleneck in both temporal data sets, the bottleneck indicated by $M$ in 1997 cannot be due to the 1998 population decline but to an older one. These populations have suffered an important lost of habitat in the past because of dam construction. Certainly, past populations were bigger than current ones because they had access to a greater area of the river basins. Moreover, ancient sea trout populations were more likely to interact with the resident form than present migratory populations. Such a historic decline could also be responsible for the detection of a bottleneck in the 2003 sample, because $M$ retains information about past demographic history for a longer period than methods that simply measure the deficit of rare alleles, such as the methods of Cornuet & Luikart (1996) and Luikart *et al.* (1998) (Garza & Williamson, 2001). In summary, it seems that the decline in catches that occurred in 1998 was not long enough to cause any significant lost of variation. Indeed, this might be expected, as genetic drift decreases heterozygosity by only $1/2N_e$ per generation (Hedrick, 2005).

Our results agree with other studies of brown trout where temporal stability has been reported (Hansen *et al.*, 2002; Charles *et al.*, 2005). Only populations living in unstable habitats seem to suffer temporal genetic changes because of frequent population turnover (Laikre *et al.*, 2002; Ostergaard *et al.*, 2003). In this regard, it is important to consider the reproductive biology of brown trout. Because of genetic drift, populations in which most adults spawn at the same age are more sensitive to temporal variation than populations where several cohorts are reproducing at the same time (Waples & Teel, 1990; Patton *et al.*, 1997). In the populations studied, three different smolt age classes were present (1+, 2+ and 3+), and the returning sea trout adults return to spawn after some months, 1 or 2 years living at sea. Furthermore, the proportion of multiple spawners in sea trout is very high (around 23% in these rivers). Therefore, all these cohorts reproducing at the same time increases the effective size of the population, helping to maintain temporal stability. Indeed, temporal stability is also common in other anadromous species with overlapping generations (Nielsen, Hansen & Loeschcke, 1997, 1999; Tessier & Bernatchez, 1999; Brown *et al.*, 2000).

### *Genetic structure and homing behaviour*

There is significant genetic structure among rivers, with restricted gene flow between neighbouring populations. This structure follows a pattern of isolation by distance, in which more distant populations are more divergent. The NJ tree, the PCO, the STRUCTURE analysis and the AMOVA, all support a genetic subdivision that corresponds very well with the three geographical areas sampled. These patterns seem quite common for anadromous populations of brown trout in Spain (Morán *et al.*, 1995; Bouza *et al.*, 1999; Ayllón *et al.*, 2006), and have also been described for other European sea trout populations (Hansen & Mensberg, 1998; Ruzzante, Hansen & Meldrup, 2001). Given this scenario, homing behaviour in these populations is apparently very high, despite the small geographical area considered. However, some straying occurred between some neighbouring rivers. In particular, straying seemed common between southern rivers (Tambre and Ulla), where most pairwise $F_{ST}$ were non-significant. The river Tambre presents a restricted accessible area which seems too small to explain its high number of captures. Therefore, it is

possible that most sea trout captured in this river were born instead in the river Ulla or other smaller neighbouring rivers. This idea is supported by the recapture in the river Tambre of individuals tagged in the river Ulla (Caballero *et al.*, 2006). On the other hand, the rivers Masma and Landro showed appreciable differences although with some gene flow. Their grouping is not well supported in the NJ tree or in the PCO. Such differences could be explained by drift and/or different selection regimes. River Masma presents different water geochemical conditions compared with the remaining rivers studied here. Interestingly, significant differences in growth rates between the Masma and Landro sea trout populations have been observed that could reflect underlying genetic differences. Differential selection occurring at a small geographical scale has been described previously in other sea trout populations (Hansen *et al.*, 2002).

### *Effective population sizes and migration rates*

The estimates obtained using TM3 and MLNE (without migration) were very imprecise. However, it is important to note that although the upper limit estimate changed considerably using both methods, the lower limit did not change much. This observation might be useful for conservation purposes, when it is especially important to know the minimum expected $N_e$. In all cases $N_e$ exceeded 50, which is the minimum short-term $N_e$ suggested to avoid inbreeding problems (Franklin, 1980). Further, given the absence of temporal variability and the reported number of captures in each river (the latter representing a fraction of the total population), we expect that the $N_e$ in these rivers should be reasonably large (Crozier & Kennedy, 2001). For instance, Hansen *et al.* (2002) estimated very large values of $N_e$ (300–500) for several brown trout populations, despite the fact that most of their temporal samples exhibited significant differences. On the other hand, when we allowed for migration in MLNE we obtained narrower confidence intervals, although the estimated $N_e$ seemed very small. The estimated migration rates (0.16–0.56) were very high, especially between the rivers Tambre and Ulla, which agrees with previous tagging experiments (Caballero *et al.*, 2006). However, the migration estimates for the remaining populations seem too high, taking into account that we have found significant genetic differences between them. Previous studies have also found unrealistically small estimates of $N_e$ and large estimates of $m$ using the same method in salmonid populations (Ford *et al.*, 2004; Consuegra *et al.*, 2005; but see Ostergaard *et al.*, 2003). Such unrealistic estimates could result from violation of model assumptions, such as discrete generations, constant migration rates or an infinitely large source population, but also could be attributable to small sample sizes.

The observed temporal variability suggests that these populations have reasonably large effective population sizes. Indeed, the existence of multiple spawners and the possibility of interbreeding with resident individuals, favoured by the different sex ratios occurring in each form (Klemetsen *et al.*, 2003), should help to maintain high population sizes. In addition, these six populations do not form a closed system; other anadromous trout populations are present in the area that could supply immigrants to these rivers (Ayllón *et al.*, 2006).

### *Genetic guidelines for management*

Hypervariable genetic markers like microsatellites nowadays provide a very useful tool for the conservation and management of populations. For example, they can assist us in deciding whether enhancement of natural populations is necessary. Although enhancement is a common practise in brown trout populations in Europe, such interventions are not recommended unless strictly necessary, as they can cause serious changes in locally adapted populations (Ryman, Utter & Laikre, 1995). These changes include extinction of local populations, introduction of domestic genetically depauperate strains, and reduction of genetic polymorphism (Ryman *et al.*, 1995; Poteaux, Beaudou & Berrebi, 1998). However, even when introductions follow proper genetic guidelines, domesticated stocks of *S. trutta* exhibit low survival rates in the marine habitat (Ruzzante *et al.*, 2004) and, consequently, anadromous systems are less affected by introgression than resident populations (Utter, 2000). In particular, introductions carried out in this area in the past have not been successful (Martínez *et al.*, 1993; Arias *et al.*, 1995). Given the temporal stability observed, we would not recommend supportive breeding programmes within these populations. Nevertheless, in the case that such interventions are needed in the future, it is

important to notice that except for the Ulla and Tambre, all rivers are genetically different. Because of this, and to preserve these different gene pools, separate stocks obtained from each river should be used.

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**Appendix** Microsatellite allele frequencies observed in each population

| SSOSL311 | U97 | U03 | T97 | T03 | Md97 | Md03 | Eu97 | Eu03 | Ln97 | Ln03 | Ms97 | Ms03 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 118 | 0.038 | 0.062 | | 0.023 | 0.098 | 0.079 | 0.112 | 0.175 | 0.065 | 0.1 | 0.16 | 0.141 |
| 122 | | | 0.071 | 0.035 | | | 0.013 | | | | | |
| 126 | | | | | | | | | | | 0.02 | |
| 128 | | | | | | | | | | | | 0.013 |
| 130 | | | 0.012 | | 0.037 | 0.026 | 0.025 | 0.025 | 0.016 | 0.025 | 0.04 | |
| 132 | | | 0.012 | 0.012 | 0.037 | 0.039 | 0.013 | | 0.016 | | | 0.09 |
| 134 | 0.288 | 0.312 | 0.238 | 0.256 | 0.073 | 0.039 | 0.087 | 0.087 | 0.194 | 0.162 | 0.1 | 0.064 |
| 136 | 0.25 | 0.203 | 0.214 | 0.302 | 0.183 | 0.237 | 0.287 | 0.112 | 0.29 | 0.25 | 0.34 | 0.423 |
| 138 | 0.115 | 0.109 | 0.131 | 0.058 | 0.195 | 0.105 | 0.213 | 0.213 | 0.016 | 0.05 | 0.04 | 0.038 |
| 140 | | 0.047 | | 0.047 | 0.061 | 0.118 | 0.062 | 0.188 | 0.097 | 0.151 | 0.1 | 0.077 |

**Appendix** (*Continued*)

| SSOSL311 | U97 | U03 | T97 | T03 | Md97 | Md03 | Eu97 | Eu03 | Ln97 | Ln03 | Ms97 | Ms03 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 142 | 0.077 | 0.109 | 0.167 | 0.081 | 0.061 | 0.118 | 0.075 | 0.075 | 0.081 | 0.15 | 0.04 | 0.013 |
| 144 | | 0.016 | 0.024 | | 0.049 | 0.026 | | | | | | 0.013 |
| 146 | 0.019 | 0.031 | 0.036 | 0.035 | 0.098 | 0.092 | 0.062 | 0.075 | 0.016 | 0.013 | 0.08 | 0.064 |
| 148 | | 0.016 | | 0.012 | | | | | | | | |
| 150 | 0.115 | 0.031 | 0.071 | 0.07 | 0.049 | 0.066 | 0.025 | 0.038 | | 0.013 | 0.04 | 0.026 |
| 152 | | | 0.024 | 0.035 | | 0.013 | | | 0.097 | 0.038 | | 0.013 |
| 154 | | 0.016 | | 0.012 | 0.024 | 0.026 | 0.013 | 0.013 | 0.048 | 0.025 | | |
| 156 | | | | 0.023 | 0.012 | | | | | | | |
| 162 | | | | | 0.012 | 0.013 | | | 0.016 | | | |
| 166 | | | | | 0.012 | | 0.013 | | 0.032 | 0.025 | 0.04 | 0.026 |
| 168 | 0.077 | 0.016 | | | | | | | | | | |
| 170 | 0.019 | 0.016 | | | | | | | 0.016 | | | |
| 172 | | 0.016 | | | | | | | | | | |
| *N* | 26 | 32 | 42 | 43 | 41 | 38 | 40 | 40 | 31 | 40 | 25 | 39 |
| *NA* | 9 | 14 | 11 | 14 | 15 | 14 | 13 | 10 | 14 | 12 | 11 | 13 |
| $H_O$ | 0.73 | 0.91 | 0.81 | 0.70 | 0.85 | 0.82 | 0.78 | 0.80 | 0.81 | 0.93 | 0.96 | 0.79 |
| $H_E$ | 0.83 | 0.84 | 0.85 | 0.83 | 0.90 | 0.89 | 0.85 | 0.87 | 0.86 | 0.86 | 0.84 | 0.79 |
| Ssa197 | | | | | | | | | | | | |
| 117 | | 0.049 | | 0.024 | 0.024 | 0.013 | 0.1 | 0.256 | 0.066 | 0.014 | 0.2 | 0.149 |
| 121 | | | | 0.012 | | | | | | | | |
| 125 | | | | | | | | | 0.016 | | | 0.014 |
| 129 | 0.077 | 0.098 | 0.131 | 0.084 | 0.11 | 0.079 | 0.112 | 0.085 | 0.164 | 0.243 | 0.08 | 0.27 |
| 133 | 0.058 | 0.098 | 0.048 | 0.036 | 0.037 | 0.092 | 0.138 | 0.134 | 0.066 | 0.135 | 0.04 | |
| 137 | 0.019 | | | 0.012 | 0.024 | 0.066 | | 0.024 | | 0.014 | 0.04 | 0.041 |
| 141 | 0.135 | 0.066 | 0.095 | 0.12 | 0.037 | 0.026 | 0.05 | 0.037 | 0.066 | 0.081 | 0.04 | 0.041 |
| 145 | 0.038 | | 0.083 | 0.036 | 0.024 | 0.039 | 0.075 | 0.024 | | | 0.12 | 0.108 |
| 149 | 0.173 | 0.131 | 0.274 | 0.349 | 0.11 | 0.118 | 0.125 | 0.085 | 0.131 | 0.081 | | 0.027 |
| 153 | 0.096 | 0.197 | 0.107 | 0.096 | 0.159 | 0.184 | 0.062 | 0.098 | 0.131 | 0.081 | 0.2 | 0.176 |
| 157 | 0.096 | 0.148 | 0.048 | 0.06 | 0.183 | 0.237 | 0.175 | 0.134 | 0.098 | 0.122 | 0.06 | 0.027 |
| 161 | 0.096 | 0.098 | 0.036 | 0.036 | 0.171 | 0.118 | 0.138 | 0.098 | 0.082 | 0.122 | 0.12 | 0.068 |
| 165 | 0.115 | 0.033 | 0.107 | 0.084 | 0.037 | 0.013 | 0.013 | | 0.016 | 0.027 | 0.08 | 0.054 |
| 169 | 0.058 | 0.049 | | 0.024 | 0.024 | | 0.013 | 0.012 | 0.131 | 0.054 | 0.02 | 0.027 |
| 173 | 0.019 | | 0.012 | 0.012 | 0.049 | 0.013 | | | | | | |
| 177 | 0.019 | 0.033 | 0.06 | | | | | 0.012 | 0.016 | 0.027 | | |
| 181 | | | | | | | | | 0.016 | | | |
| 185 | | | | | 0.012 | | | | | | | |
| 189 | | | | 0.012 | | | | | | | | |
| *N* | 26 | 31 | 42 | 42 | 41 | 38 | 40 | 41 | 31 | 37 | 25 | 37 |
| *NA* | 13 | 11 | 11 | 15 | 13 | 12 | 11 | 12 | 13 | 12 | 11 | 12 |
| $H_O$ | 0.88 | 0.97 | 0.83 | 0.83 | 0.93 | 0.79 | 0.83 | 0.90 | 0.93 | 0.84 | 0.96 | 0.92 |
| $H_E$ | 0.91 | 0.89 | 0.87 | 0.85 | 0.89 | 0.87 | 0.89 | 0.87 | 0.90 | 0.88 | 0.89 | 0.86 |
| SSOSL417 | | | | | | | | | | | | |
| 167 | 0.082 | 0.079 | 0.048 | 0.025 | | | | 0.013 | | | | |
| 169 | | 0.016 | | | | | | | | | | |
| 171 | | 0.016 | | | | | | | | | | |
| 173 | | | | | | | 0.013 | | | | | 0.013 |
| 175 | 0.041 | 0.016 | | | | | | | | | | |
| 177 | 0.184 | 0.095 | 0.071 | 0.1 | 0.024 | 0.039 | 0.013 | 0.013 | 0.018 | 0.013 | 0.04 | |
| 179 | | | | 0.025 | | 0.013 | | | | 0.038 | | |
| 181 | 0.082 | 0.063 | 0.012 | 0.038 | 0.195 | 0.053 | 0.2 | 0.1 | 0.055 | 0.038 | 0.06 | 0.051 |
| 183 | 0.02 | 0.016 | 0.095 | 0.1 | 0.037 | | 0.038 | 0.038 | | 0.038 | 0.14 | 0.077 |
| 185 | 0.02 | 0.032 | 0.012 | 0.013 | | 0.013 | | 0.038 | 0.018 | 0.025 | 0.02 | 0.013 |
| 187 | 0.143 | 0.079 | 0.107 | 0.087 | 0.122 | 0.276 | 0.062 | 0.138 | 0.2 | 0.2 | 0.32 | 0.179 |
| 189 | 0.02 | 0.032 | 0.012 | 0.038 | 0.195 | 0.158 | 0.112 | 0.025 | 0.182 | 0.175 | 0.2 | 0.321 |
| 191 | 0.02 | 0.032 | 0.012 | 0.013 | 0.024 | 0.066 | 0.062 | 0.025 | 0.055 | | | 0.038 |
| 193 | 0.02 | | | | 0.037 | | | 0.013 | 0.036 | 0.025 | 0.04 | 0.038 |

**Appendix** (*Continued*)

| SSOSL311 | U97 | U03 | T97 | T03 | Md97 | Md03 | Eu97 | Eu03 | Ln97 | Ln03 | Ms97 | Ms03 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 195 | | 0.032 | | | 0.024 | 0.026 | 0.15 | 0.05 | 0.073 | 0.038 | | 0.013 |
| 197 | 0.02 | 0.032 | 0.095 | | 0.11 | 0.039 | 0.125 | 0.225 | 0.091 | 0.112 | 0.02 | |
| 199 | 0.122 | 0.032 | 0.179 | 0.125 | 0.024 | 0.039 | 0.038 | 0.025 | | 0.087 | | 0.013 |
| 201 | 0.041 | | 0.071 | 0.05 | 0.037 | 0.013 | 0.025 | 0.087 | 0.018 | | 0.02 | 0.013 |
| 203 | 0.02 | 0.032 | 0.012 | 0.075 | | 0.066 | 0.013 | | 0.055 | | | |
| 205 | 0.041 | 0.048 | 0.036 | 0.05 | 0.024 | | 0.013 | | 0.018 | 0.013 | 0.04 | 0.077 |
| 207 | 0.041 | 0.095 | | 0.062 | | | 0.013 | 0.038 | 0.036 | 0.025 | | 0.013 |
| 209 | | 0.032 | 0.048 | 0.05 | | | | | 0.018 | | | |
| 211 | | 0.063 | | 0.038 | | | | | 0.036 | 0.038 | 0.04 | 0.038 |
| 213 | | 0.016 | | | | | | | | 0.013 | 0.02 | 0.013 |
| 215 | | 0.063 | 0.048 | 0.025 | | 0.013 | | | | 0.038 | 0.04 | 0.038 |
| 217 | | | 0.012 | 0.013 | | | | | | | | 0.026 |
| 219 | | 0.016 | | | | | 0.013 | | | 0.025 | | |
| 221 | | | | | 0.012 | 0.013 | | | 0.018 | | | |
| 223 | | | | | | | | 0.013 | 0.055 | 0.025 | | |
| 225 | | 0.032 | 0.012 | | 0.012 | | | | | | | |
| 227 | | | 0.024 | | | 0.013 | | 0.013 | | | | |
| 229 | 0.02 | | 0.036 | | 0.024 | 0.026 | 0.025 | 0.087 | | | | |
| 231 | | | | | 0.012 | | 0.013 | | | | | |
| 233 | | | | | 0.012 | 0.039 | 0.038 | 0.013 | | | | |
| 235 | | | | | | | 0.013 | 0.025 | | | | |
| 237 | | | 0.012 | | | | | | | | | |
| 239 | | | 0.012 | 0.013 | 0.012 | 0.026 | | | | | | |
| 241 | 0.041 | | | 0.025 | 0.024 | 0.026 | | | | 0.013 | | |
| 243 | | 0.016 | | 0.025 | 0.012 | | 0.025 | | | | | 0.013 |
| 245 | | | 0.036 | 0.013 | 0.012 | | | | | | | |
| 247 | | | | | 0.012 | 0.026 | | 0.025 | 0.018 | 0.025 | | |
| 249 | | | | | | 0.013 | | | | | | |
| 251 | | 0.016 | | | | | | | | | | 0.013 |
| 253 | 0.02 | | | | | | | | | | | |
| *N* | 25 | 32 | 42 | 40 | 41 | 38 | 40 | 40 | 28 | 40 | 25 | 39 |
| *NA* | 19 | 25 | 22 | 22 | 22 | 21 | 20 | 20 | 18 | 20 | 13 | 19 |
| $H_O$ | 0.92 | 0.94 | 0.98 | 0.98 | 0.85 | 0.89 | 0.88 | 0.93 | 0.89 | 0.88 | 0.88 | 0.87 |
| $H_E$ | 0.93 | 0.96 | 0.93 | 0.94 | 0.90 | 0.89 | 0.91 | 0.91 | 0.91 | 0.91 | 0.84 | 0.85 |
| SS4 | | | | | | | | | | | | |
| 174 | 0.42 | 0.359 | 0.333 | 0.321 | 0.329 | 0.237 | 0.287 | 0.256 | 0.228 | 0.304 | 0.4 | 0.324 |
| 176 | 0.04 | 0.078 | 0.024 | 0.024 | 0.061 | 0.105 | 0.038 | 0.122 | 0.14 | 0.165 | 0.08 | 0.203 |
| 178 | | | | | 0.024 | 0.026 | 0.025 | 0.012 | | | | |
| 180 | 0.14 | 0.062 | 0.167 | 0.107 | 0.061 | | 0.162 | 0.146 | 0.158 | 0.114 | 0.18 | 0.176 |
| 182 | | 0.047 | 0.024 | | 0.024 | 0.145 | 0.062 | 0.024 | 0.018 | | | |
| 184 | 0.1 | 0.219 | 0.202 | 0.274 | 0.232 | 0.171 | 0.125 | 0.171 | 0.07 | 0.089 | 0.04 | 0.081 |
| 186 | | | | | | | 0.025 | 0.037 | 0.035 | 0.025 | 0.02 | 0.014 |
| 188 | | 0.031 | | 0.048 | 0.073 | 0.105 | 0.138 | 0.098 | 0.018 | 0.063 | | |
| 190 | 0.02 | | | 0.012 | | | | | | | | |
| 192 | 0.08 | 0.031 | 0.083 | 0.048 | 0.085 | 0.053 | 0.05 | 0.098 | 0.175 | 0.076 | 0.24 | 0.054 |
| 194 | 0.08 | 0.047 | 0.012 | 0.06 | 0.061 | 0.066 | 0.062 | | 0.053 | 0.038 | | 0.027 |
| 196 | 0.06 | 0.047 | 0.071 | 0.071 | 0.024 | 0.026 | 0.025 | 0.012 | 0.053 | 0.025 | 0.04 | 0.095 |
| 198 | | | 0.048 | 0.012 | 0.012 | 0.039 | | | | 0.025 | | |
| 202 | 0.06 | 0.062 | 0.012 | 0.024 | 0.012 | | | 0.012 | 0.053 | 0.038 | | |
| 206 | | | 0.012 | | | | | | | | | |
| 214 | | | 0.012 | | | | | 0.012 | | | | |
| 216 | | | | | | 0.026 | | | | | | |
| 218 | | 0.016 | | | | | | | | | | |
| 222 | | | | | | | | | | 0.025 | | 0.027 |
| 224 | | | | | | | | | | 0.013 | | |
| *N* | 25 | 32 | 42 | 42 | 41 | 38 | 40 | 41 | 29 | 40 | 25 | 37 |

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**Appendix** (*Continued*)

| SSOSL311 | U97 | U03 | T97 | T03 | Md97 | Md03 | Eu97 | Eu03 | Ln97 | Ln03 | Ms97 | Ms03 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *NA* | 9 | 12 | 12 | 11 | 12 | 11 | 11 | 12 | 11 | 13 | 7 | 9 |
| $H_O$ | 0.84 | 0.78 | 0.95 | 0.76 | 0.80 | 0.82 | 0.90 | 0.83 | 0.96 | 0.90 | 0.80 | 0.89 |
| $H_E$ | 0.79 | 0.81 | 0.81 | 0.81 | 0.82 | 0.87 | 0.85 | 0.86 | 0.87 | 0.86 | 0.75 | 0.81 |
| SSOSL85 | | | | | | | | | | | | |
| 175 | 0.019 | | | 0.024 | 0.024 | 0.013 | 0.05 | 0.013 | 0.016 | | | 0.013 |
| 177 | 0.058 | 0.017 | | 0.024 | 0.073 | 0.092 | 0.075 | 0.09 | 0.049 | 0.038 | 0.02 | |
| 181 | 0.019 | 0.017 | 0.012 | 0.012 | | | 0.013 | 0.026 | | 0.013 | | |
| 183 | 0.019 | | 0.048 | 0.071 | | 0.066 | 0.038 | 0.103 | 0.197 | 0.19 | 0.02 | 0.065 |
| 185 | 0.038 | 0.068 | 0.071 | 0.047 | 0.061 | | 0.05 | 0.051 | 0.066 | 0.025 | 0.08 | 0.143 |
| 187 | 0.019 | 0.102 | 0.024 | 0.094 | 0.159 | 0.184 | 0.213 | 0.051 | 0.098 | 0.089 | 0.24 | 0.117 |
| 189 | 0.115 | 0.170 | 0.119 | 0.153 | 0.037 | 0.053 | 0.075 | 0.064 | 0.131 | 0.139 | 0.08 | 0.091 |
| 191 | 0.135 | 0.119 | 0.214 | 0.094 | 0.244 | 0.118 | 0.15 | 0.244 | 0.246 | 0.177 | 0.12 | 0.221 |
| 193 | 0.115 | 0.068 | 0.143 | 0.165 | 0.049 | 0.053 | 0.075 | 0.128 | | 0.114 | 0.04 | 0.013 |
| 195 | 0.115 | 0.136 | 0.048 | 0.071 | 0.195 | 0.276 | 0.125 | 0.09 | 0.098 | 0.025 | 0.26 | 0.208 |
| 197 | 0.173 | 0.169 | 0.19 | 0.094 | 0.085 | 0.105 | 0.062 | 0.077 | | 0.076 | 0.04 | 0.026 |
| 199 | 0.115 | 0.068 | 0.036 | 0.094 | 0.049 | 0.039 | 0.025 | 0.051 | 0.033 | 0.089 | 0.1 | 0.104 |
| 201 | 0.058 | | 0.095 | 0.059 | 0.012 | | | | 0.049 | 0.025 | | |
| 203 | | 0.068 | | | | | | | | | | |
| 205 | | | | | | | 0.05 | 0.013 | 0.016 | | | |
| 207 | | | | | 0.012 | | | | | | | |
| *N* | 26 | 30 | 42 | 43 | 41 | 38 | 40 | 39 | 31 | 40 | 25 | 39 |
| *NA* | 13 | 11 | 11 | 13 | 12 | 10 | 13 | 13 | 10 | 12 | 10 | 10 |
| $H_O$ | 0.69 | 0.86 | 0.60 | 0.83 | 0.78 | 0.76 | 0.63 | 0.63 | 0.73 | 0.79 | 0.80 | 0.82 |
| $H_E$ | 0.91 | 0.90 | 0.88* | 0.91 | 0.87 | 0.86 | 0.90* | 0.89* | 0.87 | 0.89 | 0.85 | 0.86 |

*N*, number of individuals; *NA*, number of alleles; $H_O$, observed heterozygotes; $H_E$, expected heterozygotes.
*Departure from Hardy–Weinberg equilibrium.

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