Deliverable Report

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Deliverable Title	Genetic characterization of different meagre captive broodstocks and evaluation of available variability			
WP No:	2		**WP Lead beneficiary:**	P3. IRTA
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Task No:	2.1		**Task Lead beneficiary:**	P2. FCPCT
Task Title:	Evaluation of the genetic variation in captive meagre broodstocks			
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SUMMARY

A total of 432 meagre (*Argyrosomus regius*) breeders were sampled from broodstocks in 13 centers from 7 countries, and studied using 18 microsatellite markers (STRI & SRTS). The arithmetic and weighted means of allele number were 3.7 and 4.13, respectively. A positive relationship between number of alleles and population size was found. As heterozygosities, both arithmetic and weighted estimates were the same (0.48) for observed heterozygosity, while the values were similar for expected heterozygosity, 0.48 and 0.49, respectively. The captive European populations of meagre had mean number of alleles and observed heterozygosities that were lower than in wild populations (around 3 times and 18% lower, respectively). These numbers of alleles and heterozygosities indicated that the variation of the populations is very similar to wild populations or has declined. Essentially these broodstocks have adequate genetic variation for a breeding program, but the decline in variability and low mean number of alleles of some broodstocks clearly indicated that these broodstocks should be enlarged with new families and stocks to ensure an optimal base population for a breeding program. Estimates of effective sizes (*Ne*) of each population ranged between 82 and 115, with a mean of 87, and was higher than the minimum recommended to minimize inbreeding depression (50), but lower than the minimum suggested for maintaining sufficient evolution capacity (500).

Therefore, the estimates of effective sizes (*Ne*) also indicated that the broodstocks probably originated from crosses between sufficient families, but the number of families in many broodstocks was at the lower limit for a base population, which again indicated that these broodstocks should be enlarged with new families and stocks to ensure an optimal base population for a breeding program. The AMOVA analysis revealed that 18.19% of the variation was found among studied populations (Fst), while the remaining 81.82% was located within populations ($P<0.0001$). A Factorial Correspondence Analysis showed two clusters correlated with the geographical distribution of populations (Atlantic and Mediterranean), and a third constituted for TU-01 population from Turkey. The significant Fst reported is indicative of a low genetic flow among captive meagre populations studied, producing a fragmentation of populations and increasing the effect of genetic stochastic processes. This high variation between population, low gene flow and fragmentation can perhaps be explained by the differences in the three groups or populations identified and associated to geographic area.

All together these analyses indicate that generally the variation is adequate in captive broodstocks. However, some broodstocks are questionable as base populations and more in depth analysis would be required to determine the suitability of the broodstock for a breeding program. It is very clear that across all broodstocks there exists more than adequate genetic variation to form a base population. This potential genetic differentiation in quantitative genes and traits would be a magnificent tool for the constitution of the best available base population for a selective breeding program on a European scale, especially because meagre as a species is currently at a clear disadvantage, in terms of its genetic starting point, compared to other important species within European aquaculture.

OBJECTIVE

To evaluate the genetic variation in the available captive broodstocks of meagre held in research centers and SMEs involved in the DIVERSIFY project and stocks held across the aquaculture industry. The results presented were obtained using a microsatellite multiplex and describe the genetic structure within and between meagre populations (number of alleles, observed and expected heterozygocity, allele range, exclusion probability, the Hardy-Weinberg equilibrium test, consanguinity, etc.). The genetic characterization of all these stocks could be used to propose strategies to improve the genetic basis for the domestication of meagre through selection for optimal sustainable culture performance.

INTRODUCTION

Meagre, *Argyrosomus regius* (Asso, 1801), is a marine species belonging to the *Scianidae* family, which is widely distributed in the Mediterranean Sea and Atlantic Ocean from Norway to the Congo (Chao, 1986) and from Iceland to the Gulf of Guinea (Quéro & Vayne, 1987). The species has also been found in the

Western Black Sea, the Sea of Marmara, and the Red Sea from the Suez Canal to the Indian Ocean (Quéro, 1989). Meagre is a pelagic species with a demersal trend (Quéro & Vayne, 1987) that can grow up to 2 meters in length and weigh more than 50 kg. The highest growth rate occurs takes place during the summer and feeding activity decreases substantially when sea temperatures fall below 13-15 °C (FAO, 2012). Adults have been described as following a reproductive migration (FAO, 2012). During mid-April, adults can be found near to the coast and in late May in estuaries for spawning (Quémèner, 2002). From mid-June to late July the adults leave estuaries to feed along the coast remaining in shallow water until early autumn, returning to deep waters during the winter. Meagre is a highly fecund species that commands medium-high market prices and is well-liked by consumers (Jimenez *et al.,* 2005). It presents the added advantage of being a euryhaline species that tolerates a wide range of salinity. Meagre fillets are a high quality product thanks to the species' organoleptic features (Poli *et al.,* 2003).

These characteristics indicate a high potential for aquaculture and meagre has recently been established as a new species for European aquaculture that can contribute to the diversification of the aquaculture industry. The first tests with wild broodstocks were carried out in the South of France and industrial production has gradually grown. The total aquaculture production of meagre in Europe and Egypt was around 15,000 tons in 2012 (**Fig. 1**) (APROMAR, 2013).

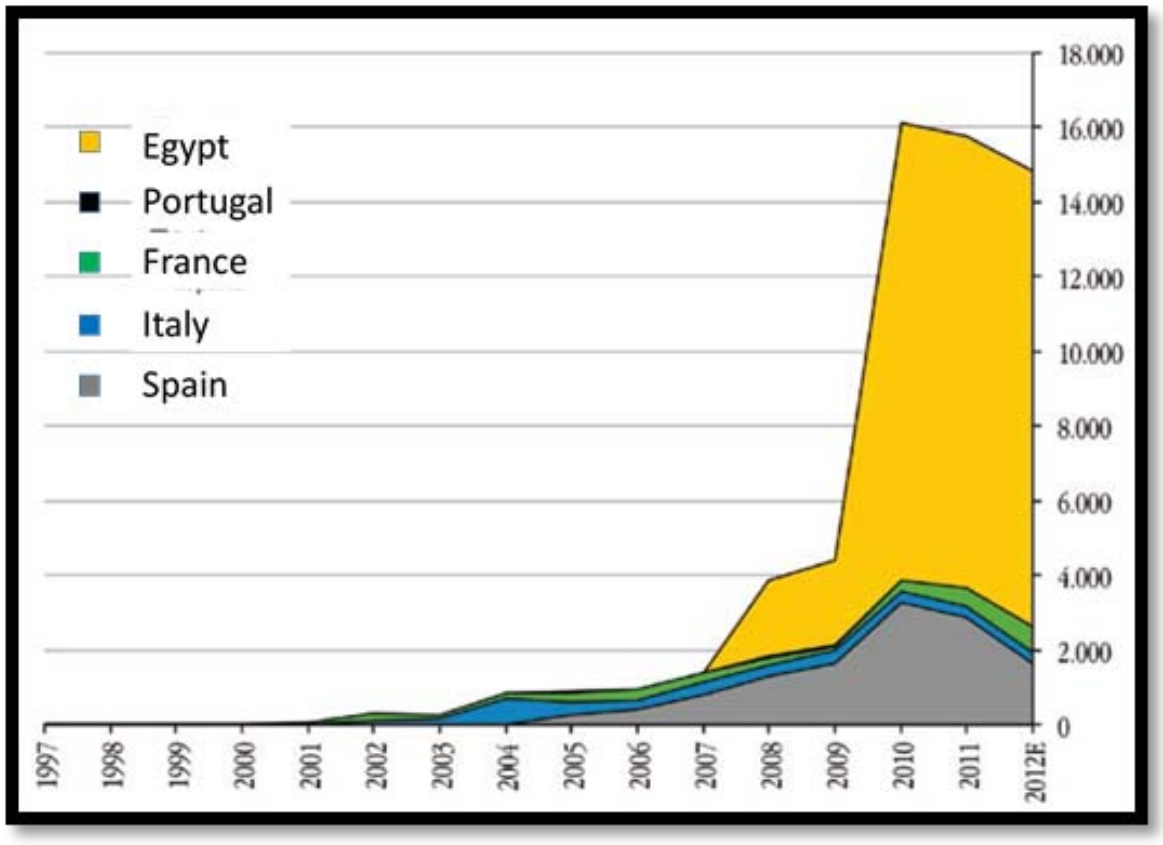

Figure 1. Evolution of meagre aquaculture production in Europe and Egypt from 1997 to 2012.

The potential for aquaculture and production levels of the meagre has promoted studies on all aspects of the life-cycle in captivity in order to understand and improve its culture. These studies have examined maturation (Schuchardt *et al.,* 2007; Mylonas *et al.*, 2013b), spawning (particularly induced with hormone treatments) and egg quality (Grau *et al.,* 2007; Duncan *et al.,* 2008, 2012, 2013; Fernández-Palacios *et al.,* 2009b, 2014, Mylonas *et al*., 2013a,b), developmental morphology and physiology (Jimenez *et al.,* 2005;

Gamsliz & Neke, 2008; Abreu *et al.,* 2009; Fernández-Palacios *et al.,* 2009a), nutrition (Hernández-Cruz *et al.,* 2007; Fernández-Palacios *et al.,* 2009a), larval feeding (Roo *et al.,* 2010), pre-fattening and fattening under various salinities (Tinoco *et al.,* 2009), temperatures (Lavie *et al.,* 2008), facilities (Jimenez *et al.,* 2005), levels of dietary lipids (Chatzifotis *et al.,* 2010) and protein resources from vegetable origin (Estévez *et al.,* 2011).

All these technical and biological characterizations are fundamental for the development of a standardized, industrial activity in meagre, making companies competitive by minimizing costs and / or adding value to their products, through fish differentiation thanks to distinctive quality and production processes. At this point, genetic tools can be introduced in order to improve productivity by selecting traits such as consistent high growth rates through genetic improvement. However, this requires that sound knowledge is needed of several factors, such as: base population variability, fish genealogy, the traits to be selected, genetic parameters, genotype-environment interactions, breeding values or batch replacement (Toro & López-Fanjul in *Genetics and Genomics in Aquaculture*, 2007).

Genetic variation in the base population is essential for developing breeding programs, because genetic variation affects the selection response in the short and long term (Falconer and Mackay, 2001). Wild populations of meagre have been studied by Haffray *et al.* (2012), in terms of genetic differentiation along the Atlantic Ocean and Mediterranean Sea, using red drum (*Sciaenops ocellatus*) microsatellite markers (Renshaw *et al.,* 2006). These authors found an average of 13.2 and 0.57 for number of alleles and observed heterozygosity, respectively. Thus, the authors estimated that the meagre has at least two very distant genetic groups: the Atlantic and the Mediterranean, in a context of six independent spawning areas, where a lower allelic richness and effective sizes of the Mediterranean populations were reported. This structuration was mainly related to interglacial phases of the Quaternary. Haffray *et al.* (2014) used the same multiplex of microsatellites (Panels-A & -B) and reported a similar, but slightly lower, genetic variation in two domestic populations of meagre from France (LPDS and FMD), with mean estimations of 8.5 and 0.52 for number of alleles and observed heterozygosity, respectively, as expected.

Soula *et al.* (2011), described two new microsatellite multiplexes (STRS & STRI), which were set up using 22 specific markers described in meagre (Porta *et al.,* 2010) and 27 microsatellites described in other species of the Scianidae family; 15 markers from *Argyrosomus japonicus* (Archangi *et al.,* 2009), 11 microsatellites from *Sciaenops ocellatus* (Turner *et al.,* 1998; O'Malley *et al.,* 2003) and 1 microsatellite from *Cynoscion acoupa* (Farias *et al.,* 2006). With both multiplexes, these authors estimated genetic parameters for growth traits (weight, length and specific growth rate) at different ages (fingerling and harvest sizes), reporting a low additive genetic variation, due to the close relationship between animals within populations.

The purpose of this study was to determine the genetic variability of meagre in research centres and university institutions in Europe, to describe the status of the bottlenecks identified through the genetic characterization of captive broodstocks, as a first step to starting meagre breeding programs.

MATERIALS AND METHODS

Samples

A total of 432 samples of meagre were collected, thanks to the collaboration of private companies and public institutions (**Fig. 2**) from Spain (SP-01 from IRTA; SP-02; SP-03 from LIMIA; SP-04 from IFAPA; SP-05 from FCPCT) Portugal (PO-01 as IPMA), France (FR-01), Italy (IT-01), Greece (GR-01; GR-02 from HCMR), Cyprus (CY-01; CY-02 from DFMR) and Turkey (TU-01 from EGE University) . Spain was the country with highest number of samples (174), followed by Greece (86), with a mean of 33 samples per institution. Only two localities sent samples from two consecutives generations, GR-01 and GR-02. All broodstocks have been used or acclimated as breeders to produce fingerlings.

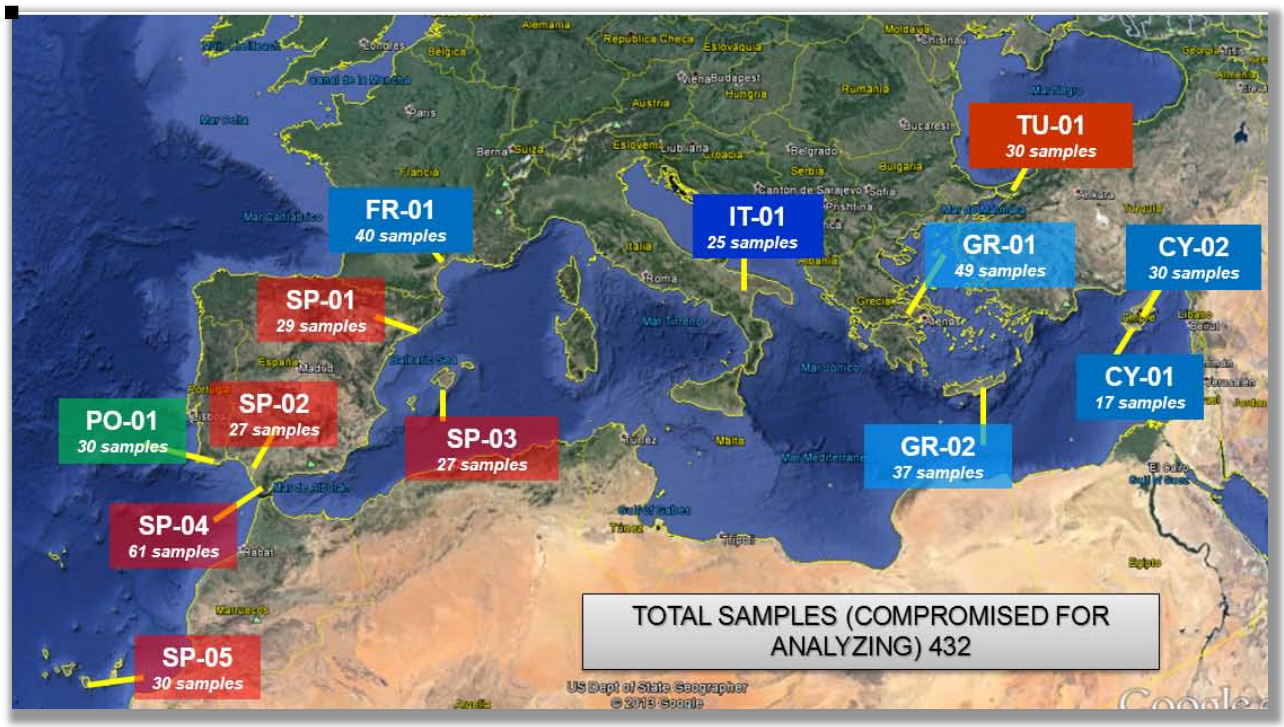

Figure 2 Geographic distribution of meagre studied samples in Europe, from the Canary Islands to Cyprus.

DNA extraction

DNA was extracted from the fin clips using the DNeasy kit (*Qiagen*). Some problematic DNA samples were extracted following the phenol-chloroform method described in Sambrook *et al.* (1989). DNA quality and quantity were determined using a NanoDrop 1000 spectrophotometerv.3.7 (*Thermo Fisher Scientific*). DNA integrity was checked by means of electrophoresis in a 1% agarose gel (8 v/cm) by GelRed™ staining (3X solution), and analysed with Quantity One software (*Bio-Rad Laboratories*), using Lambda Hind III as a molecular weight marker.

DNA quality was also important and when the template DNA was degraded or contained PCR inhibitors, BSA (0.8 µg/ µl) (Bovine Serum Albumin) as adjuvants was required to promote the multiplex PCR reactions.

Microsatellites

The samples were genotyped with 18 markers and combined in 2 multiplex reactions, one composed of 10 interspecific microsatellites (STRI) and the other of 8 specific microsatellites (STRS) (Soula *et al.,* 2011) (**Table 1**).

Table 1 *Loci* names, fluorochromes, redesigned primer sequences, primer concentration and original reference.

Locus	Fluorochrome	Redesigned forward primer sequence (5'→3')	Redesigned reverse primer sequence (5'→3')	C (µM)	Original Reference
Meagre-STRI					
Cacmic14	5' 6-FAM	TGTCCTCACTCCTCTTTTTCTTTC	GTTTAAGGCGCATCTCCAGTCTC	0.02	1*
UBA054	5' 6-FAM	CCTTGTGAGAACATTAATTTGGATG	GTTTCCAAACCCTGATAGATGGATAGTT	0.02	2*
UBA050	5' 6-FAM	GCACAACTGCATCCCTTAGAT	GTTTAGAAGTGAAGACTGCGGACTG	0.05	2*
UBA053	5' VIC	TACTTCCTTCTACCCCTAAGTCTGG	GACTTTCCAGTGTAGCTGTCGTTT	0.05	2*
Soc431	5' VIC	GTGGTAGATGAAAACGTATAAAAGGAG	GTTTCATATATATAGTGTACAGCTCCAGCTTC	0.06	2*
UBA042	5' NED	TTTCTGCCTGACTAGATGTTCTTTC	GATTGTTGCTGGTTTTTCCAAT	0.05	2*
UBA853	5' NED	CAATGCTCAAGTTACAGGAAACC	GTTTGCACTCGTTCACCCTCAC	0.02	2*
UBA005	5' NED	CATCAGGATTGGCAACTAGC	GTTTCCTCCAGGTTTATTCTTCATTGAC	0.03	2*
Soc405	5' PET	AGCCTTTTGTTTAGTTTCCCTCAT	GGGGTGTAGCAGAACCACAC	0.03	2*
UBA006	5' PET	AGCACACGTAATCACACACAGAT	GTTTCCACTAGTGCAAAACGGTGGT	0.03	2*

Locus	Fluorochrome	Redesigned forward primer sequence (5'→3')	Redesigned reverse primer sequence (5'→3')	C (µM)	Original Reference
Meagre-STRS					
GCT15	5' 6-FAM	ATCCGGGCGTTACTACAGTC	GTTTCTCCACACAGTGCTTTTCAGA	0.02	3*
GA16	5' 6-FAM	CTACACAGTCTCTCTCACTCACTCG	GTTTCTGAAACAGCGCAGCATTG	0.02	3*
GA17	5' 6-FAM	CTAGAGAAATTCATCCAGGGAAGTG	GTTTAGAGCAGAGAGTTAGCGGTTGTT	0.015	3*
CA13	5' VIC	TTTTCCTTTTTCAGTAGTCTCCTTG	GTTTATAAGGAGGACGTGAGTTTGGTAG	0.035	3*
GA6	5' NED	GTCTGATGGCGACAGACAGG	GTTTCAGCCCGCTACTTTACCTACAAC	0.02	3*
CA3	5' NED	AAGTGGAGGCTCTTACATGAAAAC	GTGACAAATTGCCTTCTGTTTCTAC	0.03	3*
CA14	5' NED	ACTGAGAGTGAAGGTGGGAAACT	GTGAGTGTCTTTGTTTTTACCAACC	0.03	3*
GA2B	5' PET	AAGTGTGGCGTCATTTCCTCT	GTATTGATGGATAGCAAGTGTCAGA	0.05	3*

C (µM) = Concentration of each primer.
1* Farias *et al.,* 2006
2*Archangi *et al.,* 2009
3*Porta *et al.,* 2010

PCR conditions

Multiplex PCRs were amplified following the recommendations of Soula *et al.* (2011). The PCR conditions consisted of an initial denaturalization at 94°C for 10 min, followed by 30 cycles at 94°C for 30 s, 60°C for 1 min and 65°C for 1 min, with a final extension of 65°C for 60 min. Reactions were carried out in a final volume of 12.5 µl with the following component concentrations: 1X GeneAmp PCR Buffer II (100 mM Tris–HCl pH 8.3, 500Mm KCl) (*Applied Biosystem*®), 3mM $MgCl_2$, 0.2 mM of each dNTP, 0.05U/µl

AmpliTaq Gold DNA polymerase (*Applied Biosystem*®), 10-40 ng of DNA template and 0.01–0.06 µM of each primer.

The samples were amplified with STRI and STRS multiplexes in order to genotype each locus, to test the genetic variability of all broodstocks sampled.

Before running multiplex reactions on an automatic sequencer, an aliquot of amplified products was checked on 2% agarose gel for 30 min (8 v cm^{-1}) to assess the correct amplification of amplicons. Subsequently, 1 µl of amplified products was mixed with 9.75 µl of Hi-Di formamide and 0.25 µl of GeneScan 500LIZ (*Applied Biosystem*®) size standard, and run on an ABI Prism-3730-XL Genetic Analyzer (*Applied Biosystem*®) with 50 cm capillary arrays and POP-7 polymer (*Applied Biosystem*®) (60°C, 2000v, 1500s). Electropherograms and genotypes were evaluated using GeneMapper (v4.0) (*Applied Biosystems, Inc*.) software.

Data analysis

To verify the existence of geographical subdivision of the populations studied, an AMOVA molecular variance analysis was conducted (Excoffier *et al.,* 1992) that takes into account the frequency of the genotypes and the distance between them. With this test, the fixation index indicates how much of the total genetic variability of meagre in Europe is due to the variability between the populations analyzed. Thus, the fixation index is the ratio of the added component to the variance between analyzed populations compared to the total variance of the meagre, considering the latter as a single population. All estimates were carried out using the ARLEQUIN program (Excoffier *et al.*, 2005).

To study the genetic variability parameters (allele number, genotypes, heterozygosities, etc..) within loci and populations, the web version of GENEPOP software was used (Raymond & Rousset, 1995; Rousset, 2008). For the biogeographic analysis or structuration of populations, GENETIX 4.05 version was used (Belkhir *et al*., 1996-2004).

RESULTS

Genetic diversity

In the present study, 13 European breeding populations of captive meagre were analyzed using 18 microsatellite markers (**Table 2**). In the analysis, broodstocks from GR-01 and GR-02 presented samples of two consecutive generations, which were considered separately, to assess the genetic variation between generations within populations.

Table 2 *Loci* and markers equivalence.

LOCUS	MARKER
1	CacMic
2	Soc 405
3	UBA50
4	UBA5
5	UBA54
6	UBA6
7	UBA853
8	UBA42
9	Soc431
10	UBA53
11	CA13
12	CA14
13	CA3
14	GCT15
15	GA16
16	GA17
17	GA6
18	GA2B

The mean number of alleles was 4 for the SP-03 population, and mean heterozygosity observed was 0.56, 9.8% higher than expected heterozygosity (**Table 3**).

Table 3 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in SP-03 population (Spain)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	54	6	0.88889	0.68903	12
2	56	3	0.42857	0.52922	8
3	56	5	0.82143	0.71429	10
4	56	3	0.82143	0.66558	4
5	54	2	0.29630	0.45283	2
6	56	8	0.92857	0.83377	16
7	56	5	0.67857	0.53442	18
8	54	3	0.29630	0.51992	6
9	46	5	0.52174	0.43575	12
11	56	6	1.00000	0.72857	20
12	56	3	0.96429	0.67273	8
13	56	3	0.07143	0.07078	8
15	50	4	0.56000	0.65551	6
16	56	5	0.78571	0.74870	12
17	56	2	0.03571	0.03571	4
18	56	8	1.00000	0.81883	16
Mean	51.667	4.000	0.56105	0.50587	9.111
s.d.	12.793	2.134	0.35707	0.27871	5.666

The mean number of alleles was 5.2 for the SP-01 population, and mean heterozygosity observed was 0.59, 2.2% lower than expected heterozygosity (**Table 4**).

Table 4 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in SP-01 population (Spain)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	58	8	0.82759	0.83061	16
2	58	4	0.72414	0.69389	10
3	58	6	0.89655	0.79734	10
4	58	4	0.65517	0.59528	6
5	58	2	0.37931	0.48699	2
6	58	7	0.68966	0.65275	18
7	58	7	0.82759	0.60980	20
8	56	3	0.17857	0.28636	6
9	58	6	0.75862	0.64489	14
10	46	6	0.39130	0.77585	18
11	56	10	0.96429	0.87662	24
12	54	4	0.29630	0.67365	10
13	56	3	0.42857	0.36039	8
14	56	2	0.17857	0.16558	3
15	28	5	0.78571	0.65608	14
16	56	4	0.60714	0.62857	10
17	56	3	0.17857	0.16818	6
18	52	10	0.76923	0.86350	22
Mean	54.444	5.222	0.58538	0.59813	12.056
s.d.	7.041	2.393	0.25625	0.21548	6.346

The mean number of alleles was 3.4 for the IT-01 population, and mean heterozygosity observed was 0.55, 9.1% higher than expected heterozygosity (**Table 5**).

Table 5 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in IT-01 population (Italy)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	50	5	0.76000	0.67918	12
2	48	3	0.54167	0.43528	10
3	48	3	0.70833	0.61082	8
4	48	3	0.29167	0.26507	4
5	50	2	0.76000	0.50694	2
6	44	5	0.81818	0.72199	14
7	48	4	0.50000	0.41755	20
8	48	2	0.41667	0.38298	6
9	46	4	0.56522	0.61932	10
10	46	3	0.39130	0.57101	18
11	50	5	0.92000	0.70122	20
12	46	4	0.21739	0.46667	10
13	50	3	0.56000	0.42204	8
14	50	2	0.16000	0.15020	3
15	12	3	0.33333	0.62121	10
16	50	4	1.00000	0.67184	10
17	50	2	0.08000	0.07837	4
18	50	5	0.84000	0.72408	8
Mean	46.333	3.444	0.54799	0.50254	9.833
s.d.	8.518	1.066	0.26371	0.18782	5.252

The mean number of alleles was 7.1 for the SP-04 population, and mean heterozygosity observed was 0.53, 11.8% lower than expected heterozygosity (**Table 6**).

Table 6. Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in SP-04 population (Spain)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	118	9	0.76271	0.78502	16
2	108	6	0.48148	0.55192	14
3	100	9	0.80000	0.85253	20
4	104	4	0.30769	0.31759	6
5	118	4	0.45763	0.53412	6
6	98	11	0.79592	0.79992	24
7	94	6	0.85106	0.58705	20
8	88	3	0.27273	0.43913	6
9	110	7	0.56364	0.63336	12
10	78	7	0.20513	0.59374	28
11	100	10	0.78000	0.81010	22
12	92	7	0.45652	0.61156	19
13	104	7	0.59615	0.49739	14
14	104	3	0.32692	0.27913	6
15	98	9	0.40816	0.76836	20
16	106	8	0.66038	0.73513	32
17	104	2	0.01923	0.01923	8
18	104	15	0.84615	0.90795	34
Mean	101.556	7.056	0.53286	0.59573	17.056
s.d.	9.488	3.153	0.24000	0.22208	8.657

The mean number of alleles was 6.3 for the SP-02 population, and mean heterozygosity observed was 0.55, 14.2% lower than expected heterozygosity (**Table 7**).

Table 7 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in SP-02 population (Spain)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	44	8	0.77273	0.78224	16
2	48	5	0.58333	0.60993	12
3	48	9	0.79167	0.82890	24
4	48	3	0.41667	0.38209	4
5	48	4	0.41667	0.52926	6
6	48	10	0.75000	0.79965	24
7	46	7	0.91304	0.70242	20
8	46	3	0.26087	0.57488	6
9	28	4	0.35714	0.71693	6
10	38	8	0.52632	0.79801	34
11	48	9	0.75000	0.82801	22
12	48	8	0.54167	0.74645	20
13	48	5	0.45833	0.39007	8
14	48	2	0.41667	0.33688	6
15	48	6	0.45833	0.66667	12
16	48	6	0.45833	0.56028	14
17	48	3	0.08333	0.08245	11
18	48	14	0.91667	0.92996	34
Mean	45.889	6.333	0.54843	0.62584	15.500
s.d.	4.965	3.000	0.22023	0.21076	9.155

The mean number of alleles was 2.8 for the CY-02 population, and mean heterozygosity observed was 0.47, 3% higher than expected heterozygosity (**Table 8**).

Table 8 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in CY-02 population (Cyprus)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	28	2	0.14286	0.47619	4
2	38	4	0.78947	0.67710	24
3	38	3	0.68421	0.56188	6
4	30	2	0.80000	0.51494	4
5	22	3	0.45455	0.64502	6
6	34	2	0.41176	0.45098	6
7	38	5	0.52632	0.55903	18
8	38	3	0.57895	0.43670	6
9	22	3	0.27273	0.25541	16
10	36	4	0.33333	0.61587	16
11	40	3	0.85000	0.59487	8
12	40	3	0.70000	0.65256	8
13	40	2	0.05000	0.05000	6
15	36	4	0.72222	0.55714	10
16	40	3	0.85000	0.67821	10
18	36	2	0.27778	0.47460	2
Mean	35.333	2.778	0.46912	0.45558	8.333
s.d.	5.774	1.030	0.28873	0.22049	6.263

The mean number of alleles was 3.1 for the CY-01 population, and mean heterozygosity observed was 0.49, 2.8% lower than expected heterozygosity (**Table 9**).

Table 9 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in CY-01 population (Cyprus)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	26	4	0.61538	0.76615	8
2	26	2	0.46154	0.51692	6
3	32	4	0.75000	0.62702	16
4	24	3	0.25000	0.23551	4
5	30	2	0.66667	0.51494	2
6	28	2	0.14286	0.13757	2
7	24	4	0.75000	0.54348	18
8	30	2	0.60000	0.43448	6
9	18	4	0.77778	0.75817	10
10	28	3	0.71429	0.55291	18
11	20	5	0.40000	0.51053	22
12	18	3	0.55556	0.69935	10
13	22	4	0.36364	0.46320	10
14	34	3	0.47059	0.60428	6
15	16	3	0.12500	0.67500	4
16	20	2	0.60000	0.44211	10
18	18	4	0.66667	0.68627	14
Mean	24.000	3.056	0.49500	0.50933	9.222
s.d.	5.375	1.026	0.22988	0.20188	6.079

The mean number of alleles was 2.6 for the TU-01 population, and mean heterozygosity observed was 0.37, 0.8% lower than expected heterozygosity (**Table 10**).

Table 10 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in TU-01 population (Turkey)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	60	4	0.03333	0.12938	6
2	60	2	0.36667	0.30452	6
3	60	4	0.53333	0.54350	8
5	54	3	0.18519	0.43396	4
6	60	5	0.96667	0.80395	10
7	60	3	0.96667	0.52373	18
8	52	2	0.23077	0.48265	6
9	54	2	0.40741	0.49825	4
11	60	2	0.60000	0.47232	8
12	58	2	0.10345	0.49909	2
13	60	3	0.90000	0.51808	12
14	60	2	0.30000	0.38136	3
15	44	3	0.04545	0.21247	6
16	60	3	0.53333	0.48418	10
18	60	3	0.53333	0.47232	16
Mean	57.889	2.556	0.37253	0.37554	6.611
s.d.	4.188	1.066	0.32370	0.21668	5.024

The mean number of alleles was 2.8 for the SP-05 population, and mean heterozygosity observed was 0.52, 16.4% higher than expected heterozygosity (**Table 11**).

Table 11 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in SP-05 population (Spain)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	60	3	0.40000	0.33672	4
2	58	3	0.93103	0.61645	10
3	58	3	0.96552	0.62613	10
4	58	2	0.44828	0.47005	4
5	60	2	0.00000	0.18305	2
6	58	3	0.44828	0.36116	8
7	58	2	0.34483	0.29038	12
8	58	2	0.44828	0.47005	6
9	60	2	0.40000	0.32542	10
10	52	2	0.38462	0.49774	4
11	60	4	0.96667	0.73503	20
12	60	3	0.96667	0.61525	10
13	60	2	0.06667	0.12655	4
15	14	5	0.57143	0.72527	16
16	60	5	0.80000	0.64633	10
17	60	2	0.40000	0.32542	4
18	60	5	0.80000	0.68136	20
Mean	56.333	2.833	0.51901	0.44624	8.556
s.d.	10.440	1.167	0.31445	0.20997	5.610

The mean number of alleles was 2.6 for the GR-01-F1 population, and mean heterozygosity observed was 0.45, 0.8% higher than expected heterozygosity (**Table 12**).

Table 12 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in GR-01-F1 population (Greece)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	28	3	0.64286	0.59524	8
2	28	3	0.71429	0.62698	10
3	28	3	0.50000	0.56878	8
4	26	2	0.38462	0.50769	4
5	26	2	0.23077	0.40923	2
6	28	2	0.42857	0.34921	6
7	28	3	1.00000	0.58201	18
8	28	2	0.28571	0.25397	6
9	26	3	0.53846	0.42769	10
10	26	3	0.30769	0.55077	12
11	28	4	0.42857	0.58466	20
12	16	2	0.12500	0.45833	8
13	28	3	0.92857	0.57407	8
15	28	3	0.42857	0.36243	4
16	28	4	0.85714	0.75397	10
18	28	2	0.35714	0.49471	2
Mean	26.889	2.556	0.45322	0.44999	7.556
s.d.	2.767	0.831	0.28232	0.19562	5.315

The mean number of alleles was 3 for the GR-01-F2 population, with a mean heterozygosity observed of 0.39, 3.8% lower than expected heterozygosity (**Table 13**). A comparison of the two consecutive generations of GR-01 population shows that F2 reported a lower observed heterozygosity than F1, as normally expected (15% lower). By contrast, the number of alleles in F2 was slightly higher than F1 (17%).

Table 13 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in GR-01-F2 population (Greece)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	52	5	0.57692	0.54299	8
2	50	3	0.72000	0.55429	10
3	50	4	0.68000	0.51592	10
4	50	3	0.36000	0.36490	6
5	44	3	0.22727	0.66490	6
6	48	3	0.29167	0.25975	8
7	52	4	0.50000	0.45400	20
8	52	3	0.11538	0.18175	12
9	38	3	0.31579	0.28023	14
10	34	2	0.17647	0.50802	4
11	68	4	0.41176	0.45698	20
12	54	2	0.07407	0.49196	8
13	68	3	0.97059	0.54917	8
15	66	3	0.24242	0.22191	4
16	68	4	0.79412	0.72388	10
18	56	3	0.53571	0.47857	20
Mean	54.778	3.000	0.38846	0.40273	9.333
s.d.	10.459	1.000	0.27401	0.20027	5.925

The mean number of alleles was 3.4 for the GR-02-F1 population, and mean heterozygosity observed was 0.47, 7.2% lower than expected heterozygosity (**Table 14**).

Table 14 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in GR-02-F1 population (Greece)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	40	6	0.70000	0.70128	12
2	38	3	0.26316	0.24324	10
3	38	3	0.57895	0.56188	8
4	38	3	0.31579	0.55619	4
5	36	2	0.50000	0.51270	2
6	38	4	0.47368	0.66714	18
7	38	3	0.78947	0.51067	18
8	38	2	0.31579	0.50071	6
9	36	3	0.38889	0.33810	10
10	38	2	0.10526	0.27312	4
11	32	5	0.68750	0.75605	20
12	22	5	0.54545	0.77922	12
13	32	2	0.68750	0.46573	8
14	32	2	0.06250	0.06250	3
15	32	3	0.62500	0.65927	4
16	32	4	0.75000	0.68347	14
17	32	2	0.12500	0.12097	4
18	30	8	0.60000	0.73333	22
Mean	34.556	3.444	0.47300	0.50698	9.944
s.d.	4.310	1.606	0.22389	0.21235	6.133

The mean number of alleles was 2.4 for the GR-02-F2 population, and mean heterozygosity observed was 0.49, 12% higher than expected heterozygosity (**Table 15**). A comparison of the two consecutive generations of the same population shows that F2 unexpectedly reported a higher observed heterozygosity than F1 (4.2% higher). By contrast, the number of alleles in F2 was lower than F1 (40%), as expected.

Table 15 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in GR-02-F2 population (Greece)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	28	4	0.85714	0.75926	12
2	32	3	0.25000	0.28427	10
3	32	2	0.25000	0.22581	6
4	30	2	0.46667	0.50805	4
5	32	2	0.43750	0.49798	2
6	30	4	0.73333	0.68276	18
7	32	2	1.00000	0.51613	18
8	32	2	0.06250	0.51411	6
9	26	3	0.61538	0.64308	10
10	34	2	0.23529	0.21390	4
11	28	3	0.57143	0.50000	20
13	32	2	1.00000	0.51613	8
15	24	4	1.00000	0.68841	6
16	32	3	0.56250	0.59879	10
18	24	4	0.83333	0.77174	18
Mean	28.111	2.444	0.49306	0.44002	8.556
s.d.	7.438	1.117	0.35008	0.25053	6.282

The mean number of alleles was 5.3 for the FR-01 population, and mean heterozygosity observed was 0.43, 17% lower than expected heterozygosity (**Table 16**).

Table 16 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in FR-01 population (France)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	56	7	0.85714	0.77078	14
2	56	4	0.67857	0.54026	10
3	58	8	0.58621	0.73382	26
4	56	3	0.46429	0.53247	4
5	50	6	0.40000	0.56980	12
6	60	8	0.63333	0.58814	20
7	60	5	0.23333	0.21921	18
8	58	4	0.13793	0.50393	9
9	22	6	0.27273	0.73160	37
10	48	6	0.41667	0.73050	18
11	80	8	0.80000	0.75570	20
12	70	5	0.57143	0.57888	14
13	78	4	0.28205	0.25175	8
14	78	3	0.05128	0.05095	6
16	80	4	0.72500	0.73323	10
17	80	2	0.02500	0.02500	4
18	78	12	0.66667	0.81785	34
Mean	59.556	5.333	0.43342	0.50744	14.667
s.d.	19.928	2.560	0.26614	0.26811	9.792

The mean number of alleles was 1.9 for the SP-03 population, and mean heterozygosity observed was 0.26, 1.1% higher than expected heterozygosity (**Table 17**).

Table 17 Number of alleles, allelic range and heterozygosities (observed and expected) on *loci*, in PO-01 population (Portugal)

Locus#	Num. gene copies	Num. alleles	Obs. Het.	Exp. Het	Allelic range
1	60	6	0.93333	0.80056	12
2	58	3	0.48276	0.56564	8
3	60	7	0.93333	0.81695	28
4	58	2	0.20690	0.24198	4
5	56	2	0.17857	0.22273	2
6	60	6	0.66667	0.60960	14
7	58	3	0.55172	0.43981	18
8	60	2	0.20000	0.47232	6
10	32	3	0.56250	0.50202	10
Mean	27.889	1.889	0.26199	0.25953	6.667
s.d.	28.530	2.307	0.32658	0.29478	7.087

Seven populations presented exclusive alleles, SP-04 and SP-02 being the populations with the most exclusive alleles, 6 and 4 respectively, followed by FR-01 with 3 alleles, CY-01 and SP-03 with 2 alleles and PO-01 and SP-05 with only 1 allele (**Table 18**). On the other hand, SP-02 and SP-04 also presented exclusive alleles versus the remaining of populations for *loci*: SOC405 (116), UBA50 (141), UBA6 (140, also shared with PO-01), CA14 (103, also shared with FR-01), GCT15 (79, also shared with FR-01), GA16 (126), GA17 (78), GA6 (120), GA2B (78).

Table 18 Exclusive alleles per population and *locus*

LOCUS	MARKER	SP-02	SP-04	CY-01	FR-01		PO-01	SP-03	SP-05
1	CacMic								
2	Soc 405								
3	UBA50	131	135	139	129		127		
4	UBA5								
5	UBA54				66	68			
6	UBA6								
7	UBA853								
8	UBA42								
9	Soc431								
10	UBA53	88	86						
11	CA13	99	95	91					
12	CA14		100						
13	CA3								
14	GCT15								
15	GA16		110						112
16	GA17							80	
17	GA6								
18	GA2B							88	

Mean alleles ranged from 2 to 7, for PO-01 and SO-04, respectively. Close to SP-04 was SP-02, with 6 alleles. As heterozygosities, both arithmetic and weighted estimates were the same (0.48) for observed heterozygosity, while the values were similar for expected heterozygosity, 0.48 and 0.49, respectively (**Table 19**).

Table 19 Total averages for number of gene copies, alleles and observed and expected heterozygosities.

	AVERAGES			
POPULATION	Nº GENE COPIES	Nº ALLELES	HET.OBS.	HET.EXP.
PO-01	27.8	1.8	0.261	0.259
SP-01	54.4	5.2	0.585	0.598
SP-02	45.8	6.3	0.548	0.625
SP-03	51.6	4	0.561	0.505
SP-04	101.5	7.1	0.532	0.595
SP-05	56.3	2.8	0.519	0.446
FR-01	59.5	5.3	0.433	0.507
IT-01	46.3	3.4	0.547	0.502
GR-01-F1	26.88	2.5	0.453	0.449
GR-01-F2	54.7	3	0.388	0.402
GR-02-FI	34.5	3.4	0.473	0.506
GR-02-F2	28.1	2.4	0.493	0.44
TU-01	57.8	2.5	0.375	0.375
CY-01	24	3	0.495	0.509
CY-02	35.3	2.7	0.469	0.455
Arithmetic mean		3.69	0.48	0.48
Weighted mean		4.13	0.48	0.49

Molecular analysis of Variance (AMOVA)

The AMOVA was run for *loci* 1-8, and it revealed that 18.19% of the variation was found among studied populations, while the remaining 81.82% is located within populations. Populations that are essentially the same exhibit 100% of the variation within the populations and 0% among populations. Therefore, an 18.19% variation among populations indicates that some or all the population present significant differences. This partition of variance was highly significant (P<0.0001), both jointly (**Table 20**) and separately (**Table 21**), denoting a high differentiation among populations studied and a low gene flow. This fixation index was independent of *loci* (**Table 21**) and ranged from 0.094 to 0.307 for loci 7 and 4, respectively.

Table 20 AMOVA for European populations of meagre

Source of variation	Sum of squares	Variance components	Percentage variation
Among populations	358.311	0.47282	18.19220
Within populations	1567.290	2.12622	81.80780
Total	1925.601	2.59905	

Average F-Statistics over all loci
Fixation Indices
FST : 0.18192

Table 21 AMOVAs in all populations for *loci* 1-8.

	Among Populations:				Within Populations:				Fixation indices:	
Locus	SSD	d.f.	Va	% variation	SSD	d.f.	Vb	% variation	FST	P-value
1	59.64035	14	0.07853	19.53170	241.68904	747	0.32355	80.46830	0.19532	0.00000
2	72.44379	14	0.09767	27.13830	195.88823	747	0.26223	72.86170	0.27138	0.00000
3	35.87438	14	0.04407	11.59604	251.61908	749	0.33594	88.40396	0.11596	0.00000
4	63.25199	14	0.08802	30.69286	144.88645	729	0.19875	69.30714	0.30693	0.00000
5	31.02726	14	0.04086	14.85683	169.30878	723	0.23418	85.14317	0.14857	0.00000
6	37.82815	14	0.04842	13.55425	226.34431	733	0.30879	86.44575	0.13554	0.00000
7	21.30366	14	0.02571	9.44332	181.19101	735	0.24652	90.55668	0.09443	0.00000
8	36.94136	14	0.04955	18.64083	156.36352	723	0.21627	81.35917	0.18641	0.00000

Effective population size

To estimate the effective size of each population (*Ne*), a mean of several scenarios was considered, in terms of two different mutation rates per generation of microsatellite markers, 4.5 10^{-3} from 8.5 10^{-3} in fish and 6 10^{-4} in high vertebrate, respectively. Thus, *Ne* estimations (**Table 22**) were realised from *Theta* estimations for each population, using the stepwise mutation model.

Table 22 Effective size (Ne) and Theta values (H) per population.

POPULATION	Theta (H)	Ne
PO-01	2,10178	115,48
SP-01	1,58012	86,82
SP-02	1,63525	89,85
SP-03	1,50028	82,43
SP-04	1,57611	86,60
SP-05	1,52339	83,70
FR-01	1,50044	82,44
IT-01	1,50005	82,42
GR-01-F1	1,52021	83,53
GR-01-F2	1,57866	86,74
GR-02-F1	1,50039	82,44
GR-02-F2	1,5292	84,02
TU-02	1,6321	89,68
CY-01	1,5007	82,46
CY-02	1,51591	83,29

The populations of meagre analysed presented a mean effective size (*Ne*) of 86.79, i.e., in an ideal population, there would be 43.4 males and the same number of females. This was higher than the minimum recommended to minimize inbreeding depression (50), but lower than the minimum suggested for maintaining sufficient evolution capacity (500). The coefficient of variation between populations was 3.1%.

Geographic structuration

The Factorial Correspondence Analysis (FCA) shows how populations are grouped in three clusters (**Fig. 4**). Within *group-a*, there are three exclusive populations (SP-03, SP-04, PO-01) from the south of the Iberian peninsula. In *group-b*, there are six exclusive populations (SP-5, IT-01, GR-01, GR-02, CY-01, CY-02). *Group-c*, was constituted only for TU-01 population. Fr-01, SP-01 and SP-02, were presented in *groups-a* and *b*. The FCA grouping was in concordance with the geographic distribution of the European meagre populations

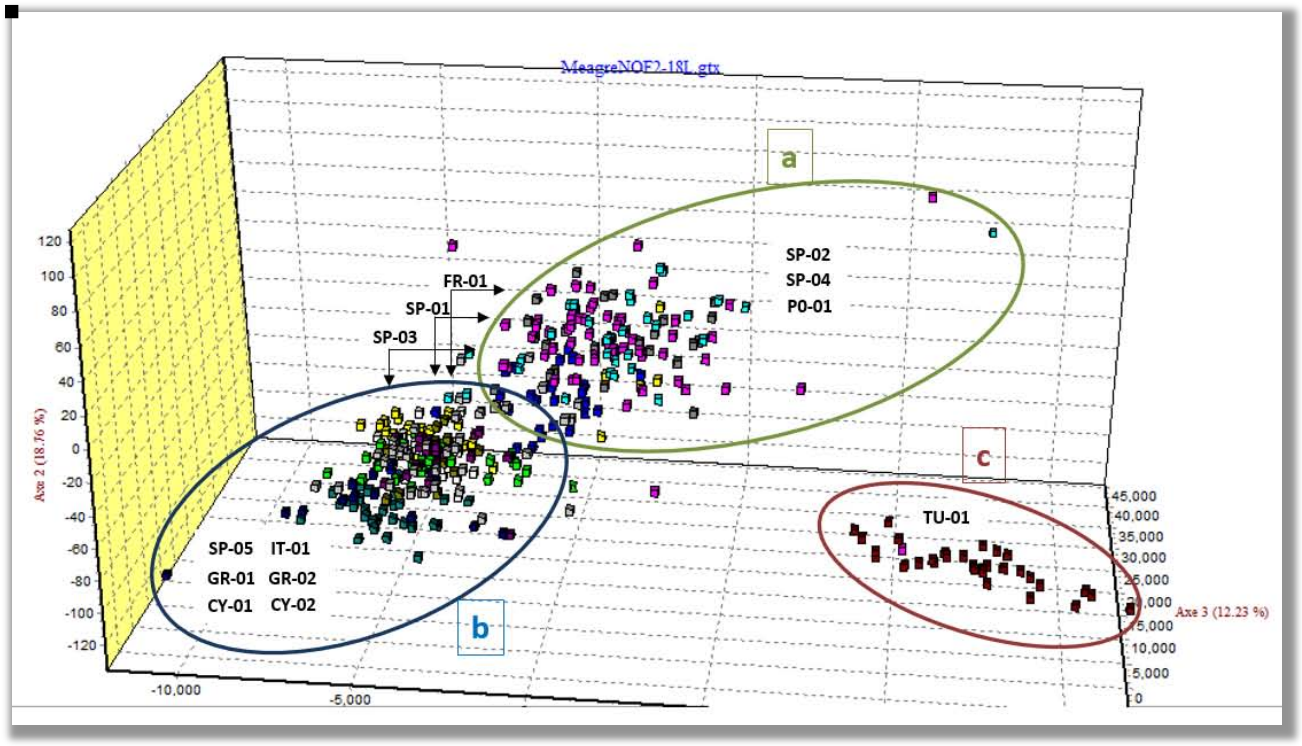

Figure 4 Graph of Factorial Correspondence Analysis from 18 loci and 376 fish (no F2) distributed in 13 Mediterranean populations of meagre.

DISCUSSION

Meagre is an important species in European aquaculture due to its high fecundity, fast growth and the fact that it is popular among consumers. A perfect understanding of the species is a prerequisite for all aquaculture species in order to optimize their productions on an industrial scale. Meagre culture is well established in the hatchery and on-growing stages of the industry. However, genetic selection offers major capacity to improve the production level of fish. The genetic gain in Atlantic salmon (*Salmo salar*) over five generations of selection for growth rate is 113% compared to the wild population (Thodesen *et al.,* 1999). Neira *et al.* (2010) and Rye *et al.* (2010) reported a revision of the impact of selective breeding programs on the production of different aquaculture species, mainly in freshwater species (Common carp, Catfish, etc.), and only in the following marine fish; turbot (*Scophthalmus maxima*), Atlantic cod (*Cadus morhua*), European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*)(**Table 23**).

Table 23 Summary of breeding programs currently underway on sea and fresh water fish (Neira *et al.,* 2010; Rye *et al.,* 2010).

Type	Nº Programs (range)	Nº families (range)	Nº Traits (range)
Fresh water (***Fw***)	72 (1-20)	147 (51-280)	2,7 (1-5,2)
Sea wáter (***Mf***)	13 (1-4)	88 (60-110)	2,9 (1-6)

To make the exploitation of genetic variation feasible and attractive, it is very important to understand the main factors affecting normal technological and biological development and processes, such as: the variation of the base population, the determination of the relationship matrix, the definition of traits to be selected, genetic parameters, quantification of genotype-environment interactions, genetic testing or replacement. So, the implementation of a breeding program requires detailed information about all these phases.

All the above-mentioned factors are important, but the variation present in the base population or starting population is essential, because genetic variation influences the response of selection in the short and long term (Falconer and Mackay, 2001). Therefore, it is relevant to know the genetic variability of species on breeding selection programs. Thus, different studies have been completed using microsatellite markers from natural and captive populations on relevant species in aquaculture. In gilthead seabream, there are studies on the genetic characterization of populations, mainly using microsatellites, indicating that the genetic variation in populations under commercial exploitation and in research centres is very high. In this regard, Batargias *et al.* (1997, 1999) determined a mean of 0.875 observed heterozygosity in a growing population, one of the highest in teleosts. Alarcon *et al.* (2004) characterized 11 stocks of gilthead seabream in the Atlantic and the Mediterranean, and found no genetic differentiation between them. Levels of expected heterozygosity in natural and culture populations were statistically equal, 0.864 and 0.845, respectively. De Innocentiis *et al.* (2005), working with four specific markers of gilthead seabream (Batargias *et al.* 1997) estimated observed heterozygosities of 0.74 and 0.79 in two populations from two Italian companies. Likewise, Borrel *et al.* (2007) characterized a population of gilthead seabream using 11 microsatellites, 6 of which were specific and 5 of which were *Pagellus bogarveo* (Piñera *et al.,* 2006), and found an observed heterozygosity of 85% in the female population and of 71.40% in the male population. These high mean heterozygosity values highlight the lack of selection pressure on gilthead seabream stocks, making them suitable to be used as the founder to start a breeding program, with the same potential as described for other species of commercial interest (**Table 24**), and for which there are remarkable and successful selection programs.

Only two studies have been conducted to determine the genetic structure of meagre. In wild populations, in Atlantic and Mediterranean Sea, by Haffray *et al.* (2012), and in captive populations, from French industry, by Haffray *et al.* (2014). These authors provided the first genetic characterisation of meagre across its natural distribution range, estimating the mean number of alleles and observed heterozygosity to be around 13.2 and 0.57, respectively. They concluded that meagre is highly structured, from a genetic point of view, with the highest level of differentiation reported in marine fish. The authors suggested that this high genetic structuration reveals that each population stems from a different genetic origin and that their management should be considered on a regional basis. In captive populations of meagre from France (LPDS and FMD), Haffray *et al.* (2014) have used the same microsatellites panels (A-B) and report a slightly lower number of alleles and observed heterozygosity (8.5 and 0.52, respectively) as compared to wild populations.

In the present work, where many captive European populations of meagre were studied in depth with 18 microsatellite markers (different to Panels A-B), the number of alleles and observed heterozygosity were lower than in wild populations (around 3 times and 18% lower, respectively). This is consistent with the results of Haffray *et al.* (2014), although these authors estimated values higher to our results in 2 times and

Table 24 Studies of genetic variability in aquaculture species of commercial interest, using microsatellite markers.

Species	*N° of samples*	*N° of loci*	*Alleles per locus*	*H.obs.*	*References*
Gilthead seabream *Sparus aurata*	932	6	16,5	0,875	Batargias *et al.* (1999)
	16	12	8	0,712*	Launey *et al.* (2003)
	270	3	15,8	0,845	Alarcón *et al.* (2004)
	98	4	17	0,74-0,79	De Innocentiis *et al.* (2005)
	32	6	16,3	0,885*	Brown *et al.* (2005a)
	397	11	18,55	0.788	Borrell *et al.* (2007)
	264	11	17,36	0.774	
	78	9	>14	>0.800	Borrell *et al.* (2011)
Red seabream *Pagrus major*	550	4	25,9	0,848	Pérez-Enríquez *et al.* (1999)
Redbanded seabream *Pagrus auriga*	42	8	14,5	0,731	Ponce *et al.* (2006)
Sole *Solea senegalensis*	21	10	10,4	0,61*	Funes *et al.* (2004)
Turbot *Scopthalmus maximus*	46	3	7	0,67*	Coughlan *et al.* (1998)
	150	12	9,6	0,74	Bouza *et al.* (2002)
	34	8	6,88	0,810	Borrell *et al.* (2004)
	28	8	6,13	0,674	
	25	8	5,00	0,650	
	26	8	6,75	0,721	
	60	8	4,13	0,641	
Atlantic salmon *Salmo salar*	270	15	17,8	0,64	Norris *et al.* (1999)
	984	12	21,8	0,70	Skaala *et al.* (2004)
Chinook salmon *Oncorhynchus tshawytscha*	2638	10	6,7	0,59	Banks *et al.* (2000)
Nile tilapia *Oreochromis niloticus*	250	6	12,34	0,70	Abdallah *et al.* (2005)
Cat fish *Ictalurus punctatus*	50-100	13	-	0,72	Waldbieser y Wolters (1999)
Commun carp *Cyprinus carpio*	-	5	5	0,365	Desvignes y Durand (2001)

* Data from captive populations

8.3% in number of alleles and observed heterozygosity, respectively. The mean numbers of alleles and heterozygosities of the different broodstocks in the present study indicated that the variation of the broodstock populations is similar to wild populations or has declined slightly. Essentially, these broodstocks

appear to have adequate genetic variation for a breeding program, but the decline in variability and low mean number of alleles of some broodstocks clearly indicated that these broodstocks should be enlarged with new families and stocks to ensure an optimal base population for a breeding program. Thus, the F2 population of GR-01 reported lower observed heterozygosity than the F1 population (GR-01-F1), as expected (15% lower). However, the number of alleles in F2 was slightly higher than F1 (17%), probably due to a larger sample size (more than twice as big) (**Fig. 3**). By contrast, the F2 population of GR-02 reported a slightly higher observed heterozygosity than F1, an unexpected result (4.2% higher), but not statistically different, which can be related with the difficulty for reading these samples. The number of alleles in F2 was lower than in F1 (40%), according to the sample size and breeders number as contributors. This high genetic structuration of captive populations of meagre is corroborated by our estimation of the fixation index, Fst, which revealed a significant statistical differentiation between the populations studied. Thus, a few effective animals are contributing as breeders in captive meagre populations in the European industry, as denoted by their low effective sizes. The presence of exclusive alleles per population may have contributed to this differentiation among populations, and where this is so the Wahlund effect may be contributing to the Fst value. Another factor that may contribute to the Fst value is the reduction in population size over successive generations, which seems expected in captive meagre as compared to wild populations. However, it is possible that it did not have any effect, if we take into account the fact that the meagre production begun only very recently (1997) and this species has a shorter generational interval (Schuchardt *et al.,* 2007) in comparison with other fast growing species. In conclusion, the significant Fst reported in this work is indicative of a low genetic flow among captive meagre populations studied, producing a fragmentation of populations and increasing the effect of genetic stochastic processes. The main consequence of reduced *Ne* in meagre populations is directly related to the inbreeding depression, reducing of evolution powerful and enabling the extinction risk. A biological explanation of this genetic structuration of meagre populations could be related to the species' high fecundity and variance in reproductive success, which may decrease *Ne*, where only small number of families survive to produce descendants. The *Ne* estimates in this study are in line with results previously reported by Haffray *et al.* (2012). Therefore, the estimates of effective sizes (*Ne*) also indicated that the broodstocks probably originated from crosses between sufficient families, but the number of families in many broodstocks was at the lower limit for a base population, which again indicated that these broodstocks should be enlarged with new families and stocks to ensure an optimal base population for a breeding program.

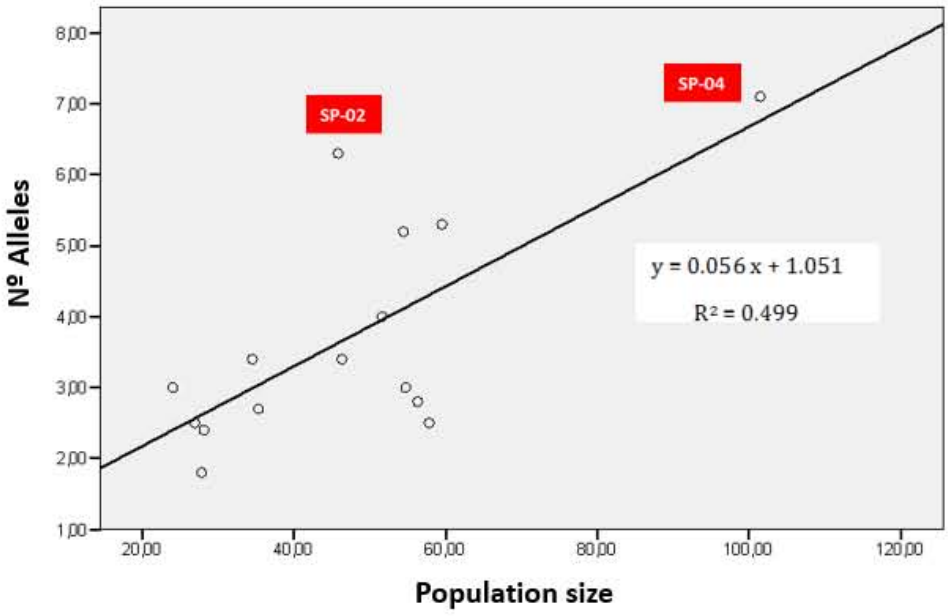

Figure 3. Relationship between number of alleles and sample size per population studied.

On the other hand, broodstock constitution policies, where different institutions share animals, may have influenced the current bottleneck in meagre. In this wide study on captive fish, we have detected how different populations share exclusive alleles at different *loci*, such as SOC405, UBA50, UBA6, CA14 or GCT15. This is consistent with the FCA results, which corroborated an interesting clustering system of the meagre populations studied within Atlantic populations (*group-a*) and Mediterranean populations (*group-b*), while the Turkish population (TU-01) is clearly separate. This is also coherent with results of Haffray *et al.* (2012), who described these three groups.

These results may be a reflection of the breeders' origin and distribution. Thus, *group-a* populations have been constituted from wild stocks that spawn in the estuary of the Guadiana River that forms the border between Spain and Portugal on the South of the Iberian peninsula (corroborated by Manuel Manchado and Narcizo Mazuelos, suppliers of SP-04 and SP-02 samples, respectively). Within the *Group-b* populations, FR-01 population seems to be the original stock that the French (LPDS) started with, and which supplied most farms as juveniles. The SP-05 population is clustered with this group, and it can be explained because breeders from this population were brought to a Canary Islands farm, which had previously imported meagre from FR-01 (Hipólito Fernández-Palacios, personal communication).

One of the most important questions is whether this genetic structuration reported on captive meagre populations has any direct consequences or effects on fish performance. Thus, it is well known that the loss of genetic diversity (low number of alleles, heterozygosity, etc.), due to effective size reduction, is affecting the heritability of quantitative traits (Franklin 1980; Frankham *et al.,* 2003), and in consequence is modifying the capacity of wild or captive populations. However, parallel studies on the performance of batches from different origins are unknown. It would, therefore, be very interesting to constitute new experiments in order to measure, under the same environmental conditions, the performance of all the lines or populations studied. This potential genetic differentiation in quantitative genes and traits would be a magnificent tool for the constitution of the best available base population for a selective breeding program on a European scale, especially because meagre as a species is currently at a clear disadvantage, in terms of its genetic starting point, compared to other important species within European aquaculture. On the other hand, it is conditioned by the interaction genotype – environment, which has to be studied as well, because we do not know whether commercially farmed meagre populations have adapted to their environmental conditions; any such adaptation would call for local genetic breeding programs rather than a breeding program on a European scale.

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