**Gradients of genetic diversity and differentiation across the distribution range of a Mediterranean coral: patterns, processes and conservation implications.**

This appendix details the calibration and standardization of allele scoring conducted in order to combine the dataset produced in this study (274 individuals genotyped with 12 microsatellites) with the dataset previously published by Casado-Amezúa et al. (2012) (381 individuals genotyped with 12 microsatellites).

The direct calibration and standardization procedures among the two studies were allowed because of the occurrence of three populations from the same locality in the two studies: PC from Casado-Amezúa et al. (2012) et ALM / ALF from this study. In the two studies, allele scoring was done based on the size of each allele following the migration of PCR fragments. It is noteworthy that 10 of the 12 loci showed repeat motif with three or four base pairs limiting potential ambiguity due to rounding of allele size during allele scoring.

The calibration was done in three steps. First, we computed the allele frequencies for each of the 12 loci using GENEPOP in each population (see Table S2-1). Then, we compared the distributions of allele frequencies for each locus (Figure S2-1) among the three populations. This second step allowed to identify the size shift during allele scoring (i.e. the difference in number of bases between the size of the alleles among the two datasets; see Table S2-2). This size shift was likely due to the different genotyping protocols applied in the two studies and resulted in lower allele size in the dataset produced in this study compared to the dataset produced in Casado-Amezúa et al. (2012). Finally, we re-scored the alleles of the dataset produced in this study by adding, for each allele in each locus, the number of bases corresponding to the allele size shift.

Genetic diversity parameters and patterns of genetic structure were consistent before and after calibrations (see Table S2-3).

Table S2-1: Allele frequencies for each of the 12 loci for the three populations belonging to the same location (Ceuta). PC was genotyped following the genotyping protocol in Casado-Amezúa et al. (2012) while ALM and ALF were genotyped following the genotyping protocol developed in this study.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L10 |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |   | 237 | 241 | 243 | 247 | 255 | 259 |
| Population | ALF | 0.639 | 0 | 0.069 | 0 | 0.292 | 0 |
| ALM | 0.838 | 0 | 0.025 | 0 | 0.138 | 0 |
| PC | 0 | 0.74 | 0 | 0.04 | 0 | 0.22 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L11 |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |   | 279 | 282 | 284 | 285 | 287 | 288 | 290 |
| Population | ALF | 0.333 | 0.333 | 0 | 0.333 | 0 | 0 | 0 |
| ALM | 0.438 | 0.212 | 0 | 0.338 | 0 | 0.013 | 0 |
| PC | 0 | 0 | 0.36 | 0 | 0.32 | 0 | 0.32 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ac-L22 |  |  |  |  |  |
|  |  | Alleles |
|  |  | 189 | 193 | 195 | 199 |
| Population | ALF | 0.056 | 0.944 | 0 | 0 |
| ALM | 0.113 | 0.887 | 0 | 0 |
| PC | 0 | 0 | 0.14 | 0.86 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L23 |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 265 | 269 | 272 | 276 | 277 | 284 |
| Population | ALF | 0.53 | 0.409 | 0 | 0 | 0.061 | 0 |
| ALM | 0.516 | 0.453 | 0 | 0 | 0.031 | 0 |
| PC | 0 | 0 | 0.696 | 0.261 | 0 | 0.043 |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L33 |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 310 | 316 | 366 | 372 | 378 | 386 | 390 | 392 | 396 |
| Population | ALF | 0.653 | 0 | 0.306 | 0 | 0.014 | 0.028 | 0 | 0 | 0 |
| ALM | 0.675 | 0 | 0.212 | 0 | 0 | 0.087 | 0.025 | 0 | 0 |
| PC | 0 | 0.66 | 0 | 0.26 | 0 | 0 | 0 | 0.04 | 0.04 |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Ac-L34 |  |  |  |  |  |
|  |  | Alleles |
|  |  | 196 | 199 | 203 | 206 |
| Population | ALF | 0.514 | 0.486 | 0 | 0 |
| ALM | 0.537 | 0.463 | 0 | 0 |
| PC | 0 | 0 | 0.5 | 0.5 |

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L37 |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 185 | 190 | 197 | 202 | 206 | 211 | 223 | 224 | 230 | 232 |
| Population | ALF | 0.681 | 0 | 0.139 | 0 | 0.139 | 0 | 0 | 0.028 | 0.014 | 0 |
| ALM | 0.4 | 0 | 0.35 | 0 | 0.225 | 0 | 0 | 0.025 | 0 | 0 |
| PC | 0 | 0.5 | 0 | 0.14 | 0 | 0.22 | 0.02 | 0 | 0 | 0.12 |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L7 |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 134 | 149 | 155 | 161 | 164 | 170 | 173 | 179 | 182 |
| Population | ALF | 0.097 | 0.014 | 0.139 | 0.611 | 0 | 0.056 | 0.083 | 0 | 0 |
| ALM | 0.1 | 0 | 0.175 | 0.625 | 0.013 | 0.025 | 0.062 | 0 | 0 |
| PC | 0 | 0 | 0 | 0.08 | 0.08 | 0.64 | 0 | 0.02 | 0.18 |

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-7E-AC |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 190 | 192 | 194 | 196 | 198 | 200 | 202 | 204 | 208 |
| Population | ALF | 0.056 | 0.194 | 0.639 | 0.097 | 0 | 0.014 | 0 | 0 | 0 |
| ALM | 0.15 | 0.15 | 0.375 | 0.275 | 0 | 0 | 0 | 0 | 0.05 |
| PC | 0 | 0 | 0 | 0.1 | 0.2 | 0.54 | 0.14 | 0.02 | 0 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L18 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 240 | 246 | 248 | 250 | 254 | 256 | 258 | 260 | 262 | 264 | 266 | 272 | 274 | 276 | 278 | 280 | 282 | 284 |
| Population | ALF | 0.014 | 0.181 | 0 | 0 | 0.097 | 0.083 | 0.069 | 0.083 | 0.014 | 0 | 0 | 0.069 | 0.083 | 0.25 | 0.042 | 0.014 | 0 | 0 |
| ALM | 0 | 0.138 | 0.025 | 0 | 0.1 | 0.025 | 0.1 | 0.163 | 0 | 0 | 0 | 0.062 | 0.05 | 0.275 | 0.062 | 0 | 0 | 0 |
| PC | 0 | 0 | 0 | 0.146 | 0 | 0 | 0.062 | 0.083 | 0.167 | 0.042 | 0.104 | 0 | 0 | 0 | 0.042 | 0.104 | 0.188 | 0.062 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L20 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 328 | 335 | 336 | 338 | 340 | 342 | 343 | 344 | 345 | 346 | 347 | 348 | 349 |
| Population | ALF | 0.086 | 0 | 0.314 | 0.071 | 0.186 | 0.029 | 0 | 0.014 | 0 | 0.114 | 0 | 0.014 | 0 |
| ALM | 0.113 | 0 | 0.138 | 0.075 | 0.3 | 0.025 | 0 | 0.013 | 0 | 0.188 | 0 | 0.062 | 0 |
| PC | 0 | 0.02 | 0 | 0 | 0 | 0 | 0.22 | 0 | 0.06 | 0 | 0.18 | 0 | 0.06 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L20 suite |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 350 | 351 | 352 | 353 | 354 | 355 | 357 | 359 | 363 | 367 | 373 | 378 | 380 | 384 |
| Population | ALF | 0.071 | 0 | 0.014 | 0 | 0.043 | 0 | 0 | 0 | 0 | 0 | 0 | 0.029 | 0 | 0.014 |
| ALM | 0.062 | 0 | 0.013 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.013 | 0 |
| PC | 0 | 0.04 | 0 | 0.18 | 0 | 0.06 | 0.1 | 0.02 | 0.02 | 0.02 | 0.02 | 0 | 0 | 0 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Ac-L25 |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Alleles |
|  |  | 95 | 102 | 104 | 107 | 110 | 111 | 113 | 114 | 116 | 117 | 120 | 123 |
| Population | ALF | 0.194 | 0 | 0.181 | 0.111 | 0.306 | 0 | 0.083 | 0 | 0.125 | 0 | 0 | 0 |
| ALM | 0.15 | 0 | 0.1 | 0.212 | 0.45 | 0 | 0.05 | 0 | 0.037 | 0 | 0 | 0 |
| PC | 0 | 0.083 | 0 | 0 | 0 | 0.271 | 0 | 0.167 | 0 | 0.375 | 0.042 | 0.062 |

Figure S2-1: Allele frequencies for each locus in the three populations from the same location.



Ac-L7E

Ac-L7

Ac-L10

Ac-L11

Ac-L18

Ac-L20

Ac-L22

Ac-L23

Ac-L25

Ac-L33

Ac-L34

Ac-L37

Table S2-2: Allele size shift for each locus between the two datasets. These shifts were identified based on the comparisons of allelic frequencies. For instance, the allele size was smaller by 4 bases between our dataset and the dataset of Casado- Amezúa et al. 2012 (see Figure S2-1). The repeat motif of each locus is also shown. Most of the repeats are irregular and involving more than 2 bases limiting ambiguity due to rounding of allele size during allele scoring.

|  |  |  |
| --- | --- | --- |
| Locus name | Allele size shift | Repeat motif |
|
| Ac-L10 | +4 | (GAT)xGGT(GAT)x |
| Ac-L11 | +5 | (TCA)x |
| Ac-L18 | +6 | (CA)xCG(CA)x |
| Ac-L20 | +7 | (TG)x |
| Ac-L22 | +6 | (AGAC)x |
| Ac-L23 | +7 | (AGAC)xAGTC(AGAC)xAGAAA(AGAC)x |
| Ac-L25 | +7 | (CAT)x |
| Ac-L33 | +6 | (GAYA)xGACGCACA(GAYA)xCAGTA(GYA)xTA(CAYA)x |
| Ac-L34 | +7 | (GAT)xN(GAT)x |
| Ac-L37 | +5 | (GRT)xGAC(GRT)xGTTGAC(GRT)xGTT(GRT)x |
| Ac-L7 | +9 | (GAT)x |
| Ac-L7E | +6 | (AC)x |

Table S2-3: Genetic diversity parameters computed on our dataset before and after the calibration and standardization processes. *Ho*: observed heterozygosity; *He*: gene diversity (Nei 1973); *Ar(10)*: rarefied allelic richness considering a minimum of 10 genes at a locus in a population; *f*: Weir & Cockerham (1984) estimator of *FIS*.

|  |  |  |
| --- | --- | --- |
|  | Before standardization | After standardization |
| Sample Name | *Ho* | *He* | *Ar(10)* | *f* | Ho | He | *Ar(10)* | *f* |
|
|
| AAA | 0.69 | 0.75 | 4.6 | 0.08 | 0.69 | 0.74 | 4.6 | 0.08 |
| AIK | 0.58 | 0.69 | 4.25 | 0.15 | 0.58 | 0.68 | 4.25 | 0.12 |
| ALF | 0.6011 | 0.58 | 3.37 | -0.03 | 0.60 | 0.58 | 3.37 | -0.03 |
| ALM | 0.60 | 0.59 | 3.33 | -0.01 | 0.60 | 0.59 | 3.33 | -0.01 |
| BBE | 0.56 | 0.67 | 3.92 | 0.16 | 0.56 | 0.67 | 3.93 | 0.16 |
| CAD | 0.67 | 0.71 | 4.36 | 0.07 | 0.67 | 0.71 | 4.36 | 0.07 |
| CAS | 0.61 | 0.73 | 4.59 | 0.17 | 0.61 | 0.72 | 4.59 | 0.17 |
| CGD | 0.66 | 0.71 | 4.48 | 0.07 | 0.66 | 0.71 | 4.48 | 0.07 |
| LAM | 0.65 | 0.73 | 4.43 | 0.11 | 0.65 | 0.72 | 4.43 | 0.11 |
| MTS | 0.53 | 0.63 | 3.71 | 0.12 | 0.56 | 0.63 | 3.73 | 0.12 |
| NHS | 0.57 | 0.66 | 3.95 | 0.14 | 0.57 | 0.66 | 4 | 0.14 |
| TUN | 0.61 | 0.71 | 4.32 | 0.13 | 0.61 | 0.70 | 4.32 | 0.13 |
| ZEM | 0.67 | 0.72 | 4.44 | 0.07 | 0.67 | 0.72 | 4.44 | 0.07 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|   | Na | Ho | Hs | r |
| Ac-L10 | 20 | 0.4203 | 0.4723 | 0.05 |
| Ac-L11 | 14 | 0.5183 | 0.5250 | 0.03 |
| Ac-L18 | 28 | 0.8508 | 0.8258 | 0 |
| Ac-L20 | 39 | 0.7046 | 0.7510 | 0.04 |
| Ac-L22 | 19 | 0.2137 | 0.3767 | 0.12 |
| Ac-L23 | 5 | 0.4308 | 0.4595 | 0.17 |
| Ac-L25 | 18 | 0.6114 | 0.6560 | 0.03 |
| Ac-L33 | 19 | 0.5462 | 0.5750 | 0.05 |
| Ac-L34 | 6 | 0.4494 | 0.5341 | 0.08 |
| Ac-L37 | 23 | 0.6028 | 0.6473 | 0.04 |
| Ac-L7 | 19 | 0.4652 | 0.4723 | 0.02 |
| Ac-L7E | 25 | 0.7123 | 0.7487 | 0.03 |

Table S2-4: Genetic diversity parameters computed for each locus after standardization. *Na:number of alleles; Ho*: observed heterozygosity; *He*: gene diversity (Nei 1973); *r:* null allele frequency estimated with FREENa