PARENTAGE ANALYSIS OF HATCHERY STOCKS FROM THE EUROPEAN HAKE *Merluccius merluccius*

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Introduction

The European hake *Merluccius merluccius* is a demersal fish that exhibits high growth rate and has an important market value (Lloris et al., 2005). Those properties together with the overexploitation of its Atlantic fishery make this species a promising candidate for marine aquaculture (Engelsen et al., 2007). This species is at its very early stages of domestication, the unique world stock being harvested at the Spanish Institute of Oceanography (Iglesias et al., 2010). Early steps of domestication require conjugation of various population genetic criteria to skip founding effects and monitoring reproductive schemes of cultured stocks. On the one hand, the population genetic background of the donor Atlantic population is well known after a wide range studies using different markers (e.g. Pita et al., 2011). On the other hand, little is known on the reproductive behaviour of this species or on the genetic load of the European hake stock maintained at the Spanish IEO facilities (Vigo, Spain).

Materials and methods

The IEO domestic broodstock (9 males, 11 females) was sampled by non-invasive methods. Samples were kept in 96% ethanol upon collection. DNA from broodstock and 2 larval batches Me42.10 and Me43.10 from 2010 (42 individuals) and Me0.13 from 2013 (44 individuals) were extracted following standard phenol:chloroform method (Pérez and Presa, 2011). Selection of a subset of high polymorphic microsatellites from 16 new EST-diverted microsatellites developed herein were used in a fast multiplexed PCR set of 8 microsatellites as 5 EST-diverted markers plus 3 neutral ones. Individuals were genotyped and the broodstock reproductive structure was inferred after genetic assignment of year-round spawning batches performed using Colony 2.0.5.0 (Jones and Wang, 2010) and Vitassign (Vandeputte et al., 2006) software. Genetic diversity estimators were calculated with Cervus 3.0.

Results

The combined set of EST-derived microsatellites plus neutral markers showed good technical resolution and a large polymorphism, ranging from 3 to 22 alleles with expected heterozygosity values ranging from 0.188 to 0.910. The mean proportion of individuals genotyped was 0.959 and the polymorphic information content (PIC) ranged 0.634 - 0.899. The average probability of non-exclusion for a candidate parent pair ranged 0.057 - 0.344.

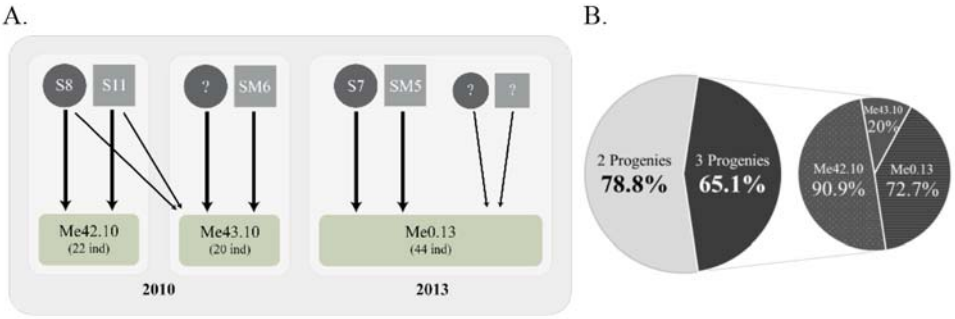

Figure 1. Schematic representation of parentage assignment of European hake *Merluccius merluccius*. *A*. Inferred pedigree for the three progenies analysed. Filled circles and squares indicate dams and sires, respectively, arrows show parentage relationships. *B*. Percentage of assignment for combined and single progenies.

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Preliminary parentage assignment was determined for 56 out of 86 individuals and three main clusters of families were recognized (Figure 1A). From those latter, two groups with single pairs of dams and sires were identified as majority breeders for each, Me42.10 and Me0.13 progenies. A single parent was assigned to the second larval batch of 2010 (Me43.10). Probabilities of full-sib families from 2010 and 2013 were higher than 98% and 79%, respectively.

Percentage of assignment for 3 larval batches was 65.1%, a value lesser than expected, likely due to genotyping errors, drop out, null alleles and the lack of samples from some putative parents for Me43.10. Nonetheless, that assignment value increased to 78.8% when analysis was performed only with progenies Me42.10 and Me0.13 (Figure 1B).

The best cluster configuration using maximum likelihood for progenies Me43.10 and Me 0.13 suggests that other less likely parent pairs could also be involved. In Me43.10, 4 out of 22 individuals shared the same couple of parents assigned to Me42.10 what generates a slight full-sib families' relationship within the 2,010 batches.

Discussion and conclusion

Parentage allocation in the present test was quite successful due to the polymorphism of the marker set designed herein. Moreover, the combined exclusion power allowed to trace back the parents of larvae and to test the performance of each locus for parentage inference using the families obtained. The application of this molecular tool to individual identification and parentage assignment is useful to assess the genetic processes underlying reproduction and management of the hake broodstock, as well as to assist in planning strategies aiming to maintain the genetic diversity of domestic hake stocks. The calibration of this genetic tool is obligatory to set up a well-based genetic broodstock of this new candidate species for aquaculture.

References

Engelsen, R., F. Asche, F. Skjennum and G. Adoff. 2007. New species in aquaculture: Some basic economic aspects. p.487-515. In: Culture of cold-water marine fish. Moksness, E., E. Kjørsvik and Y. Olsen. (Eds.). Blackwell Publishing Ltd, Oxford, UK. doi: 10.1002/9780470995617.ch12.

Iglesias, J., M.J. Lago, F.J. Sánchez and R. Cal. 2010. Capture, transport and acclimatization to captivity of European hake, *Merluccius merluccius*: preliminary data on feeding and growth. Aquaculture Research 41(4): 607-609.

Jones, O.R. and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular ecology resources 10, 551-555.

Lloris, D., J. Matallanas and P. Oliver. 2005. Hakes of the world (Family Merlucciidae). An annotated and illustrated catalogue of hake species known to date. FAO Species Catalogue for Fishery Purposes. No. 2. Rome. FAO. 57 pp.

Pérez M., and P. Presa. 2011. FENOSALT: un método sintético para la extracción de ADN de peces y moluscos. p.79-87. In: Métodos y Técnicas en investigación marina. Tecnos. García-Estévez J.M. et al. (Eds.). Tecnos, Madrid. 404p.

Pita, A., M. Pérez, S. Cerviño and P. Presa. 2011. What can gene flow and recruitment dynamics tell us about connectivity between European hake stocks in the Eastern North Atlantic? Continental Shelf Research 31, 376-387.

Vandeputte, S., M. Mauger, and M. Dupont-Nivet. 2006. An evaluation of allowing for mismatches as a way to manage genotyping errors in parentage assignment by exclusion. Molecular Ecology Notes, 6: 265-267.