

## COMBINING VITASSIGN AND COLONY FOR PEDIGREE RECONSTRUCTION IN A CASE OF FACTORIAL MATING WITH MISSING PARENTAL GENOTYPES

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### Introduction

The contribution of parentage assignment in selective breeding of aquaculture species is undeniable [1, 3, 7]. However, breeding programs often face practical management problems and it is not uncommon that some broodstock genotypes miss because of premature death, traceability problems or sample quality problems [5, 6, 7]. This may lead to unexpectedly low pedigree recovery [5, 6] and decreases markedly the potential of genetic improvement. The parentage assignment is based on two computation methods, exclusion-based methods and likelihood-based methods [3]. Exclusion is very simple and makes no hypotheses other than Mendelian segregation of alleles, but is very sensitive to genotyping errors while likelihood methods use a different approach with probabilities. Likelihood methods generally give higher assignment rates than exclusion with low power marker sets but sometimes give inconsistent results. In this study, we explored the potential of combining two softwares, VITASSIGN (exclusion) and COLONY (likelihood) for obtaining parentage assignment in the case of a few missing parental genotypes in a full factorial mating design.

### Materials and methods

In this study, 60 wild sires were crossed with 9 wild dams in a full factorial mating scheme and 2000 offsprings were reared in a single batch. The caudal fins or sperm of parents were collected directly during the artificial mating while the caudal fins of the 2000 offsprings were collected at five months post-harvest. All were sent to LABOGENA (Jouy-en-Josas, France) for DNA extraction and genotyping of 12 microsatellite markers. VITASSIGN, an exclusion-based parentage assignment software, was run as described by Vandeputte et al. 2006 [5], allowing for up to two allelic mismatches between parents and offsprings. COLONY, a maximum likelihood parentage software, was run as described by Jones and Wang, 2010 [2, 9]. The reconstruction of missing genotypes and correction of genotyping errors was proceeded when posterior probabilities of genotypes inferred by COLONY were equal to 1.

### Results and discussion

Due to low sample qualities, 2 dams, 2 sires and 9 offspring could not be genotyped. Therefore, only 7 dams, 58 sires and 1991 offsprings were used for first pedigree assignment trials using VITASSIGN and only 40.8% of the offsprings were assigned to a single parent pair with perfect match (55.8% allowing up to 2 mismatches). In order to identify the missing genotypes and genotyping errors, the same data set was processed with COLONY, but if highly probable pedigree was obtained for only 52.6% of the offsprings, this run allowed identifying 252 additional potential dam genotypes. Two genotypes among those, displaying likelihood probabilities equal to 1, were suspected to correspond to the 2 missing dams. The following runs, including the 2 dam genotypes inferred by COLONY, resulted in 78.0% of perfect match with VITASSIGN (92.4% allowing up to 2 mismatches) and in 77.1% of assignment with COLONY (figure 2). Later genotyping of alternative samples of the two missing dams, confirmed that the genotypes inferred by COLONY were exact. However, due to residual missing loci and genotyping errors, the parental assignment remained lower than expected through VITASSIGN simulations [5]. Therefore, sires and dams were corrected based on the genotypes inferred by COLONY (1 dam and 11 sire genotypes were corrected or completed, for a total of 48 corrected alleles). Finally, using VITASSIGN, 96.4% of the offsprings were uniquely assigned with assignment power  $>0.99$  (86.1% with perfect match and 96.4% with up to 2 mismatches allowed). Only 3.4% of the offsprings could not be assigned (figure 2).

### Conclusion

We demonstrated the power of combining VITASSIGN and COLONY for significantly improving pedigree assignments when parent genotypes are missing. The proportion of parentage assignment was increased from 40.8% to 96.4%. This improvement was allowed by combining the successful reconstructions of missing genotypes and genotyping-errors corrections using likelihood posterior probabilities calculated by COLONY and the exclusion-based assignment power of VITASSIGN.

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