

In silico mining of microsatellites to explore the mating system of *Rhynchophorus ferrugineus* (Coleoptera, Curculionoidea, Dryophoridae) – Silvia Belvedere & Alessio De Biase

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Abstract

The presented project aims at developing microsatellite markers through a combined approach of Next Generation Sequencing and bioinformatic analysis to study particular aspects of the mating system of Red Palm Weevil (*Rhynchophorus ferrugineus*), an invasive species of Coleoptera Dryophoridae. Our research is part of a joined IAEA-FAO cooperation program aimed to evaluate the Sterile Insect Technique for the integrated management of this pest.

R. ferrugineus is in fact a big and polyphagous phytophagous insect, associated to a large number of Arecaceae species, among which a lot of palms of economically interest, like the date palm and the Canary Island date palm, very widespread in the whole Mediterranean area. The larval stages of the insect bore deep galleries into the stem and the crown of the host, generally leading to its death when the tunnels reach the meristematic tissues. The species, native of southern Asia and Melanesia, spread by trade of infected palms firstly in the Middle East (in the '80s) and then in nearly all the countries of the Mediterranean basin (included Italy) since the early '90s.

Because of the ineffectiveness of the integrated pest management strategies actually employed to control the infestation, the hypothesis of use a biological control approach named Sterile Insect Technique (SIT) is currently under study. The SIT consists in the sterilization of males (generally through gamma radiation) to be released in the field, with the aim of drastically reduce the population's birth rate. However, to successfully employ SIT is essential a good knowledge of the biology of the target species, and in particular of its reproduction and mating systems. Main goals of the project are to verify the presence of polyandrous behaviors (if females mate with more males) and of cryptic-choice mechanisms by which females can select sperm after multiple copulations.

The chosen methodological approach relies on the parentage analysis of mating individuals to estimate the average number of paternal contributions for reproductive event. Genetic analyses will be done on microsatellite loci, which have been isolated *de novo* for the Red Palm Weevil by means of an advanced strategy. The procedure relies on a first step of deep sequencing by 454 technology (Roche GS-FLX Titanium platform) and a second step of microsatellite mining and primer design by bioinformatic analysis.

Our sequencing and bioinformatic results show that even with only 1/8 of the sequencer plate we isolated a large number of microsatellites, with high reduced costs in terms of time and money, respect to the classic procedure based on cloning and Sanger sequencing. Our results represent also the first significant contribution to the knowledge of the genome of the Red Palm Weevil that is a non-model organism.

Afterwards the more suitable microsatellites will be selected in terms of amplification success and polymorphism and next their allelic frequencies will be estimated on natural population samples. We planned two different series of genotyping experiments with the chosen microsatellites, which will be conducted even in a preliminary phase of tuning of the method (on individuals from laboratory programmed crosses), and then on natural population samples. During the first series of experiments, genotyping assays will be done on DNA extracted from pregnant females spermatecae, to verify the presence of sperms belonging to different males. For the second experiments set we planned real paternity tests to be done on males, pregnant females and their progeny reared in the laboratory. Statistical analyses of scored genotypes will allow to estimate the overall number of alleles assignable to different males within each reproductive event. Therefore, it will be possible to verify the hypothesis of polyandry and, by comparing the results of the two experimental series, it will be possible to check the presence of post-copulation female cryptic-choice.