Precedenza spermatica dell'ultimo maschio nella specie dannosa *Rhynchophorus ferrugineus*: un approccio molecolare allo studio del *mating system*

Last-male sperm precedence in the pest species Rhynchophorus ferrugineus*: a molecular approach to the mating system study.*

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Abstract

The Red Palm Weevil (RPW), *Rhynchophorus ferrugineus*, Olivier (Curculionoidea, Dryophthoridae), is an invasive pest species from southeastern Asia and Melanesia. Since the '80, the phytophagous colonized through the Middle East almost all the countries of the Mediterranean Basin, including Italy. Unfortunately, actual actions of integrated pest management revealed to be inadequate and the stem-boring larva of this pest is causing huge damages to several palm species of the family Arecaeae. Many of those palms are economically important for agricultural and ornamental purpose. Therefore, a great attention has been recently focused in studying this species for finding sustainable and effective eradication strategies, like biological control ones.

Connecting the RPW rapid spread with its high reproductive success, the aim of this study was to shed light on particular aspects of its mating system, such as the presence of polyandry and post-copula sexual selection mechanisms. For this purpose, we developed 16 polymorphic microsatellite loci, 14 of them *de novo* isolated through an innovative approach based on 454 Next Generation Sequencing and bioinformatics mining (and 2 additional ones picked from literature). We then evaluated their reliability for paternity determination in laboratory cross experiments and we also discussed their use for more in depth mating system studies through simulations and power analyses. In order to maximize the probability of exposing females to males carrying different alleles, thus improving the resolution power of our system, we also performed a preliminary analysis of genetic variability on natural populations of RPW from its primary and secondary distribution areas. Our aim was in fact to identify well differentiated source populations for the collection of the individuals to be crossed. To investigate whether the female behavior of multiple mating really produces progeny from different males, we conducted two laboratory experiments crossing a single virgin female to two males carrying different genotypes at selected loci. We then reared and genetically characterized the filial generation in order to assess the paternity of progeny from comparison with potential parental genotypes.

Our results suggest that, even if multiple mating occurs frequently, it is the last male that almost exclusively contributes to the progeny (P_2-value=99.2%). This is probably due to a kind of post-copulatory sexual selection mechanism based on last male sperm precedence, like some evidences from mating experiments with sterilized and fertile males also suggest. Such a phenomenon, widespread in many insect orders, could be produced by sperm competition between males or even by female cryptic choice. Certainly, our findings require more in depth studies on these aspects of the mating system of RPW, and should be carefully considered in the planning of biological control programs against the pest, such as Sterile Insect Technique.