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Use of molecular markers to correct grape breeding errors and determine the identity of novel sources of resistance to *Xiphinema index* and Pierce's Disease

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• In 1988 renowned breeder Harold Olmo performed a series of crosses at the University of California, Davis, that were to make history. He crossed *Vitis rupestris* with *Muscadinia rotundifolia* in an effort to develop hybrids that would combine the ease of rooting and grafting of *V. rupestris* with the very high pest resistance of *M. rotundifolia*. The way breeders do these crosses is: 1) they first isolate the feminine flower organ (pistil) –for instance, with a paper bag; 2) then they inseminate it with the pollen from the male flower organ (stamen)– for instance, rubbing it with a brush, 3) and finally, they allow the pistillate plant –or "mother" plant- to develop berries and they extract the seeds to generate the cross-bred plants. In this way, Dr. Olmo generated 161 seedlings potentially exhibiting both an excellent disease resistance and ability to graft.

• Two of the above 161 seedlings were later selected to continue producing crosses. What happened next was that researchers started noticing that many of the individuals of this progeny had morphological features other than those expected from a *V. rupestris* x *M. rotundifolia* cross. (For instance, they had hairy leaves and stems, instead of smooth –glabrous- ones). This progeny was also mostly fertile, and possessed 38 chromosomes, whereas a cross between *V. rupestris* (38 chromosomes) and *M. rotundifolia* (40 chromosomes) is expected to produce progeny with and odd number of chromosomes (39 chromosomes), which would make it infertile. But it was not until molecular tools were applied to the study of this progeny that it was revealed beyond a doubt that ***M. rotundifolia* could not possibly be the actual male parent**.

• So if *M. rotundifolia* was not the parent, who was? Given the incredible value of this material for pest-resistance breeding programs, the current authors decided it was important to investigate the true parentage of these selections. This paper describes the steps they took to identify those parents.

• The authors went back to the original vineyard block and collected plant material from all potential sources of pollen within the block, that is, from all staminate and hermaphroditic grapevines that flowered at the same time as *V. rupestris*, the original "mother" plant. After extracting the DNA from each of these plants, they used 15 molecular markers –specific sequences of DNA that are publicly available and that can identify specific *Vitis* species- to compare the DNA of the progeny with the DNA of all the "potential parents". Briefly, 1) they first amplified those portions of DNA of each species that matched these molecular markers. 2) Then, they separated the amplified products by size. By comparing the amplified fragment sizes of the progeny with the amplified fragment sizes of the potential parents, they gained information about which vines could and which could not have been a parent. Only those plants showing a perfect segregation for all of the molecular markers could be a potential parent.

• **Results**. Of the 161 seedlings originally believed to be "fathered" by *M. rotundifolia*, the authors found that:
- most of them (55 seedlings) had as male parent another *V. rupestris* source (*V. rupestris* Pillans, instead of a *V. rupestris* A. de Serres, like the mother);
- the next largest group (46 seedlings) had as male parent *V. arizonica*, from northern Mexico (which was growing about 7 meters from the mother);
- of the remaining seedlings, a few, indeed, had *M. rotundifolia* as a male parent, some others had *V. vinifera*, and very few could not be identified.

• In what could be considered a separate experiment, and to get a feeling of how much *V. rupestris* tends to cross-pollinate with nearby vines, in 2005 the authors collected 192 seeds from an open-pollinated cluster of *V. rupestris* A. de Serres –potentially recreating what had happened with Dr. Olmo- and analyzed it with the same molecular markers as before. What they found was that the neighboring *V. rupestris* Pillans was the male parent of 154 (80%) of the seeds. But the remaining 38 seeds (20%) had indeed been fathered by 5 other vine species, growing some 11 meters away from the mother plant. (Aha!) What this showed is that ***V. rupestris* A. de Serres naturally tends to cross with several species**.

• In their discussion, the authors explain how, even though Dr. Olmo placed paper bags over the female vines to prevent contamination from pollen other than that belonging to *M. rotundifolia*, we cannot ignore the role of wind as a pollination agent. When the authors checked the temperature on record for the month and year in which Dr. Olmo was likely to have performed the crosses (April 1988), they found out that it had been a rather warm month (high average temperature of 26.6°C). As the authors explain, these high temperatures may have induced early pollen maturation, accelerated pollen drying, and increased the pollen's ability to travel in the wind and potentially be deposited on unprotected female flowers of *V. rupestris* before they bloomed. Alternatively, wind could have deposited pollen on the female flowers the split second that the protective bags were removed to perform the pollination.

• In conclusion, the authors discovered that *V. arizonica* and its relatives are the true male parents of many of Dr. Olmo's original progeny. It is really remarkable how Dr. Walker and his team "nailed" the identity of all of these parents. This reveals a new and precious source of plants containing "resistant genes" that can now be crossed with other *Vitis* species to obtain new material resistant to nematodes and diseases such as Pierce's Disease. Additionally, and despite previous studies reporting that grape pollen shows limited wind dispersal because of its lack of ornamentation, this study demonstrates that grape pollen can, indeed, be easily moved by wind. You may want to read the discussion of the original article for yourself, which is quite entertaining and reads like a mystery tale. Fortunately, the authors figured out the culprits, and the story had a happy ending.

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