WHITE PAPER

HYBRIDS:
WE'RE ALL A BIT MIXED UP

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Hybrids, by definition, are the offspring of two organisms of different species or varieties and while hybrids are a natural occurrence, humans have largely contributed to additional hybridization, referred to as artificial hybridization. The first intentional hybrid was grown by Thomas Fairchild in 1717, beginning the art and science of artificial hybridization [8]. His work has been continued by others for centuries and has produced many commonly used plants, such as peppermint. Hybrids bring immense diversity and intriguing insights into plant mechanics. However, they also pose particularly difficult issues in commercial, ecological, and scientific settings by making morphological, genetic, and chemical identification difficult. This article will explain the ins-and-outs of hybrids and what that means to the food and dietary supplement industry and their consumers.

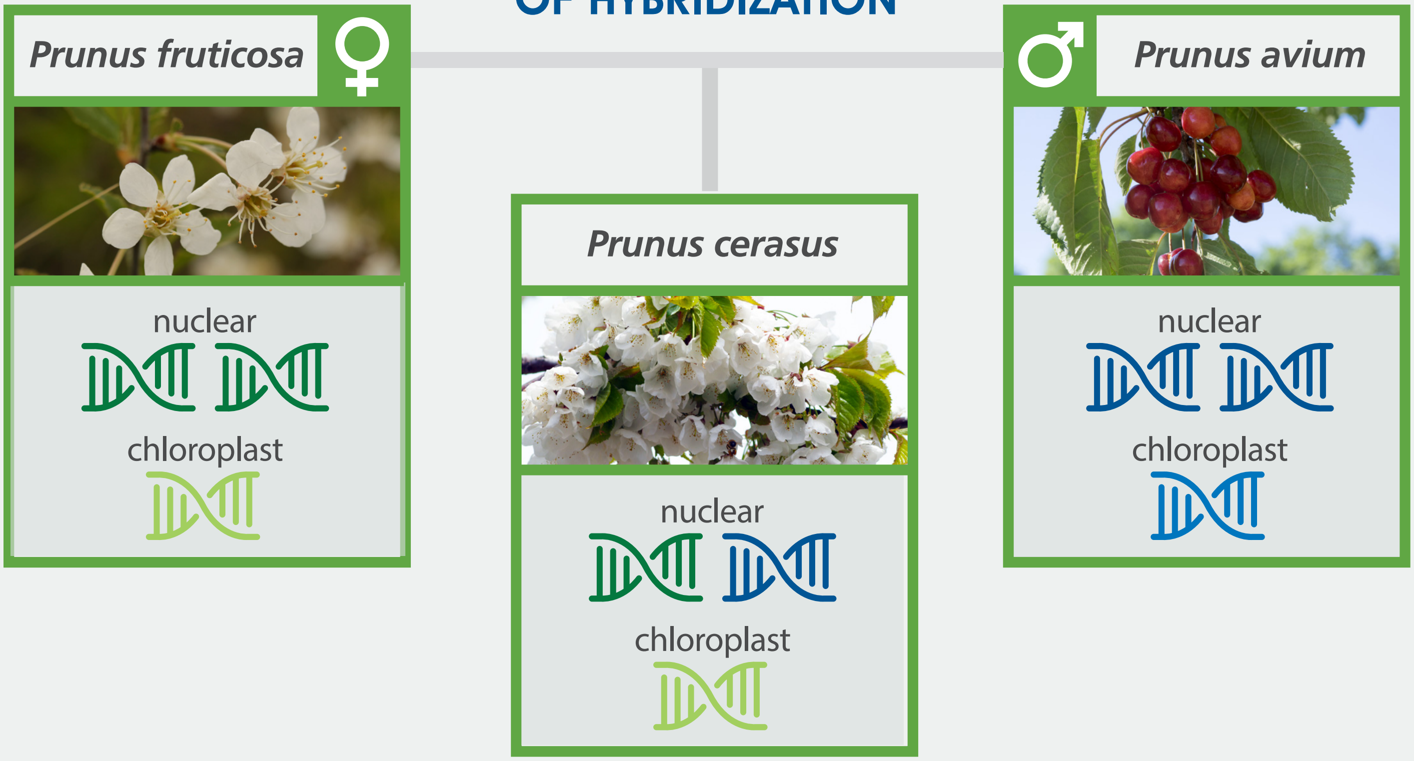
Approximately twenty-five percent of plant species hybridize with at least one relative, whether distant or close. This number, while widely debated due to the potential for mistaken identity, is in contrast to the 10 percent of animals that can hybridize with a related species [1].

Hybridization is limited by several factors: geographical location, chromosomal count, sexual compatibility, and timing [1]. These limiting factors mean that the chances are low that an individual will naturally hybridize within its lifetime. Hybridization rates must be low in order for natural selection to continue because the differences between species must be maintained [9]. On the other hand, if hybridization occurs, more diversity is invested into a genus than mutation alone would provide [8].

Whether intentionally or by accident, humans have utilized hybridization in the pursuit of artificially selecting desirable traits. In regard to horticulture, selecting for physical characteristics of interest like larger fruit size, improved taste, or resistance to pests - humans have created more purposeful plants through hybridization. The benefits of hybrid crops, however, were slow to be utilized on a large scale. It wasn't until 1930 that a handful of U.S. companies began to actually introduce hybrid corn seeds to the market, but by 1965 approximately 95% of U.S. corn was hybrid in origin [4]. Utilizing hybrid crops, especially corn, increased production dramatically throughout the twentieth century [12].

Humans have become incredibly creative with hybridization over the years, even going so far as to utilize a concept dubbed "introgression". This concept involves finding a trait of interest and selectively breeding individuals with that trait for generations. The hybrid can then be subjected to repeated backcrossing with one of its parents to introduce the desirable characteristic back into the original population [6]. Naturally, this method is a slow process, but humans have been able to utilize it in horticulture to obtain relatively immediate results in a few generations.

THE PROCESS OF HYBRIDIZATION



Introgression has the potential to create new varieties by introducing unique traits into a commonly used plant. For example, work by Eshed et al. (1995) utilized an "Introgression Line" of tomatoes to breed out characteristics like sterility from an inbred population [3]. Utilizing a small amount of wild type genome has the possibility to eliminate issues like disease sensitivity, sterility, and increase soluble-solids (Brix) content [10]. Another concept of use to industries is heterosis. While one may expect a hybrid's phenotype to lie evenly between a mixing of both parent's traits, the phenomenon of heterosis results in many hybrid plants being much larger and considerably more robust than either parent [12].

A USDA assessment of heterosis of corn hybrids in the early 1990's documents a nearly ten-fold increase in bushels per acre of U.S. corn yield since the Civil War. Single cross and double cross hybridizations are responsible for this logarithmic increase in yield compared to open-pollenated corn varieties [12]. While corn production has arguably benefitted the most from hybridization, it is not the only crop to do so. Crops like beans, tomatoes, peanuts, rice, spinach, sunflowers, onion, and broccoli all profit from hybridization to some extent.

While the concept of hybrid plants may sound alarming, in reality most of the plants we have utilized for centuries are products of multiple, intentional hybridization acts. In fact, there is strong evidence that our very species has come about through several hybridization events with other closely related species. Taking advantage of the benefits that hybridization has to offer in an agriculture sense, is yet another way humans have conquered our environment.

To recognize how hybrids can affect authentication and identification testing, it is necessary to understand the underlying biology of hybrid species. In terms of genetics, a hybrid offspring receives DNA from both parent species. However, the ratio of DNA from each parent is rarely equal. One reason for this is due to uniparental inheritance. Mitochondria and chloroplasts of plants each have their own small genomes both of which were originally believed to come from the maternal parent [2]. However, in some cases, chloroplasts were also shown to be paternally inherited, and in rarer cases, inherited from both parents. To further complicate the matter, parental inheritance can vary within a genus depending on the relation of species involved in the cross[5]. Although there are certainly exceptions, the general result of a hybrid offspring is at least two sets of nuclear chromosomes (at least one from each parent species), and only one chloroplast genome from either the maternal or paternal plant species.

Identifying plant species has traditionally been through morphological identification but with new technologies, it can become easier to find the true identity of a plant, simply by looking at the genes inherited. One of the more commonly used methods is DNA barcoding with Sanger, or chain-termination DNA sequencing [7]. This method targets and amplifies a gene region that is then compared to references for species identification. However, this method has several limitations that are especially prevalent when testing hybrid species. Firstly, if there are multiple genotypes within a sample, the resulting sequence can have ambiguous bases or be largely unreadable. This can make it nearly impossible to identify any species, let alone assess whether the starting material was a blend of multiple species or a hybrid. In contrast, when testing within the chloroplast, or other uniparentally inherited gene regions, hybrids would likely go undetected, as only one parent's genotype would be present [11]. Thus, it is important to consider which genes you are targeting and how a hybrid may present itself using DNA barcode testing.

NSF AuthenTechnologies offers routine testing using a Next Generation Sequencing (NGS) method that can overcome some of the issues from Sanger sequencing, but also has some limitations to consider when testing potential hybrid species. NGS has the ability to evaluate thousands to millions of sequences concurrently. This allows for testing of blends and hybrids, where different sequences can be evaluated separately. However, because the sequences from a mixture of two species would appear identical to a hybrid of the same two species, reporting is limited to "hybrid or mixture" for such samples. In some cases, an additional test using a chloroplast gene region can indicate that a sample is a hybrid, if the chloroplast gene only detects one species, but the nuclear gene test detects two. It must be noted that rare cases of biparental chloroplast inheritance limits one from confirming a mixture when multiple genotypes are found in both the chloroplast and nuclear genes.

Ranging from purposeful selective breeding or accidental crosspollination, hybrid species have a common presence within the food and dietary supplement industries. While a hybrid species may be desired within supplied starting material or its avoidance is preferred, understanding the biology behind hybrid species is necessary when interpreting quality testing results and what that means for the final product. Genetic testing is a useful tool for confirming the identity of a species within a material, especially when its strengths and limitations are understood. By taking advantage of the strengths of a test and bolstering its limitations with other testing tools, one can ensure that quality material is utilized in the manufacturing of the final product.

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