SHORT, NON-REFEREED PAPER

A NEW ORIGIN OF SUGARCANE: THE UNDISCOVERED SPECIES

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Abstract

The *Saccharum sensu stricto (s.s)* genus consists of three recognised species: *S. spontaneum, S. officinarum and S. robustum,* and three subspecies: *S. sinense, S. barberi and S. edule*. Currently, limited knowledge is available about the precise descent of these species from one another. Modern sugarcane arose through the 'Nobilization' process, and it is believed that *S. officinarum* and *S. spontaneum* were involved in an interspecific cross with subsequent backcrossing to *S. officinarum*. Molecular phylogenetic studies reveal the need to partition *Saccharum s.s* into four species: *S. narenga*; *S. spontaneum*; *S. officinarum* and the lineage of modern hybrid cultivars which are derived from a cryptic founder species that the authors formally name *Saccharum cultum sp. nov.* Lloyd Evans and Joshi, 2016. This necessitates the re-evaluation of the origins of sugarcane cultivation. This study integrates genome science, history, archaeology, geophysics, ethnobotany and ethnolinguistics to reveal a new history of sugarcane from its wild origins to modern cultivars. These findings overturn the current models of sugarcane taxonomy and sugarcane origins and reveal that South African *Miscanthidium* is the most closely related genus to *Saccharum*. Moreover, this study reveals a new, integrated, model of sugarcane origins that will pave the way towards more effective introgression breeding by accurately determining the effective window for hybridization outside the *Saccharum* genus. In light of these findings, the origins of sugarcane cultivars is re-evaluated and the claim that New Guinea is the site of sugarcane's origin is refuted.

Keywords: molecular evolution, *Saccharum cultum*, sugarcane cultivation, sugarcane origins, sugarcane taxonomy

Introduction

Taxonomy, from the Greek τάξις (arrangement) and -νομία (method) is the science of arranging groups of species on the basis of shared characteristics. Modern molecular taxonomy allows for the combination of DNA analysis with taxonomy to characterise the evolutionary relationship between organisms. This is crucial for informed breeding, as the closer two species are to one another, the more likely they are to yield viable crosses.

Modern sugarcane cultivars are complex hybrids between several members of the *Saccharum* genus. This view was first developed by Artshwager and Brandes (1958), who proposed that the majority of sugarcane species originated in New Guinea before spreading out through China to India. Their classification was further developed by Clayton and Renvoize (1986), with additional enhancements by the 2014 Grass Nomenclature Committee (Clayton *et al*. 2014). As a result, only three species within the genus *Saccharum* are validly recognised: *Saccharum spontaneum, Saccharum officinarum* and *Saccharum robustum* (three formerly recognised species being re-classified as subspecies: *Saccharum officinarum* subsp. *sinense* and cultigens: *Saccharum edule* and *Saccharum barberi*).

Until now, no large-scale molecular systematic and phylogenetic analyses have been applied to genus *Saccharum*. Indeed, the current taxonomy of *Saccharum* has remained largely unchallenged for over 50 years.

All plants carry three genomes within their cells: the eukaryotic nuclear genome, the bacterial based chloroplast genomes (organelles that convert sunlight and carbon dioxide to sugars) and mitochondrial genomes (the organelles that provide the cell with energy). Organelle genomes evolve in concert, but slower than, the nuclear genome and as they are maternally inherited, they allow for a more ancient view on the origins of species that is not obscured by the effects of hybridization and modern breeding (Lloyd Evans and Joshi, 2016).

By combining whole chloroplast and whole mitochondrial phylogenetic analysis with phenotypic analysis, geophysical data, oceanographic data, ethnobotanical, ethnobiological, ethnolinguistic, human migration studies and ancient text analyses the authors present a new taxonomy and a new history of sugarcane's origins that overturns all previous studies. Also described is a novel species of *Saccharum*, *Saccharum cultum*, which was key to the development of modern sugarcane hybrids. It is demonstrated that New Guinea could not have been the centre of origin for any of the *Saccharum* species.

Materials and Methods

Chloroplast assembly, and phylogenetic analyses were performed as described previously (Lloyd Evans and Joshi, 2016). Finishing and polishing of the chloroplast genome was also performed as described previously (Martin *et al*. 2017). Searches for herbarium specimens and historical sugarcane drawings were performed with a custom Internet search spider written in LibWWWPerl.

Results and Discussion

Prior to this study, only two sugarcane chloroplast genomes had been assembled and were available in the public domain. Using SASRI-generated, UK BBSRC generated, and public data from the NCBI (ncbi.nlm.nih.gov) complete chloroplast genome sequences were assembled from genus *Saccharum*, as well as several potentially related genera. In addition, a complete chloroplast genome was constructed from transcriptomic data for a possible sugarcane relative, *Narenga porphyrocoma*.

Each chloroplast is about 140 000 bases in length, giving a total alignment matrix of over 5.3 million characters. After sequence alignment and optimisation, phylogenetic analyses were performed (about four weeks processing on a server).

The final phylogeny (Figure 1) revealed that the currently recognized *Saccharum* species separated into three groups: *Saccharum spontaneum*, *Saccharum officinarum* (which includes former *S. robustum*, *S. barberi* and *S. sinense*) as well as a novel grouping indicating the presence of a new species. Analysis of the cultivars in this new group showed that they were descended from the Polynesian sugarcanes discovered during the 1770s (Forster, 1777). When global herbaria collections were queried, a single specimen, GOET012507, from the University of Göetingen herbarium, which was collected by Georg Forster on Cook's second voyage (1772–1775), was identified. Treating this specimen as a fossil, it was introduced into the phylogeny and emerged as ancestral to the new species, which is described as *Saccharum cultum* (cultivated sugarcane) *sp. nov.*, Lloyd Evans and Joshi, 2016.

When the floral characteristics of the three *Saccharum* species were compared (Figure 1) it became evident that these are different species. Thus, modern sugarcane hybrids are a complex mix of the genomes of *Saccharum cultum*, *Saccharum officinarum* and *Saccharum*

spontaneum. Indeed, it was only when *Saccharum cultum* was introgressed with *Saccharum spontaneum* (and with the earlier *Saccharum* x *officinarum*) that modern, fertile, 'noble' canes were developed.

Analysis of *Narenga porphyrocoma*, (Figure 1) shows that this species lies within the *Saccharum* portion of the phylogeny and is ancestral to *Saccharum spontaneum*. Thus, *Narenga* is a true *Saccharum* and should be named *Saccharum narenga*. This is a very drought tolerant species and could act as an invaluable source in introgressing stress-resistance traits into the sugarcane genome.

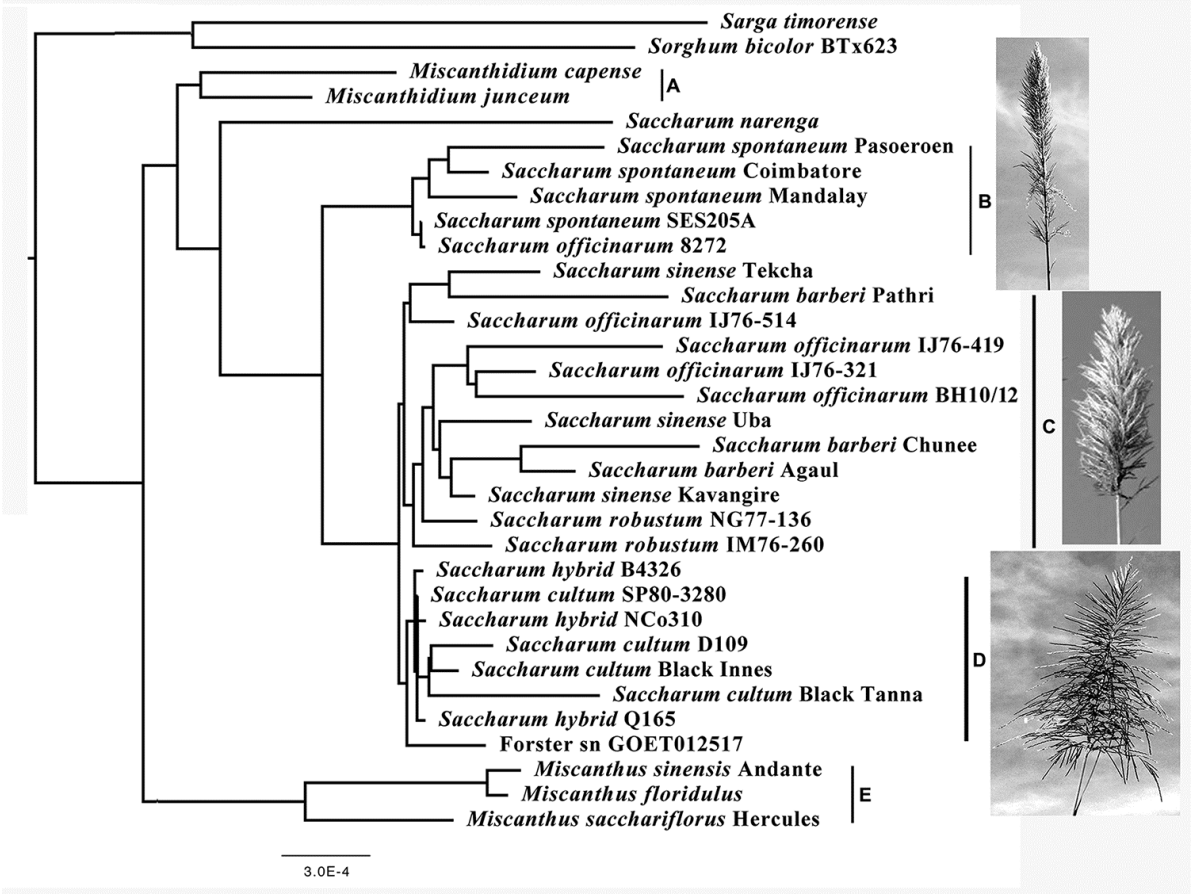

Figure 1. Phylogeny of *Saccharum* and allied genera. Presented is a maximum likelihood phylogeny for sugarcane and closely related genera. All species names are given, and the major genera are defined. The scale bar at the base gives the expected number of substitutions per site. The main species or genera on the phylogeny represented by vertical bars are: A, *Miscanthidium*; B, *Saccharum spontaneum*; C, *Saccharum officinarum*; D, *Saccharum cultum* and E, *Miscanthus*. The typical panicle forms are shown next to clades B, C and D, with the *S. spontaneum* panicle being dense and lanceolate (spear shaped, B), the *S. officinarum* panicle being dense and ovate or ovoidal (egg-shaped or oval, C) and the *S. cultum* panicle, which is totally different, being sparse and conoidal (cone or pyramid shaped, D). Images B and D © D Lloyd Evans, photographed at the South African Sugarcane Research Institute, image C photographed at Karnatka, India © Itslife.in. All images modified from the original and reproduced with permission.

Surprisingly, it was discovered that the southern African *Miscanthidium* species (*M. capense* and *M. junceum*) were the next closest species to sugarcane, followed by true *Miscanthus*. Thus, both *Miscanthus* and *Miscanthidium* should be considered in sugarcane breeding (a polyploid form of *Miscanthus sacchariflorus* is known to hybridize with sugarcane in the wild).

Knowing that there are two high sucrose ancestors of modern sugarcane, and knowing that these two species have different floral characteristics, the history of sugarcane's spread across the planet can now be re-evaluated.

Sugarcane did not enter Han China until the third century CE, though there are reports of sugarcane growing as part of kitchen garden crops in the Yüeh (Barbarian) regions of South Asia as chewing cane and pig fodder (Needham *et al*. 1996). The history of sugarcane in India is older, with animal powered cane mills known to exist at least 2500 BCE. This is confirmed by Alexander the Great's expedition into India in 325 BCE which reports the presence of reeds that 'make honey without bees'.

From India, sugarcane reached Persia about 500 CE, and it was subsumed into the Arab world with the conquest of Persia, about 600 CE. The plant soon became a mainstay of the Arab cropping system and Arab traders introduced it to East Africa soon after (al-Idrisi, mentions it amongst East Africa's products (al-Idrisi, 1150)). Umayyad conquests of North Africa and Mediterranean Europe (c. 900 CE) brought sugarcane to these regions. These are sugarcanes of the *Saccharum officinarum* type, as images from European and Arab texts of the 14th century demonstrate (D. Lloyd Evans, personal communication). These were typically sterile and it was these cultivars that were introduced into the New World with Columbus' second voyage. Indeed, these were the only cane types grown globally until after 1780.

It was only in 1690 that *Saccharum cultum* entered the scientific record, with Rumphius' discoveries in Indonesia (Rumpf, 1741) and only in 1780 were cultum-type canes introduced globally from Polynesia. Having higher sucrose content, they soon supplanted the old *S. officinarum* hybrids, but were disease susceptible. In the 1860s it was discovered that the new sugarcane types were fertile and experiments with hybridization started. These led to the first 'noble' canes.

This study presents a completely new phylogeny of *Saccharum* species, discovering a previously undefined species (*Saccharum cultum*) and define a new origin of sugarcane. The authors re-interpret the history of sugarcane in the light of these findings and overturn all previous hypothesis regarding sugarcane's origins.

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