Genetic Diversity and Relationships in Native Hawaiian Saccharum officinarum Sugarcane

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Abstract

Commercial sugarcane hybrid cultivars currently in production are high-yielding, disease-resistant, millable canes and are the result of years of breeding work. In Hawaii, these commercial hybrids are quite distinct from many Saccharum officinarum canes still in existence that were brought to the islands and cultivated by the native Polynesians. The actual genetic relationships among the native canes and the extent to which they contributed to the commercial hybrid germplasm has been the subject of speculation over the years. Genetic analysis of 43 presumed native Hawaiian S. officinarum clones using 228 DNA markers confirmed them to be a group distinct from the modern hybrid cultivars. The resulting dendrogram tended to confirm that there were several separate S. officinarum introductions that, owing to selections of somatic mutations, diverged into a number of cluster groups. When the “Sandwich Isles” were discovered by Captain James Cook in 1778, the Hawaiians were found to be growing sugarcane, S. officinarum (Cook 1785). Sugarcane (ko, in the Hawaiian language) appeared in a variety of stalk and leaf colors, often with stripes (the “ribbon canes”). In the interest of preserving this historic germplasm, a collection was assembled in the 1920s by Edward L. Caum of the Hawaiian Sugar Planters’ Association and W. W. G. Moir of American Factors. Histories and descriptions of the canes were reported by Moir (1932). Moir (1932) arranged the native Hawaiian cultivars into groups and families based purely on their morphological characteristics (Table 1). Wilfong (1883) stated that Ualalehu, Ualalehu maoli (native), Honuaula, Laukena (Laukona), Kea (Kokea), Papa, and Ohua were indigenous natives, while Lahaina, Palani, Hou, Manulele, Uala, and others were brought here from abroad. Although Lahaina has a native Hawaiian name, it was imported from the Marquesas in 1854 (Wilfong 1883; Hawaiian Sugar Planters’ Association records). Mangelsdorf (1956) surmised that the native canes might all be selections of somatic mutants of a single Saccharum officinarum introduction, but this is lost to history. This project attempted to clarify the actual genetic relationships of the native Hawaiian S. officinarum varieties that still exist and their relationship, if any, to the commercial Saccharum hybrids.

Early attempts to profitably produce sugar from the original Hawaiian canes proved unsuccessful because they were too soft for milling and were susceptible to introduced diseases (Mangelsdorf 1956). The native Hawaiian S. officinarum canes were thought to be infertile, although they do produce flowers and recent research has shown that a percentage of the pollen of some of them is viable (Nagai et al. 1990). Other S. officinarum cultivars and interspecific hybrids were imported for commercial production and for breeding (Mangelsdorf 1956). As a result, modern commercial Hawaiian sugarcanes are hybrids of S. officinarum, Saccharum robustum, Saccharum barberi, and Saccharum spontaneum species. Detailed records were kept and the parentage of the present commercial canes can be traced back to the original imports (Tew 1987). Mangelsdorf (1956) stated that “All of the present major varieties of Hawaii include in their ancestry 32-8560, itself until recently the leading variety in the Territory. The mother of 32-8560 is the Indian variety CO 213, a seedling of P.O.J. 213. The father of 32-8560 is the Java variety P.O.J. 2878.” Other early cultivars included Lahaina (S. officinarum), H-109 (a progeny of Lahaina and an unknown parent), and Yellow Caledonia (S. officinarum imported in 1881 from an unknown location) (Tew 1987). All of these cultivars still exist and were included in this study. It is not known whether any of the native Hawaiian S. officinarum cultivars contributed germplasm to the commercial breeding program.
The taxonomy and speciation within the genus *Saccharum* is complex and uncertain. Although most authors agree that *S. spontaneum* and *S. robustum* are ancestral wild species, and the whole group—*S. spontaneum*, *S. robustum*, *S. officinarum*, *S. barberi*, and *Saccharum sinense*—is interfertile (Berding and Roach 1987; Irvine 1999). Chromosome number and ploidy vary greatly and there is much overlap between them. Recent advances in genetic technology, notably the application of restriction fragment length polymorphism (RFLP) markers, has begun to shed some light on this complex and diverse genus (Jannoo et al. 1999; Lu et al. 1994a,b). Interspecific crosses were the basis for the development of the modern sugarcane industry, with its many high-yielding, disease-resistant cultivars adapted to various environmental conditions worldwide.

**Materials and Methods**

**Sugarcane Cultivars**

The sugarcane cultivars used in this project were taken from plots in the Hawaii Agriculture Research Center (formerly Hawaiian Sugar Planters’ Association) breeding station at Maunawili, Hawaii, or from the Waimea Arboretum, Haleiwa, Hawaii, and other locations around the islands. The cultivars with Hawaiian names such as Akoki, Pakaweli, Manulele, etc., are the same clones as those collected by Moir and Caum (Moir 1932). Their appearance matches Moir’s description. The cultivars collected from Waimea Arboretum were previously given to it by Hawaiian Sugar Planters’ Association and are also the same clones that were collected by Moir and Caum and maintained since that time. Others were found in various locations and their original names are unknown. A total of 43 Hawaiian *S. officinarum* accessions and three later *S. officinarum* imports were collected for DNA fingerprinting, along with two early *Saccharum* hybrids (circa 1925), five current commercial hybrids, and one sample each from *S. robustum* and *S. spontaneum* added as outgroups.

**DNA Extraction**

DNA was extracted from leaf blade tissue that was chopped, lyophillized, and ground to powder. Genomic DNA extraction was slightly revised from Tai and Tanksley (1990). Each sample was digested with *EcoRI* and *HindIII*. About 7.5 μg of DNA per lane were run on a gel. Southern blotting, radioactive labeling, and autoradiography were as described (Chittenden et al. 1994).

**DNA Probes**

Thirty-eight genomic and cDNA probes derived from sugarcane and sorghum were selected for fingerprinting the native Hawaiian sugarcane collection, including 28 sugarcane cDNA probes derived from cell suspension culture, germinating lateral buds, and germinating set roots; 1 sugarcane genomic probe; and 9 sorghum genomic probes. The sorghum genomic DNA probes were obtained from the Center for Applied Genetic Technology, University of Georgia, Athens, Georgia. These probes were distributed throughout the sugarcane genome based on their position on the *Saccharum* consensus genetic map (Ming et al. 2002).

**Data Analysis**

For each probe, the polymorphic restriction fragments found from all varieties were numbered from high to low molecular weight. Each fragment (marker) was scored as 1 (present) or 0 (absent) in a spreadsheet. The data were formatted for the NTSYSpc (version 2.1) cluster analysis software (Exeter Software Co., Setauket, NY). Monomorphic markers were not scored. The RFLP marker data were used to compute pairwise Dice coefficients (Dice 1945). Cluster analysis was performed on the similarity matrix using the “unweighted pair group method using arithmetic means” (UPGMA) algorithm (Sneath and Sokal 1973) provided in the software package NTSYSpc. The cophenetic correlation coefficient was calculated to test the goodness-of-fit between the similarity and the cophenetic matrices. A 50% majority rule consensus tree was calculated from the most parsimonious trees using PAUP 4.0 (Swofford 2002). Bootstrap values were calculated from 100 replicates.

**Results and Discussion**

A total of 228 polymorphic DNA markers were detected by the 38 sugarcane and sorghum DNA probes and scored for phylogenetic analysis. Each probe detected a discrete hybridization pattern and produced one to nine polymorphic markers with an average of six markers per probe. Each probe detected 5 to 17 DNA fragments, with most of them being polymorphic. One hundred eighty-five DNA fragments were too close to be discretely scored. Thirty monomorphic markers were detected by 26 probes. A few markers showed differences in the band intensity, possibly

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Groups and families of <em>S. officinarum</em> based on Moir (1932)</th>
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</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td><strong>Group II</strong></td>
</tr>
<tr>
<td>Akilolo family</td>
<td>Laukona family</td>
</tr>
<tr>
<td>Nanahu</td>
<td>Laukona</td>
</tr>
<tr>
<td>Pakaweli</td>
<td>Lahia</td>
</tr>
<tr>
<td>Akoki family</td>
<td>Uahiapete family</td>
</tr>
<tr>
<td>Akoki</td>
<td>Uahiapete</td>
</tr>
<tr>
<td>Uala</td>
<td>Pohina</td>
</tr>
<tr>
<td>Manulele family</td>
<td>Kea family</td>
</tr>
<tr>
<td>Manulele</td>
<td>Kea (Kokea)</td>
</tr>
<tr>
<td>Honuaula</td>
<td>Miscellaneous</td>
</tr>
<tr>
<td>Awela family (seedlings, mutants or imports)</td>
<td>Awela (Puaole)</td>
</tr>
<tr>
<td>Ulahu</td>
<td>Lahia</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Lehu (closely related to above families)</td>
</tr>
<tr>
<td>Ohia</td>
<td>Waiohia</td>
</tr>
</tbody>
</table>

The sugarcane cultivars used in this project were taken from plots in the Hawaii Agriculture Research Center (formerly Hawaiian Sugar Planters’ Association) breeding station at Maunawili, Hawaii, or from the Waimea Arboretum, Haleiwa, Hawaii, and other locations around the islands. The cultivars with Hawaiian names such as Akoki, Pakaweli, Manulele, etc., are the same clones as those collected by Moir and Caum (Moir 1932). Their appearance matches Moir’s description. The cultivars collected from Waimea Arboretum were previously given to it by Hawaiian Sugar Planters’ Association and are also the same clones that were collected by Moir and Caum and maintained since that time. Others were found in various locations and their original names are unknown. A total of 43 Hawaiian *S. officinarum* accessions and three later *S. officinarum* imports were collected for DNA fingerprinting, along with two early *Saccharum* hybrids (circa 1925), five current commercial hybrids, and one sample each from *S. robustum* and *S. spontaneum* added as outgroups.
Table 2. Supposed origins of *S. officinarum* clones in Hawaii

| Akilolo (no longer exists) | *S. officinarum* introduced by original Hawaiian immigrants
|---------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Akoki | *S. officinarum* introduced by original Hawaiian immigrants
| Cavengerie (Icic) | Native Hawaiian *S. officinarum*; Early commercial *Saccharum* hybrid, progeny of White Mexican
| H52 | *S. officinarum* introduced by original Hawaiian immigrants
| H109 | *S. officinarum* introduced by original Hawaiian immigrants
| Halalili | Native Hawaiian *S. officinarum*; Introduced by Europeans
| HC62 | Unknown Hawaiian *S. officinarum*
| HC63 | Unknown Hawaiian *S. officinarum*
| Honuaula | Native Hawaiian *S. officinarum*, mutant of Manulele
| Ieic (Cavengerie) | Native Hawaiian *S. officinarum*
| Kalaoa | *S. officinarum*
| Keauhou | *S. officinarum*
| Kokea (Kea) | *S. officinarum* introduced by original Hawaiian immigrants
| Koula (Ula) | *S. officinarum*
| Lahaina | Introduced from the Marquesas in 1854, parent of H109
| Lahi (Ulalehu) | Mutant of Laukona
| Laukoa | *S. officinarum*
| Laukona | *S. officinarum* introduced by original Hawaiian immigrants
| Lehu | Introduced by Europeans
| Mahaula | *S. officinarum*
| Manulele | *S. officinarum* introduced by original Hawaiian immigrants
| Moano | Introduced by Europeans
| Nanahu | Native Hawaiian *S. officinarum*, mutant of Akilolo
| Not Kokea (original name lost) | *S. officinarum*
| Obia | Native Hawaiian *S. officinarum*
| Pakaweli | Native Hawaiian *S. officinarum*, mutant of Akilolo
| Pohina | Native Hawaiian *S. officinarum*, almost identical to Uahiapele
| Pokapua | *S. officinarum*
| Puaole (Awela) | *S. officinarum* introduced by Europeans; native Hawaiian
| Uahiapele | Native Hawaiian *S. officinarum*, almost identical to Pohina
| Uala | Introduced by Europeans; native Hawaiian mutant of Akoki
| Ulalehu (Lahi) | Mutant of Laukona
| Ulu | Native Hawaiian *S. officinarum*
| Ululuhui | Native Hawaiian mutant of Awela

Table 2. Continued

| Akilolo (no longer exists) | *S. officinarum* introduced by original Hawaiian immigrants
|---------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Waimea | *S. officinarum*
| Waiohia | Native Hawaiian *S. officinarum*
| Yellow Caledonia | Introduced in 1881 for breeding

*Moir (1932).*  
*Kamakea (1872).*  
*HSPA breeding records.*  
*Wilfong (1883).*

reflecting the dosage effect. This type of marker was not included in the cluster analysis.

Forty-six accessions of *S. officinarum* clones thought to be Hawaiian natives or early imports were collected and analyzed (Table 2). The numbers following some of the names indicate individual accessions. Clones with the same name should therefore be alike genetically, but this was not always the case. In some samples there was no known name: HC (for “Hawaiian cane”), unknown, and Maui cane are some of these. In addition, we also included some of the early imports and two of the first cultivars (H52 and H109) bred in Hawaii and grown commercially. The dendrogram of cluster analysis based on Dice similarity coefficients is shown in Figure 1. Seven clusters with two or more closely related accessions can be distinguished with the outgroup samples *S. robustum (MOL 5829)* and *S. spontaneum* (Burma). A cluster was defined as sharing 90% or more identical markers. A second dendrogram was generated using Wagner parsimony, and the bootstrap values for all seven clusters were 100 (Figure 2).

A cluster of very closely related clones, all with historic Hawaiian names, can be seen at the top of the diagram (cluster I). All of the names within this cluster are cited in one or more of the references to be of native Hawaiian origin or to be mutants of them (Table 2). Only the names Kalaoa, Keauhou, and Koula are not mentioned by any of the early authors. It is possible that cluster I represents a series of somatic mutants from a single original introduction by the ancient immigrants to the Hawaiian islands, as Mangelsdorf (1956) suggested.

Some of the other canes that were thought to be of native Hawaiian origin shared less than 80% of the markers with the above-mentioned cluster. These include Manulele, Waiohia, Laukona-15, Kokea, and Honuaula. Whether we still have the correct canes so named or have other misnamed canes is unknown. Our Kokea sample is so different that it may not even be *S. officinarum*. Moir (1932) cited an ancient Hawaiian legend concerning Manulele (“flying bird”) and considered it to be a native, as did Kamakea (1872). However, our results support Wilfong (1883), who listed it as a later import.

Other than the “core” group, there is quite a diversity among the Hawaiian clones, indicating that they represent many different introductions. Most of Moir’s “family” groupings do appear to be justified genetically (Table 1). Moir grouped Nanahu and Pakaweli, Akoki and Uala, Ululuh
and Puaole, Ohia and Lauloa, and Lahi and Laukona. However, Uahiapele and Pohina are not clustered together. Uahiapele is genetically unrelated to Uahiapele-50, nor are they similar in appearance. It is unlikely that our so-called Uahiapele is actually the correct clone. Moir included Manulele and Waiohia among the ancient Hawaiian clones, whereas our cluster analysis showed that Manulele and Waiohia shared more than 90% of their markers with the later import, Yellow Caledonia (cluster V). As expected, the known foreign imports of *S. officinarum*, Lahaina and Yellow Caledonia, as well as the early commercial hybrids H52 and H109, share less than 80% of their markers with the “core” cluster. Lahaina and H109 share nearly 80% of their markers and are known to be parent and progeny.

Cluster analyses of the modern commercial Hawaiian *Saccharum* hybrids show them to be clearly distinct as a group from the native Hawaiian canes. Kokea, as well as the early Hawaiian commercial hybrid H52, also segregates with the modern commercials. *S. officinarum* is thought to have originated from *S. robustum*, although probably not directly (Lu et al. 1994b). It is clear from the dendrogram that *S. officinarum* is more closely related to the *S. robustum* clone than it is to the *S. spontaneum* clone tested in this study.

One of our hypotheses was that the native Hawaiian canes represented an isolated and distinct group of *S. officinarum* that were nearly identical to each other, but significantly different from all other *S. officinarum* clones. While there was a closely related cluster of Hawaiian clones (cluster I), most were genetically quite diverse. The results tended to confirm that there were several separate *S. officinarum* introductions into Hawaii that, owing to selections of somatic mutations, diverged into a number of cluster groups. Mangelsdorf (1956) stated that the ancient Hawaiian canes were infertile and therefore could not have contributed to the germplasm of the commercial cultivars. Our results support, but do not prove, this conclusion.

**Acknowledgments**

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