

## POLYPLOID AND HYBRID ORIGINS OF PACIFIC ISLAND SANDALWOODS (*SANTALUM*, SANTALACEAE) INFERRED FROM LOW-COPY NUCLEAR AND FLOW CYTOMETRY DATA

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It has been argued that polyploids are better adapted than diploids for long-distance dispersal to and establishment on oceanic islands. To address this issue in a molecular phylogenetic framework, the extensive history of auto- and allopolyploidization in *Santalum* (Santalaceae), the sandalwood genus, was studied by sequencing the low-copy nuclear gene *waxy* and investigating the ploidy level of all 16 species. Ploidy level was estimated by measuring the C value (total amount of DNA per nucleus) using flow cytometry and calibrating it by known chromosome numbers and new root-tip chromosome counts of several taxa. Results indicate four ploidy levels in *Santalum*: diploid ( $n=10$ ), tetraploid ( $n=20$ ), hexaploid ( $n=30$ ), and octoploid ( $n=40$ ). The *waxy* phylogeny suggests that at least six independent polyploid events occurred in the history of *Santalum*: two allopolyploid events between distantly related species and four putatively autopolyploid events. An additional hybrid event between two tetraploid Hawaiian clades evidently produced the tetraploid species *S. boninense*, endemic to the Bonin Islands. By finding more than twice as many long-distance island colonizations from polyploid as from diploid ancestors, this study provides novel evidence for the role of polyploidy in plant colonization throughout the Pacific Islands.

**Keywords:** hybridization, island biogeography, phylogeny, polyploidy, *Santalum*, *waxy*.

**Online enhancement:** appendix table.

### Introduction

Polyploidy is a widespread, evolutionarily important process in plants, producing both novel traits and new taxa (Stebbins 1950; Rieseberg 1997; Jiang et al. 1998; Adams and Wendel 2005). The increased genetic diversity and DNA content of polyploids, both auto- and allopolyploids, has been demonstrated to have a variety of physiological effects in plants, such as changes in cell and organ size (Stebbins 1950). Polyploid plants have also been shown to have enhanced vigor (Comai 2005) and higher tolerance for environmental stress (MacGillivray and Grime 1995) and low nutrient availability (Levin 1983), as well as lower rates of inbreeding depression (Soltis and Soltis 2000) and broader ecological tolerances (Bottini et al. 2000; Soltis and Soltis 2000), than their diploid relatives. Many invasive plant species have been determined to be polyploids (Thompson 1991; Pandit et al. 2006).

Polyploidy has been argued to be particularly advantageous in long-distance dispersal to and subsequent establishment on oceanic islands (Stebbins 1950; Carr 1998; Barrier et al. 1999). Several studies have demonstrated that polyploids have been successful colonizers of oceanic islands, including *Glycine tabacina* and *Glycine tomentella* (Fabaceae) in the west-

central Pacific (Doyle et al. 2000, 2002) and the silversword alliance (Asteraceae) in the Hawaiian Islands (Barrier et al. 1999). A number of oceanic island floras, such as those of the Hawaiian Islands (Carr 1978, 1998; Kiehn 2005), the Juan Fernandez Islands (Sanders et al. 1983; Stuessy and Crawford 1998), and Norfolk Island (de Lange and Murray 2003), contain large numbers of polyploids. However, plants of other island archipelagos, such as the Canary Islands, have low incidences of polyploidy, as do their mainland relatives (Suda et al. 2003). Stuessy and Crawford (1998) argued that high levels of polyploidy in island floras are due to a high frequency of polyploid colonists and that in situ polyploidization is much less important in island plants because of the short timescale of island radiations and selection for the retention of trait complexes that led to colonization and establishment. Therefore, polyploidy may have different levels of importance to the evolution of island floras, depending on the age of the island or island lineage (Whittaker 1998) and the ploidy level of potential colonists in mainland source areas.

Molecular phylogenetic analyses to investigate the role of polyploidy in the colonization or establishment of plants to oceanic islands are lacking. The sandalwood genus *Santalum* (Santalaceae), commonly known for its fragrant heartwood oil, is a good study system in which to test the hypothesis that oceanic island taxa are more likely to have arisen from polyploid ancestors than from diploid ancestors, because it has a history of repeated dispersals to Pacific islands and more than one ploidy level. *Santalum* is one of the most widely distributed Pacific Basin plants, naturally occurring from Australia

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to the Hawaiian Islands (Harbaugh and Baldwin 2007). A previous phylogenetic analysis of *Santalum* using a combination of two nuclear ribosomal spacer regions (ITS and ETS) and one chloroplast gene region (3' *trnK* intron) gave evidence for at least five naturally occurring long-distance dispersal events from Australia to Pacific islands, including the extinct *S. fernandezianum*, endemic to the Juan Fernandez Islands (Harbaugh and Baldwin 2007). Previous chromosome counts indicate that at least two ploidy levels occur in the genus: the well-known commercial sandalwood, *S. album*, which is distributed throughout India, Indonesia, and Australia, is diploid (Goldblatt and Johnson 2000), while the two Hawaiian clades are tetraploid (Carr 1978; Harbaugh and Baldwin 2007). Incongruence between nuclear and chloroplast trees in the positions of two taxa, *S. boninense* and *S. macgregorii*, shows that hybridization and possibly allopolyploidization may have played a role in the evolution of the genus (Harbaugh and Baldwin 2007).

To help understanding of the pattern of polyploidization in the genus, the ploidy levels of all *Santalum* species and nearly all varieties were determined or estimated in this study by flow cytometry (C value) and root-tip chromosome counts, and patterns of this trait were examined in a phylogenetic context. In this study, phylogenetic relationships in all 16 extant *Santalum* species, including the recently recognized cryptic Australian species *S. leptocladum* (Harbaugh 2007b), were studied by using an additional nuclear region, the 3' end of the low-copy gene *waxy* (granule-bound starch synthase [GBSSI]), to resolve instances of auto- and allopolyploidization and to provide additional insights into long-distance dispersal patterns in the genus. In combination with results of the flow cytometry analysis and root-tip chromosome counts, the *waxy* phylogeny was used to test the hypothesis that colonizers of oceanic islands tend to be polyploid rather than diploid in *Santalum*.

## Material and Methods

### Flow Cytometry Estimation of Ploidy Levels

Flow cytometry has been used to identify the presence of polyploidy in a number of plant groups (Amsellem et al. 2001; Suda et al. 2003; Dart et al. 2004). To assess ploidy levels across *Santalum* in the absence of fresh floral bud or root-tip material, DNA flow cytometry was used to estimate the absolute DNA amount per nucleus (C value) in 15 of the 16 extant *Santalum* species. (C values could not be determined from the available *S. spicatum* material, so root-tip chromosome counts were performed to estimate its ploidy level, as described in "Root-Tip Chromosome Counts.") Twenty-nine specimens were included in this analysis (see table A1 in the online edition of *IJPS*); sampling was designed to cover as much as possible of the taxonomic and genetic diversity identified in the previous phylogenetic analysis (Harbaugh and Baldwin 2007), given availability of freshly (silica-)dried leaf material. Samples consisting of a single leaf were analyzed at the Flow Cytometry Facility, at Iowa State University, Ames, using the Suda and Trávníček (2006) procedure for dried leaf material. Three measurements were taken for each specimen, along with an internal standard (chicken erythrocyte nucleus [CEN]) to account for variation in the preparation of the sample as

well as random error in the flow cytometer (Dolezel and Bartos 2005). The nuclear DNA content (C value), measured in pg DNA, of *Santalum* samples was estimated using the equation

$$\text{nuclear DNA content} = 2.33(\text{Santalum sample DNA mean} / \text{CEN sample DNA mean}),$$

where 2.33 was the known nuclear DNA content (in pg DNA) of the internal standard, CEN.

### Root-Tip Chromosome Counts

Chromosome counts for two previously unreported species, *S. acuminatum* and *S. spicatum*, were made from root tips. Wild-collected seeds were obtained for germination (B and T World Seeds, Pagnignan, France); *S. acuminatum* was from commercial collections made in Western Australia, and *S. spicatum* was from South Australia. Positive identification of each species was based on the unique morphological characteristics (i.e., endocarp texture) of the seeds. Seeds were germinated in a greenhouse at the University of California, Berkeley, in 100% perlite, after endocarp removal and treatment with 250 ppm gibberellic acid.

Shortly after germination, root tips were harvested and pretreated in a saturated aqueous solution of PDB (paradichlorobenzene, 1,4-dichlorobenzene) for 18–20 h at 4°C. The roots were fixed in 3 : 1 ethanol : glacial acetic acid for 24 h at 4°C and then stored in 70% ethanol at –20°C. Root tips were squashed on a glass slide in a drop of 1.0 M hydrochloric acid, stained with acetocarmine, and scanned for mitotic cells under an LM with phase optics. Chromosome number determinations were based on counts from at least five cells of each taxon.

### Estimating Unknown Ploidy Levels

Data from the flow cytometry analysis were used to estimate ploidy level of taxa for which chromosome counts were unavailable. Published chromosome counts (Carr 1978; Goldblatt and Johnson 2000), along with those from this study, were used as internal calibrations to determine the amount of DNA per nucleus (pg DNA) for a given ploidy level. The amount of DNA was plotted for each sample, and a multiple-range test (Tukey-Kramer) was run to determine discrete breaks in the data (different ploidy levels) using the statistics software JMP, version 6.0 (SAS Institute, Cary, NC). Once chromosome numbers were predicted for each specimen, a regression analysis was performed to test for a linear relationship between ploidy level and amount of DNA per nucleus.

### Phylogenetic Analysis of the Low-Copy Nuclear Gene *waxy*

**Taxonomic and molecular character sampling.** Representatives of all 16 extant species, including any major clades within species, and all putatively hybridizing taxa identified in the *Santalum* phylogeny of Harbaugh and Baldwin (2007) were sampled. Thirty-six specimens were used in this analysis, including 34 ingroup and two outgroup taxa (appendix table A1), all of which were identified by unique morphological characters used to delimit taxa in *Santalum* (Harbaugh 2007a). Of the 34 *Santalum* specimens, 24 were the same samples

used in the flow cytometry analysis. The nuclear gene *waxy* (GBSSI) was selected to provide molecular characters for this phylogenetic analysis, in part because such single- or low-copy nuclear genes are less prone to concerted evolution than multigene families (such as ribosomal DNA) and therefore often provide evidence for hybridization (Raymond et al. 2002; Sang 2002; Small et al. 2004). In addition, *waxy* has been demonstrated to have utility across a wide range of plant groups for resolving relationships, including hybrid origins, of taxa in young clades (Mason-Gamer et al. 1998; Miller et al. 1999; Evans et al. 2000; Peralta and Spooner 2001; Walsh and Hoot 2001; Baumel et al. 2002; Ingram and Doyle 2003; Guo and Li 2004; Levin et al. 2005).

**Gene amplification, cloning, and sequencing.** Total genomic DNA was extracted from silica-dried leaf material using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA), after the leaves had been ground to a fine powder. Symmetric PCR amplification of the 3' end of the *waxy* (GBSSI) gene, from exon 10 to exon 13, was performed using the primers GBSSI-13R (Tank 2006) and GBSSI-10F (AYTGCYGGNGCTGAT-TTTRTG); the latter was modified from Tank (2006), using *Musgravea* (Proteaceae) and *Macadamia* (Proteaceae) sequences from GenBank, to make the primer more widely useful. The 3' end of *waxy* was amplified using Bioneer AccuPower PCR Pre-Mix tubes (Bionexus, Oakland, CA) in 20- $\mu$ L reactions including 5  $\mu$ L genomic DNA and 12  $\mu$ L distilled water on a thermal cycler with the following parameters: 95°C for 2 min followed by 30 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min. The cycling ended with 72°C for 5 min. Amplified products were cloned by using the Zero Blunt TOPO TA Cloning Kit for sequencing (Invitrogen Corporation, Carlsbad, CA).

For each specimen, up to 10 colonies were examined for inserts to screen for multiple alleles in heterozygotes and homeologous loci in allopolyploids (see table A1 for numbers of clones sequenced per specimen). In a diploid individual, sampling five colonies results in a >93% chance of sequencing both alleles of a locus (Mort and Crawford 2004). Inserts were screened using the standard M13F and M13R plasmid vector primers with the following cycling parameters: 94°C for 12 min followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min. The cycling ended with 72°C for 7 min. Products of the appropriate length were then cleaned using the Exo-Sap Pre-Sequencing Kit (USB, Cleveland, OH) followed by standard cycle-sequencing protocols using the ABI Prism BigDye Terminator kit, version 3.1 (Applied Biosystems, Foster City, CA). Direct sequencing of both strands was done at the University of California, Berkeley, DNA Sequencing Facility. Sequences were visualized and edited using the software Sequence Navigator (Applied Biosystems) and then (easily) aligned by eye in PAUP\* 4.0b10 (Swofford 2002).

**Phylogenetic analyses.** A maximum parsimony (MP) heuristic search of the *waxy* data set was performed in PAUP\* with 100 random-addition sequences, TBR (tree bisection reconnection) branch swapping, and MulTrees on. Identical sequences and uninformative characters were removed before the analysis. Parsimony bootstrap support values for clades were calculated using PAUP\*. In total, 500 bootstrap replicates were run with a full heuristic search, 10 random-addition sequences per replicate, TBR branch swapping, and MulTrees on.

A maximum likelihood (ML) analysis was run on the data set in PAUP\* using the best-fit model HKY+G, as chosen from likelihood ratio tests in Modeltest, version 3.6 (Posada and Crandall 1998). A heuristic search was performed with 10 random-addition sequences and TBR branch swapping. Identical sequences were removed before analysis. ML bootstrap values were computed in PAUP\* by running 100 replicates with a full heuristic search using 10 random-addition sequences per replicate, nearest-neighbor-interchange branch swapping, and MulTrees on.

The conservative Shimodaira-Hasegawa (S-H) test was used to examine the significance of alternative hypotheses of evolution in *Santalum*, including the origination from a single lineage of the putative allopolyploids and hybrids (*S. boninense*, the *S. ellipticum*/*S. paniculatum* clade, and *S. macgregorii*; Shimodaira and Hasegawa 1999; Goldman et al. 2000). Separate S-H tests for each hypothesis were conducted in PAUP\* using resampling estimated log-likelihood (RELL) optimization and a one-tailed test, with 1000 bootstrap replicates and the same likelihood settings as in the ML analysis. An additional test, in which all hypotheses were tested simultaneously, was also conducted with the same PAUP\* settings.

## Results

### *Chromosome Numbers and Estimated Ploidy Levels*

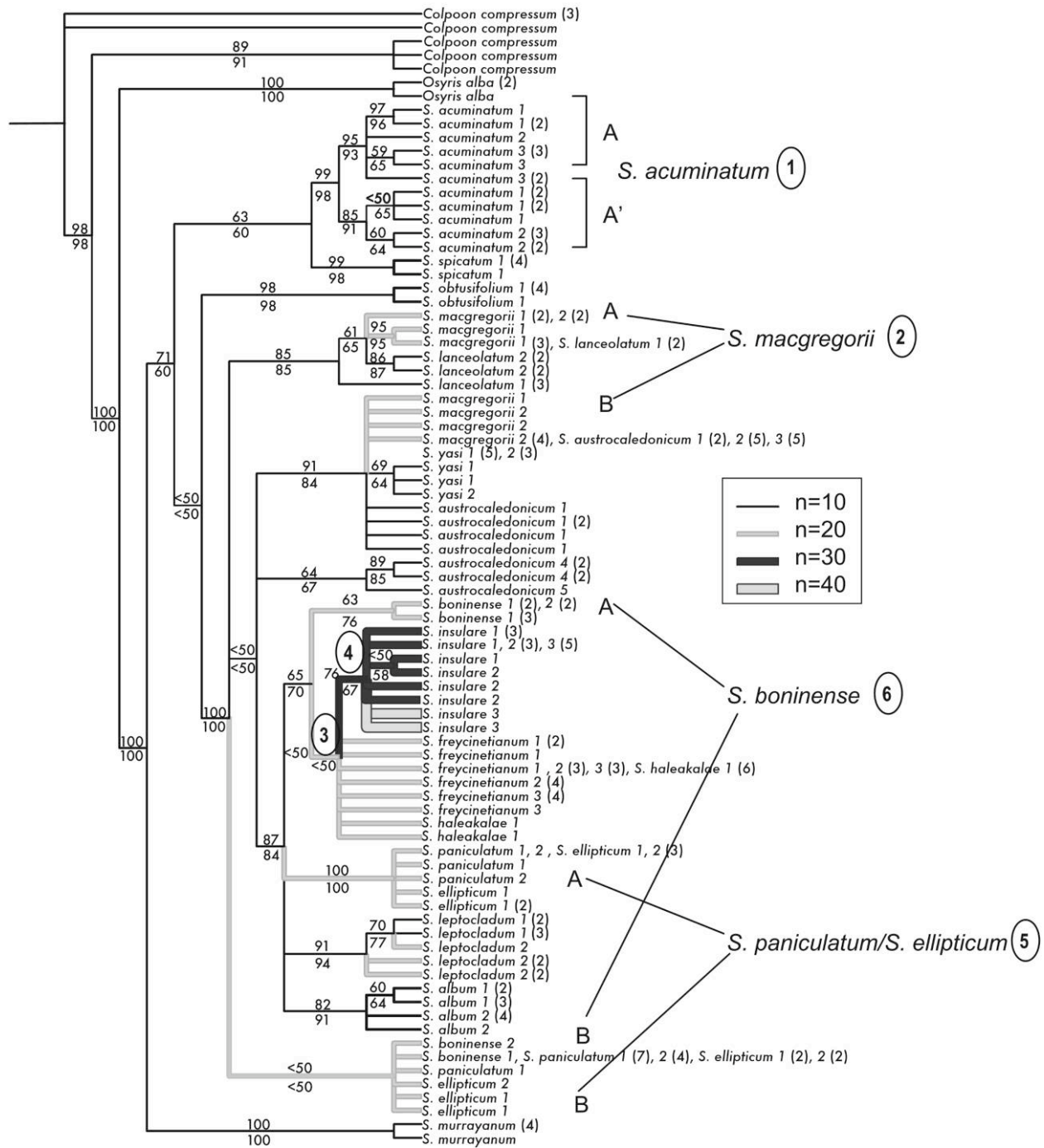
Root-tip counts show that *S. acuminatum* and *S. spicatum* each had a somatic diploid chromosome number of  $2n \approx 20$ . The count for *S. acuminatum* and previously published chromosome numbers for other *Santalum* taxa were used to calibrate the DNA content data (C value) from the flow cytometry analysis (DNA content data were unavailable for *S. spicatum*). A multiple-range test on the DNA content data showed four discrete groups of values that are significantly different from one another ( $P = 0.05$ ; fig. 1A). With calibrations of known ploidy levels based on chromosome counts for four taxa (fig. 1A), four ploidy levels were estimated to have the following ranges of DNA content:  $n=10$ , 0.61–0.98 pg DNA;  $n=20$ , 1.17–1.57 pg DNA;  $n=30$ , 1.83–1.97 pg DNA;  $n=40$ , 2.33–2.66 pg DNA. A regression analysis of DNA content data on chromosome number showed a significant linear relationship ( $df = 86$ ,  $P < 0.0001$ ,  $R^2 = 0.96$ ; fig. 1B; data in table A1).

### *Phylogenetic Analysis of the Low-Copy Nuclear Gene waxy*

The total length of the aligned *waxy* sequences was 689 characters, of which 183 were parsimony informative (26.6%). For the 36 specimens included in the analysis, an average of 5.72 colonies were sequenced, resulting in a total of 206 *waxy* sequences. MP analysis resulted in one tree island having 44,541 shortest trees (286 steps, consistency index = 0.82, retention index = 0.96). The ML analysis resulted in only one tree from one tree island (fig. 2;  $-\ln L = 3234.8372$ ), which is congruent with but better resolved than the strict consensus from the MP analysis. The MP and ML bootstrap values were similar (fig. 2).

The tree (fig. 2) shows several instances of polyphyletic *waxy* isolates from the same individual, consistent with a history of hybridization and allopolyploidization in *S. macgregorii*, *S.*





**Fig. 2** *Santalum waxy* maximum likelihood tree. Numbers above the branches represent maximum likelihood bootstrap values, while those below are maximum parsimony bootstrap values. Numbers after taxon name refer to specimen numbers in table A1. Numbers in parentheses after taxon names indicate the number of isolates sharing identical *waxy* sequences. Large numbers in ovals indicate events discussed in “Polyploidization, Hybridization, and Long-Distance Dispersal of *Santalum* taxa.” Line thickness represents the ploidy level mapped onto the tree, with the thinnest line for diploids (n=10) and thickest line for octoploids (n=40). The clades labeled A and A’ for *S. acuminatum* refer to the two loci produced from a putative gene duplication event. The clades labeled A and B for *S. macgregorii*, *S. boninense*, and *S. ellipticum/S. paniculatum* refer to the two loci inferred to be the result of hybridization.

inferred to be tetraploid or of higher ploidy. All of the Hawaiian taxa (*S. ellipticum*, *S. freycinetianum*, *S. haleakalae*, and *S. paniculatum*) had chromosome numbers or DNA content that indicated tetraploidy. The varieties of *S. insulare* from

French Polynesia had the highest amounts of DNA, with *S. insulare* var. *marchionense* from the Marquesas Islands being a putative hexaploid and *S. insulare* var. *raiateense* from the Society Islands being a putative octoploid.

### Phylogeny and Biogeography Inferred from Waxy Analysis

The *waxy* phylogeny (fig. 2) is similar in topology to the combined ITS, ETS, and 3' *trnK* intron phylogeny of Harbaugh and Baldwin (2007). As in the previously published phylogeny (Harbaugh and Baldwin 2007), the *waxy* tree resolved a basal grade of Australian taxa; however, *S. murrayanum* is attached closer to the base of *Santalum* in the *waxy* analysis but with weak support (fig. 2). These data support the earlier findings based on nuclear ribosomal and chloroplast data (Harbaugh 2007b; Harbaugh and Baldwin 2007) that there are two distantly related clades within the formerly circumscribed *S. lanceolatum*, which now includes the morphologically cryptic species *S. leptocladum*. In the *waxy* analysis, the sister relationship between the morphologically similar *S. yasi* and *S. album* seen in the tree of Harbaugh and Baldwin (2007) was not recovered (fig. 2); this difference in topologies could have resulted from either homoplasy or lineage sorting (Maddison 1997). The remaining incongruities between the phylogeny of Harbaugh and Baldwin (2007) and the *waxy* analysis may be explained by an extensive history of allopolyploidization and hybridization in the genus, as discussed in the next section.

#### Polyloidization, Hybridization, and Long-Distance Dispersal of *Santalum* Taxa

Six hybridization or polyploidization events in the evolution of *Santalum* taxa can be inferred from the *waxy* tree and ploidy data. At least one autopolyploidization event within another Australian species, *S. leptocladum*, must be postulated to account for the presence of both diploid and tetraploid specimens. In addition, two well-supported sister clades of sequences in the diploid *S. acuminatum* from Australia evidently reflect a gene duplication event ("1" in oval, fig. 2); the presence of five distinct *waxy* sequences among clones from one individual plant of (*S. acuminatum* 1) suggests at least two heterozygous loci (clades A and A'; one sequence must be the result of PCR or sequencing error).

The inferred tetraploidy of the New Guinean *S. macgregorii* appears to have resulted from an allopolyploidization event ("2" in oval, fig. 2) that occurred in Australia between a progenitor of a (diploid) *S. lanceolatum* clade (clade A) and an ancestor of (diploid) *S. austrocaledonicum* (clade B). A hybrid origin of *S. macgregorii* is consistent with results of the S-H test, which showed two independently evolving *waxy* loci, and with results from Harbaugh and Baldwin (2007) in which nuclear ribosomal and chloroplast trees were incongruent in their placement of this taxon. It is also consistent with RFLP banding patterns in this taxon (C. Jones, University of Western Australia, personal communication). Hybridization may be invoked over lineage sorting on the basis of the sympatry and distant relationship of the proposed progenitors (Maddison 1997).

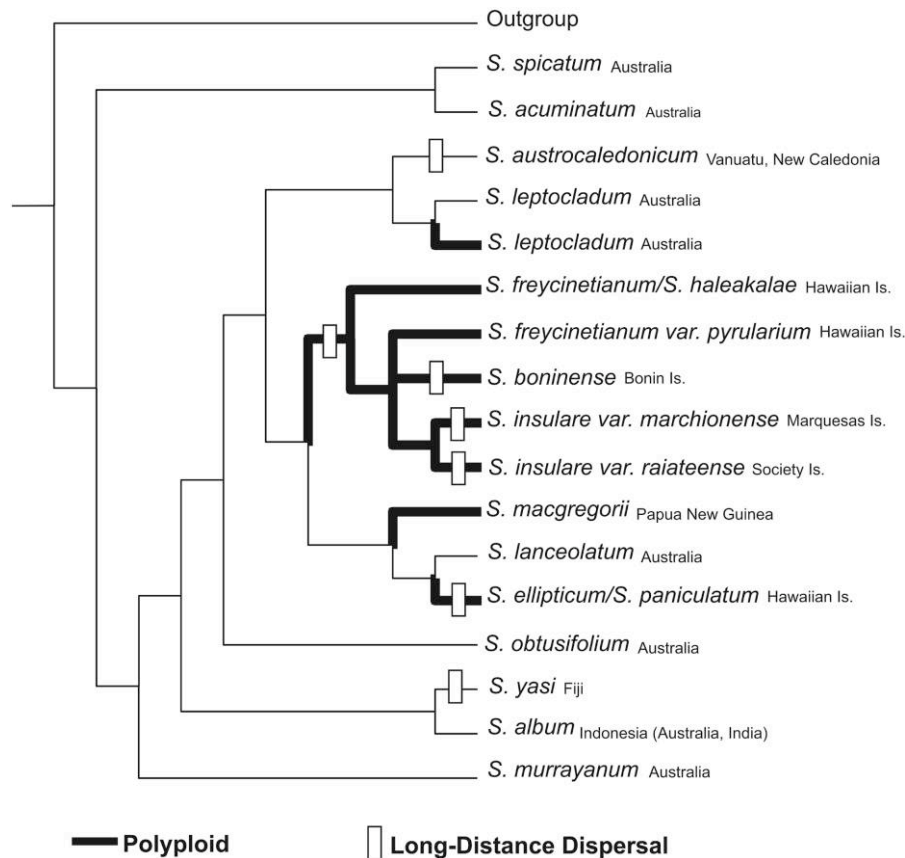
The Hawaiian *S. freycinetianum* and *S. haleakalae*—both tetraploids—evidently stem from a common autopolyploid ancestor of Australian descent ("3" in oval, fig. 2) and represent the earliest sandalwood colonization of the archipelago (Harbaugh and Baldwin 2007). Successive autopolyploidization events ("3" and "4" in ovals, fig. 2) are inferred for *S. insulare*, resulting in the putative hexaploidy of var. *marchionense* from

the Marquesas Islands and the putative octoploidy of var. *raiateense* from the Society Islands. The source area for dispersal of *S. insulare* to Polynesia has not been reconstructed; however, results from Harbaugh and Baldwin (2007) for the combined nuclear ribosomal and chloroplast tree indicated that *S. insulare* dispersed from either the Hawaiian or the Bonin Islands to Polynesia. The previous hypothesis of dispersal from Hawaii to Polynesia (Harbaugh and Baldwin 2007) is consistent with a stepwise increase in ploidy from its tetraploid Hawaiian ancestor.

The second sandalwood colonization of the Hawaiian Islands is represented by the *S. paniculatum*/*S. ellipticum* clade (Harbaugh and Baldwin 2007). On the basis of the *waxy* phylogeny, this clade ("5" in oval, fig. 2) may have resulted from an allopolyploidization event between two diploid progenitors (clades A and B), likely in Australia before colonization of the Hawaiian Islands, consistent with results of the S-H test, although the parents are unresolved. Allopolyploidization may be invoked over lineage sorting because of the extensive divergence between the two *waxy* lineages of *S. paniculatum*/*S. ellipticum* and the increase in ploidy from diploid to tetraploid.

Inferred tetraploidy of the Bonin Islands endemic, *S. boninense* ("6" in oval, fig. 2), is most parsimoniously interpreted as the result of a hybridization event between the two major, tetraploid Hawaiian clades (clades A and B). This hypothesis is consistent with results of Harbaugh and Baldwin (2007), where *S. boninense* was associated with the *S. freycinetianum* clade in the nuclear ribosomal trees and with the *S. paniculatum* clade in the chloroplast tree. Results of the S-H test also provide evidence for the presence of two independent *waxy* loci in *S. boninense*, consistent with hybridization. The findings presented here from cytogenetic and *waxy* data provide additional evidence for dispersal out of Hawaii to the Bonin Islands, which was hypothesized from the nuclear ribosomal and chloroplast data in Harbaugh and Baldwin (2007).

When the data from this study are combined with the results of the previous phylogenetic analysis of *Santalum* based on nuclear ribosomal and chloroplast sequence data (Harbaugh and Baldwin 2007), there is abundant evidence to suggest that *Santalum* island colonists tend to be polyploids, although observations are too few to provide statistically significant results from  $\chi^2$  or phylogenetic concentrated-changes tests. The results from this study, examined in light of the pruned nuclear ribosomal and chloroplast tree of Harbaugh and Baldwin (2007), indicate five instances of long-distance dispersal events to oceanic islands resulting from polyploid ancestors (*S. freycinetianum*/*S. haleakalae*, *S. ellipticum*/*S. paniculatum*, *S. insulare* var. *marchionense*, *S. insulare* var. *raiateense*, and *S. boninense*), more than twice the number from diploid ancestors (*S. austrocaledonicum*, *S. yasi*; fig. 3). Evidence from this study suggests that the polyploid events leading to *S. freycinetianum*/*S. haleakalae* and *S. ellipticum*/*S. paniculatum* were ex situ, most likely occurring in Australia or Papua New Guinea (which was connected to Australia at the time of dispersal; see Harbaugh and Baldwin 2007) before the two dispersal events to the Hawaiian Islands. This is consistent with the findings of Stuessy and Crawford (1998), who argued that ex situ polyploid formation may be more important than in situ formation because of the short timescale of island radiation and selection favoring the retention of trait complexes that led to colonization



**Fig. 3** Polyplodization and island colonization of *Santalum*. Pruned phylogeny of *Santalum* from the combined ITS, ETS, and 3' *trnK* intron phylogeny of Harbaugh and Baldwin (2007). Polyplod lineages are mapped onto the phylogeny with thick black lines. Long-distance dispersal events from mainland (Australia) to oceanic islands, or between distant island archipelagos, are marked by boxes. The native geographical ranges of each taxon are noted to the right of the taxon name, and the putative human distribution of *S. album* is noted in parentheses (Harbaugh and Baldwin 2007).

and establishment. There are two instances of polyplod formation that have not led to dispersal to oceanic islands (*S. leptocladum*, *S. macgregorii*); however, this provides evidence that polyplod formation does occur in mainland areas, from which the island colonists could arise.

Results from this study provide evidence for the tendency of island colonists in *Santalum* to be polyplods, although further investigation is required to understand the exact mechanisms by which *Santalum* polyplods may be better suited than diploids for long-distance dispersal and successful establishment on oceanic islands. Polyplody may have provided novel fruit and seed characteristics, such as decreased seed size or increased endocarp thickness, making polyplod *Santalum* taxa better adapted for long-distance dispersal by frugivorous birds; the fruit and seed sizes of island *Santalum* taxa are significantly smaller than those of mainland taxa (Harbaugh 2007a). Alternatively, polyplody may have provided increased fitness (Soltis and Soltis 2000; Comai 2005) to those fruits or seeds that successfully colonized the Pacific Islands by providing seedlings with higher tolerance for the environmental stress (MacGillivray and Grime 1995) and low nutrient availability (Levin 1983) on volcanic islands. In addition, polyplody may have lowered rates of inbreeding depression (Soltis and Soltis 2000)

in *Santalum* island populations, increasing the likelihood of their establishment and long-term survival across the islands of the Pacific.

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