ARAUCARIA IN THE URBAN LANDSCAPE: A NOVEL LEANING PATTERN AND EVIDENCE OF CULTIVATED HYBRIDIZATION

A Thesis
presented to
the Faculty of California Polytechnic State University,
San Luis Obispo

In Partial Fulfillment
of the Requirements for the Degree
Master of Science in Biological Sciences

by
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January 2017

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ABSTRACT

Araucaria in the urban landscape: A novel leaning pattern and evidence of cultivated hybridization

Jason Johns

Our understanding of the natural world is constantly evolving and strengthening as more observations are made and experiments are performed. For example, we understand that tree stems grow toward the light (positive phototropism; Darwin 1880, Loehle 1986, Christie et al. 2013) and against gravity (negative gravitropism; Knight 1806, Hashiguchi et al. 2013). We also know that plants respond to mechanical stimulus and perturbation (thigmotropism; Braam 2005). Genes and their resulting proteins have been described to uncover some of the mechanisms for these environmental responses, but relatively speaking, we have just scratched the surface (Wyatt et al. 2013). While the discovery of the molecular mechanisms responsible for these behaviors is certainly dependent on the ever-improving lab technology available, every molecular discovery is dependent on a macroscopic observation.

In this manuscript I present the two novel macroscopic observations I made on members of *Araucaria* in the urban forest. The first describes a hemisphere-dependent lean in *A. columnaris*, and the second provides genetic and morphological evidence that hybrids exist between *A. columnaris* and *A. heterophylla*.

Araucaria columnaris (J.R. Forst.) Hooker, or the Cook Pine is a conifer with a narrow native range that has been cultivated worldwide and grows unlike any other tree known. The initial observation we made was that trees in California and Hawaii lean south, and trees in California lean to a greater extent than trees in Hawaii. Measuring 250 trees in 16 regions worldwide, however, produced

statistically significant evidence for a hemisphere dependent directional leaning pattern. Trees in the northern hemisphere lean south, and trees in the southern hemisphere lean north. Additionally, the lean becomes more pronounced at greater distances from the equator.

We also gathered morphological and genetic evidence in the California urban forest that *A. columnaris* and *A. heterophylla* (Salisb.) Franco are hybridizing. Many individuals have intermediate characteristics of both species, which originally led me to believe that hybrids exist in cultivation. After analyzing several individuals with microsatellite genetic markers, I have enough evidence to conclude that hybrids between *A. columnaris* and *A. heterophylla* exist. This is an important observation mainly for municipalities and arborists interested in properly identifying trees in the urban forest. Knowing the proper identity of trees is imperative to informing decisions about their protection or removal.

As we continue to ask questions about the inner workings of nature we will continue to gain a better appreciation for what we still do not know. The evidence provided in this manuscript better informs our future questions about a leaning pattern in *A. columnaris* and about the history of the cultivation of *Araucaria*.

ACKNOWLEDGMENTS

I would like to thank the Wertman Foundation and Cal Fire for the generous funding provided. My lab mates and fellow botany students were instrumental in supporting my mental health and facilitating my growth as an academic. This research would not have been possible without the numerous skilled botanists around the world who took careful measurements for my data set. Thank you to Dr. Boris Igic who helped with statistical analyses and made beautiful figures for me in R. Without Dean Nicolle's keen and observant eye, we would not have known that Cook pines lean north in the southern hemisphere

Jenn Yost was one of the first educators to inspire me and she has continued to dedicate herself not only to my success as a botanist and a student, but as a contributing member of the community.

I am forever grateful to Matt Ritter for his influence and mentorship. He facilitated my admission into the program, made the crucial observation that would lead to the publication of a novel finding in science, and facilitated my growth as a human being. I had an exciting, fulfilling masters project because of his ever discerning and curious mind. I am forever grateful for Matt's unmatched desire to help make me better person with a more fulfilling life.

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CHAPTER 1

INTRODUCTION

A tree's form is determined by a complex interaction of genetics and environmental stimuli (Braam 2005; Rowe and Speck 2005; Reed and Stokes 2006). Under most conditions, trees grow vertically in response to the opposing influences of light and gravity (Loehle 1986). In challenging environments, where competition for light or mechanical stress is intense, trees may grow in a non-vertical fashion (Braam 2005; Telewski 2006). Although these growth responses have been studied for over 100 years, there is still much to discover about the mechanisms driving them (Darwin and Darwin 1880; Sinnott 1952; Bamber 2001, Wyatt and Kiss 2013). Never before has a hemisphere-dependent leaning pattern been documented across a tree species in the absence of environmental influence.

Vertical growth in shoots is maintained by a negative relationship with gravity (negative gravitropism; Knight 1806; Hashiguchi et al. 2013) and a positive relationship with their light source (positive phototropism; Darwin and Darwin 1880; Loehle 1986; Christie and Murphy 2013). Non-vertical shoot growth can be caused by mechanical perturbation from wind (Telewski 1995) or snow (Petty and Worrell 1981) or by a phototropic response to a light source that is not directly above the shoot (Fielding 1940; Tomlinson 1983). Mechanistic studies in *Arabidopsis thaliana* have identified several gene families whose regulation effects phototropic and gravitropic growth in plants (Wyatt and Kiss, 2013). However, the mechanisms controlling the expression of these genes and the interactions between them are not well understood (Christie and Murphy 2013), especially for woody species.

The leading hypothesis for the molecular mechanism of gravitropism is that sedimentation of amyloplasts on actin microfilaments activates a signal transduction pathway resulting in asymmetric transport of auxin in the stem, causing it to straighten itself parallel with the gravity vector (Knight 1806, Hashiguchi et al. 2013). This hypothesis, like phototropism, is supported by the identification of genes whose proper expression is necessary for observing a gravitropic phenotype (Toyota and Gilroy 2013).

Phototropism acts principally during primary growth in shoot tips to maintain an upright form in trees (Wilson and Gartner 1996; Herrera et al. 2010, Speck and Burgert 2011). Once secondary growth begins in stems, trees correct asymmetrical growth by forming reaction wood (Sinnott 1952; Du and Yamamoto 2007). Reaction wood results from asymmetric growth in the vascular cambium and can cause a leaning stem to correct itself to vertical. Although the mechanisms are different in gymnosperms (compression wood) and angiosperms (tension wood), trees can re-establish vertical growth after external forces cause leaning (Plomion and Leprovost 2001; Yamamoto et al. 2002).

Araucaria columnaris (J.R. Forst.) Hooker is a conifer endemic to New Caledonia, which has been planted in temperate, subtropical, and tropical areas throughout the world (Kershaw and Wagstaff 2009). When planted outside of its native range, this species has a pronounced lean, so ubiquitous that it is often used as the identifying characteristic for the species (Figure 1; Farjon 2013).

Figure 1. **Typical cultivated stand of *A. columnaris* at the University of California, Irvine campus** (33.65 degrees north, 117.84 degrees west). All individuals pictured are leaning south.

MATERIALS AND METHODS

Tree Measurement Protocol

We collected data from 256 trees in 18 different regions (localities greater than 500 kilometers apart) between 7-35˚ N and 12-42˚ S. Data collected from each tree included: height, diameter at breast height (ca. 1.5m above ground), azimuth degree direction of lean, extent of lean, and GPS position. Each tree was also photographed. Any tree whose growth appeared to be affected by an external object (another tree, building, telephone pole, etc.) was excluded from the data set.

All measurements were taken on trees in their current state, disregarding any changes in growth direction over its life. To determine the height of each tree, measurers used either laser range finders or a low-tech measuring technique (see Extended Data online for details). A compass was used to determine the current azimuth direction of lean. The extent of lean was measured by standing at the point at which a straight line could be drawn from the apical meristem to the ground. The distance from the measurer's feet to the base of the trunk was the extent of lean.

Statistical Analysis

We performed all statistical analyses in the R programming language (R Core Team, 2016). We calculated circular summary statistics using implementations in the R package 'circular' (Agostinelli and Lund, 2013).

Lean Azimuth Analyses

We used Rao's spacing tests and Kuiper's one-sample tests to determine whether the tree lean azimuth data were likely to have been drawn from a uniform circular distribution.

We also performed a Rayleigh test of uniformity, with a specified mean direction, to determine if the data were drawn from a unimodal distributions with an expected mean azimuth direction (axial north and south). We specified the mean direction for northern hemisphere trees as π radians (south), and specified the mean direction for the southern hemisphere as zero radians (north). Specifically, we were interested in the axial lean component vectors, or the extent of lean in the two cardinal directions, south and north, by trees in opposite hemispheres. The axial lean component vector was calculated as the magnitude of the lean multiplied by the cosine of lean azimuth.

Finally, Watson's tests indicated that our data were not drawn from a single von Mises distribution (significantly different concentration parameters, and mean directions). Consequently, we estimated the confidence intervals of the median by bootstrapping the original data for each hemisphere. In a separate test, we rotated only the northern hemisphere azimuths (π rad), and assessed whether the calculated means are homogeneous for the rotated northern and raw southern hemisphere tree lean azimuths.

Axial Lean vs. Latitude

From preliminary data we hypothesized that the magnitude of the lean may have a positive relationship with latitude. We performed a simple linear regression to analyze this relationship.

RESULTS

We measured 256 trees in 18 different regions (distinct areas more than 500 kilometers from each other), on five continents spanning 7-35º N and 12-42º S, including the species' native range in New Caledonia (21º S). On each tree we recorded height, trunk diameter at 1.5 m above ground, azimuth direction of lean, and the extent of lean. We defined the extent of lean as the horizontal distance on the ground from directly beneath the apical meristem to the base of the trunk. The magnitude of lean is the extent of the lean divided by the tree's height, which accounts for how hard the tree leans. We consequently used magnitude of lean for downstream analyses. The median lean for all trees measured is 2.42 m away from the base, and the median tree height is 18 m, resulting in a 8.05˚ lean angle (95% CI 7.50˚ - 8.50˚).

We uncovered a surprisingly consistent pattern of hemisphere-dependent directional leaning in a worldwide sample of Cook pines. In the northern hemisphere, trees lean south (median azimuth of 151˚, 95% CI 144˚-157˚), and in the southern hemisphere they instead lean north (median azimuth of 0˚, 95% CI -15˚-10˚; Figure 2). This pattern is consistent, without exception, in all regional samples (18/18; Sign Test, $p<<0.001$). Fewer than 9% of individual trees lean away from their predicted direction.

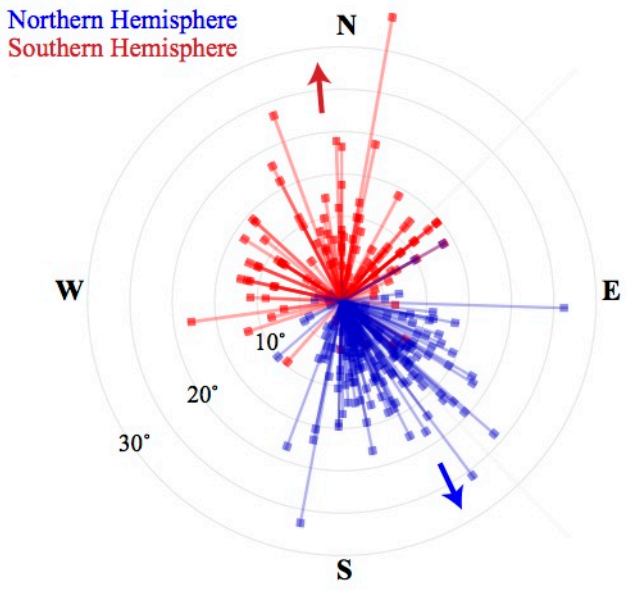

Figure 2. **Hemisphere dependent magnitude and azimuth of lean of each measured tree** (n=256). The circular position of each point gives the compass azimuth. The magnitude of lean is given by lines radiating from the center and measured in degrees from vertical, as indicated by concentric circles. Cardinal directions are marked. Red points represent trees from the southern hemisphere, blue points represent trees from the northern hemisphere. Arrows indicate mean direction for trees in each hemisphere.

We also examined the relationship between magnitude of lean and latitude (Figure 3). For our analysis, we converted magnitude of lean to axial lean, which adjusts the magnitude of lean to account for the direction of lean. This is calculated by multiplying the magnitude of lean by the cosine of the azimuth direction of lean. We found that a simple linear regression explains 54% of the

variance, suggesting that trees lean harder the further they are from the equator (R^2=0.543, $F_{1,254}$=304.4, p<<0.001).

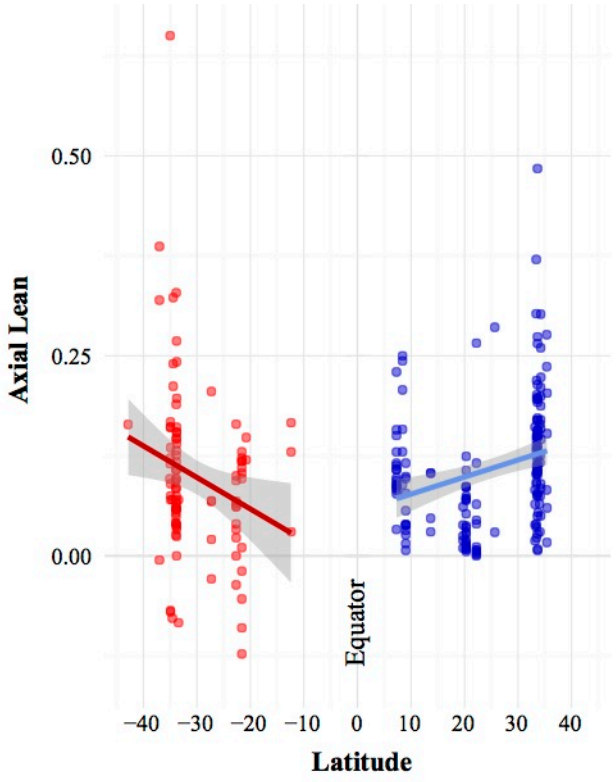

Figure 3. **Axial lean versus latitude** (n=256). Axial lean provides a measure of magnitude of lean along the north-south azimuth. It is the magnitude of lean multiplied by the cosine of the lean azimuth. Latitude is a strong predictor of axial lean (R^2=0.543, $F_{1,254}$=304.4, p<<0.001). For clarity, we show the absolute value of axial lean separated for each hemisphere, so that increasing values indicate greater axial lean.

DISCUSSION

The mechanisms underlying how *Araucaria columnaris* leans toward the equator may be related to a tropic response to annual light, gravity, magnetism, or any combination of these external forces (Loehle 1986; Hangarter 1997). It is interesting that *A. columnaris* has this characteristic lean when other species of *Araucaria*, including others native to New Caledonia, are not known to have a noticeable lean. Regardless of which processes are acting on *A. columnaris* to cause its lean, we have discovered a unique phenomenon that needs further study. A better understanding of what causes the dramatic global leaning pattern in this species will lead to new discoveries about the underlying mechanisms of plant responses to their environment.

CHAPTER 2 INTRODUCTION

Trees make urban environments more comfortable for humans (Fuller 2007). For this reason we go to great lengths to maintain them in order to reap the benefits of their service. The urban forest is a harsh place for any tree to live, and they must be carefully maintained so that they can survive and we can enjoy their presence (Tyrväinen et al. 2003). Part of this maintenance is making decisions about which trees to maintain and which to remove, and the most fundamental information to making these decisions is proper tree identification (Nowack et al. 2001). Most of the time we can use morphological differences to distinguish between closely related species. Occasionally, however, it can be difficult to distinguish between two close relatives, especially when hybrids between them exist (Cogolludo-Agustín et al. 2000).

Historically, the majority of taxonomic work has been focused on organisms in their natural environment, as there are greater ecological implications there. However, it is also important to be able to properly distinguish one species from another in the urban forest. While the taxonomic identity of urban forest trees is often known before they are planted, trees are commonly misidentified, or their true identity is cryptic and must be determined by an expert. Most common urban forest species in California are imported, and seed sources are not always well-tracked. This leads to common confusion about the true identity of many individual trees in the urban forest.

Another complication with the urban forest is that trees come into contact that are geographically isolated in the wild, thus providing a novel opportunity for them to hybridize. If two species are grown side-by-side that are wind pollinated and genetically

compatible, they will almost certainly produce hybrid seed. Hybrid seeds would be mixed with pure seeds and distributed throughout the nursery trade. In several cases, such as in *Ulmus, Platanus*, or *Eucalyptus,* hybrids have been identified both morphologically and genetically, and can be distinguished by experts (Cox et al 2014; Besnard et al. 2002; Grattapaglia et al. 2012). In other cases, such as the one presented here, little or no investigation has been done and species identification falls upon the inclinations of "experts." Here I present an investigation I carried out with two species of two commonly cultivated species of *Araucaria* whose history of hybridization has never been well-established (Buck and Imoto 1982).

Araucaria is a genus of conifers native to the southern hemisphere. Most (17 of 19) of the extant members are endemic to the South Pacific, while the other two (*A. angustifolia* and *A. araucana*) come from South America (Kershaw 2009). Captain James Cook was the first European to record observations of the *Araucaria* species in the South Pacific (Cameron 1964). A few species intrigued him enough to collect seed for cultivation, noting that their trunks might be useful to make masts out of. Among these select species were *A. columnaris* (Cook pine) and *A. heterophylla* (Norfolk Island pine). Since their discovery by Captain Cook at the end of the 18th century, these two species have been grown (mostly horticulturally) throughout the world. While the history of the worldwide cultivation of these two species is not well known, we do know that both of these species were introduced to Hawaii in about 1860 and subsequently grown for seed (Buck and Imoto 1982; Scowcroft 1987).

Because *A. columnaris* and *A. heterophylla* have been grown together for many years in close proximity throughout the world, there would have been opportunities for them to hybridize. In the wild, they are separated by almost 1000km of ocean and their natural reproduction cycles are misaligned, thus precluding them from interbreeding (Little, Jr. and Skolmen 1989). However, this can change when they are grown beside one another in the urban forest. Plants in general alter their reproductive cycles when cultivated horticulturally, thus allowing that *A. columnaris* and *A. heterophylla* could interbreed (Jochner and Menzel 2015). While there is some anecdotal morphological evidence that these species can hybridize (Buck and Imoto 1982), it has never been explored genetically.

As juveniles, *A. columnaris* and *A. heterophylla* are indistinguishable. Their leaves, bark, and overall architecture are identical until they mature into adult trees. An adult *A. columnaris* has a dense, columnar shaped canopy with dark grey bark that peels in larger sheets. As an adult, *A. heterophylla* has an open, pyramidal shaped canopy with orange bark that peels in small patches. Their leaves remain identical as adults (Figure 4). Putative hybrids have some variation between the two in varying forms (Figure 5). Because they are impossible to distinguish morphologically as juveniles, the two species are often mixed up in the nursery. *Araucaria columnaris* is often sold as *A. heterophylla* in 1-5 gallon pots. These juveniles look enough like *A. heterophylla* until they mature into leaning columns. Many places worldwide have seemingly randomly arranged mixed stands of the two species, likely from misidentification in the nursery (Figure 6).

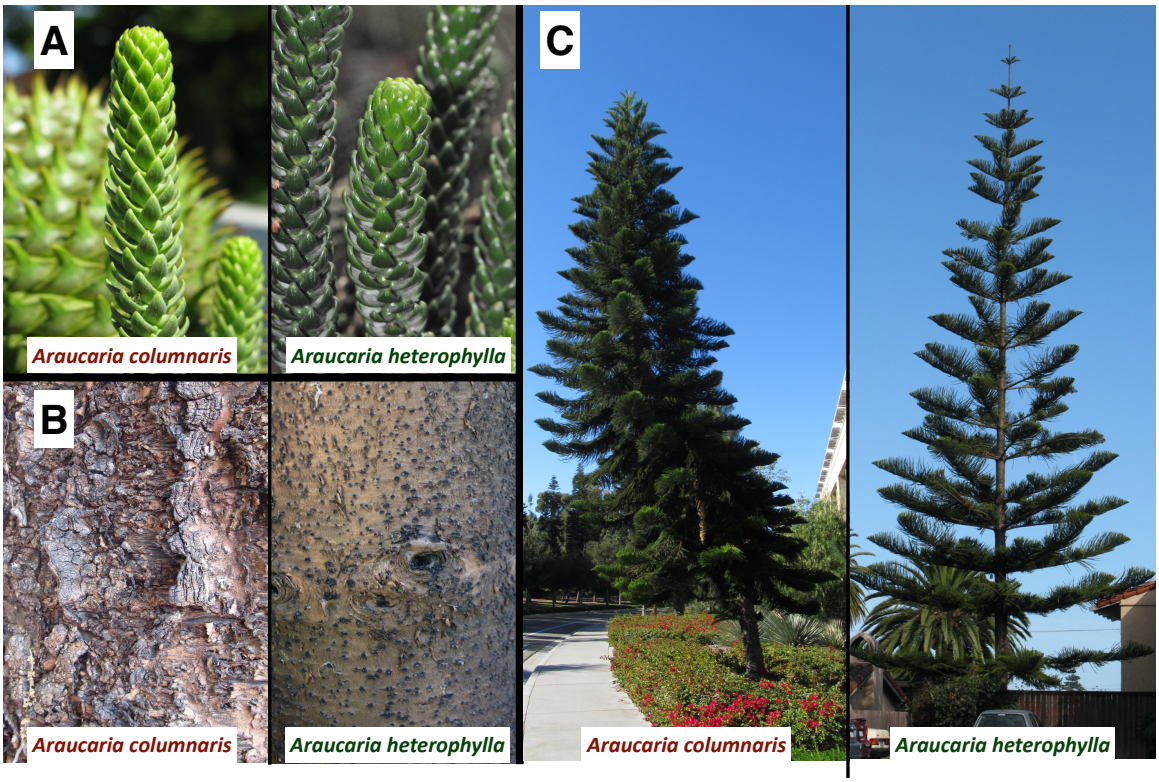

Figure 4. Morphological comparison of *A. columnaris* and *A. heterophylla*. They are entirely indistinguishable by their leaves (A), however their bark and architecture are distinct as adults. The bark of *A. columnaris* peels off in large sheets and is darker in color than the bark of *A. heterophylla*, which tends to be orange and barely peel (B). Their architecture is clearly distinct as adults, where *A. columnaris* has a dense, columnar canopy and leans, and *A. heterophylla* has an open, pyramidal canopy and does not lean.

Figure 5. Representative morphological variation in *A. columnaris* × *A. heterophylla* architecture, canopy, and bark. *Araucaria columnaris* × *A. heterophylla* in the California urban forest display a spectrum of intermediate qualities between their parent species. There is a continuum of variation between the parents in architecture, canopy, and bark.

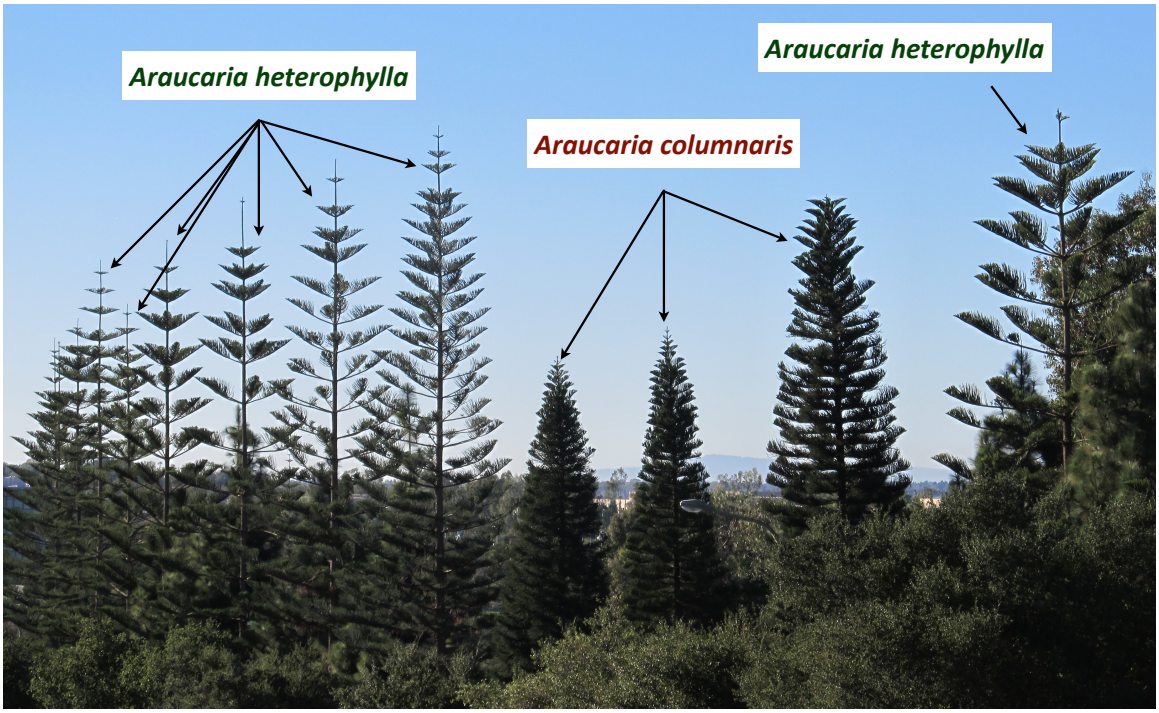

Figure 6. Mixed stand of *A. columnaris* and *A. heterophylla*. Typical mixed stand in cultivation with seemingly random placement. The photo was taken on the UC Irvine campus.

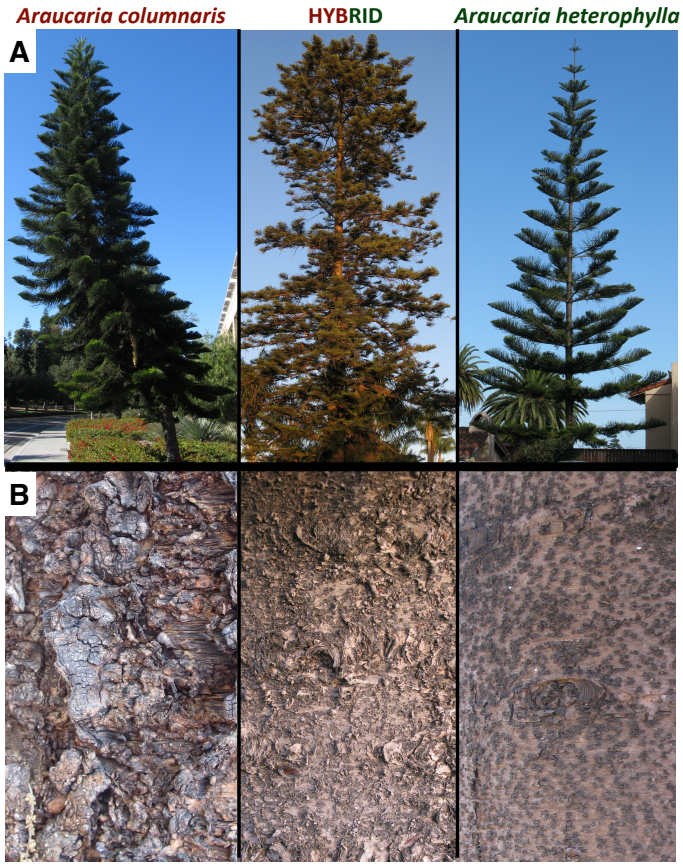

Figure 7. Morphological comparison of *A. columnaris*, *A. columnaris x A. heterophylla*, and *A. heterophylla* (left to right). (A) Side-by-side comparison shows the distinct differences in architecture and canopy between *A. columnaris* and *A. heterophylla*. The hybrid expresses intermediate characters between the two. (B) The same side-by-side comparison is shown with bark characters.

As is the case with gymnosperms in general, there is not a great deal of genetic variation within *Araucaria* (Setoguchi 1998). Thus, it has been difficult to build a well-resolved phylogeny that elucidates intraspecific relatedness among species of Araucaria (Hollingsworth et al. 2009). There is, however, enough genetic diversity to distinguish them from each other genetically using microsatellite markers (Ruhsam et al. 2015). One

of the most effective ways to elucidate this variation is by targeting parts of the genome that are more susceptible to mutation and not under selection (Kalia et al. 2007). Regions commonly used for this analysis are microsatellites, tandem repeats within the genome of all prokaryotic and eukaryotic organisms that are not only variable among closely related species, but often among different populations of the same species (Zane 2002, Ellegren 2004). Thus, they are commonly used for distinguishing between two closely related species or individuals, depending on the amount of genetic variation in that group.

Mutations in microsatellite regions are thought to be more common because of the nature of their sequence. DNA polymerase, the enzyme responsible for copying DNA, is more likely to slip or stutter while copying the DNA, thus resulting in a mutated copy of the region that either has more tandem repeats, due to stuttering, or fewer tandem repeats, due to slippage (Jarne 1996). Mutations due to slippage in microsatellites are three orders of magnitude more common than point mutations in coding regions of the genome (Jarne 1996). The regions flanking the microsatellites are more highly conserved than the microsatellite sequences themselves, making them useful for primer development and amplification across a genus (Gupta and Varshney 2000).

The ability to inexpensively link genetic variation to morphological variation would be valuable for any entity interested in confidently distinguishing between *A. columnaris*, *A. heterophylla*, and hybrids between the two. Historically, interested parties have relied on the opinions of arborists for distinguishing between the species, which has no genetic support. Phenotypic plasticity in the two species and their hybrids makes morphology

alone a weak distinguishing factor. However, in this study we present a method for obtaining genetic evidence that supports previous morphological evidence for distinguishing between *A. columnaris*, *A. heterophylla*, and hybrids between them.

MATERIALS AND METHODS

Tissue collection

I collected tissue from 6 *A. columnaris,* 8 *A. heterophylla*, and 19 putative hybrids between the two from individuals in California (Table A.1). I collected more individuals of putative hybrids in order to generate a stronger representation of genetic variation from individuals that have characteristics of both species, in varying degrees (Figure 5). I labeled trees as putative hybrids if they expressed morphological characters of both species, or intermediate characters between the two. The characters I considered in identification were canopy shape and/or density and bark (Figure 7). These distinguishing morphological characters care from Aljos Farjon's An Atlas of the World's Conifers. I took two photographs of each tree, illustrating the architecture and bark.

DNA extraction and amplification

I extracted genomic DNA from leaf tissue of plants using DNAeasy Plant Maxi Kit (Qiagen; Valencia, CA, USA). I tested 15 polymorphic microsatellite markers to assay sequence loss among different species (Robertson et al. 2004; Scott et al. 2003). Markers tested were Aru1, AS152, AS190, AS110, AS167, CRCAc1-10 (Table A.2). AS167 was the only marker able to distinguish between *A. columnaris* and *A. heterophylla* via polyacrylamide gel electrophoresis, and thus was used for subsequent analysis. I amplified fragments using the following conditions: 1.0 U of GoTaq Flexi DNA Polymerase (Promega Corporation; Madison, WI, USA), 2.25 mM $MgCl_2$, 1X Green GoTaq Flexi Buffer, 2.5 μM of dNTPs, 10 μM each of forward and reverse primers, 10 ng of plant DNA, and

dH_2O to final volume of 20 µl. PCR conditions were as follows: 94°C for 5 min; ten cycles of 94°C for 30 sec, 58°C for 45 sec, 72°C for 45 sec; twenty cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 45 sec; eight cycles of 94°C for 30 sec, 53°C for 45 sec, 72°C for 45 sec; 72°C for 10 min. I separated fragments using a 6.0% polyacrylamide gel. 8 µL of PCR product were loaded into each lane and electrophoresed for 2 hours 45 minutes at 175V. I soaked the gel in a 15µg/ml ethidium bromide bath for 20 minutes, and visualized the fragments using a BIO RAD ChemiDoc XRS+ UV transilluminator and analyzed using Image Lab software (BioRad, Hercules, CA, USA).

RESULTS

The AS167 microsatellite region was the only one that produced differential amplification between *A. columnaris*, *A. heterophylla*, and *A. columnaris x A. heterophylla*, thus we used it for all genetic comparisons between *Araucaria* individuals (Table A.2; Figure 8). The amplification of the AS167 region in *Araucaria columnaris* produces a consistent 4-band pattern: a bright band at ~180bp, and dimmer bands at ~200bp, ~220bp, and ~260bp. *Araucaria heterophylla* consistently makes faint bands at ~180bp, ~210bp and ~240bp. I amplified 12 putative hybrids and found evidence of hybridization between *A. columnaris* and *A. heterophylla*. Five putative hybrids made bands that match both species at ~210bp, ~220bp, ~240bp, and ~260bp, confirming them as *A.columnaris x A. heterophylla* (Table A.3; Figure 8). Additionally, two individuals that I originally identified as *A. heterophylla* amplified as *A.columnaris x A. heterophylla*.

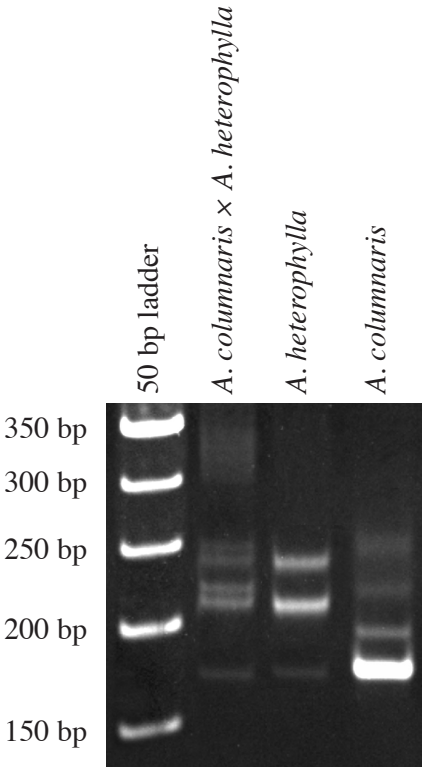

Figure 8. PCR amplification of *A. columnaris*, *A. heterophylla*, and *A. columnaris* × *A. heterophylla* using the AS167 primer pair. *A. columnaris* makes a bright band at ~180bp, and dimmer bands at ~200bp, ~220bp, and ~250bp. *A. heterophylla* makes faint bands at both ~210bp and ~240bp. *Araucaria columnaris x A. heterophylla* make bands that match both species at ~210bp, ~220bp, ~240bp, and ~260bp.

Six putative hybrids produced bands that did not match the confirmed hybrids nor the pure species, suggesting there may be genetic influence from some other species of *Araucaria* (Table A.3; Figure 9). We amplified the AS167 region in three individuals of *A. cunninhamii*, another commonly cultivated species to determine whether it was the source of the unidentified PCR product. This marker did not amplify consistently across individuals, nor did it match any of our unknown bands in the putative hybrids, making it unsuitable for comparison with *A. columnaris* and *A. heterophylla* in this analysis (Figure 10).

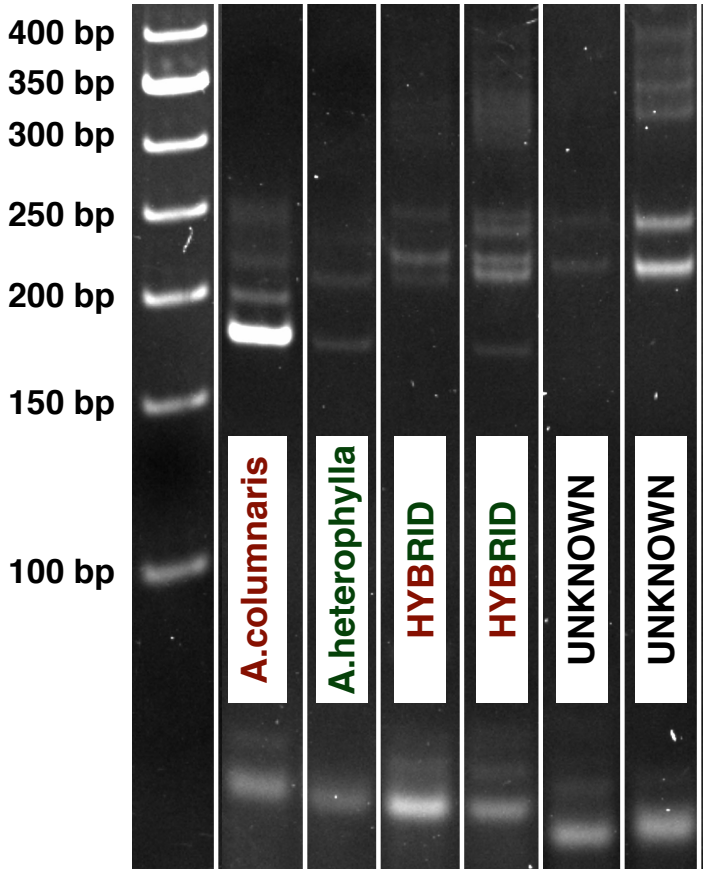

Figure 9. Unidentified *Araucaria* hybrids. The bands on both "UNKNOWN" samples do not match that of pure *A. columnaris* nor pure *A. heterophylla,* thus leading us to believe that they contain DNA from some other closely related species of *Araucaria*.

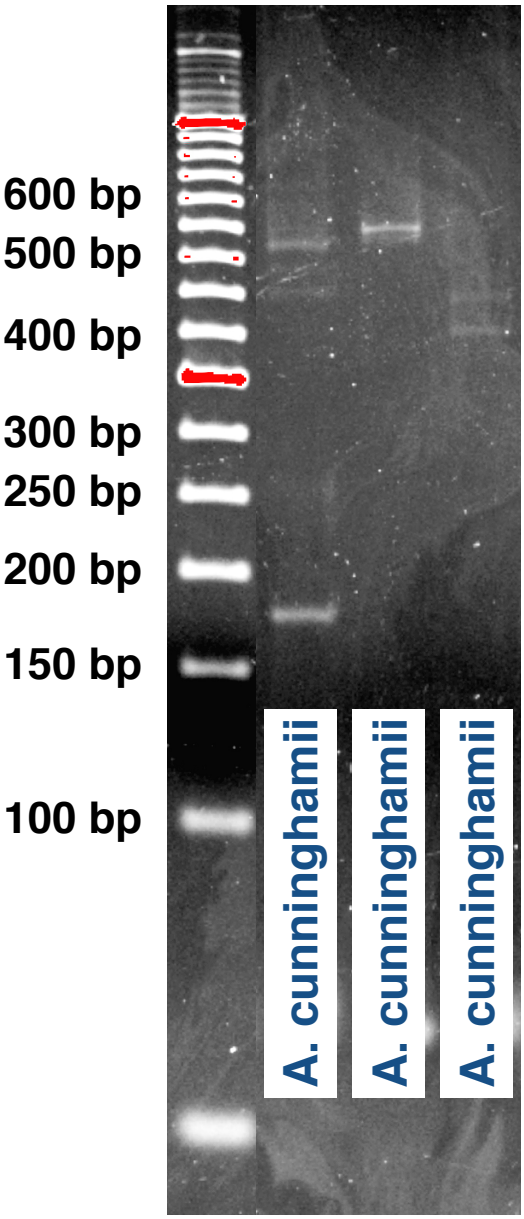

Figure 10. Amplification of pure *A. cunninghamii* with AS167. This marker does not amplify consistently across the species, making it unsuitable for comparison in this study.

DISCUSSION

I have developed a relatively simple and inexpensive way of genetically distinguishing between *A. columnaris*, *A. heterophylla*, and hybrids between them. This is a cost and time effective means of genetic identification when compared to sequencing techniques. It is also favorable when compared to the cost of hiring "experts" to identify trees based on highly variable anecdotal morphological traits, and the subsequent legal battles the ensue over whose opinion is more accurate. This will be valuable for parties interested in identifying trees in the urban forest such as municipalities and private landowners. Rather than using morphological characteristics alone, when the identity of an individual is in question, the AS167 locus can be amplified and the individual can be genetically identified.

Additionally, *A. columnaris* and *A. heterophylla* can now be distinguished as juveniles. Nurseries who have been selling misidentified juvenile *Araucaria* now have a means for properly labeling trees. Any city that wants to avoid planting a mixed row of *Araucaria* on their streets can now gather relatively quick and inexpensive genetic evidence for properly identifying their juvenile trees.

While I was able to successfully distinguish between *A. columnaris*, *A, heterophylla*, and hybrids between them, there is clearly genetic influence from another unknown source (Table A.3; Figure 9). This source is likely another widely cultivated species of *Araucaria*, such as *A. cunninghamii* or *A. bidwilii*,

however the AS167 regions that I used to distinguish between *A. columnaris* and *A. heterophylla* is not useful to clarify this. Comprehensive genetic identification of cultivated *Araucaria* would have to involve the discovery of another genetic marker that amplifies consistently within a species and differentially across species of *Araucaria*.

The results of this study will lead to a better knowledge of prominent members of the temperate and subtropical urban forests worldwide. Thus, their identity, and subsequent management can be better informed by evidence that is inexpensive to obtain and genetically backed.

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APPENDIX. SUPPLEMENTARY TABLES AND FIGURES

Table A.1. Sample collection localities

Phenotypic ID	Collection #	City	Lat	Long	Phenotypic ID	Collection #	City	Lat	Long
columnaris	60	Laguna Niguel, CA	33.476034	-117.717067	hybrid	262	Santa Barbara, CA	34.434703	-119.714454
columnaris	62	Dana Point, CA	33.463220	-117.703411	hybrid	263	Santa Barbara, CA	34.426248	-119.705977
columnaris	63	Dana Point, CA	33.463339	-117.703308	hybrid	264	Santa Barbara, CA	34.422308	-119.707844
columnaris	69	San Clemente, CA	33.429027	-117.624814	hybrid	265	Santa Barbara, CA	34.422308	-119.707844
columnaris	70	San Clemente, CA	33.421540	-117.618218	hybrid	266	Laguna Beach, CA	33.528285	-117.770607
columnaris	75	Oceanside, CA	33.190208	-117.348231	hybrid	267	Laguna Beach, CA	33.550266	-117.804519
heterophylla	278	San Luis Obispo, CA	35.269407	-120.662124	hybrid	268	Manhattan Beach, CA	33.899861	-118.399074
heterophylla	279	San Luis Obispo, CA	35.272838	-120.663058	hybrid	269	Camarillo, CA	34.216596	-119.018215
heterophylla	280	San Luis Obispo, CA	35.275468	-120.656458	hybrid	270	Camarillo, CA	34.216596	-119.018215
heterophylla	281	San Luis Obispo, CA	35.296405	-120.661597	hybrid	271	Camarillo, CA	34.217306	-119.018248
heterophylla	282	San Luis Obispo, CA	35.298196	-120.663372	hybrid	272	Camarillo, CA	34.217306	-119.018248
heterophylla	283	San Luis Obispo, CA	35.294229	-120.649979	hybrid	273	Ventura, CA	34.256120	-119.193231
heterophylla	284	San Luis Obispo, CA	35.293719	-120.648948	hybrid	274	Ventura, CA	34.252594	-119.204172
heterophylla	285	San Luis Obispo, CA	35.294144	-120.650942	hybrid	275	Ventura, CA	34.252594	-119.204172
hybrid	259	Santa Barbara, CA	34.434336	-119.712014	hybrid	276	Ventura, CA	34.256913	-119.210354
hybrid	260	Santa Barbara, CA	34.434336	-119.712014	hybrid	277	Ventura, CA	34.276660	-119.219506
hybrid	261	Santa Barbara, CA	34.431319	-119.709677					

Table A.2. Microsatellite loci used and summary of results

Locus	Repeat motif	Forward (5'-3')	Reverse (5'-3')	Source	Summary of Result
Aru1	(GA)24	GGGACCTTAGATATGTGGCATGA	TGATAGTTTACAAATGGGTGACATTG	Robertson et al. 2004	Amplified in *A. columnaris*; no amplification in *A. heterophylla*
AS152	(TC)30	TGTTTGCTCAATCGGTCAAATCT	AGTATGGAGTGTGTCTCGCTCAAG	Robertson et al. 2004	No differential amplification in hybrids
AS190	(AT)8(GT)12	TGACAACACAGTATGAGGCAACA	TTTGTAGGTCGAGGGGCATTAT	Robertson et al. 2004	No amplification
AS110	(AT)8(GT)12(GA)11	TCATGACACATGTGAAAGAGATGAAT	GCATATGCCTTGATCTCCTCATT	Robertson et al. 2004	No differential amplification
AS167	(CA)9	ACCCTTGTGAAGACACCTTCTTG	GGTTTCCAACAATGGAAAGAGTG	Robertson et al. 2004	Differential amplification between species
CRCAc1	(GA)19	ACAACATGGAAAACATATGAG	CCGTGTAACATTATGACTGC	Scott et al. 2003	Amplified in *A. columnaris*; no amplification in *A. heterophylla*
CRCAc2	(GA)23	ATGCATGACTAGGATGAACA	ATAGTTCTGCTTATCACATCT	Scott et al. 2003	No differential amplification
CRCAc3	(TTC)19	TGAGTGAGGAACATAAACCAT	GCAGGTCGACTCTAGAGGA	Scott et al. 2003	No amplification
CRCAc4	(GA)14	AAGAATCTTGCACGCATGAG	TCGTAACCTTGATCGTGGCT	Scott et al. 2003	Faint differentiation; variable w/in *A. columnaris*
CRCAc5	(GT)8(GA)11	CTAACATAAGGCAGAGGGT	CAAATAACGCTCTCCTTCA	Scott et al. 2003	No amplification in *A. columnaris*; amplified in *A. heterophylla*
CRCAc6	(GA)16	AGAGAACCTAGCAGACCACA	TCAGGAGTGCTGGTTCTTAA	Scott et al. 2003	No amplification in *A. columnaris*; amplified in *A. heterophylla*
CRCAc7	(GA)23^imp	CAATACAAATATGATGACTTC	GGTGATAGGTGGCTACTAA	Scott et al. 2003	No differential amplification
CRCAc8	(CA)9	CCATGCAAGTAGTCTGAAG	AGTGGCATGTGCAAGTTGT	Scott et al. 2003	No differential amplification
CRCAc9	(TG)9^imp(GA)15	CTTAAGTCTTCAATCTTACAT	GAAGTTTAACTTCATCTCAT	Scott et al. 2003	No differential amplification
CRCAc10	(GA)33	ACAAGTGAGGCCCATACAA	GAGGCGCGTGTGTGCAT	Scott et al. 2003	No differential amplification

Table A.3. Amplification of the AS167 in individuals of *A. columnaris*, *A. heterophylla*, and putative hybrids between them

Phenotypic id	Genetic id	Collection #	Amplification fragment sizes	Phenotypic id	Genetic id	Collection #	Amplification fragment sizes
co	unknown	60	no amplification	co x he	unknown hybrid	262	220, 260
co	co	62	180, 200, 220, 260	co x he	co x he	263	210, 220, 240, 260
co	co	63	180, 200, 220, 260	co x he	co x he	264	210, 220, 240, 260
co	co	69	180, 200, 220, 260	co x he	co x he	265	210, 220, 240, 260
co	unknown	70	no amplification	co x he	co	266	180, 200, 240, 260
co	unknown	75	no amplification	co x he	unknown hybrid	267	210, 220, 230
co	co	81	180, 200, 240, 260	co x he	unknown hybrid	268	210, 230
he	he	278	180, 210, 240	co x he	unknown hybrid	269	210, 230
he	he	279	180, 210, 240	co x he	co x he	270	210, 220, 240, 260
he	he	280	180, 210, 240	co x he	co x he	271	210, 220, 240, 260
he	he	281	180, 210, 240	co x he	unknown hybrid	272	220, 260
he	co x he	282	210, 220, 240, 260	co x he	unknown hybrid	273	210, 230
he	he	283	180, 210, 240				
he	co x he	284	210, 220, 240, 260				