Genetic diversity and structure of Moreton Bay fig (Ficus macrophylla): Potential for genetic contamination of Lord Howe Island world heritage area.

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Abstract

Genetic contamination of natural populations through human dispersal of plants has the potential to erode or change the genetic diversity of wild plant populations. This is a concern for the Moreton Bay fig (*Ficus macrophylla*), which has two distinct forms, *macrophylla* found in eastern Australia and *columnaris* in Lord Howe Island (LHI). Here we undertook genetic analysis using microsatellite markers from nuclear DNA and chloroplast DNA sequences of mature trees sampled from across the entire known distribution of *F. macrophylla* from eastern Australia and LHI to determine the species' genetic structure and diversity. We went on to genotype trees with the macrophylla growth form found on LHI to confirm their origin, along with seedlings emerging on LHI to identify potential genetic contamination from pollen and seeds. Trees on LHI were clearly genetically distinct from those in eastern Australia, forming two discrete clusters in our analyses, validating the recognition of separate mainland and LHI forms. On the mainland, the northern population has the greatest genetic diversity and is most likely the ancestral population. The southern mainland population is connected with gene flow from the northern population through a series of intermediate populations along the east coast. In contrast, the low level of genetic variation detected in the LHI population suggest it has gone through a genetic bottleneck. The genetic identity of trees with macrophylla form on LHI confirm that they are planted trees of mainland origin. Given that we recorded high phenological overlap in reproductive stages between the two forms in co-planted trees in Sydney, and that they shared the same pollinator wasp, there appears to be no premating barrier to genetic exchange between the two forms where they co-occur, e.g. planted macrophylla form and native *columnaris* form on LHI. Moreover, mainland nuclear and chloroplast variants were detected in some LHI seedlings, confirming genetic exchange via pollination to and from planted *macrophylla* trees on LHI. Given the world heritage status, high levels of endemism and unique biological processes on Lord Howe Island, preventing genetic contamination of endemic forms is an important environmental, social and economic issue.

Introduction

The Moreton Bay fig (*Ficus macrophylla*) occurs naturally from central Queensland to southern New South Wales and on the World Heritage Site, Lord Howe Island. The island is approximately 600km offshore from Port Macquarie New South Wales and was created approximately 6.4 to 6.9 million years by the activity of nine underwater volcanoes (Savolainen et.al, 2006). As a result, it has given rise to unique and endemic species found exclusively on Lord Howe Island and consequently has created interest in better understanding of sympatric speciation (Savolainen et.al, 2006).

The Moreton Bay fig exists in two forms, the Australian mainland form (*Ficus macrophylla macrophylla*), a singular free-standing trunk and the Lord Howe Island form (*Ficus macrophylla columnaris*), a buttressing root system called "banyans" (Dixon, 2001). However, given the morphology of leaves and fruits are similar, and the two forms share the same fig-wasp pollinator (*Pleistodontes froggatti*) the mainland and Lord Howe Island forms haven't been elevated to subspecies level (Dixon, 2001).

The Moreton Bay fig is a key species among the Australian ecosystem because it plays a major role in providing Australian fauna with food provisions when other fruits are not in season. As a consequence, the seed from the fruit has the potential to be dispersed over long distances and new locations during the process of digestion and defecation. In addition, the fig wasps can travel several hundred kilometres via wind dispersal to transport pollen from one fig tree to another.

Even though these two forms share a common pollinator, their phenologies may create a barrier to gene flow. Currently phenological surveys have recorded the stages of the fig life cycle over an extended period of time and it is possible to identify at what times of the year the fig wasp (*Pleistodontes froggatti*) are able to access receptive fig fruits for pollination of both forms of *Ficus macrophylla* (Jia et al. 2008, McPherson, 2005). However, these were surveys were about the reproductive cycles of the fig wasp (*Pleistodontes froggatti*) and its related species rather than the reproductive cycle of the fig (Jia et al. 2008, McPherson, 2005). Recently, the topic of genetic contamination has been raised in relation to the maintenance of Lord Howe Island's Moreton Bay fig genetic diversity. Two planted Moreton Bay figs with the mainland characteristics have been identified and National Parks rangers have requested genetic verification prior to removal. Therefore, identifying the phenological overlap and reproductive effort of the Moreton Bay fig while determining the success and potential presence of hybrids associated with human plantings. Furthermore, the Lord Howe Island local government implemented 'The Weed Management Strategy' in an effort to control possible contamination of the *Ficus macrophylla columnaris* population by the *Ficus*

macrophylla. The *Ficus macrophylla* is categorised as an alert species (sleeper weed) that has the potential to hybridise with the island population with possible loss of genetic diversity. It is currently unknown whether genetic contamination has occurred because it has not yet been confirmed whether the plant individuals of each form have reproduced with each other. Until now there has only been a hypothesis that the *Ficus macrophylla columnaris* and the *Ficus macrophylla macrophylla* could have hybridised but there is yet any conclusive evidence of the existence of ongoing hybridisation between the two forms.

Therefore, the aim of this study is to provide a better description of the structure and differentiation of genetic variation within the Moreton Bay fig. Therefore, this study aims to elucidate whether the mainland and Lord Howe island populations are genetically distinct? Does gene flow occur between mainland and Lord Howe island populations? And what is the impact of the planted material on the integrity of Lord Howe Island?

Methods

Sampling

Plant material was collected from natural-growing *F. macrophylla* trees in across its natural distribution (Figure 1). This sampling design involved collecting plant material from naturally occurring trees that represented most of the geographical distribution of the species and which met one or more of the following criteria: being 150 years old or more with known lineage, grown as stranglers, emergent tree from an old growth forest, a remnant tree on pasture land or local source material planted by bushland regenerators. Leaves from the selected individual figs were collected when available otherwise a cambium sample was collected of the desired individual fig using a 10mm diameter hollow leather punch. The leaves obtained were mature leaves, deep green in colour and still turgid in structure. A total 204 trees were sampled and dried with silica beads for long term storage at room temperature.

The four locations were focused on to explore the distribution and abundance of genetic variation across the entire distribution of species (Figure 1). in these trees were sampled include Illawarra, Lismore, Lord Howe Island and Coast (between Illawarra and Lismore). The locations Illawarra, Lismore, Lord Howe Island were more densely sampled with the intention of providing power in estimating allele frequencies and observed heterozygosity. The coast was sampled in a random stratified method at intervals of 50 to 150 kilometres to ensure adequate representation over the large distance between the locations Illawarra and Lismore. In this study a subsample of 114 trees the four locations were selected for genotyping (31 for Illawarra, 30 for Lismore, 33 Lord Howe Island and 20 coastal).

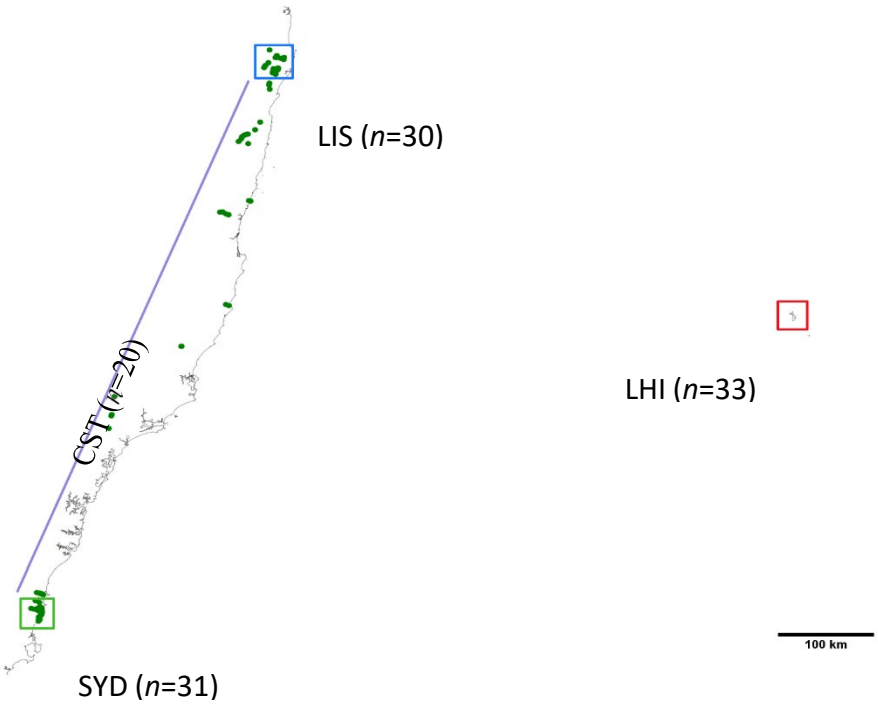


Figure 1: Sampling locations of *F. macrophylla* trees in four sampling locations across its natural distribution in eastern Australia and LHI. Each dot is an individual GPS-recorded tree that was sampled for genetic material.

Additional sampling was conducted on LHI following the detection of a planted macrophylla tree in the central valley. This tree along with 74 seedlings distributed across the central valley of the island were sampled for leaf material to test for genetic origin. Specifically genotyping with nuclear microsatellite markers and DNA sequencing chloroplast loci to determine the potential of genetic contamination from pollen or seed.

Genotyping

The CTAB extraction protocol was used to extract DNA from cambium or leaves of individual fig trees (Doyle and Doyle, 1987). Minor modifications were made to the protocol to deal with the latex content of the *Ficus* tissue. These modifications included adding an extra 200uL of CTAB buffer and an extra chloroform: isoamyl alcohol extraction (steps 9 to 12 of the protocol). The extracted DNA was stored in 100ul of TE buffer or DNase/RNase free water at -20 °C.

Multi-locus PCR (polymerase chain reaction) amplification was performed using a QIAGEN Multiplex master mix (QIAGEN #206145) per the manufacturer's recommendations. The PCR reactions volumes were 10 µL per sample with 1 µL of 1:20 DNA (concentrations

standardised) and 0.1uL of Forward primer and Fluorophore and 0.2uL Reverse primer at 10 pmol concentration. The Hot-start annealing Cycling conditions used were 95 °C denaturation for 2 minutes, 30 cycles of 94 °C melt for 30 seconds, 60 °C annealing for 1 min, and 72 °C extension for 1:30 minutes, with a final 72 °C extension period for 10 minutes followed by a 4°C cool down. All PCR thermocycling was performed on a Biorad Dyad System thermocycler.

Fragment analysis was carried out on an Applied Biosystems 3500 Genetic Analyser with the addition of the LIZ600 internal size standard. The fragment analysis output data was visualised in the program GeneMapper 5 (v5.0 Applied Biosystems) using an automated allele binning function which used the information about the base pair range and the repeat motif of each microsatellite used. The automated allele binning function then uses the information created to score the intensity (height) and the size (number base pairs) of each peak that appeared for each sample that was run with its corresponding fluorescent marker. In addition, the results for the automated function were also checked manually for any incorrect selection of alleles.

DNA barcoding

Samples were DNA barcoded to ensure that they were Ficus macrophylla, provide additional data regarding population history and structure, and provide a point of reference for existing and future work. The ITS2 region and the trnH-psbA primer pair were chosen as barcoding loci based on the previous work of Li et al. (2012) and Rønsted et al. (2008). The ITS2 region was chosen based on amplification success in a small panel of F. macrophylla DNA and because it is more informative than ITS1 (Baraket et al. 2009).

Barcode loci were amplified in Promega GoTaq Colorless master mix (Promega, #M7132) in 30 µ L reaction volumes with 1 µ L of 1:20 DNA and 10 pmol of each Forward and Reverse primer. Cycling conditions were 94 °C denaturation for 2 minutes, 30 cycles of 94 °C melt for 30 sec, 55 °C annealing for 30 sec, and 72 °C extension for 30 sec, with a final 72 °C extension period for 10 minutes. Products were cleaned up with ExoSAP (Appendix 6) before shipping to Macrogen (S. Korea) for standard bi-directional sequencing.

Genetic analyses

The data was then imported into the program Microchecker (Van Oosterhout et al., 2004) to determine the quantity of existing null alleles in each of the four populations sampled for this experiment. In addition, the program uses four statistical methodologies to calculate the frequency of null alleles within the dataset. These methodologies vary in their calculations

because it depends whether the samples have failed to amplify or whether the nonamplified samples are null homozygotes. Furthermore, this is an indication of null alleles are a result of a significant number of observed homozygotes within the highlighted loci.

GenAlEx (a Genetic Analysis cross-platform package for population genetic analyses that runs within Microsoft Excel) (Peakall et.al, 2012) was used to conduct frequency and distance-based analysis. The analyses performed were F-statistics and the observed heterozygosity was also calculated. The F-statistics are used to identify the quantity of inbreeding within the population, amongst the individuals and between populations. In addition, the observed heterozygosity is used to identify the quantity of heterozygosity in the population.

STRUCTURE analysis was run to identify patterns of population structure from the individuals sampled. The specific parameters were run length: 10000, burn in MCMC: 10000, K=1-4 with 5 repetitions of each K and the ancestral model used was admixture and allele frequency were correlated. The Evanno method is used to find the K-value (Evanno et.al, 2005). The visual representation of the STRUCTURE output firstly began by collating the multiple runs for each K value using CLUMPP and then Distruct was used to create Figure 2 showing individual bar graphs partitioned according to the estimated relation to each of the K clusters (Jakobsson et.al, 2007, Evanno et.al, 2005, Rosenburg, 2003).

In addition, a discriminant analysis of principal components was performed using an R script on R. The R function uses the input data to perform discriminant analysis of principal components is performed using the retained principal components (Jombart et al. 2015). In this study, the parameters used in the R script were 20 principal components were retained and 2 discriminant components were retained (Jombart et al. 2015). Following these steps, a scatter plot is produced to visually represent the results produced by the analysis. In addition, to the graph a summary can also be produced (Jombart et al. 2015).

To assess the evolutionary history and demographic processes shaping the mainland and Lord Howe Island populations a suite of analyses was performed. BayesAss is designed to determine the amount and direction of ‘recent’ gene flow among populations (Wilson, Rannala, 2003). Using the mixing parameters, the methodology was optimised to ensure an accurate convergence of the population data. The parameters set for each were the except the seed number which changed twice. The parameters were as follows, delta A - 0.3, delta F - 0.3, delta M - 0.2, iterations - 10000000, burnin - 1000000, sampling - 100, seed number - 113, 100,1000 and input file (Wilson, Rannala, 2003).

Flowering phenology

A phenological survey was performed according to the methodology presented by Jia et al. (2008). Moreton Bay fig trees within the Sydney Royal Botanic Garden were surveyed every fortnight for a period of 24 weeks, including six planted macrophylla and three columnaris mature trees. The developmental phases A–E (Figure 2) of a high and low branch on each of the four cardinal points on the tree we recorded. This included flowering opening (stage B) when female wasp pollinator *Pleistodontes froggatti* enter to lay eggs and pollinate flowers, as well as mature fruits when wasps emerge (stage D).

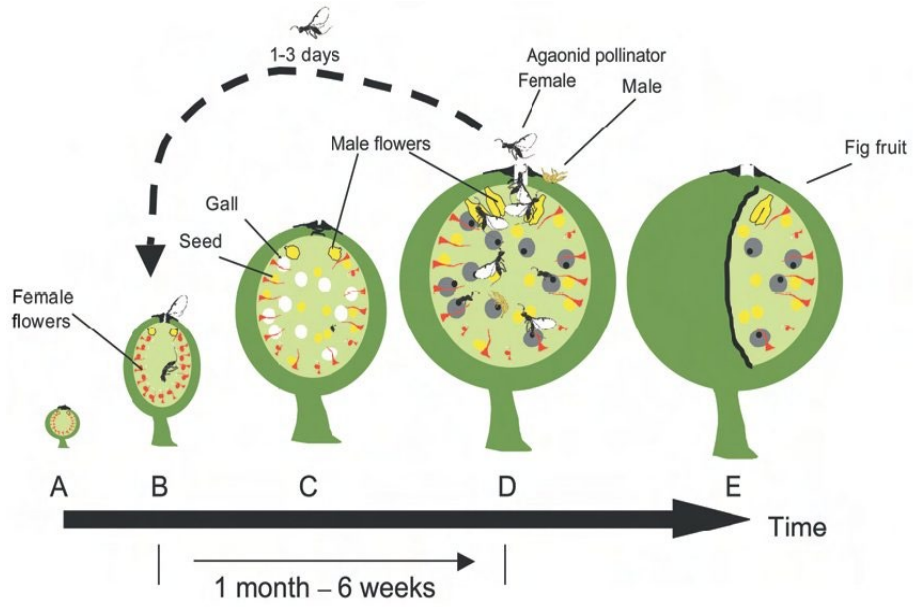


Figure 2: Development stages in Ficus macrophylla (Cranston and Gullan, 2010)

The data was used to estimate the potential for self-pollination, cross-pollination within form and hybridization among forms. Pollination may occur only when there is phenological overlap between stage B (open flower) and D (wasp emergence).

Seed viability

The fig fruit were collected from the Royal Botanic Gardens Sydney from macrophylla and columnaris trees, as well as columnaris trees on LHI. These samples were all stored in silica to remove excessive moisture that can cause mould to develop. Once these samples were ready the seed were removed from the husk and were planted in all-purpose potting mix with slow release fertiliser. 70 macrophylla fruits, 26 columnaris (Sydney) and 22 columnaris (LHI). Once the seeds were planted the pots were placed in a shade house facility with automated sprinkler system for irrigation. The seedlings were monitored on a fortnightly basis to remove weeds and to observe the quantity of emergence. This monitoring continued over the next 18 months till a definitive number of emerged seedlings were able to be counted.

Results

Molecular and ecological datasets were collected and analysed to determine the level of genetic diversity, structure, migration, population size and splitting times among *F. macrophylla* populations from the mainland and Lord Howe island populations.

Genetic diversity

A total of 18 nuclear microsatellite markers were genotyped for 114 adult trees from across the geographic distribution of *F. macrophylla*. Three markers were removed from further analysis, because of missing data due to poor amplification, which is an indication of null alleles. 21 individuals had greater than 50% of missing markers were removed from the analysis. The remaining 93 individuals genotyped for 15 markers averaging 16% missing data among all markers (25% had less than 6% missing data; 75% of the population had greater than 20% missing data) overall variation in the 15 SSR markers had a minimum of 5 alleles and maximum of 11 alleles (mean 7.2 +/- 0.5 SE)

The Microchecker analysis highlighted loci 1_03fb, micr1, 1_23fy, 2_15fr, 2_33, frub415 and 2_01 with possible null alleles, and deviation from Hardy Weinberg equilibrium. The 1_03fb, micr1, 2_33, and frub415 were highlighted with potential null alleles in all three populations (Illawarra, Lismore, LHI). In addition, 1_23 and 2_15 were highlighted loci with possible null alleles in the Illawarra population and the Lord Howe island population had the 2_01 loci highlighted as a possible null allele. Microchecker is not sensitive to whether the null alleles have failed to amplify or whether the nonamplified samples are null homozygotes. Therefore, these null alleles maybe a result of a significant number of observed homozygotes.

The levels of genetic diversity were different among the sampled populations (Table 2). The Lord Howe Island population had lower levels of genetic diversity than the mainland populations with effective number of alleles detected in Lord Howe Island (1.81 +/- 0.19) was lower than that detected in Lismore (2.62 +/- 0.30), Coastal (2.66 +/- 0.27), and Illawarra (2.43 +/- 0.25). Similarly, Shannon's diversity index (Table 2) and the unbiased estimate of expected heterozygosity was lower in Lord Howe Island (0.39 +/- 0.05) compared to the mainland populations (0.57 +/- 0.05, 0.60 +/- 0.06, 0.53 +/- 0.06). Overall, there was no difference in the levels of genetic diversity detected in the three mainland populations.

Table 1: The populations genetic metrics estimated for four locations of F. macrophylla. Values are mean (standard error).

Population	N	Ne	I	Ho	uHe	F
LIS	17.64 (1.26)	2.62 (0.30)	1.02 (0.11)	0.40 (0.05)	0.57 (0.05)	0.23 (0.09)
CST	12.79 (0.94)	2.66 (0.27)	1.05 (0.11)	0.37 (0.06)	0.60 (0.06)	0.32 (0.10)
ILL	25.79 (0.54)	2.43 (0.25)	1.02 (0.11)	0.38 (0.06)	0.53 (0.06)	0.27 (0.07)
LHI	22.50 (1.33)	1.81 (0.19)	0.71 (0.10)	0.33 (0.07)	0.39 (0.05)	0.24 (0.12)
Total	19.68 (0.84)	2.38 (0.13)	0.95 (0.06)	0.37 (0.03)	0.52 (0.03)	0.26 (0.05)

N is the number of individuals samples. Ne is the number of effective alleles. I Shannon's index. Ho is the observed heterozygosity. uHe is the unbiased expected heterozygosity. F is the inbreeding coefficient.

The overall levels of observed heterozygosity were less than that expected based on Hardy Weinberg Equilibrium, such that the inbreeding coefficient for *F. macrophylla* was moderate (F = 0.26 +/- 0.05; Table 2). All three populations have reduced levels of heterozygosity (comparison between H_o and uH_e; Table 2), resulting in the inbreeding coefficient (F) being greater than 0. This indicates the system is not randomly mating, and there is evidence for partial self and/or biparental inbreeding (F = 0.24 to 0.32) in all populations. The inbreeding coefficient in the Coastal sample was marginally elevated compared to the other populations, given adults were sampled from Sydney to Brisbane (~1000km) this may be due to non-random mating and population substructure (Wahlund Effect).

Genetic structure

The visual representation of the STRUCTURE output firstly began by collating the multiple runs for each K value using CLUMPP and then Distruct was used to create Figure 3 showing individual bar graphs partitioned according to the estimated relation to each of the K clusters (Jakobsson et.al, 2007, Evanno et.al, 2005, Rosenburg, 2003). The reason for showing Figure 2 in such manner was to provide a comparison between having 2 and 3 genetic distinct clusters.

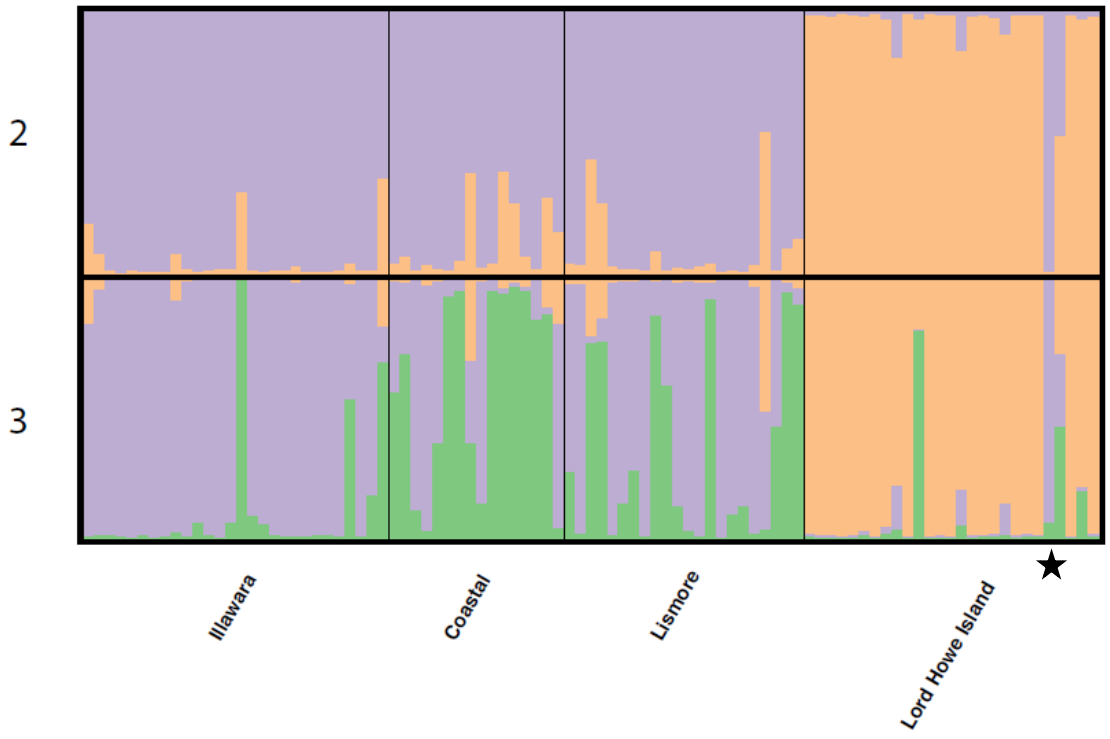


Figure 3: Bar plots from STRUCTURE constructed using Distruct and CLUMPP showing K=2 and K=3 showing the ancestral composition for each individual from mainland and LHI populations of *Ficus macrophylla*. The star indicates the planted macrophylla on LHI.

The genetic differentiation among populations estimated as F_{ST} value from a molecular variance analysis was 0.188. While, the pairwise F_{ST} values between the three populations were Illawarra and Lismore 0.103, Lord Howe Island and Lismore 0.162, and Lord Howe Island and Illawarra 0.219.

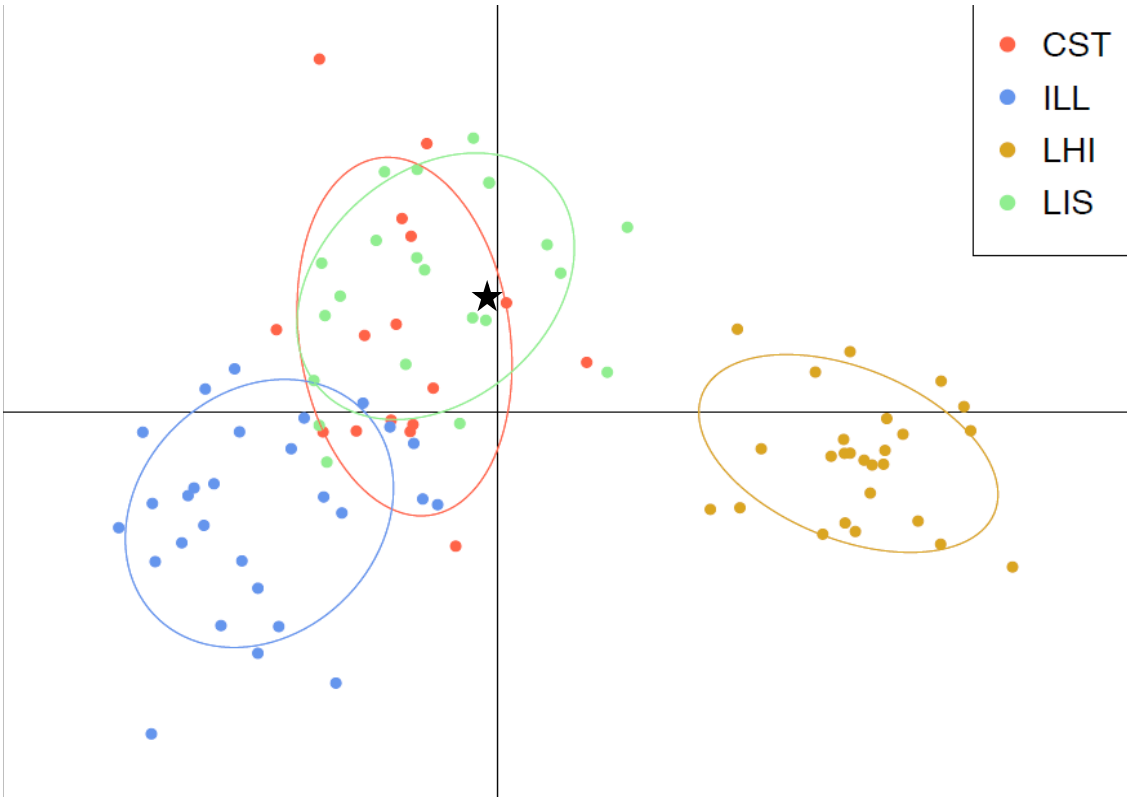


Figure 4: Discriminant Analysis of principal components (DAPC) of *F. macrophylla* populations from the Australian mainland (Illawarra, coastal, Lismore) and Lord Howe Island.

A discriminant analysis of principal components shows clusters of genetically related individuals clearly separating Lord Howe Island from the mainland populations. The mainland southern (ILL) and northern (LIS) populations are connected with a series of intermediate (CST) populations. The discriminant analysis of principal components explains 78.5% of the variation between the two axes (Figure 4).

Migration

The results from the BayesAss analysis illustrate that the greatest amount of gene flow is unidirectional towards Illawarra from Lismore (Figure 5). The level of gene flow to/from Lord Howe island was an order magnitude lower. In addition, there was relatively low level of gene flow estimated towards Lismore from Illawarra.

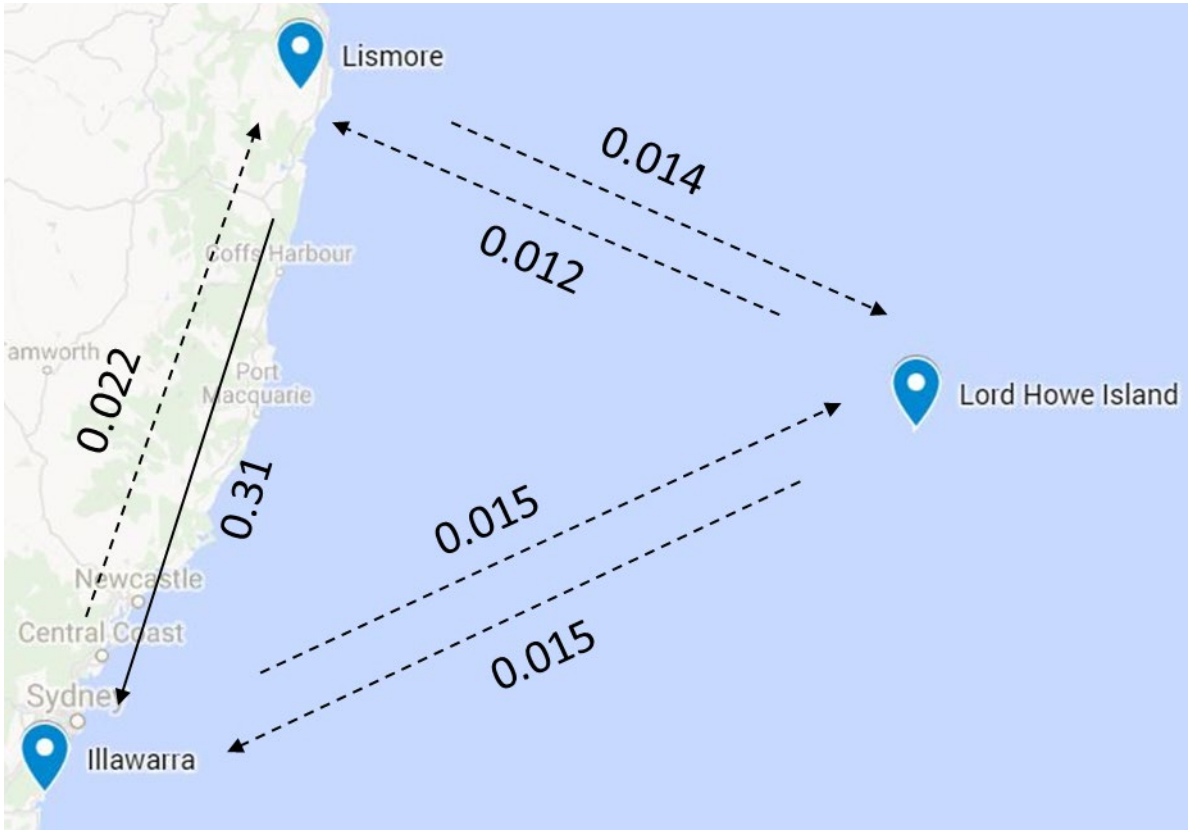


Figure 5: The mean rate of gene flow among populations along eastern Australia and LHI.

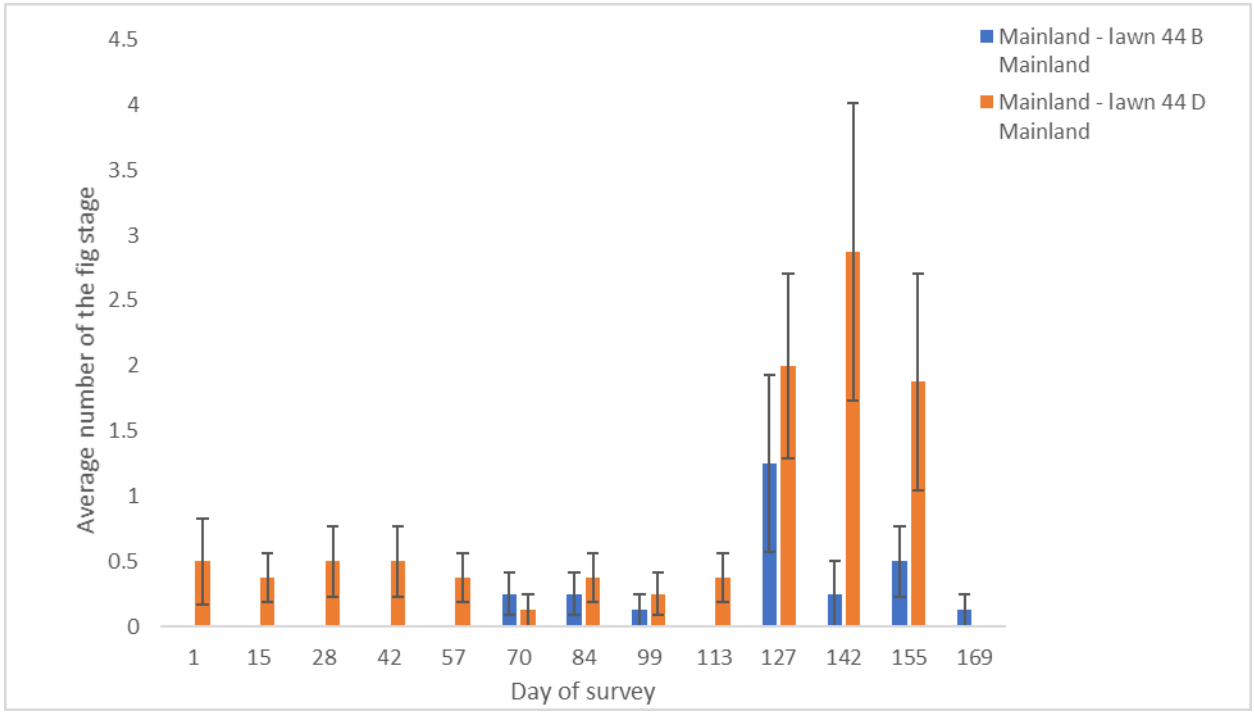
DNA barcoding

We found no intraspecific variation in the DNA chloroplast barcoding region *ITS2*, however the *trnH-psbA* ribosomal region presented two polymorphic sites that distinguish the Lord Howe Island form *columnaris* from the mainland form *macrophylla*, reinforcing the island/mainland break.

Flowering phenology

The phenological surveys highlight that it is possible for both self-pollination and hybridisation to occur between the two forms due to the simultaneous availability of receptive figs for pollination (developmental stages B) and the release of fig wasps with pollen (stages D). In addition, the phenological surveys have shown greater activity of the fig tree and the pollinator fig wasp under warmer conditions rather than colder conditions throughout the peak in flowering during the spring and summer seasons.

Seed germination trials confirmed fruit collected from the *columnaris* trees in the Sydney Royal Botanic Garden has similar levels of seed viability as both the *macrophylla* (Sydney) and *columnaris* (LHI) trees. This provides further support that hybridization among forms may lead to genetic contamination of natural populations.



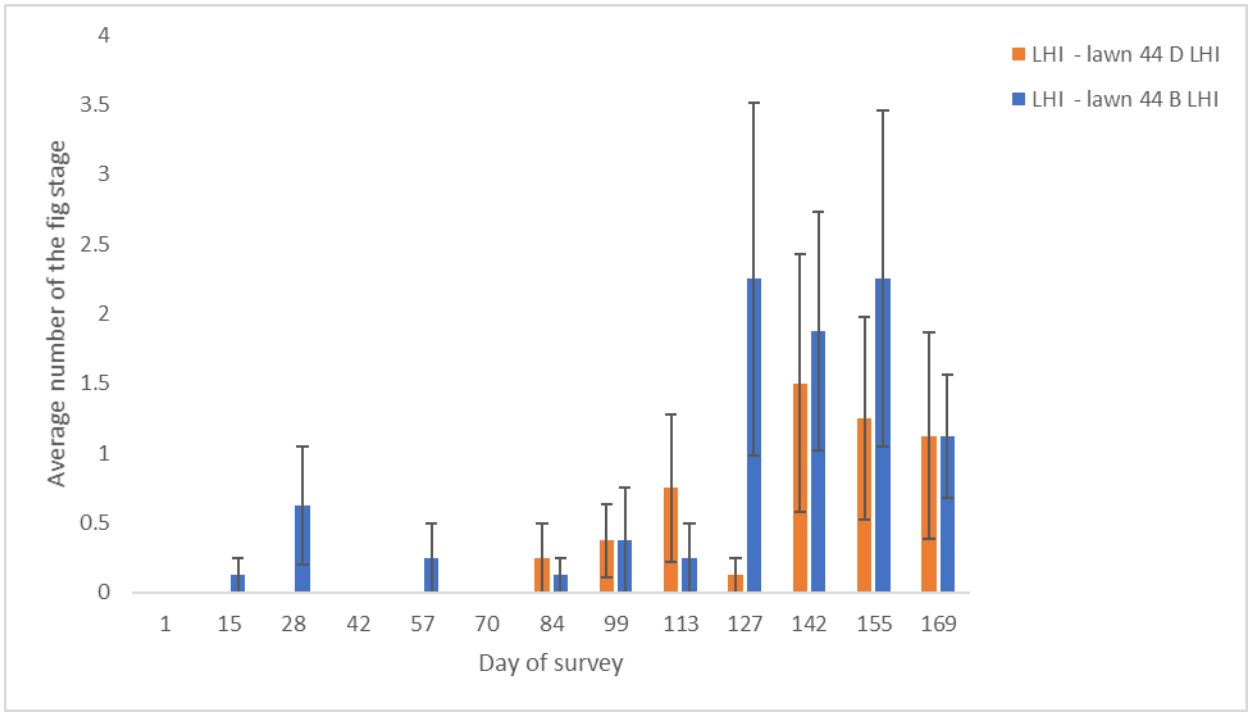


Figure 1: Phenology survey showing the average (+/- standard error) presence of receptive figs (stage B) and emerging wasps (stage D) for *macrophylla* (mainland) and *columnaris* (LHI).

Genetic contamination

The genetic identity of the planted macrophylla tree on LHI was confirmed through nuclear microsatellite markers (star noted in Figure 3 & 4), and the trnH-psbA sequence matched the mainland macrophylla form.

The genetic screening of 75 seedlings on LHI revealed evidence of both pollen and seed contamination. Six seedlings under the planted macrophylla tree had the macrophylla trnH-psbA sequence directly confirming the seed origin. An additional seedling approximately 170 m from the mainland tree had the macrophylla trnH-psbA sequence, indicating long distance dispersal. The microsatellite markers classified the six seedlings under the planted macrophylla tree, and the additional seedling with the macrophylla trnH-psbA sequence, as hybrid origin. This confirms cross pollination among the forms results in viable seed. There were two additional seedlings with macrophylla alleles, which were also classified as hybrid origin. One seedling was 570 m from the planted macrophylla tree, while the other seedling was 120 m away. It should be note that other seedlings may have hybrid origin but were not able to be detected because of the genetic similarity among forms with a high frequency of common alleles.

Discussion

The maintenance of natural biodiversity is critically important for the maintenance of ecosystem function and the continuity of ecological and evolutionary processes. This study has characterised the genetic diversity and structure in Moreton Bay fig (*F. macrophylla*) incorporating populations from across its entire distribution in eastern Australia and LHI. While the mainland populations are connected through gene flow LHI is genetically distinct and isolated. The planted mainland macrophylla form on LHI was documented to have genetic exchange through both pollen and seed. As such genetic contamination poses a risk to the World Heritage Area.

Island Biogeography

The *F. macrophylla* population on Lord Howe Island had low levels of gene flow with the Australian mainland populations which was genetically differentiated from Lord Howe island. The Lord Howe island population had low diversity, low effective population size and lower expected heterozygosity in comparison to the mainland populations. In contrast, to the mainland populations that showed greater expected heterozygosity, effective population size and diversity among the three populations. However, the inbreeding coefficient indicated there is non-random mating occurring and therefore is evidence for partial self and/or biparental breeding ($F = 0.24 – 0.32$) in all populations. The overall level of gene flow indicates it occurs most predominantly from north to south on the mainland while the rest of the gene flow interactions between the populations occur at an order of magnitude lower.

The size of the Lord Howe island greatly impacts the genetic structure of the *Ficus macrophylla columnaris* population. As a result, it would be expected the population structure of the LHI *columnaris* population would see changes to the observed and unbiased expected heterozygosity and the inbreeding coefficients. Furthermore, the distance between the mainland and LHI affects the genetics of the two Ficus populations via geneflow. The oceanic barrier between the two landmasses and the low rate of genetic exchange have resulted in alterations to the genetic structure and diversity on LHI and the Australian mainland. Consequently, we have shown the populations to be genetically distinct. A founder effect when the *Ficus macrophylla* first established on LHI may have resulted in the limited genetic variation on the island population.

Speciation

Currently there are two forms of *Ficus macrophylla* which were taxonomically described by Dixon (2001). As a result, the concerning question is, when is the *Ficus macrophylla columnaris* form likely to permanently separate from the *macrophylla* form? While the forms are distinctly different in habit, they are similar in leaf and flower structure and share a common wasp pollinator. Our phenological surveys show they share a similar flowering phenology with a large overlap in the release of fig wasp pollinators and the availability of receptive figs for pollination, which suggest that the possibility of cross pollination as well as self-pollination. Our genetic analysis confirms the forms are not reproductively isolated and may be fully compatible with each other. The only barrier appears to be the 650 km of Pacific Ocean separating the mainland and LHI. Geographic isolation and different environmental and climatic conditions on LHI are the essential ingredients for local adaptation and speciation. Indeed, LHI has a high degree of endemism supporting some of the best documented cases of sympatric speciation (Howea palms; Wieslaw et.al 2015). Future research building on this study would be fruitful on the genetic determinants of the banyan habit of the LHI *columnaris* form.

Potential for genetic contamination

The experiments performed make it clear that genetic contamination can affect the genetic diversity of the respective *Ficus macrophylla* populations. Currently, due to human activity there are fig trees from the mainland population established on Lord Howe island and vice versa. Overcoming the historic, oceanic barrier to natural gene flow. The results of the phenological surveys and seedling emergence experiment support the possibility for hybridisation or self-pollination producing viable seed. Furthermore, the genetic surveys of

seedlings on LHI have documented macrophylla introgression from pollination in both directions. Seed and pollen have dispersed from the planted macrophylla tree up to 570 m and 170 m, respectively. This poses a significant risk to the integrity of the LHI World Heritage Area.

The potential for genetic contamination the mainland form has on the population on Lord Howe island has resulted in the local government having policies that entailing how to manage weeds and invasive species on the island (Lord Howe Island Board, 2016). As a result of these policies by the local government and the oceanic barrier between the mainland and the island are currently the most effective management strategies. Removal of the macrophylla tree along with seedlings identified with macrophylla variants should be a priority. This may avoid potential long term ecological and evolutionary impacts on the LHI ecosystem.

Conclusion

This study has confirmed the two forms of *Ficus macrophylla* are genetically distinct with LHI being genetically isolated from the mainland. The origin of the species is likely in the Lismore region with migration south to Sydney and the Illawarra. Experimental and genetic evidence has supported the potential for hybridization among the two forms, and genetic contamination through pollen and seed. Given the shared pollinator wasp and fleshy fruit dispersed by birds and mammals it will be critically important for macrophylla to be eradicated from LHI before there is too much introgression and the problem becomes too hard to handle.

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