# *Fontainea oraria* conservation genomics report

**October 2021**

**FINAL REPORT**

*Research Centre for Ecosystem Resilience*

*Australian Institute for Botanical Science*


This report was prepared for the Saving Our Species initiative by the Department of Planning, Industry and Environment (DPIE).



**Acknowledgements**

We acknowledge the Traditional Custodians of the land on which the plant species in this study are found on, and pay respects to Elders past and present. We acknowledge all collectors who conducted the field sampling.

**Citation**

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# *Fontainea oraria* conservation genomics report (October 2021)

*prepared by the Research Centre for Ecosystem Resilience*

*(Royal Botanic Garden Sydney)*

## Background

As part of ongoing monitoring of translocation success of the Critically endangered *Fontainea oraria*, a follow up conservation genomic study from a previous study (August 2017) was conducted to assess the relative contribution of diversity by the current seedling crop from translocated sites compared to the existing adults and if necessary, provide advice on further steps to ensure translocated populations have maximum genetic diversity (and fitness) and therefore are more viable in the long term. A secondary aim was to detect whether the mislabelling issue identified in the previous study persists (consequently a number of adults were also included in the analyses).

## Results

We report results based on analyses of genomic data sourced from adult and seedling samples of *Fontainea oraria*, an extremely rare species with only 10 known wild mature plants remaining around Lennox Head. The overall numbers has recently been increased through a genetically informed translocation program (Fig. 1). Sampling included seedlings and adults collected from the wild, at translocation sites, and Coffs Harbour Botanic Gardens. The overall analyses were also supplemented by baseline data from the previous study to verify identities and assess genetic representativeness of *F. oraria* progenies (Table 1). Findings include:

- A high-quality genome scan based on 11,817 genome-wide markers (SNPs) of 138 samples, including 59 new samples (Table 1).
- A cluster-based network summarises the relatedness among the adults and progenies, identifying the parents for most seedlings except for NSW1093671. For the latter only one parent could be identified (422).
- A summary of the number of adults and seedlings in the wild and translocation sites (Table 2). The data show that some translocation sites, especially C and E, generated seedlings from unique combinations of parents in comparison to wild seedlings. No seedlings were available for testing from sites B, F, G and N, possibly because the individuals were more recently planted. Future crosses could focus on boosting the paternal contribution of male 325 by crossing with the females, 403, 404 and 418, as it has the least reproductive output. It is important to remember that reproductive success may vary across seasons.
- Interestingly site A contained seedlings, 324 x 401, that are derived from the adult, 324 which is not planted at the site (Table 2). With no indication that these seedlings have been planted, this suggests that gene flow between sites is taking place which is a highly positive outcome, and an important consideration for future planting strategies.
- Principal Component Analysis (Fig. 3) supports a high coverage of genomic diversity by the seedlings at the translocation sites compared to the wild site. In aggregate (wild

and translocations) the evolutionary representativeness of all seedlings is greater than that of the adults, suggesting that sexual reproduction is not only maintaining existing genetic diversity, but also establishing novel and unique combinations of the parental genomes.

- Misidentification remains an issue, with the genomic data determining the individual NSW1093652 as 325 despite being labelled “A420 Planted”, NSW1093674 as 420 but labelled “E325 seedling”, and seedlings (NSW1093645, NSW1093675) mislabelled as having 422 as a parent but genomic data suggests 420 x 418. This result demonstrates the importance of ongoing screening of plant identities, to aid the continued review of plant production mechanisms, record keeping and translocation processes to mitigate mislabelling issues.

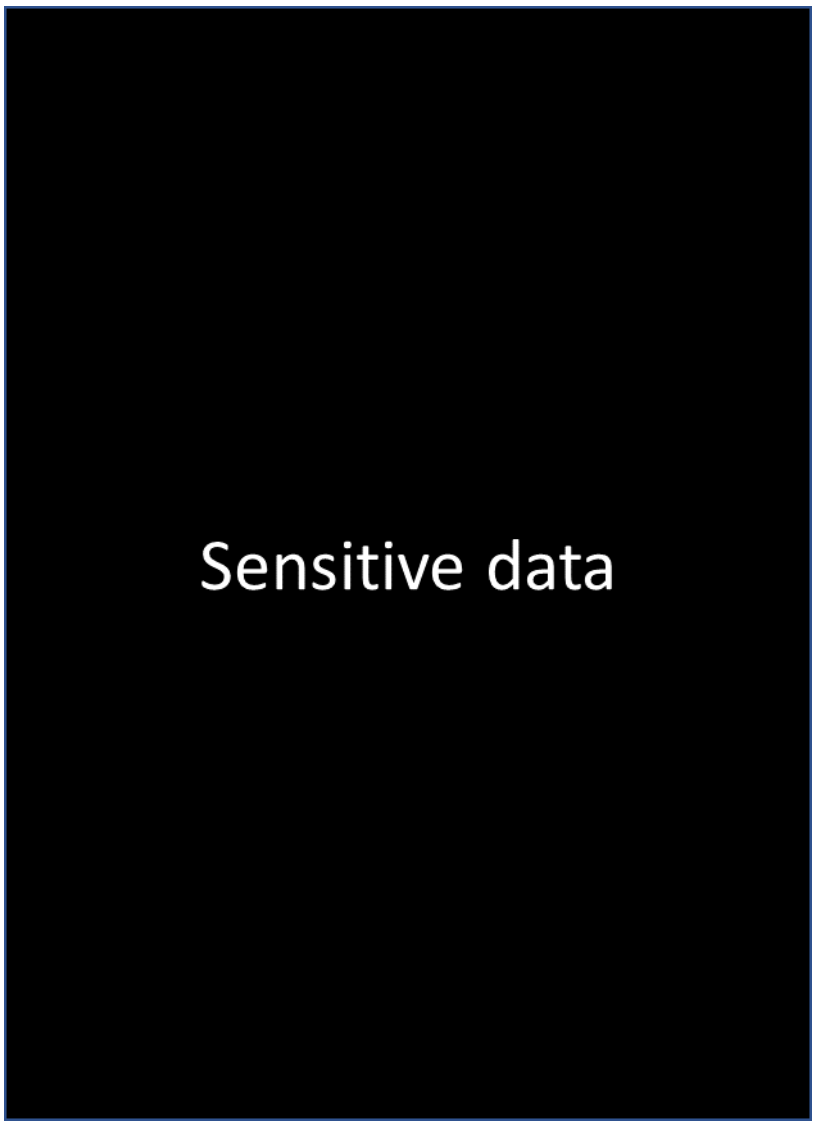



Figure 1: Location of *Fontainea oraria* wild and translocation sites near Lennox Head in north-east NSW. Each red dot indicates where the original mature plants were observed (i.e. Wild) and each yellow dot is a translocation site where propagated cuttings of the mature plants and subsequent seedling crop occur.

**Table 1**: Genomic determinations of *Fontainea oraria* adult plants and seedlings in the wild, translocated sites, the Australian Botanic Garden Mt Annan, and Coffs Harbour Botanic Garden. This table combines information from previous samples from August 2017 and from current samples. Genomic identity is indicated for each plant and kinship (parentage) for each seedling based on our kinship analysis. See Fig. 1 for a network of relatedness among adults and seedlings.

| NSW # | Old/New | Original Details | Genomic identity / kinship | Wild or Translocation site? (extracted from original details) |
|---|---|---|---|---|
| NSW1018783 | Aug-17 | 558242/6812260 Wild tree 324 site 3 | 324 | Wild |
| NSW1018768 | Aug-17 | C324 | 324 | siteC |
| NSW1019510 | Aug-17 | D324 | 324 | siteD |
| NSW1018794 | Aug-17 | F324 | 324 | siteF |
| NSW1018777 | Aug-17 | G324 m9 | 324 | siteG |
| NSW1018753 | Aug-17 | 558039/6812167 Wild tree 325 Ballina Council Castle Drive Sth Site 1 | 325 | Wild |
| NSW1018859 | Aug-17 | B420 | 325 | siteB |
| NSW1018756 | Aug-17 | B420(02) | 325 | siteB |
| NSW1018860 | Aug-17 | B420(1) | 325 | siteB |
| NSW1019520 | Aug-17 | C420(1) | 325 | siteC |
| NSW1018779 | Aug-17 | C420(2) | 325 | siteC |
| NSW1018771 | Aug-17 | 558120/6813137 Wild tree 401 Amber Drive single tree | 401 | Wild |
| NSW1018748 | Aug-17 | C401 | 401 | siteC |
| NSW1019509 | Aug-17 | D401 | 401 | siteD |
| NSW1018795 | Aug-17 | F401 | 401 | siteF |
| NSW1018778 | Aug-17 | G401 | 401 | siteG |
| NSW1018789 | Aug-17 | 55816/6812318 Wild tree 402 | 402 | Wild |
| NSW1018759 | Aug-17 | B402 | 402 | siteB |
| NSW1018754 | Aug-17 | C402 | 402 | siteC |
| NSW1018785 | Aug-17 | D402 | 402 | siteD |
| NSW1018786 | Aug-17 | D403 | 402 | siteD |

| | | | | |
|---|---|---|---|---|
| NSW1018853 | Aug-17 | F402 translocation | 402 | siteF |
| NSW1018793 | Aug-17 | F403_1 | 402 | siteF |
| NSW1019521 | Aug-17 | G402 | 402 | siteG |
| NSW1019527 | Aug-17 | G403 | 402 | siteG |
| NSW1018767 | Aug-17 | 558161/6812319 Wild tree 403 | 403 | Wild |
| NSW1018755 | Aug-17 | C403 | 403 | siteC |
| NSW1018758 | Aug-17 | F403 | 403 | siteF |
| NSW1018770 | Aug-17 | 558161/6812317 Wild tree 404 | 404 | Wild |
| NSW1018760 | Aug-17 | B404 | 404 | siteB |
| NSW1018751 | Aug-17 | C404 | 404 | siteC |
| NSW1018784 | Aug-17 | D404 | 404 | siteD |
| NSW1018854 | Aug-17 | F404 translocation | 404 | siteF |
| NSW1019540 | Aug-17 | G404 | 404 | siteG |
| NSW1018772 | Aug-17 | 558161/6812320 Wild tree 418 | 418 | Wild |
| NSW1018761 | Aug-17 | B418 | 418 | siteB |
| NSW1018750 | Aug-17 | C418 | 418 | siteC |
| NSW1019528 | Aug-17 | D418 | 418 | siteD |
| NSW1018852 | Aug-17 | F418 translocation | 418 | siteF |
| NSW1019541 | Aug-17 | G418 | 418 | siteG |
| NSW1022283 | Aug-17 | 20100631AA (Mt Annan) | 420 | ABGMA |
| NSW1018788 | Aug-17 | 558159/6812319 Wild tree 420 | 420 | Wild |
| NSW1018857 | Aug-17 | B325 | 420 | siteB |
| NSW1018762 | Aug-17 | C325 | 420 | siteC |
| NSW1019512 | Aug-17 | D325 | 420 | siteD |
| NSW1018799 | Aug-17 | F325 | 420 | siteF |
| NSW1018791 | Aug-17 | F420(1) | 420 | siteF |
| NSW1018780 | Aug-17 | G325 | 420 | siteG |
| NSW1018775 | Aug-17 | G420(2) | 420 | siteG |
| NSW1018774 | Aug-17 | 558160/6812318 Wild tree 421 | 421 | Wild |
| NSW1018858 | Aug-17 | B401 | 421 | siteB |
| NSW1018757 | Aug-17 | B421 | 421 | siteB |
| NSW1018747 | Aug-17 | C421 | 421 | siteC |
| NSW1018787 | Aug-17 | D421 | 421 | siteD |
| NSW1018792 | Aug-17 | F421 | 421 | siteF |
| NSW1019.45 | Aug-17 | G421 m1 | 421 | siteG |
| NSW1018773 | Aug-17 | 558156/6812315 Wild tree 422 | 422 | Wild |
| NSW1018763 | Aug-17 | B422 | 422 | siteB |
| NSW1018749 | Aug-17 | C422 | 422 | siteC |
| NSW1019519 | Aug-17 | D422 | 422 | siteD |
| NSW1018687 | Aug-17 | F422 translocation | 422 | siteF |
| NSW1018781 | Aug-17 | G422 | 422 | siteG |

| | | | | |
|---|---|---|---|---|
| NSW1022288 | Aug-17 | 20151894 (Mt Annan) | 324 x 401 | ABGMA |
| NSW1022273 | Aug-17 | 20151895 (Mt Annan) | 324 x 401 | ABGMA |
| NSW1018855 | Aug-17 | Wild seedling under C401 | 324 x 401 | siteC |
| NSW1018800 | Aug-17 | Wild seedling under C401 | 324 x 401 | siteC |
| NSW1018856 | Aug-17 | Wild seedling under C401 | 324 x 401 | siteC |
| NSW1018744 | Aug-17 | 558159/6812318 Wild seedling 333 Near 422 or 420 | 324 x 421 | Wild |
| NSW1023626 | Aug-17 | Only germinated seedling from 2017 seeds | 402 x 418 | ABGMA |
| NSW1022278 | Aug-17 | 20150334 (Mt Annan) | 402 x 421 | ABGMA |
| NSW1022298 | Aug-17 | 20001422 (Mt Annan) | 420 x 421 | ABGMA |
| NSW1018797 | Aug-17 | 558160/6812308 Wild seedling 322 East of 421 | 420 x 421 | Wild |
| NSW1018798 | Aug-17 | 558161/6812314 Wild seedling 425 near 421 | 420 x 421 | Wild |
| NSW1018745 | Aug-17 | 558161/6812317 Wild seedling 326 | 420 x 421 | Wild |
| NSW1018752 | Aug-17 | 558163/6812319 Wild seedling 443 near 421 | 420 x 421 | Wild |
| NSW1018766 | Aug-17 | Seedling 411 in compound with 401, unsure if wild or planted, Amber Drive | 420 x 421 | Unsure |
| NSW1018765 | Aug-17 | Seedling 412 in compound with 401, unsure if wild or planted | 420 x 421 | Unsure |
| NSW1018769 | Aug-17 | Seedling 413 in compound with 401, unsure if wild or planted | 420 x 421 | Unsure |
| NSW1018776 | Aug-17 | G420(1) | 325 | siteG |
| NSW1090633 | Oct-21 | B324 Planted 4m | 324 | siteB |
| NSW1090643 | Oct-21 | C324 Planted 1.5m | 324 | siteC |
| NSW1093660 | Oct-21 | E324 Planted male buds + flowers | 324 | siteE |
| NSW1093652 | Oct-21 | A420 Planted | 325 | siteA |
| NSW1090625 | Oct-21 | B325 Planted (was originally labelled F1) | 325 | siteB |
| NSW1093696 | Oct-21 | Wild 325 | 325 | Wild |
| NSW1093637 | Oct-21 | A401 Planted | 401 | siteA |
| NSW1090634 | Oct-21 | C401 Planted (female flowers) 4m next to C420 male flowers | 401 | siteC |
| NSW1093701 | Oct-21 | D401 | 401 | siteD |
| NSW1093640 | Oct-21 | E401 Planted 4m | 401 | siteE |

| | | | | |
|---|---|---|---|---|
| NSW1093682 | Oct-21 | J401 Planted 3.2m | 401 | siteJ |
| NSW1090628 | Oct-21 | C4012 planted 2m male flowers | 402 | siteC |
| NSW1093657 | Oct-21 | D402 Planted | 402 | siteD |
| NSW1093665 | Oct-21 | E402 Planted 3.2m | 402 | siteE |
| NSW1082893 | Oct-21 | Fontainea oraria "N3" Lennox | 402 | siteN |
| NSW1090644 | Oct-21 | C403 Planted 5m seedling under | 403 | siteC |
| NSW1093700 | Oct-21 | E403 Planted 3m | 403 | siteE |
| NSW1093676 | Oct-21 | Wild 403 | 403 | Wild |
| NSW1090631 | Oct-21 | C404 Planted | 404 | siteC |
| NSW1093661 | Oct-21 | Wild 404 | 404 | Wild |
| NSW1093642 | Oct-21 | A418 Planted 4m tall | 418 | siteA |
| NSW1093690 | Oct-21 | E418 newly planted (replacement plant) | 418 | siteE |
| NSW1093641 | Oct-21 | Wild 418 | 418 | Wild |
| NSW1090640 | Oct-21 | C420 Planted male 3.5m next to C401 (big seedlings) | 420 | siteC |
| NSW1093662 | Oct-21 | D420 Planted | 420 | siteD |
| NSW1093674 | Oct-21 | E325 seedling | 420 | siteE |
| NSW1082904 | Oct-21 | Fontainea oraria "N1" Lennox | 420 | siteN |
| NSW1093711 | Oct-21 | D421 Planted | 421 | siteD |
| NSW1093635 | Oct-21 | E421 Planted 5m | 421 | siteE |
| NSW1093636 | Oct-21 | Wild 421 | 421 | Wild |
| NSW1093691 | Oct-21 | Wild 422 | 422 | Wild |
| NSW1093677 | Oct-21 | A401a seedling | 324 x 401 | siteA |
| NSW1093692 | Oct-21 | A401b seedling | 324 x 401 | siteA |
| NSW1093687 | Oct-21 | A401c seedling | 324 x 401 | siteA |
| NSW1090638 | Oct-21 | C401b seedling | 324 x 401 | siteC |
| NSW1090626 | Oct-21 | C401c seedling | 324 x 401 | siteC |
| NSW1093706 | Oct-21 | D401a seedling | 324 x 401 | siteD |
| NSW1093647 | Oct-21 | D401b seedling | 324 x 401 | siteD |
| NSW1090624 | Oct-21 | C401a seedling | 325 x 401 | siteC |
| NSW1090635 | Oct-21 | E401 seedling | 401 x 420 | siteE |
| NSW1093670 | Oct-21 | E401a seedling | 401 x 420 | siteE |
| NSW1093694 | Oct-21 | E401b seedling | 401 x 420 | siteE |
| NSW1093672 | Oct-21 | J401 seedling | 401 x 420 | siteJ |

| | | | | |
|---|---|---|---|---|
| NSW1093667 | Oct-21 | J401 seedling | 401 x 420 | siteJ |
| NSW1090639 | Oct-21 | C403b seedling | 402 x 403 | siteC |
| NSW1093695 | Oct-21 | E403 seedling | 402 x 403 | siteE |
| NSW1090629 | Oct-21 | 404b seedling | 402 x 404 | site C |
| NSW1090645 | Oct-21 | C403a seedling | 403 x 324 | siteC |
| NSW1090630 | Oct-21 | C404a seedling | 404 x 402 | siteC |
| NSW1093664 | Oct-21 | E404 Planted 2m | 404 x 420 | siteE |
| NSW1093645 | Oct-21 | E422 seedling | 418 x 420 | siteE |
| NSW1093675 | Oct-21 | under E422 seedling (may not be from E422) | 418 x 420 | siteE |
| NSW1093686 | Oct-21 | ?324? Wild no tag | 420 x 421 | Wild |
| NSW1093659 | Oct-21 | E421 seedling | 420 x 421 | siteE |
| NSW1093666 | Oct-21 | E421 seedling | 420 x 421 | siteE |
| NSW1082888 | Oct-21 | Fontainea oraria (planted) Coffs Harbour Botanic Gardens | 420 x 421 | CoffsBG |
| NSW1093716 | Oct-21 | original Wild plant X334 1.2m | 420 x 421 | Wild |
| NSW1093681 | Oct-21 | Wild 420 | 420 x 421 | Wild |
| NSW1093671 | Oct-21 | E422 Planted 2.2m no flowers/fruits | 422 x unk | siteE |

**Table 2:** Summary of *Fontainea oraria* adults (top) and seedlings (below) and their numbers in the wild and at each translocation site analysed in this study.

| Genet | Wild | siteA | siteB | siteC | siteD | siteF | siteG | siteE | siteJ | siteN | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 324 | 1 | | 1 | 2 | 1 | 1 | 1 | 1 | | | **8** |
| 325 | 2 | 1 | 4 | 2 | | | 1 | | | | **10** |
| 401 | 1 | 1 | | 2 | 2 | 1 | 1 | 1 | 1 | | **10** |
| 402 | 1 | | 1 | 2 | 3 | 2 | 2 | 1 | | 1 | **13** |
| 403 | 2 | | | 2 | | 1 | | 1 | | | **6** |
| 404 | 2 | | 1 | 2 | 1 | 1 | 1 | | | | **8** |
| 418 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | **9** |
| 420 | 1 | | 1 | 3 | 2 | 2 | 2 | 1 | | 1 | **13** |
| 421 | 2 | | 2 | 1 | 2 | 1 | 1 | 1 | | | **10** |
| 422 | 2 | | 1 | 1 | 1 | 1 | 1 | | | | **7** |
| **Total** | **16** | **3** | **12** | **18** | **13** | **11** | **11** | **7** | **1** | **2** | **94** |
| **N Genets** | **10** | **3** | **8** | **10** | **8** | **9** | **9** | **7** | **1** | **2** | |

| Genet | Wild | siteA | siteB | siteC | siteD | siteF | siteG | siteE | siteJ | siteN | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 324x401 | | 3 | | 5 | 2 | | | | | | **10** |
| 324x421 | 1 | | | | | | | | | | **1** |
| 325x401 | | | | 1 | | | | | | | **1** |
| 401x420 | | | | | | | | 3 | 2 | | **5** |
| 402x403 | | | | 1 | | | | 1 | | | **2** |
| 402x404 | | | | 1 | | | | | | | **1** |
| 402x418 | | | | | | | | | | | **0** |
| 402x421 | | | | | | | | | | | **0** |
| 403x324 | | | | 1 | | | | | | | **1** |
| 404x402 | | | | 1 | | | | | | | **1** |
| 404x420 | | | | | | | | 1 | | | **1** |
| 418x420 | | | | | | | | 2 | | | **2** |
| 420x421 | 7 | | | | | | | 2 | | | **9** |
| 422x? | | | | | | | | 1 | | | **1** |
| **Total** | **8** | **3** | **0** | **9** | **2** | **0** | **0** | **10** | **2** | **0** | **34** |

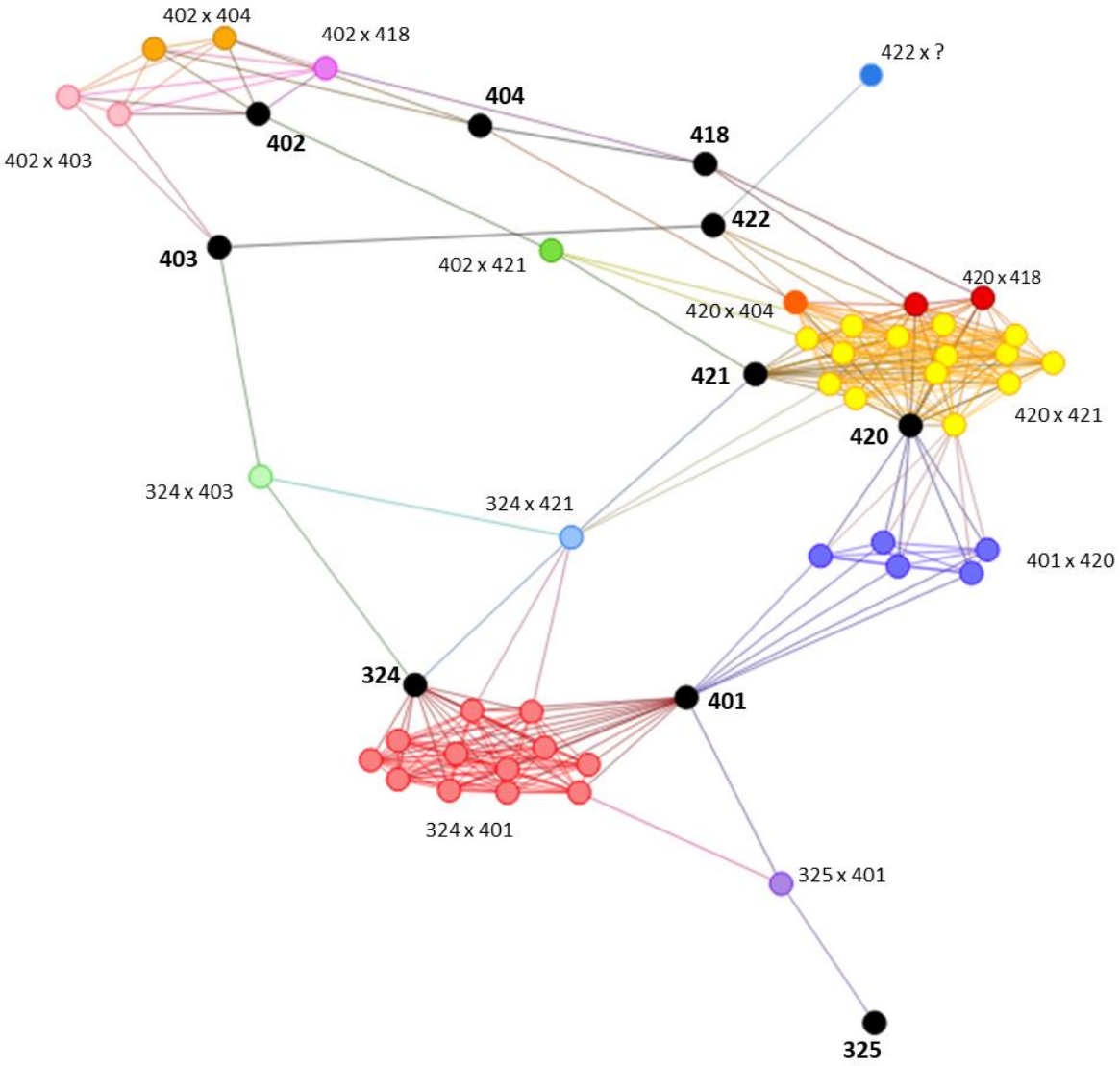
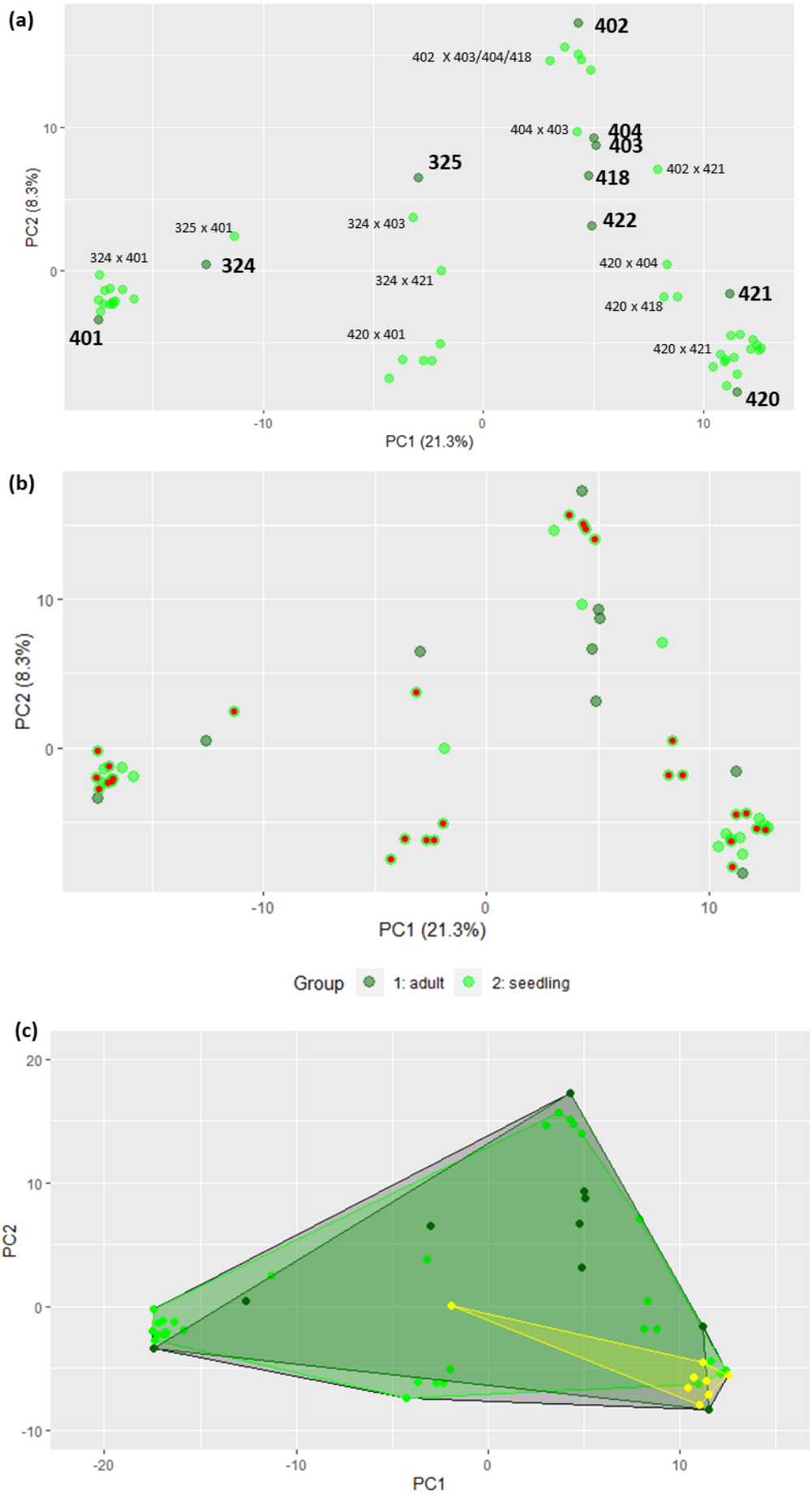



**Figure 2**: A cluster-based network summarising parentage data generated from the SNP data of *Fontainea oraria*. This network shows only one genet of each adult and excludes low levels of relatedness (i.e. half-sibs or less, kinship below 0.1) to highlight relatedness between adults and seedlings (a line indicates relatedness, amount of relatedness between two individuals not shown in network). Each node (circle) is a genet, nodes in black are adults and all other coloured nodes are seedlings. For the seedlings, nodes of the same colour indicate they originate from the same pair of parents.


(a)
402
402 X 403/404/418
404 x 403
404
403
325
418
402 x 421
325 x 401
324 x 403
422
324 x 401
324
420 x 404
324 x 421
421
420 x 418
401
420 x 401
420 x 421
420
PC2 (8.3%)
PC1 (21.3%)
(b)
PC2 (8.3%)
PC1 (21.3%)
Group
1: adult
2: seedling
(c)
PC2
PC1

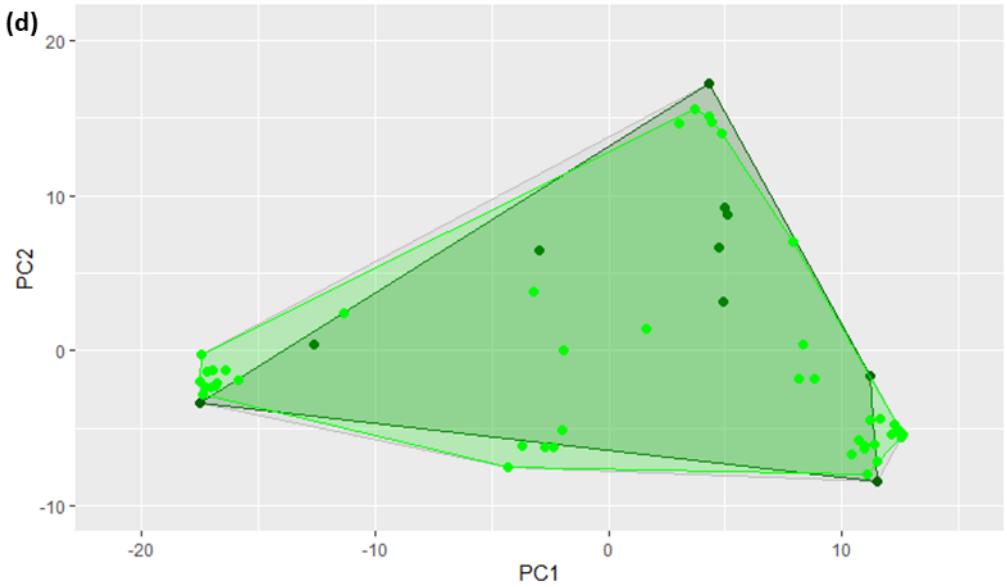



**Figure 3**: Principal Component Analysis of Single Nucleotide Polymorphism (SNP) data showing representativeness of genetic diversity by the *Fontainea oraria* adults and all known seedlings (wild, translocation site, ABGMA, Coffs Harbour BG, unknown).

All four PCA plots are the same except labels were added to (a), and in (c, d), convex hull analyses were produced to assess representativeness in genomic variation by seedlings vs adults. The convex hull analyses in (c) shows that the wild seedlings (yellow convex hull with an area of 25.48) has a small coverage compared to the seedlings at translocation sites (light green convex hull with an area of 374.09) or the adults (dark green convex hull with an area of 397.28). The analysis in (d) identified the area of convex hull for all seedlings is greater than that of the adults (412.51 vs 374.09), showing that sexual reproduction between adults results in progeny that not only maintains original genetic variation but increases overall variability of the species by way of each offspring inheriting new and unique combinations of the parental genomes. Red dots in (b) indicate the new batch of seedlings.

**Table 3**: Summary of all known offspring and their parents. The percentage of seedlings contributed by each adult is indicated in the "Total" column/row.

The gender of selected adults was recorded during sampling based on observations of male/female flowers or fruits at the time (i.e, 324 Male, 401 Female, 402 Male, 420 Male from Table 1). This information together with the kinship data allowed us to infer their maternal/paternal identity. For example, if a known male was observed to share offspring with another adult with an unknown gender, we were able to infer the latter adult as the mother plant. This is except for adult 422 (not in table below) whose gender is unclear because it is only related to one other individual sampled in this study, which was determined to be its offspring (kinship value around 0.27), NSW1093671. The other parent of this offspring could not be determined, suggesting it might have since perished.

A study tracking flowering and fruiting of *F. oraria* found that it is primarily dioecious but monoecy has been observed in a number of individuals (Brown et al. 2016 APC article). The adults that were found to always produced female / male flowers corresponded well with the inferred gender of the adults. For example, we detected 401, 404 and 421 are females that the study found always produced female flowers during each monitoring event. Given this information, we can be certain that the male adults, 324 and 402 which were reported to produce a small number of female flowers were the fathers to offspring shared with the females 401, 404 and 421. We also detected 325 and 420 as males that the previous study found as producing male flowers, so we could be sure that 418 which was observed to produce male and female flowers on the same branch was the mother to the offspring shared with 325 and 420.

Note that this table contains seedlings from ABGMA and Coffs Harbour Botanic Gardens. ABGMA: 2 324 x 401, 1 402 x 418, 402 x 421 and 420 x 421; Coffs Harbour BG: 420 x 421. Among the 14 420 x 421 seedlings, the location of 3 seedlings is unknown (as per previous report) and so they were not accounted for in Table 2.

| | | **Putative father** | | | | Total |
|---|---|---|---|---|---|---|
| | | **402** | **324** | **325** | **420** | |
| **Putative mother** | **404** | **402 x 404** 2 | | | **420 x 404** 1 | 3 (6.97%) |
| | **418** | **402 x 418** 1 | | | **420 x 418** 2 | 3 (6.97%) |
| | **401** | | **324 x 401** 12 | **325 x 401** 1 | **420 x 401** 5 | 18 (41.86%) |
| | **421** | **402 x 421** 1 | **324 x 421** 1 | | **420 x 421** 14 | 16 (5.33%) |
| | **403** | **402 x 403** 2 | **324 x 403** 1 | | | 3 (6.97%) |
| | Total | 6 (13.95%) | 14 (32.55%) | 1 (2.33%) | 22 (51.17%) | 43 (100%) |