Final report to the Australian Flora Foundation
on the project

Hybridisation in three sympatric *Persoonia* species: *P. chamaepitys, P. myrtelloides* and *P. levis.*

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Summary

Although hybridisation is commonly used to develop plants more suited for commercial use, we have little understanding of hybridisation in nature. The genus *Persoonia* contains a number of species which appear to have evolved in the same location (sympatric speciation), and which produce intermediates which may be hybrids. The species provide good material to examine the processes of natural hybridisation. The three *Persoonia* species chosen for study were *P. chamaepitys, P. myrtilloides* and *P. levis* all of which occur in the Blue Mountains region in eastern Australia (200 km west of Sydney). *Persoonia chamaepitys* is not known to hybridise in the wild, whereas *P. levis* and *P. myrtilloides* both appear to generate hybrids when in sympatry with other *Persoonia* species.

This project, supported by the *Australian Flora Foundation,* was a study to examine two questions: (i) Can putative hybrids be identified in the field, based on vegetative and floral characteristics? (ii) What is the potential for interspecific pollination to produce fruits and therefore hybrid offspring?

It was found that there appears to be considerable potential for hybridisation between *Persoonia myrtilloides* and *P. levis* in areas of the Blue Mountains where these species co-occur. In some sites, there are adult plants with morphological characteristics that are intermediate between plants of either species in pure stands. Leaf characters, especially leaf length, provided the greatest discrimination. Hand pollination studies confirmed that plants in these two *Persoonia* species are self-incompatible and therefore require pollinators to transfer pollen between plants.

The potential for gene exchange between these two species in creating a hybrid zone appears to be asymmetrical. Tthere was a greater likelihood of pollination and fertilisation being successful with *P. levis* as mothers than *P. myrtilloides.*

Introduction

How limited is hybrid zone establishment and what type of environmental conditions and/or circumstances can promote its formation in nature? Although hybrid formation is widespread in plants, and has provided an important platform for the development of the horticulture industry, we have limited understanding about the processes producing and maintaining hybrid zones in nature.

The genus *Persoonia* offers unique opportunities to understand hybridisation processes. Among the species in eastern NSW, many are sympatric. Among these, some pairs appear to produce intermediate forms that are putative hybrids, while others so not. The three *Persoonia* species chosen for study were *P. chamaepitys, P.*

myrtilloides and *P. levis* all of which occur in the Blue Mountains region in eastern Australia (200 km west of Sydney). *Persoonia chamaepitys* is not known to hybridise in the wild, whereas *P. levis* and *P. myrtilloides* both appear to generate hybrids when in sympatry with other *Persoonia* species (Weston & Johnson 1991; Bernhardt & Weston, 1996).

One key process that would limit hybrid zone establishment is barriers to interbreeding. Thus, the main overall aim of this research project was to examine potential reproductive barriers to interspecific gene flow amongst *P. chamaepitys, P. myrtilloides* and *P. levis* plants by quantifying flowering phenology, pollinator behaviour and movements, and pre- and post-zygotic barriers to interbreeding.

Previous work focussed on flowering phenologies and pollination ecologies of the three species. This revealed that there was very limited overlap in flowering time between *P. chamaepitys* and either of the other species at several sites over several years (1998-2000) but that *P. myrtilloides* and *P. levis* flowering times overlapped substantially. There was limited information on pollinator behaviour, but the same suite of native bee species was observed visiting flowers of both *P. myrtilloides* and *P. levis,* indicating that interspecific pollen flow was possible. This part of the research, supported by the *Australian Flora Foundation*, was a study to examine two questions: (i) Can putative hybrids be identified in the field, based on vegetative and floral characteristics? (ii) What is the potential for interspecific pollination to produce fruits and therefore hybrid offspring?

***Putative hybridisation between* Persoonia myrtilloides *and* P. levis**

Two sites were chosen: Blue Mountains National Park (BMNP) approximately 8 km east of Blackheath, and Newnes State Forest (NSF). Within each site, two sympatric populations of *P. myrtilloides* and *P. levis*. One of the sympatric populations contained putative hybrids in addition to plants that appeared to be true *P. myrtilloides* and *P. levis*, whilst the other lacked any apparent hybrids (these are referred to as 'reference populations' for the two parental species).

Fifteen individuals of *P. myrtilloides* and 15 of *P. levis* were randomly selected in each location for morphological characterisation. Four branches, each oriented to a different point on the compass were randomly selected from each plant. From each branch, the most mature leaf nearest to the base of the branch was sampled and the flower nearest to the tip of the branch was sampled (these are one-day old flowers). Because of variation in flowering among sites, the BMNP populations were compared using both floral and leaf characters, whereas only leaf characters could be employed for the Newnes State Forest. Quantitative measurements of six leaf and seven floral characters were conducted, and each character was recorded as an average figure of the four individual leaf or floral samples from each plant. The characters used were: leaf area, petiole length, leaf length, leaf width at widest point, leaf width/length ratio, anther length, filament length, nectary length, nectary width, style length, tepal length.

The morphological data confirmed the existence of hybrids between *Persoonia myrtilloides* and *P. levis* in the Blue Mountains and Newnes State Forest sites. Individuals from the putative hybrid zone populations clustered between distinct groups of *P. myrtilloides* and *P. levis* for both leaf and floral characters in the Blue Mountains, and leaf characters at the Newnes State Forest.

Tests for compatibility in crosses between **Persoonia myrtilloides** ***and*** **P. levis**

At NSF, ten plants were randomly selected from each of the *P. myrtilloides* and *P. levis* morphological groups. To confirm that these plants were indeed 'parentals' and not themselves hybrids, the six leaf dimensions were recorded for four randomly selected mature leaves from each tree and these measurements were compared with the results described above. Flowers were hand-pollinated using the following pollination treatments: outcrossed within the species, hybridised between the species, selfed, untreated.

At BMNP, 12 *P. myrtilloides*, 5 intermediates and 10 *P. levis* plants were randomly selected. Once again, the PCA analyses described above were usesd used to confirm the morphological group to which each plant belonged. Flowers were hand-pollinated using the following pollination treatments: outcrossed, hybridised, selfed, untreated (as for NSF). In addition, some of the flowers on intermediate plants were allocated to each of two backcrossing treatments (x *P. myrtilloides*and x *P. levis*) and some flowers on each of the parental species were allocated to crosses with the intermediate plants.

Some flowers from each treatment were sampled 14 days after hand pollination and examined using fluorescence microscopy to detect pollen tubes in the style, and others were simply tagged and observed through to fruit set.

Persoonia myrtilloides was self-incompatible (only 2 out of 161 selfed flowers over two sites had pollen tubes to the ovary) and there was no fruit set resulting from selfing (Fig. 2a). *Persoonia levis* also appeared to be self-incompatible: only one out of 75 selfed flowers resulted in a mature fruit (Fig. 2b). None of the bagged and untreated flowers set fruits indicating that there is no automatic self hybridisation or apomyxis in these species.

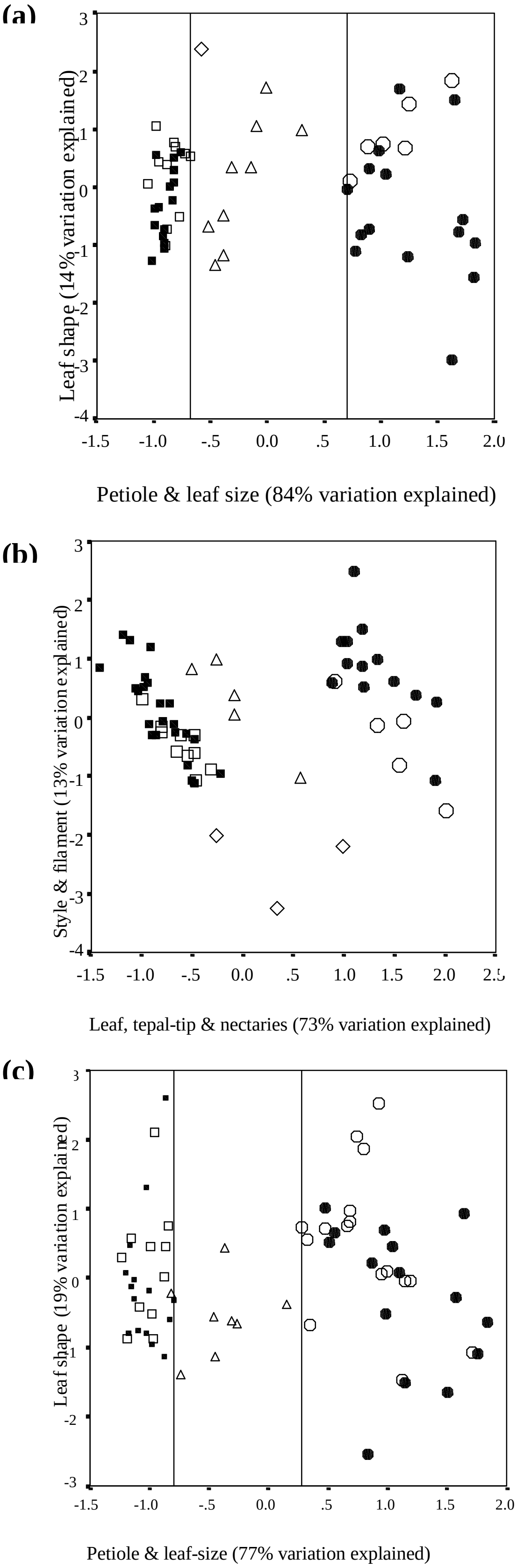
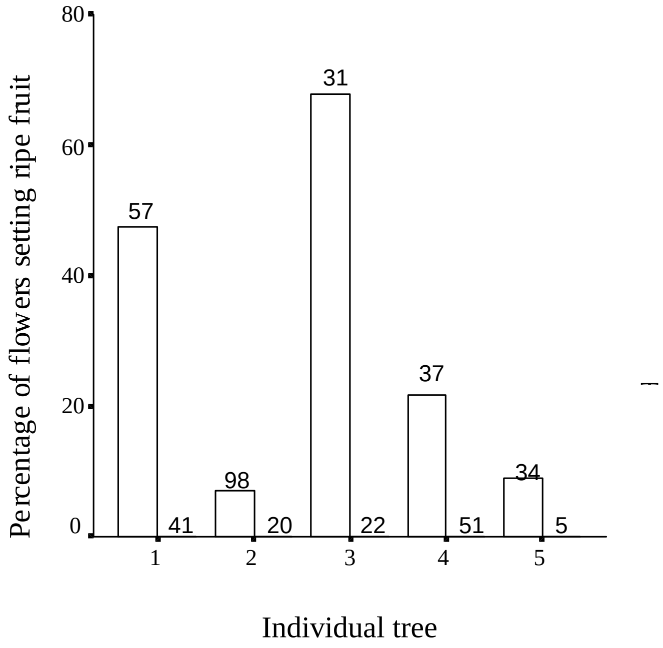


Figure 1: Principal Components Analysis plots in sites containing both *P. myrtilloides* and *P. levis* for (a) BMNP leaf characters only, (b) BMNP both leaf and flower characters, and (c) NSF leaf characters only. Solid symbols represent plants in the 'reference populations' (no putative hybrids present).

Open symbols represent plants in the populations with putatitve hybrids present. The triangle symbols represent the plants that were classified as hybrids based on the degree of separation from either cluster of parental-type plants. Diamond symbols represent plants that did not cluster with either parental type but were not located between them – these were referred to as 'outliers'.

(a)



(b)

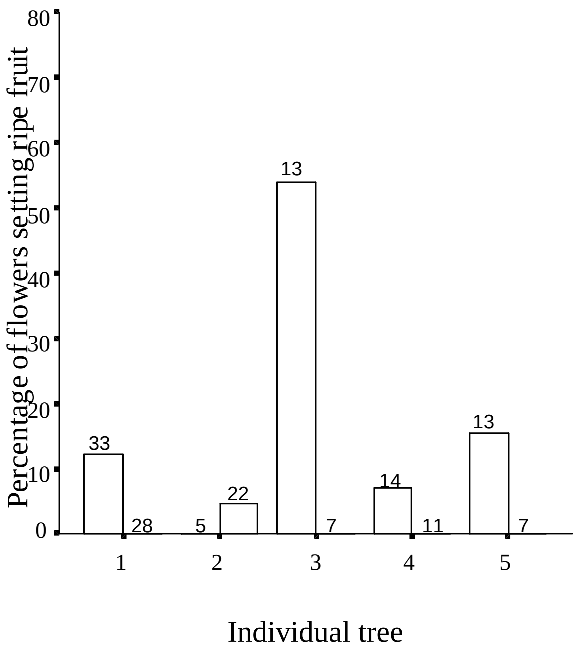


Figure 2: Percent fruit set for outcrossing (left bar for each plant) and selfing (right bar) hand-pollination treatments in 5 *P. myrtilloides* plants (a) and 5 *P. levis* plants (b). Numbers above bars represent numbers of flowers treated.

Both species showed some potential for accepting pollen from the other, and possibly setting fertile fruits. Pollen tube data show that there is potential for hybridisation in all directions. Some flowers on plants of all three types (*P. levis, P. myrtilloides* and intermediates) contained pollen tubes when hand pollinated with pollen from each type (Fig. 3). It is clear, however, that *P. myrtilloides* flowers are only highly likely to be pollinated when *P. myrtilloides* pollen is provided, while intermediate plants and *P. levis* plants can be readily pollinated whatever the pollen source. Thus gene flow appears more likely to occur from *P. myrtilloides* to *P. levis* than *vice versa.*

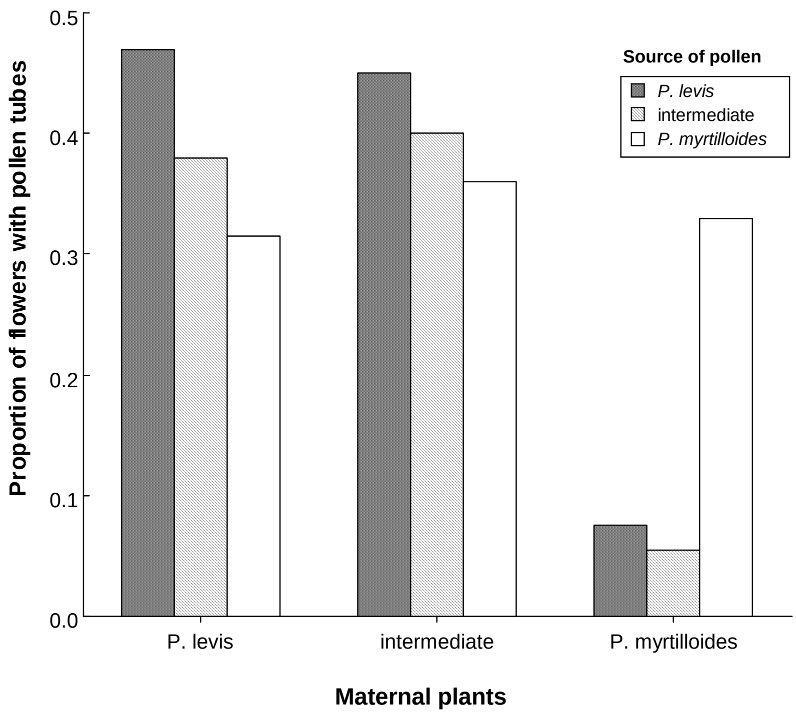


Fig. 3: Proportion of flowers of each type ("maternal plants") with pollen tubes to half way down the style following hand-pollination with pollen from each type.

Evidence from fruit-set data supports this result of the skewed nature of potential hybridisation. For *P. myrtilloides*, very few flowers produced fruits following hand pollination with pollen from *P. levis*, compared to the rates of fruit set following intra-specific outcrossing (Fig. 4a). For *P. levis*, there was substantial fruit set following pollination using *P. myrtilloides* pollen (Fig. 4b).

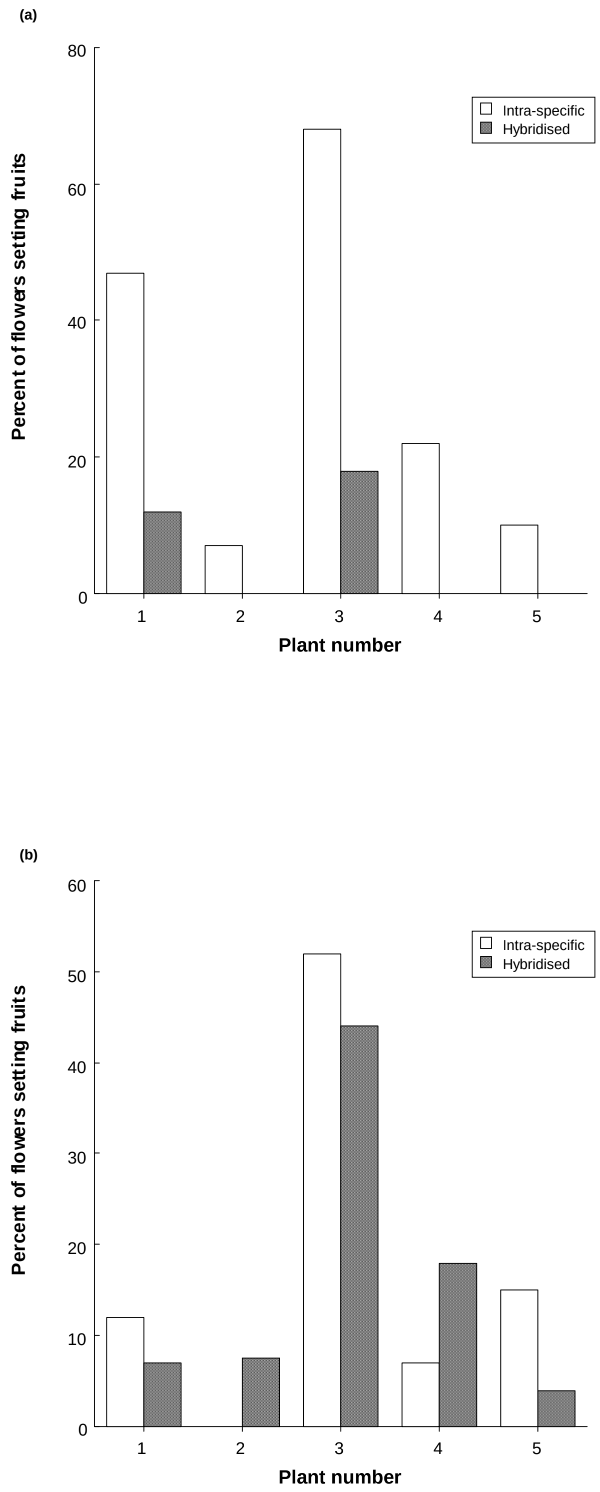


Figure 4: Comparisons of percent fruit set following intra-specific outcrossing and interspecific crossed (‘hybridised) for (a) *Persoonia myrtilloides* and (b) *P. levis.*

Conclusions

There appears to be considerable potential for hybridisation between *Persoonia myrtilloides* and *P. levis* in areas of the Blue Mountains where these species co-occur. In some sites, there are adult plants with morphological characteristics that are intermediate between plants of either species in pure stands. Leaf characters, especially leaf length, provided the greatest discrimination. Hand pollination studies confirmed that these two *Persoonia* species are self-incompatible and therefore require pollinators to transfer pollen between plants.

The two species can both be pollinated by the other, as pollen tubes were found growing down the styles of flowers of each species that had pollen from the other species deposited on the style. Hybrids were also able to be both pollen donors and recipients, with pollen tubes found in the styles of both species following hand pollination with pollen from hybrids, and in the styles of hybrid plants following hand pollination from either parental.

The potential for gene exchange between these two species in creating a hybrid zone appears to be asymmetrical. With both fruit set and pollen tube date, there was a greater likelihood of pollination and fertilisation being successful with *P. levis* as mothers than *P. myrtilloides.*

References

Bernhardt, P. and Weston, P. H. (1996) The pollination ecology of *Persoonia* (Proteaceae) in eastern Australia. *Telopea* **6**, 775-802.

Weston, P.H. and Johnson, L.A.S (1991) Taxonomic changes in *Persoonia* (Proteaceae) in New South Wales. *Telopea* **4**, 269-306.

Images

Persoonia chamaepitys in flower. This species is a sprawling shrub which can cover areas up to several square meters. Fruits are borne on the undersides of branches, beneath the mat of vegetative growth.

Source: www.anbg.gov.au/gnp/interns-2005/persoonia-chamaepitys.html

Persoonia levis is a tall shrub to small tree. It has flaky bark that may be important in protecting the plant from fires, as this species appears to be able to resprout from epicormic buds underneath the bark.

Source: www.grevilleapark.org/static_images/2049/doneIMG_6113.JPG

Source: www.anbg.gov.au/images/photo_cd/630930713442/045.html, Photographer – D. Grieg ANBG Photo No.: a.10267