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Title: Comparison of host susceptibility to native and exotic pathogens provides evidence for pathogen imposed selection in forest trees

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Summary

- The extent to which spatial structuring of host resistance in wild plant populations reflects direct pathogen imposed selection is a subject of debate. To examine this issue, genetic susceptibility to an exotic and co-evolved native fungal pathogen were compared using two Australian host tree species.
- Damage to common host germplasm of *Corymbia citriodora* subsp. *variegata* (CCV) and *Eucalyptus globulus*, caused by recently introduced (*Austropuccinia psidii*) and

native (*Quambalaria pitereka* and *Teratosphaeria sp.*) pathogens was evaluated in common-garden experiments.

- There was significant additive genetic variation within host species for susceptibility to both the exotic and native pathogens. However, susceptibility to *A. psidii* was not genetically correlated with susceptibility to either native pathogen, providing support for pathogen specific rather than general mechanisms of resistance.
- Population differentiation (Q_{ST}) for susceptibility to the native pathogens was greater than neutral expectations (molecular F_{ST}), arguing for divergent selection. Coupled with lower native, but not exotic, pathogen susceptibility in host populations from areas climatically more prone to fungal proliferation, these findings suggest that pathogen imposed selection has directly contributed to a geographic mosaic of host resistance to native pathogens.

Key words

Host resistance, forest tree, Pathogen imposed selection, *Eucalyptus globulus*, *Corymbia citriodora* subsp. *variegata*, *Austropuccinia psidii* (myrtle rust), *Quambalaria pitereka*, *Teratosphaeria*.

Introduction

The extent to which spatial structuring of host resistance in wild plant populations reflects direct enemy imposed selection is a subject of ongoing debate (Parker, 1991; Thompson & Burdon, 1992; Laine *et al.*, 2011; O'Reilly-Wapstra *et al.*, 2014; Carmona *et al.*, 2015; Toor & Best, 2016). Plants constantly interact with a multitude of animals, bacteria, viruses and fungi (Walters, 2011). These interactions may have a genetic basis, occur at many levels from individual plants to entire species, and vary through time and space (Whitham *et al.*, 2012; Burdon & Thrall, 2014). It is argued that differences in such interactions throughout the native range of a species will produce complex 'mosaics of coevolution', resulting in geographically structured differences in coevolved traits between host and pathogen populations (Thompson, 1999). While there are well-cited examples of co-evolution in the wild (Soubeyrand *et al.*, 2009) and agricultural systems (Flor 1972), providing evidence that specific biotic interactions have shaped the evolutionary trajectory of even one of a species

pair is challenging (Smith *et al.*, 2011; Desprez-Loustau *et al.*, 2016; Stenlid & Oliva, 2016), particularly when this involves long-lived hosts such as forest trees.

Consistent with co-evolutionary theory, most studies assessing host resistance in the wild report spatial variation (reviewed by Salvaudon *et al.*, 2008; Laine *et al.*, 2011). However, such genetic differentiation could also occur through non-adaptive processes, such as random drift arising from bottlenecks and founder effects (Thompson & Burdon, 1992). It could also arise indirectly, through a correlated response to selection by other biotic or abiotic factors (Close & McArthur, 2002; Leimu & Koricheva, 2006; O'Reilly-Wapstra *et al.*, 2014). In the case of pathogens, for example, there are general resistance mechanisms (van der Hoorn & Kamoun, 2008; Cook *et al.*, 2015) which would make it difficult to differentiate pairwise selection due to a specific pathogen from diffuse (multispecies) selection (Leimu & Koricheva, 2006). We apply a novel approach to help unravel these possibilities. Forest systems worldwide are increasingly being impacted by exotic pathogens (Stenlid & Oliva, 2016). By comparing the genetic architecture of host resistance to native (co-evolved) and exotic pathogens, as recently outlined by Perry *et al*. (2016), we provide evidence that the evolutionary trajectory of a temperate (*Eucalyptus globulus* Labill.) and a subtropical (*Corymbia citriodora* subsp. *variegata* Bean and McDonald; hereafter termed CCV) eucalypt species has been shaped by pathogen imposed selection.

Eucalypts belong to the Myrtaceae family and comprise the genera *Eucalyptus, Corymbia* and *Angophora* (Grattapaglia *et al.*, 2012). They are foundation species which dominate most Australian forest ecosystems and many have become economically important plantation species (Doughty, 2000). In two host species, we quantified variation in susceptibility to both the recently introduced pathogen *Austropuccinia psidii* (G. Winter) Beenken (myrtle rust or guava rust; formally *Puccinia psidii* Winter - Beenken, 2017) and the most significant native pathogens of the host species; *Teratosphaeria* spp. (Syd and P. Syd) Crous (Teratosphaeria leaf disease) in *E. globulus* and *Quambalaria pitereka* (J. Walker and Bertus) J.A. Simpson (Quambalaria shoot blight) in CCV. Host resistance to native pathogens was assessed following natural infection in field trials and, in the case of CCV, artificial inoculations.

We first establish that there is significant additive genetic variation and population divergence in host resistance within each pathosystem. We then hypothesise, that if specific pathogen imposed selection has played a significant role in the evolution of population divergence in host susceptibility to native pathogens: (i) there should be no genetic

correlation between susceptibility to native and exotic pathogens; (ii) population differentiation in host susceptibility to the pathogen should exceed that expected through drift (i.e. $Q_{ST} > F_{ST}$ Leinonen *et al.*, 2013) for the native but not the exotic pathogen; and (iii) variation in home-site climate variables related to disease risk would be significantly correlated with host population susceptibility to the native (White *et al.*, 2007; Perry *et al.*, 2016) but not the exotic pathogen.

Materials and Methods

Study systems

Hosts

Eucalyptus globulus is native to Tasmania and coastal regions of mainland south-eastern Australia (Jones *et al.*, 2013) and has become an important plantation species in temperate regions worldwide (Doughty, 2000). Within its native range in south-eastern Australia, *E. globulus* has been variously classified as a species or subspecies of four closely related taxa with core distributions that are geographically and morphologically distinct (Jones *et al.*, 2013). There is significant quantitative genetic variation across the geographic range of *E. globulus* in virtually all traits studied, which has been used to partition the native populations of *E. globulus* into 13 geographic races (Dutkowski & Potts, 1999).

Corymbia citriodora subsp. *variegata* (CCV) is the most important taxa for hardwood plantations in subtropical Australia (Lee, 2007). It is one of a complex of four closely-related spotted gum taxa (*C. citriodora* subsp. *variegata*, subsp. *citriodora*, *C. henryi* and C. *maculata*) which occur as a latitudinal replacement series on the east coast of Australia (Shepherd *et al.*, 2012). CCV has a wide natural distribution spanning coastal and sub-coastal regions in northern New South Wales and South-eastern Queensland. Commercial plantations have more recently been derived from blight tolerant provenances, such as the Woondum population that was a focus for this study (Johnson *et al.*, 2009; Lee *et al.*, 2010).

Pathogens

Exotic pathogen

Austropuccinia psidii (Phylum Basidiomycota, Order Pucciniales, family Sphaerophragmiaceae) is a pathogen of global significance. Native to South America, it is now rapidly spreading worldwide with multiple biotypes identified (Stewart *et al.*, 2017). The pathogen has a remarkably broad host range within the *Myrtaceae*, a predominantly southern hemisphere plant family with many economically important species, including the eucalypts (Giblin & Carnegie, 2014). It affects young actively growing leaves, shoots, flower buds and fruits, with repeated infection leading to death of highly susceptible plants (Coutinho *et al.*, 1998; Carnegie *et al.*, 2016; Pegg *et al.*, 2014a; Pegg *et al.*, 2017). The potential risk to Australia has long been recognised, due to its rich Myrtaceous flora (Glen *et al.*, 2007). Following the first detection in Australia in 2010, it spread rapidly across the east coast from Victoria to Northern Queensland within a year. It was reported on the island of Tasmania by early 2015 and most recently west to the Northern Territory.

A wide range of *Eucalyptus* and *Corymbia* species are susceptible to *A. psidii*, as evidenced by controlled inoculation and field infection (Zauza *et al.*, 2010; Pegg *et al.*, 2014a; Potts *et al.*, 2016).

Native pathogens

Quambalaria pitereka (Phylum Basidiomycota, Order Microstromatales, Family Quambalariaceae; Simpson, 2000) causes the disease Quambalaria shoot blight in *Corymbia*, *Blakella* and *Angophora* in Australia (Pegg *et al.*, 2011b) and China. It infects foliage and juvenile stems, which can lead to losses in leaf area and negatively impacts stem form (due to loss of apical dominance) and growth (Pegg *et al.*, 2009; Johnson *et al.*, 2009). Native to the east coast of Australia (Old 1990), *Q. pitereka* has also been introduced into Western Australia, where it has been reported from plantations of *C. maculata* as well as native forests (*C. calophylla*) (Paap *et al.*, 2008). In China, it has been reported on *C. citriodora* subsp. *citriodora* (Zhou *et al.*, 2007) and the other three spotted gum taxa (Brawner pers. obs.; 2017 Guangxi). Teratospheria leaf disease (TLD, formerly Mycosphaerella Leaf Disease) caused by *Teratospheria cryptica* and *T. nubilosa* (Phylum Ascomycota, Order Capnodiales, Family Teratosphaeriaceae) is the most significant foliar pathogen in *Eucalyptus globulus* plantations

in temperate regions of the world (Park *et al.*, 2000; Mohammed *et al.*, 2003). TLD lesions reduce photosynthetic capacity, cause leaf necrosis and defoliation which can be highly detrimental to tree growth and form (Mohammed *et al.*, 2003).

Genetic material, raising seedlings and trial designs

In both *E. globulus* and CCV, susceptibility to *A. psidii* was assessed using artificial inoculation of open-pollinated families derived from wild populations in Australia. Most families used had been previously assessed for native disease damage following natural infection of field trials and, in the case of CCV the same plants that were inoculated with *A. psidii* were also artificially inoculated with *Q. pitereka*. Artificial inoculations and assessment of disease symptoms were performed at the Ecosciences Precinct, Brisbane (Queensland, Australia).

Austropuccinia psidii

Susceptibility of *E. globulus* to *A. psidii* was assessed using 191 open-pollinated families from 13 native populations, representing the races of Dutkowski & Potts (1999). The families were initially grown in glasshouse facilities at the Queensland Department of Agriculture, and Fisheries in Gympie Queensland, following the procedure outlined in Lee et al. (2015). Plants were arranged in a randomized incomplete block design with 20 replicates, each comprising 4 incomplete blocks of 40 plants in a 5 x 8 arrangement. Randomization occurred at the family-level and each family was represented once per replicate, except where poor germination or mortality limited the number of available seedlings per family. Seedlings were transferred to the screening facility in Brisbane for rust inoculation in two batches comprising 10 replicates each, at two and three months of age. At this stage, 79% of the families were represented in both batches. The first batch was screened in October/November 2013, when plants were three months old. Due to a lack of active growth (flushing) of the second batch, and thus poor infection of the plants in the initial inoculation (at three and a half months of age), the plants were cut back to remove any diseased tissue and leave approximately two leaves on the main stem to produce a flush of new leaves for inoculation. Plants were then re-inoculated in January 2014 (at 6 months of age) when most plants had two pairs of fully expanded new leaves and data from this second inoculation was analysed. When *A. psidii*

inoculations are successful there is high repeatability of damage scores obtained before and after cutting back seedlings (Butler *et al.*, 2016). Overall, there were 2,597 flushing seedlings assessed for *A. psidii* damage, with 9-38 families represented per population and a mean of 13.7 seedlings per family.

Susceptibility of CCV to *A. psidii* was assessed in 125 families from the Woondum provenance from the coastal region of southeastern Queensland. The experimental design and growth of seedlings was essentially the same as described above for *E. globulus*, except 125 families were represented in each replicate with 5 incomplete blocks of 25 plants. The first batch of 10 replicates was screened in June 2013 when plants were 3.5 months old and the second batch in December 2013 when plants were 10 months old and most had susceptible new growth. Both batches were successfully infected following a single inoculation.

Quambalaria pitereka

Following the assessment of susceptibility of CCV to *A. psidii*, the seedlings were cut back and allowed to regrow as described for *E. globulus* above. In this case seedlings had to be cut back twice to ensure the majority of plants had uniform new growth for inoculation (see below) with *Q. pitereka* in January and February 2014.

The susceptibility of CCV to *Q. pitereka* was also quantified following natural infection of a field trial (25.76˚S, 152.68˚E; Bakers in Brawner *et al.*, 2011), which included the same 125 families that were used in the artificial inoculations (as well as two additional families from the same provenance). The field trial was established with 5-month old-seedlings (King, 2004) with seven replicates of each family represented in 4-tree line plots. The trial also contained seedlots from other *C. variegata* provenances, but only the Woondum families were used in this study. A severe outbreak of *Q. pitereka* was first noted 4 months after planting and, in April 2000, nine months after planting the percentage of crown damage on each tree was visually scored using a 6-point scale (1 = 0%, 2 = 1-10%, 3 = 11-25%, 4 = 26-50%, 5 = 51-75%, 6 = 76-100%) These data formed part of the study of Brawner *et al.* (2011).

Teratosphaeria leaf disease

The susceptibility of *E. globulus* to TLD was assessed in 247 open-pollinated families from 13 populations (as described above), based on natural infections in five common garden field trials planted between 2005 and 2008. The severity of TLD damage was assessed as the percentage leaf area necrosis on the juvenile canopy in spring of 2007, when plants were one to two years old, with the exception of one trial assessed in spring 2010 (at three years of age). A quantitative genetic analysis of these TLD data has been reported in Hamilton et al. (2013). Of the families we assessed for rust damage, 81% were also assessed for TLD; and of those assessed for TLD, 96% were also assessed for rust.

Inoculum, inoculation and assessment of disease symptoms

Austropuccinia psidii

The procedures for collecting inoculum, rust inoculation and assessment of disease severity largely followed Pegg *et al.* (2014b). In brief, frozen urediniospores from the *A. psidii* biotype present in Australia were used for inoculation. These urediniospores were derived from a pustule isolate of *A. psidii*, collected from a susceptible host *Rhodamnia sessiliflora* growing in Brisbane.

Seedlings were assessed 20 (CCV) and 25 (*E. globulus*) days after inoculation for the severity of infection on new shoots and leaves using a disease rating scale modified from Junghans *et al.* (2003). This scale was: 1 = no symptoms, or minor yellow flecking evident; 2 = presence of a hypersensitive reaction (HR) with flecking or necrosis; 3 = small pustules, <0.8 mm diameter, with one or two uredinia; 4 = medium-sized pustules, 0.8–1.6 mm diameter with about 12 uredinia; 5 = large pustules, >1.6 mm diameter, with 20 or more uredinia on leaves, petioles and/or shoots (Pegg *et al.*, 2014b). Only seedlings which were actively growing and had new shoots and leaves at inoculation were assessed.

Quambalaria pitereka

Selected isolates of *Q. pitereka* were obtained from the Brisbane Plant Pathology (BRIP) storage collection (BRIP samples 48368, 48387, 48343, 48424) and cultured onto Potato

Dextrose Agar (PDA) for 2 to 3 weeks in the dark at 25°C. To ensure the effects of storage had not impacted pathogenicity, each isolate was re-inoculated onto CCV seedlings and new cultures of each isolate were established. A suspension containing an equal concentration of spores from all four isolates was used for artificial inoculation following the general procedure outlined in Pegg *et al*. (2009). Seedlings were assessed 20 days after inoculation using a 1-5 scale similar to that used for *A. psidii*.

Data analysis

E. globulus pathosystems

Genetic analyses of the *A. psidii* data collected from *E. globulus* focused on the estimation of heritabilities (h^2), degree of population differentiation (Q_{ST}), (Latta, 1998; Leinonen *et al.*, 2013) and the extent to which susceptibility to *A. psidii* was genetically correlated (r_g) with the susceptibility to TLD reported in Hamilton *et al*. (2013). Following Hamilton *et al.* (2013), variance components for estimating narrow-sense heritability and Q_{ST} were obtained from restricted maximum likelihood (REML) mixed model analyses implemented with ASReml 4.0 (Gilmour *et al.*, 2014), and fitting the following linear model:

$$Y = \mu + REP + POPULATION + IBLK(REP) + TREE + RESIDUAL,$$

where Y is the pathogen damage score, μ is the mean, REP is the fixed replicate effect, *POPULATION* is the random population effect, *IBLK(REP)* is the random incomplete block within replicate effect, *TREE* is the within population additive genetic effect, and *RESIDUAL* is the error. This model was fitted separately for each screening batch and the combined data. The tree term allowed the estimation of the pooled additive genetic variance within populations (σ_a^2). This analysis used a multi-generation pedigree file to define the additive relationship matrix for parents and open-pollinated offspring assuming a selfing rate of 0.3 (Dutkowski *et al.*, 2001; Gilmour *et al.*, 2014).

The narrow-sense open-pollinated heritability (h_{op}^2) and Q_{ST} were estimated as:

$$h^2_{op} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_e}$$

$$Q_{ST} = \frac{\sigma^2_p}{\sigma^2_p + 2\sigma^2_a}$$

where σ^2_p is the *population* variance and σ^2_e is the residual variance.

The significance of the population and additive genetic variances was tested with a 'one-tailed' likelihood ratio test (Gilmour *et al.*, 2014). To test whether the h^2_{op} estimates differed between batches, a bivariate extension of the above linear model (with each batch treated as different variables) was fitted allowing for covariation among populations and additive genetic effects (Gilmour *et al.*, 2014). The common heritability that achieved the maximum log likelihood value was identified iteratively by varying the common additive to residual variance ratio, and comparing the log likelihood with that of the unconstrained model where independent variances were estimated for each batch. This comparison was done using a two-tailed likelihood ratio test (LRT) with 1 degree of freedom (Gilmour *et al.*, 2014). Following Hamilton *et al*. (2013), 'two-tailed' likelihood ratio tests (Gilmour *et al.*, 2014) were used to test the significance of the difference of the Q_{ST} estimate for *A. psidii* damage from (i) a previously published estimate of F_{ST} (0.09) which was based on eight microsatellite markers and used a similar group of *E. globulus* populations (Steane *et al.*, 2006) and (ii) previously published estimates of Q_{ST} for TLD damage (Hamilton *et al.*, 2013).

To estimate the population and additive genetic correlations between exotic (*A. psidii*) and native (TLD) pathogen damage, the *A. psidii* inoculation data was combined with previously analysed TLD data (Hamilton *et al.*, 2013). This was done by treating TLD damage at the various field sites and the inoculation experiment as separate traits linked through a common pedigree. The correlations were estimated using bivariate analyses fitting the model terms detailed in Hamilton *et al.* (2013) for the TLD damage and terms described in the above linear model for *A. psidii* damage, and allowing for covariation among random population and additive genetic effects. These random genetic effects were common to both the field trial and inoculation data. Standard errors of parameters were estimated from the average information matrix, using a standard truncated Taylor series approximation (Gilmour *et al.*,

2014). Population means for plotting spatial trends and for path analyses (see below) were predicted by treating population as a fixed effect in univariate analyses with the above linear models.

Corymbia citriodora subsp. *variegata pathosystems*

The analysis of the CCV inoculation data followed that described for *E. globulus*, except that the population term was excluded from the model as only a single population of CCV was studied. In addition, as the families were planted as 4-tree line plots in the field trial, a random plot term was fitted for the analysis of *Quambalaria* field damage. For each pathogen, a bivariate model was used to estimate genetic correlations among the different inoculation batches, and the significance of these correlations deviating from one was tested using a LRT. The bivariate model was then extended to the multivariate level to estimate all the genetic correlations among the *A. psidii* damage and the *Quambalaria* field and inoculation damage scores. This was a five-trait analysis, as the two glasshouse inoculation batches were treated as separate traits for both pathogens. As the same CCV plants were sequentially screened with the exotic and native inoculum, it was necessary to allow for covariation among their residuals and incomplete block terms in the multivariate model. As the additive genetic correlation between the two batches was not significantly different from one in the bivariate analysis of *Quambalaria*, this correlation was fixed to one in the multivariate analysis and other paired correlations involving these two batches constrained to be equal. To test the homogeneity of the genetic correlations across field and inoculation studies, a further constraint was applied which required the correlations of both batches of rust screening with the field and inoculation screenings with *Quambalaria* to all be equal. The difference in log likelihoods of the two models was then tested with a LRT.

Path analysis

The relationship of population home-site geographic and climate variables with population variation in disease susceptibility was modelled in each pathosystem using the path analysis framework of PROC CALIS of SAS (Version 9.4). Susceptibility was linked to variation in the key climate variables expected to affect disease risk – precipitation and temperature. For consistency with Hamilton *et al*. (2013), the climatic data used for the *E. globulus* populations was derived from the SILO Data Drill

(http://www.longpaddock.qld.gov.au/silo/datadrill/index.frames.html) and modelling undertaken with population mean annual precipitation (mm) and mean daily maximum temperature (˚C) based on data from 1908 to 2007. For CCV, modelling used 1976-2005 mean annual precipitation (RANN, mm) and mean annual temperature (TANN,˚C) derived from BIOCLIM climate surfaces (ANUCLIM version 6.1). For the native pathosystems we used published population data on susceptibility from field trials showing the greatest population differentiation (*E. globulus*/ TLD - trial TEM06, Hamilton *et al*. (2013); CCV/*Quambalaria* – mean tip damage from trial 451c, Brawner *et al.*, 2011). For the exotic pathogens we used population means for *E. globulus* (Figure 1) and for *Corymbia* we used the average disease severity ratings published in Pegg *et al*. (2014b) for nine spotted gum populations (that encompassed *C. henryi* and two subspecies of *Corymbia citriodora*), five of which were CCV.

Results

Eucalyptus globulus

Significant genetic variation in damage due to *A. psidii* was observed both within (i.e. $h^2_{op} > 0$, Table 1) and among (LRT [$Q_{ST} = 0$] $P < 0.001$) populations of *E. globulus*. As similar levels of additive and population variation were evident in the two different batches (data not shown) and high additive genetic (0.93 ± 0.05; LRT from 1 $P = 0.070$) and population level (0.84 ± 0.14 LRT from 1, $P = 0.007$) correlations were found across batches, batch data were combined into a single trait. The heritability of *A. psidii* damage across the artificial screenings was 0.65, nearly 2-fold higher than that of even the highest value obtained following natural TLD field infection of comparable germplasm (Table 1). In contrast, the quantitative inbreeding coefficient (Q_{ST}) among populations for TLD damage was on average (0.14) over two-fold greater than that for *A. psidii* damage (0.06 ± 0.03)(Table 2). In the field trial with the highest TLD damage (Temma06), where population differences were most expressed, the Q_{ST} for TLD was significantly ($P < 0.01$) greater than that observed for the rust (Table 2) and significantly ($P < 0.05$; Hamilton *et al*., 2013) greater than neutral expectation based on the published F_{ST} from neutral molecular markers of 0.09. The Q_{ST} for *A. psidii* damage was less than and not significantly different ($P = 0.417$) from F_{ST}. The

genetic correlation between *A. psidii* and TLD damage was not significantly different from zero for any of the field trials at either the population or additive genetic levels (Table 2).

At the population level, the non-significant correlation between *A. psidii* and TLD damage was a result of their different patterns of spatial variation across the native range of *E. globulus* (Fig. 1a,b). Specifically, there was a distinct latitudinal cline in TLD damage (Fig. 1a) with the most susceptible population found in southern Tasmania and resistance increasing northward into mainland Australia (see also Hamilton *et al.*, 2013). These trends are associated with changes in climate with the path analysis showing that populations originating from warmer and wetter environments are less susceptible to TLD (Fig. 2a). This is consistent with an expectation of an increased disease risk in these environments. By contrast the only significant path coefficient detected for *A. psidii* susceptibility was with precipitation (Fig. 2b), but in this case the association was positive. This association was mainly driven by differences in the proportion of plants in disease class 1 (no symptoms or mild necrotic flecking) ($r=-0.73$, $P<0.004$), suggesting that provenances from wetter regions have a greater proportion of plants with symptoms indicative of host leaf penetration by the pathogen. This result is inconsistent with pathogen induced selection having shaped population divergence in susceptibility, as the populations originating from the wetter areas (i.e. higher disease risk) were more susceptible. While *A. psidii* susceptibility decreased northward on the east coast of Tasmania, this trend did not extend to mainland populations as it did with TLD (Fig. 1). Indeed, the western Victorian populations which were the most resistant to TLD were the most susceptible to *A. psidii*.

Corymbia citriodora subsp. *variegata* (CCV)

Significant additive genetic variation was observed within the Woondum population of CCV for susceptibility to *A. psidii* (i.e. $h^2_{op} > 0$) and, as with *E. globulus*, heritability estimates were high (Table 3). Similar levels of additive genetic variation were evident in the two batches (data not shown), however, the different batches were treated as separate traits as bivariate analysis showed that the additive genetic correlation between batches was lower than for *E. globulus* and highly significantly different from 1 (0.77 ± 0.08; LRT from 1 $P < 0.001$).

When the same CCV plants were artificially inoculated with *Q. pitereka* the heritability of damage estimates ($h^2_{op} = 0.08$-0.12; Table 3) was markedly lower than that observed for *A.*

psidii (h^2_{op} = 0.59-0.63; Table 3). It was also lower than observed following other inoculations of CCV with *Q. pitereka* (h^2_{op} = 0.33; Supplementary Table S1 and Note S1). In a bivariate analysis the genetic correlation between batches was effectively 1 (estimate was at the boundary of the parameter space) and the different batches were therefore treated as a single trait. While the individual batch heritability estimates were not significantly greater than zero, the pooled estimate was ($P<0.001$). Despite the low heritability observed under artificial inoculation, the same families exhibited a high heritability for *Q. pitereka* damage under natural infection in the field (h^2_{op} = 0.42 ± 0.06; Table 3), and the estimates from natural and artificial inoculation were highly correlated at the additive genetic level (r_a = 0.72 ± 0.15, LRT from zero $P < 0.001$; Table 4).

As with *E. globulus*, no genetic association between susceptibility to the native and introduced pathogen was detected in CCV. This result was consistent across natural and artificial inoculations as well as the different CCV batches screened for *A. psidii* (Table 4) and another smaller-scale confirmation inoculation of CCV (Supplementary Note S1). While there was a slightly positive correlation of susceptibility to the different pathogens under artificial inoculation, this was not observed with the natural infection of *Q. pitereka*, and the LRT constraining the four native/exotic genetic correlations to be the same indicated they were not significantly different (pooled r_a = -0.13 ± 0.10; LRT for homogeneity with 4 df, P = 0.190).

Integration and re-analysis of the published data on population variation in *C. citriodora* revealed that the population differences in susceptibility were significantly associated with home-site climate for the native (Fig. 2c) but not the exotic (Fig. 2d) pathogen. The associations were somewhat consistent with those observed in *E. globulus*. In both native pathosystems, increased population susceptibility was associated with decreasing home-site precipitation. However, in contrast to the relationship between temperature and susceptibility observed in the temperate *E. globulus* native pathosystem, the populations originating from warmer sites within the subtropical CCV range (i.e. sites further north, or further inland) tended to be more susceptible to *Q. pitereka* damage. No significant associations between climate and population susceptibility to the exotic pathogen *A. psidii* were detected (Fig. 2d).

Discussion

Three key results relevant to our initial hypotheses emerged. First, despite significant additive genetic variation for susceptibility within host populations, host susceptibility to the native and exotic pathogens is genetically uncorrelated, suggesting that pathogen specific defence mechanisms are involved. Second, host populations are more differentiated in their susceptibility to native than exotic pathogens in cases exceeding that expected through drift for the native, but not the exotic pathogen. Third, where testable, population differentiation in native and exotic pathogen susceptibility is also uncorrelated and susceptibility generally decreases to the native, but not the exotic pathogens where climate increased disease risk. Together, these findings provide strong evidence that pathogen imposed selection has shaped the natural distribution of host resistance to native pathogens in these tree species.

Significant genetic variation was detected within populations for susceptibility to the native and exotic pathogens in both host species. In the case of the native pathogens, this finding is consistent with previous reports for TLD in *E. globulus* (Costa e Silva *et al.*, 2013; Hamilton *et al.*, 2013; Balmelli *et al.*, 2014) and *Q. pitereka* in CCV (Brawner *et al.*, 2011; Pegg *et al.*, 2014b). Such genetic variation in host susceptibility is common in native pathosystems in which spatial and temporal fluctuations in selection pressure from pathogens are likely to maintain variation in host resistance (Burdon *et al.*, 2006; Laine *et al.*, 2011). Indeed, for the native pathogens in this study, seasonal variation in epidemics related to climatic differences within and between years have been documented (Pinkard *et al.*, 2010) and is accentuated by host developmental and ontogenetic variability in susceptibility (Park, 1988a; Park, 1988b; de Little *et al.*, 2008; Hunter *et al.*, 2009). Variation in disease risk and host susceptibility has also been documented over finer spatial scales than our sampling (Wilkinson, 2008; Pinkard *et al.*, 2010), which may contribute to the genetic variability within populations. In contrast, native hosts often lack significant resistance to exotic pathogens (Burdon *et al.*, 2013). Thus the significant genetic variation in susceptibility to the exotic *A. psidii* evident in both hosts (present study, Pegg *et al*., 2014b) is somewhat unexpected, since they are unlikely to have been exposed to this pathogen in their recent evolutionary history (Tobias *et al.*, 2016).

Consistent with our findings, variation in susceptibility to *A. psidii* has been reported in numerous Australian Myrtaceae species (Morin *et al.*, 2012). As well as the existence of genetic variation for susceptibility in a range of taxa, the nature of the resistance response to the exotic *A. psidii* is intriguing, as in some cases it appears to involve a highly specific

response normally indicative of pathogen recognition (Thumma *et al.*, 2013; Tobias *et al.*, 2016; Hsieh *et al.*, 2017). This raises the questions: what genetic mechanisms confer variation in susceptibility to the exotic *A. psidii* in the Australian flora and is this variation related to susceptibility to native pathogens? Past studies employing artificial inoculation in eucalypt (Butler *et al.*, 2016; Potts *et al.*, 2016) and other taxa (Morin *et al.*, 2012) have shown resistant genotypes may display no symptoms/mild necrotic flecking, suggesting a lack of host penetration or cell invasion, as well as symptoms consistent with a hypersensitive response implying the pathogen has entered the host cells and been recognised by the host (Jones & Dangl, 2006).

Among the potential explanations for specific resistance to *A. psidii* in the Australian flora, Tobias *et al.* (2016) argue that the surveillance of host integrity (Cook *et al.*, 2015), as proposed by the guard or decoy models (van der Hoorn & Kamoun, 2008), is likely to play an important role. Under this hypothesis resistance to *A. psidii* may be attributable to a common Myrtaceae 'effector hub' which, when modified, leads to host recognition and response. This implies exotic pathogens may produce similar 'invasion patterns' (Cook *et al.*, 2015) to those produced by co-evolved pathogens. Thus, selection by co-evolved pathogens could account for the variation in resistance to *A. psidii* in Australian Myrtaceae. The absence of significant genetic correlations between host susceptibility to the native pathogens in this study and susceptibility to the exotic *A. psidii* would argue against the common effector hub hypothesis. In the case of *E. globulus*, this is consistent with the observation that none of the QTL identified for *A. psidii* resistance were co-located with QTL for TLD (Freeman *et al.*, 2008; Butler *et al.*, 2016). Together, the lack of significant genetic correlations, or common QTL in the case of *E. globulus*, suggest that the genetic mechanisms influencing susceptibility to the native and exotic pathogens within each host are largely independent and therefore selection by the native pathogens studied has not substantially impacted resistance to *A. psidii*.

Our findings also support the contention that host population divergence in resistance to the native pathogens is not due to diffuse (multispecies) selection arising from a pathogen general resistance mechanism (Leimu & Koricheva, 2006) or variation in other foliar traits that indirectly impact general pathogen susceptibility. However, our native pathogens are quite phylogenetically distant from *A. psidii* (*Teratospheria* is in a different phylum and *Quambalaria* a different order, but the same phylum). Thus, in terms of diffuse selection, we cannot dismiss the possibility that the variation in susceptibility of our host species to *A. psidii* is more similar to phylogenetically closer native pathogens than those in our study.

If diversifying pathogen-imposed selection has shaped the evolution of wild host species, we expect population differentiation (measured by the quantitative inbreeding coefficient, Q_{ST}) in host susceptibility should exceed that expected through drift (i.e. $Q_{ST} > F_{ST}$; Leinonen *et al.*, 2013) for the native, but not the exotic pathogens. This was the case in *E. globulus*, where population differentiation in susceptibility to the native pathogen was on average more than two-fold greater than that to the exotic pathogen (Table 2); in the trial with the highest TLD damage this significantly exceeded neutral marker F_{ST} (average 0.09, Steane *et al.*, 2006; Hamilton *et al.*, 2013). Similar trends are likely in CCV. Using genetic parameters from Brawner *et al*. (2011) and Pegg *et al*. (2011a), we derived Q_{ST} values for the susceptibility of CCV to the native pathogen *Q. pitereka* (Supplementary Material Note S2). Regardless of the manner in which host susceptibility was assessed the average Q_{ST} value (Supplementary Material Table S1), was (0.17) about twice the average neutral marker F_{ST} values reported for CCV (0.07 – 0.09; Ochieng *et al.*, 2010; Dillon *et al.*, 2012), providing additional evidence that pathogen imposed selection has shaped population divergence in susceptibility to the native pathogen.

Our final line of evidence for pathogen imposed selection is the population level associations between susceptibility to the native pathogens and climatic gradients related to disease risk in both host species. In each case, population level susceptibility is significantly associated with home-site temperature and precipitation (Figure 2) and populations from environments with greater climatic suitability for disease outbreaks are generally less susceptible to the native pathogens. Such trends have been reported previously in *E. globulus* (Hamilton *et al.*, 2013) and other forest trees (Ades *et al.*, 1992; Perry *et al.*, 2016). In *E. globulus*, physiological tolerance (Park, 1988b) and disease risk modelling (Pinkard *et al.*, 2010) suggest that the probability of infection by TLD will increase with increasing temperature and precipitation in the near-coastal region of South-eastern Australia, which *E. globulus* inhabits. Therefore, the negative relationships of susceptibility to TLD with home-site temperature and precipitation, as well as modelled bioclimatic risk (Pinkard *et al.*, 2010), are consistent with historical pathogen imposed selection influencing population level susceptibility to TLD as proposed by Hamilton *et al*. (2013).

Population-level susceptibility of CCV to *Q. pitereka* is also significantly associated with climatic and geographic factors related to disease risk. Past studies have reported provenance level variation in susceptibility with mean annual rainfall (Dickinson *et al.*, 2004), latitude (Johnson *et al.*, 2009) and in coastal versus inland provenances (Self *et al.*, 2002; Johnson *et*

al., 2009). Our analysis highlights a strong relationship with precipitation and an association between susceptibility and temperature. While climatic risk modelling has not been undertaken for this pathosystem, the requirements for *Q. pitereka* conidia germination are similar to *A. psidii* (Pegg *et al.*, 2009). The modelled climate envelope for *A. psidii* is mostly confined to near coastal regions within the natural range of CCV, as inland regions are too hot and dry (Kriticos *et al.*, 2013). In this subtropical region, high rather than low temperatures (as in the case of the temperate *E. globulus*) are a limiting factor. The decreased susceptibility in wetter and cooler populations (Figure 2) is therefore consistent with increased pathogen imposed selection.

In contrast, host susceptibility to the exotic *A. psidii* did not decrease in provenances from areas with a greater disease risk. In fact, the opposite association with rainfall was observed in *E. globulus*, whereby susceptibility to *A. psidii* tended to be higher in populations originating from wetter areas where disease risk is likely greater (Figure 2). Such population differences in susceptibility may reflect an indirect response associated with adaptation of functional traits to rainfall gradients across the range of *E. globulus*. Indeed, increased susceptibility to *A. psidii* in populations originating from wetter areas, where disease risk would be expected to be higher, was also reported in *E. cloeziana* in Queensland (Lee *et al.*, 2015), consistent with pre-adaptation to precipitation gradients influencing susceptibility at the population level. Such pre-adaptation could impact susceptibility to specific pathogens and involve constitutive morphological, anatomical or chemical traits (Niinemets, 2001; Close *et al.*, 2007; Smith *et al.*, 2006; Smith *et al.*, 2017).

A number of factors may be confounded in our comparison of susceptibility to native and exotic pathogens. These include the comparison of results from artificial inoculation with a single strain of *A. psidii* with natural infections by the native pathogens, which may reflect multiple strains and multiple species in the case of TLD. Hence, findings such as the higher heritability estimates for susceptibility to *A. psidii* than the native pathogens in both host species, for example, require further verification. The lower heritability estimates for the native pathogens may reflect an increased error variance in field-based estimates due to factors such as host escapes from infection, variability in the pathogen and greater assessment error. However, such factors are unlikely to affect the relative differences in Q_{ST} as this is a ratio of genetic variances. In addition, the heritability for field-based infection by *Q. pitereka* ($h_{op}^2 = 0.42$) was actually higher than for artificial inoculation ($h_{op}^2 = 0.08$-0.12), although the latter may have been reduced by the fact plants were inoculated with *A. psidii* prior to

inoculation with *Q. piterka*, as well as issues with variation in the vigour of reshooting plants. Nonetheless, the high correlation between estimates of CCV susceptibility to *Q. pitereka* from natural infections and artificial inoculation and the fact that neither were genetically correlated with susceptibility to *A. psidii* reinforces our findings (Table 4). Similarly, the stability of TLD damage across five different trial sites (Hamilton et al., 2013) and the fact that none of these damage estimates were genetically correlated with susceptibility to *A. psidii* at the additive or population level provides further evidence these key findings are robust.

In conclusion, comparison of the genetic architecture of susceptibility to native and exotic pathogens provides multiple lines of evidence for direct pathogen imposed selection. The independence of susceptibility to native and exotic pathogens indicates different resistance mechanisms are involved, and suggests a general pathogen recognition/resistance mechanism does not play a significant role. The greater population divergence in native disease susceptibility is consistent with divergent selection, which quantitative genetic independence argues is pathogen specific. At the population-level, reduced susceptibility in areas climatically more suitable for disease outbreaks was evident for the native but not exotic pathogen, arguing against an indirect abiotic basis to population divergence in susceptibility to the native pathogens. Taken together, our findings provide strong evidence that historic pathogen imposed selection has directly shaped the evolutionary trajectory of these forest-tree gene pools and contributed to a geographic mosaic of host resistance to native pathogens.

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Author Contributions

J.T.B and all other authors contributed to the experimental design, critical review and approved the final manuscript. P.A.T managed seed collections and prepared figures. D.J.L. organised seedling production. G.S.P performed the controlled inoculations and disease assessments. M.G.H and B.M.P analysed the data. J.S.F and B.M.P. drafted the manuscript.

REFERENCES

Ades PK, Simpson JA, Eldridge KG, Eldridge RH. 1992. Genetic variation in susceptibility to Dothistroma needle blight among provenances and families of *Pinus muricata*. *Canadian Journal of Forest Research* **22**(8): 1111-1117.

Balmelli G, Simeto S, Torres D, Castillo A, Altier N, Diez JJ. 2014. Susceptibility to *Teratosphaeria nubilosa* and precocity of vegetative phase change in *Eucalyptus globulus* and *E. maidenii* (Myrtaceae). *Australian Journal of Botany* **61**(8): 583-591.

Beenken L. 2017. *Austropuccinia* : a new genus name for the myrtle rust *Puccinia psidii* placed within the redefined family Sphaerophragmiaceae (Pucciniales). *Phytotaxa* **297**(1): 53-61.

Brawner JT, Lee DJ, Hardner CM, Dieters MJ. 2011. Relationships between early growth and *Quambalaria* shoot blight tolerance in *Corymbia citriodora* progeny trials established in Queensland, Australia. *Tree Genetics & Genomes* **7**(4): 759-772.

Burdon J, Thrall P. 2014. What have we learned from studies of wild plant-pathogen associations?—the dynamic interplay of time, space and life-history. *European Journal of Plant Pathology* **138**(3): 417-429.

Burdon JJ, Thrall PH, Ericson L. 2006. The current and future dynamics of disease in plant communities. *Annual Review of Phytopathology* **44**: 19-39.

Burdon JJ, Thrall PH, Ericson L. 2013. Genes, communities & invasive species: understanding the ecological and evolutionary dynamics of host–pathogen interactions. *Current Opinion in Plant Biology* **16**(4): 400-405.

Butler JB, Freeman JS, Vaillancourt RE, Potts BM, Glen M, Lee DJ, Pegg GS. 2016. Evidence for different QTL underlying the immune and hypersensitive responses of *Eucalyptus globulus* to the rust pathogen *Puccinia psidii*. *Tree Genetics & Genomes* **12**(3): 1-13.

Carmona D, Fitzpatrick CR, Johnson MTJ. 2015. Fifty years of co-evolution and beyond: integrating co-evolution from molecules to species. *Molecular Ecology* **24**(21): 5315-5329.

Carnegie AJ, Kathuria A, Pegg GS, Entwistle P, Nagel M, Giblin FR. 2016. Impact of the invasive rust *Puccinia psidii* (myrtle rust) on native Myrtaceae in natural ecosystems in Australia. *Biological Invasions* **18**(1): 127-144.

Close DC, Davidson NJ, Shields CB, Wiltshire R. 2007. Reflectance and phenolics of green and glaucous leaves of *Eucalyptus urnigera*. *Australian Journal of Botany* **55**(5): 561-567.

Close DC, McArthur C. 2002. Rethinking the role of many plant phenolics - protection from photodamage not herbivores? *OIKOS* **99**(1): 166-172.

Cook DE, Mesarich CH, Thomma BP. 2015. Understanding plant immunity as a surveillance system to detect invasion. *Annual Review of Phytopathology* **53**: 541-563.

Costa e Silva JC, Potts BM, Bijma P, Kerr RJ, Pilbeam DJ. 2013. Genetic control of interactions among individuals: contrasting outcomes of indirect genetic effects arising from neighbour disease infection and competition in a forest tree. *New Phytologist* **197**(2): 631-641.

Coutinho T, Wingfield M, Alfenas A, Crous P. 1998. *Eucalyptus* rust: a disease with the potential for serious international implications. *Plant Disease* **82**(7): 819-825.

de Little DW, Foster SD, Hingston TL. 2008. Temporal occurrence pattern of insect pests and fungal pathogens in young Tasmanian plantations of *Eucalyptus globulus* Labill. and *E. nitens* maiden. *Papers and Proceedings of the Royal Society of Tasmania* **142** 61-69.

Desprez-Loustau M-L, Aguayo J, Dutech C, Hayden KJ, Husson C, Jakushkin B, Marçais B, Piou D, Robin C, Vacher C. 2016. An evolutionary ecology perspective to address forest pathology challenges of today and tomorrow. *Annals of Forest Science* **73**(1): 45-67.

Dickinson G, Lee D, Huth J. 2004. Early plantation growth and tolerance to Ramularia Shoot Blight of provenances of spotted gums (*Corymbia citriodora* subsp. *citriodora*, *C. citriodora* subsp. *variegata* and *C. henryi*), grown on a range of sites. *Australian Forestry* **67**: 122–130.

Dillon SK, Brawner JT, Meder R, Lee DJ, Southerton SG. 2012. Association genetics in *Corymbia citriodora* subsp *variegata* identifies single nucleotide polymorphisms affecting wood growth and cellulosic pulp yield. *New Phytologist* **195**(3): 596-608.

Doughty RW. 2000. *The Eucalyptus. A natural and commercial history of the gum tree*. Baltimore and London: The Johns Hopkins University Press.

Dutkowski GW, Gilmour AR, Borralho NMG 2001. Modification of the additive relationship matrix for open pollinated trials. *Developing the Eucalypt of the Future*. Valdivia, Chile: INFOR.

Dutkowski GW, Potts BM. 1999. Geographic patterns of genetic variation in *Eucalyptus globulus* ssp. *globulus* and a revised racial classification. *Australian Journal of Botany* **47**(2): 237-263.

Freeman JS, Potts BM, Vaillancourt RE. 2008. Few Mendelian genes underlie the quantitative response of a forest tree, *Eucalyptus globulus*, to a natural fungal epidemic. *Genetics* **178**(1): 563-571.

Giblin F, Carnegie A 2014. *Puccinia psidii* (myrtle rust)- global host list. . *https://www.anbg.gov.au/anpc/resources/MyrtleRust.html*

Gilmour AR, Gogel BJ, Cullis BR, Welham SJ, Thompson R. 2014. *ASReml User Guide Release 4.0*. Hemel Hempstead, HP1 1ES, UK: VSN International Ltd.

Glen M, Alfenas A, Zauza E, Wingfield M, Mohammed C. 2007. *Puccinia psidii*: a threat to the Australian environment and economy–a review. *Australasian Plant Pathology* **36**(1): 1-16.

Grattapaglia D, Vaillancourt RE, Shepherd M, Thumma BR, Foley W, Kuelheim C, Potts BM, Myburg AA. 2012. Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. *Tree Genetics & Genomes* **8**(3): 463-508.

Hamilton MG, Williams DR, Tilyard PA, Pinkard EA, Wardlaw TJ, Glen M, Vaillancourt RE, Potts BM. 2013. A latitudinal cline in disease resistance of a host tree. *Heredity* **110**(4): 372-379.

Hsieh J-F, Chuah A, Patel HR, Sandhu K, Foley WJ, Külheim C. 2017. Transcriptome profiling of *Melaleuca quinquenervia* challenged by myrtle rust reveals differences in defense responses among resistant individuals. *Phytopathology* **early view**: 10.1094/PHYTO-1009-1017-0307-R.

Hunter GC, Crous PW, Carnegie AJ, Wingfield MJ. 2009. *Teratosphaeria nubilosa*, a serious leaf disease pathogen of *Eucalyptus* spp. in native and introduced areas. *Molecular Plant Pathology* **10**(1): 1-14.

Johnson I, Carnegie A, Henson M. 2009. Growth, form and *Quambalaria* shoot blight tolerance of spotted gum in north-eastern New South Wales, Australia. *Silvae Genetica* **58**(4): 180-191.

Jones JD, Dangl JL. 2006. The plant immune system. *Nature* **444**(7117): 323-329.

Jones RC, Steane DA, Lavery M, Vaillancourt RE, Potts BM. 2013. Multiple evolutionary processes drive the patterns of genetic differentiation in a forest tree species complex. *Ecology and Evolution* **3**(1): 1-17.

Junghans DT, Alfenas AC, Maffia LA. 2003. Escala de notas para quantificação da ferrugem em *Eucalyptus*. *Fitopatologia Brasileira* **28**(2): 184-188.

King R. 2004. *Spatial structure and population genetic variation in a eucalypt species complex.* Griffith University Brisbane, Australia.

Kriticos DJ, Morin L, Leriche A, Anderson RC, Caley P. 2013. Combining a climatic niche model of an invasive fungus with its host species distributions to identify risks to natural assets: *Puccinia psidii* sensu lato in Australia. *PLoS One* **8**(5): e64479

Laine AL, Burdon JJ, Dodds PN, Thrall PH. 2011. Spatial variation in disease resistance: from molecules to metapopulations. *Journal of Ecology* **99**(1): 96-112.

Latta R G. 1998. Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. *The American Naturalist* **151**(3): 283-292.

Lee D, Huth J, Osborne D, Hogg B. 2010. Selecting hardwood taxa for wood and fibre production in Queensland's subtropics. *Australian Forestry* **73**(2): 106-114.

Lee DJ. 2007. Achievements in forest tree genetic improvement in Australia and New Zealand 2: Development of Corymbia species and hybrids for plantations in eastern Australia. *Australian Forestry* **70**(1): 11-16.

Lee DJ, Brawner JT, Pegg GS. 2015. Screening *Eucalyptus cloeziana* and *E. argophloia* populations for resistance to *Puccinia psidii*. *Plant Disease* **99**(1): 71-79.

Leimu R, Koricheva J. 2006. A meta-analysis of genetic correlations between plant resistances to multiple enemies. *American Naturalist* **168**(1): E15-E37.

Leinonen T, McCairns RS, O'hara RB, Merilä J. 2013. QST-FST comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews. Genetics* **14**(3): 179.

Mohammed C, Wardlaw T, Smith A, Pinkard E, Battaglia M, Glen M, Tommerup I, Potts B, Vaillancourt R. 2003. Mycosphaerella leaf diseases of temperate eucalypts around the southern pacific rim. *New Zealand Journal of Forestry Science* **33**: 362-372.

Morin L, Aveyard R, Lidbetter JR, Wilson PG. 2012. Investigating the host-range of the rust fungus *Puccinia psidii* sensu lato across Tribes of the Family Myrtaceae present in Australia. *PLoS One* **7**(4): e35434.

Niinemets Ü. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* **82**(2): 453-469.

O'Reilly-Wapstra JM, Hamilton M, Gosney B, Whiteley C, Bailey JK, Williams D, Wardlaw T, Vaillancourt RE, Potts BM 2014. Genetic correlations in multi-species plant/herbivore interactions at multiple genetic scales: implications for eco-evolutionary dynamics. In: MoyaLarano J, Rowntree J, Woodward G eds. *Advances in Ecological Research, Vol 50: Eco-Evolutionary Dynamics*. London, UK: Academic Press, 267-295.

Ochieng JW, Shepherd M, Baverstock PR, Nikles G, Lee DJ, Henry RJ. 2010. Two sympatric spotted gum species are molecularly homogeneous. *Conservation Genetics* **11**(1): 45-56.

Old KM 1990. Diseases caused by fungi. In: Cremer KW ed. *Trees for Rural Australia*. Melbourne: Inkata Press, 210-216.

Paap T, Burgess TI, McCOMB JA, Shearer BL, Hardy GESJ. 2008. *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of *Corymbia* species in the southwest of Western Australia. *Mycological Research* **112**(1): 57-69.

Park RF. 1988a. Effect of certain host, inoculum, and environmental factors on infection of *Eucalyptus* species by two *Mycosphaerella* species. *Transactions of the British Mycological Society* **90**(2): 221-228.

Park RF. 1988b. Epidemiology of *Mycosphaerella nubilosa* and *M. cryptica* on *Eucalyptus* spp. in south-eastern Australia. *Transactions of the British Mycological Society* **91**(2): 261-266.

Park RF, Keane PJ, Wingfield MJ, Crous PW 2000. Fungal diseases of eucalypt foliage. In: Keane PJ, Kile GA, Podger FD, Brown BN eds. *Diseases and Pathogens of Eucalypts*. Collingwood, Australia: CSIRO Publishing, 153-239.

Parker MA. 1991. Nonadaptive evolution of disease resistance in an annual legume. *Evolution* **45**(5): 1209-1217.

Pegg G, Brawner J, Lee D. 2014b. Screening *Corymbia* populations for resistance to *Puccinia psidii*. *Plant Pathology* **63**(2): 425-436.

Pegg G, Carnegie A, Wingfield M, Drenth A. 2011a. Variable resistance to *Quambalaria pitereka* in spotted gum reveal opportunities for disease screening. *Australasian Plant Pathology* **40**(1): 76-86.

Pegg G, Giblin F, McTaggart A, Guymer G, Taylor H, Ireland K, Shivas R, Perry S. 2014a. *Puccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. *Plant Pathology* **63**(5): 1005-1021.

Pegg G, Nahrung H, Carnegie J, Wingfield M, Drenth A. 2011b. Spread and development of *Quambalaria* shoot blight in spotted gum plantations. *Plant Pathology* **60**(6): 1096-1106.

Pegg G, Taylor T, Entwistle P, Guymer G, Giblin F, Carnegie A. 2017. Impact of *Austropuccinia psidii* (myrtle rust) on Myrtaceae-rich wet sclerophyll forests in south east Queensland. *PLoS One* **12**(11): e0188058.

Pegg GS, Webb R, Carnegie A, Wingfield M, Drenth A. 2009. Infection and disease development of *Quambalaria* spp. on *Corymbia* and *Eucalyptus* species. *Plant Pathology* **58**(4): 642-654.

Perry A, Brown AV, Cavers S, Cottrell JE, Ennos RA. 2016. Has Scots pine (*Pinus sylvestris*) co-evolved with *Dothistroma septosporum* in Scotland? Evidence for spatial heterogeneity in the susceptibility of native provenances. *Evolutionary Applications* **9**(8): 982-993.

Pinkard E, Kriticos D, Wardlaw T, Carnegie A, Leriche A. 2010. Estimating the spatio-temporal risk of disease epidemics using a bioclimatic niche model. *Ecological Modelling* **221**: 2828-2838.

Potts BM, Sandhu KS, Wardlaw T, Freeman J, Li H, Tilyard P, Park RF. 2016. Evolutionary history shapes the susceptibility of an island tree flora to an exotic pathogen. *Forest Ecology and Management* **368**: 183-193.

Salvaudon L, Giraud T, Shykoff JA. 2008. Genetic diversity in natural populations: a fundamental component of plant–microbe interactions. *Current Opinion in Plant Biology* **11**(2): 135-143.

Self N, Aitken E, Dale M. 2002. Susceptibility of provenances of spotted gums to Ramularia shoot blight. *New Zealand Plant Protection* **55**: 68-72.

Shepherd M, Henson M, Lee DJ. 2012. Revisiting genetic structuring in spotted gums (genus *Corymbia* section Maculatae) focusing on *C. maculata*, an early diverged, insular lineage. *Tree Genetics & Genomes* **8**(1): 137-147.

Simpson J. 2000. *Quambalaria*, a new genus of eucalypt pathogens. *Australasian Mycologist* **19**(2): 57-62.

Smith AH, Pinkard EA, Hunter GC, Wingfield MJ, Mohammed CL. 2006. Anatomical variation and defence responses of juvenile *Eucalyptus nitens* leaves to Mycosphaerella leaf disease. *Australasian Plant Pathology* **35**(6): 725-731.

Smith AH, Potts BM, Ratkowsky DA, Pinkard EA, Mohammed CL. 2017. Association of *Eucalyptus globulus* leaf anatomy with susceptibility to *Teratosphaeria* leaf disease. *Forest Pathology*: efp.12395.

Smith DS, Bailey JK, Shuster SM, Whitham TG. 2011. A geographic mosaic of trophic interactions and selection: trees, aphids and birds. *Journal of Evolutionary Biology* **24**(2): 422-429.

Soubeyrand S, Laine A-L, Hanski I, Penttinen A. 2009. Spatiotemporal structure of host-pathogen interactions in a metapopulation. *The American Naturalist* **174**(3): 308-320.

Steane DA, Conod N, Jones RC, Vaillancourt RE, Potts BM. 2006. A comparative analysis of population structure of a forest tree, *Eucalyptus globulus* (Myrtaceae), using microsatellite markers and quantitative traits. *Tree Genetics and Genomes* **2**(1): 30-38.

Stenlid J, Oliva J. 2016. Phenotypic interactions between tree hosts and invasive forest pathogens in the light of globalization and climate change. *Philosophical Transactions of the Royal Society B-Biological Sciences* **371**(1709): 20150455.

Stewart JE, Ross-Davis AL, Graça RN, Alfenas AC, Peever TL, Hanna JW, Uchida JY, Hauff RD, Kadooka CY, Kim MS, *et al*. 2017. Genetic diversity of the myrtle rust pathogen (*Austropuccinia psidii*) in the Americas and Hawaii: Global implications for invasive threat assessments. *Forest Pathology* **48**: e12378.

Thompson JN. 1999. Specific hypotheses on the geographic mosaic of coevolution. *The American Naturalist* **153**(S5): S1-S14.

Thompson JN, Burdon JJ. 1992. Gene-for-gene coevolution between plants and parasites. *Nature* **360**(6400): 121-125.

Thumma B, Pegg G, Warburton P, Brawner J, Macdonell P, Yang X, Southerton S. 2013. *Molecular tagging of rust resistance genes in eucalypts. Final Report to Plant Health Australia Ltd*. CSIRO Plant Industry, Canberra.

Tobias PA, Guest DI, Külheim C, Hsieh JF, Park RF. 2016. A curious case of resistance to a new encounter pathogen: myrtle rust in Australia. *Molecular Plant Pathology* **17**(5): 783-788.

Toor J, Best A. 2016. Evolution of Host Defense against Multiple Enemy Populations. *American Naturalist* **187**(3): 308-319.

van der Hoorn RA, Kamoun S. 2008. From guard to decoy: a new model for perception of plant pathogen effectors. *The Plant Cell* **20**(8): 2009-2017.

Walters D. 2011. *Plant Defense: Warding off Attack by Pathogens, Herbivores and Parasitic Plants*: John Wiley & Sons.

White TL, Adams WT, Neale DB. 2007. *Forest Genetics*. Wallingford UK: CABI.

Whitham TG, Gehring CA, Lamit LJ, Wojtowicz T, Evans LM, Keith AR, Smith DS. 2012. Community specificity: life and afterlife effects of genes. *Trends in Plant Science* **17**(5): 271-281.

Wilkinson GR. 2008. Population differentiation within *Eucalyptus obliqua*: implications for regeneration success and genetic conservation in production forests. *Australian Forestry* **71**(1): 4-15.

Zauza EA, Alfenas AC, Old K, Couto MM, Graça RN, Maffia LA. 2010. Myrtaceae species resistance to rust caused by *Puccinia psidii*. *Australasian Plant Pathology* **39**(5): 406-411.

Zhou X, De Beer ZW, Xie Y, Pegg GS, Wingfield MJ. 2007. DNA-based identification of *Quambalaria pitereka* causing severe leaf blight of *Corymbia citriodora* in China. *Fungal Diversity* **25**: 245-254.

Figure legends

Figure 1 Host population variation in susceptibility to (a) the native pathogen *Teratosphaeria* species (TLD) and (b) the exotic rust *Austropuccinia psidii* across the native range of *Eucalyptus globulus* in South-eastern Australia, including Tasmania and the Bass Strait Islands. Least-square mean damage for each population is shown, with the bigger the triangle or circle the greater the host tree disease damage is above or below the overall mean, respectively. Small circles and triangles represent values close to the overall mean. The damage from *Teratosphaeria* infection is shown for the field trial exhibiting greatest population differentiation (Q_{st}) in damage following natural infection at 1 year of age (Temma06 - Table 1; Hamilton *et al*. 2013). The rust damage was assessed following artificial inoculation of seedlings, mainly from the same open-pollinated *E. globulus* families.

Figure 2 Path diagram depicting the relative effect of home-site climate on host susceptibility in the different pathosystems in this study. (a) *Eucalyptus globulus* / *Teratosphaeria*, (b) *Eucalyptus globulus* / *Austropuccinia psidii*, (c) *Corymbia citriodora* subsp. *variegata* (CCV) / *Quambalaria pitereka,* (d) *Corymbia citriodora* subsp. *variegate* /*Austropuccinia psidii*. To standardise interpretation, response data were adjusted such that increasing values reflected increasing provenance susceptibility. While all paths were fitted in the model, only significant ($P<0.05$) paths and their standardised coefficients reflecting the relative effect size and their significance were plotted. R^2 refers to the proportion variation in susceptibility explained by the full model. Significance levels of path coefficients are: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Supporting Information

Table S1 Estimates of the quantitative inbreeding coefficient (Q_{ST}) and narrow-sense heritability (h^2_{op}) for native and exotic pathogens in *Corymbia citriodora* subsp. *variegata* and *Eucalyptus globulus.*

Notes S1 Analysis of a second artificial inoculation trial of *Corymbia citriodora* subsp. *variegata* (CCV) with *Austropuccinia psidii and Quambalaria pitereka.*

Notes S2 Calculation of quantitative inbreeding coefficients (Qst) from information presented in Brawner *et al.* (2011).

Table 1 Narrow sense heritability (h^2_{op}) estimates for *Austropuccinia psidii* and Teratospheria leaf disease (TLD) damage in *Eucalyptus globulus*.

Pathogen and assessment[a]	h^2_{op}	SE	P[b]
A. psidii			
Batch 1	0.63	0.09	<0.001
Batch 2	0.70	0.10	<0.001
Combined[c]	0.65	0.07	<0.001
TLD[d]			
Site:			
Tog05	0.22	0.05	<0.001
SR05	0.17	0.04	<0.001
Temma06	0.26	0.05	<0.001
SR06	0.13	0.03	<0.001
GC08	0.35	0.06	<0.001
AVERAGE	0.23		

[a]Indicates the screening batch for *A. psidii* artificial inoculations and trial site for TLD natural infections.
[b]Significance of the additive genetic variance from zero.
[c]Combined data with the difference between batches included in the modelled replicate effect.
[d]Results are from Hamilton *et al*. (2013).

Table 2 Quantitative inbreeding coefficients (Q_{st}) for Teratospheria leaf disease (TLD) and *Austropuccinia psidii* (RUST) damage in *Eucalyptus globulus*, and correlations with susceptibility to *A. psidii* damage for TLD in each field trial at the population and additive levels.

						Population correlations (r_p)			Additive genetic correlations (r_a)		
Pathogen and Trial[a]	n fams	n	Q_{ST}	SE	P (Q_{ST}TLD = Q_{ST}rust)	r_p	SE	P (r_p = 0)	r_g	SE	P (r_a = 0)
TLD											
Tog05	140	2295	0.05	(0.03)	0.655	-0.36	(0.41)	0.417	-0.10	(0.17)	0.610
SR05	146	2727	0.12	(0.06)	0.173	-0.38	(0.34)	0.313	0.08	(0.16)	0.624
Temma06	124	2771	0.25	(0.09)	0.005	0.06	(0.35)	0.888	0.21	(0.15)	0.182
SR06	140	2863	0.12	(0.06)	0.168	0.29	(0.35)	0.431	0.16	(0.17)	0.354
GC08	141	2236	0.16	(0.07)	0.057	0.49	(0.30)	0.164	-0.04	(0.15)	0.806
Average			0.14			0.02			0.06		
RUST	189	2597	0.06	(0.03)							

[a]n fams indicates the number of families, and n the number of individuals, used for TLD damage estimates in each field trial.

Table 3 Narrow-sense heritability (h_{op}^2) estimates for *Austropuccinia psidii* and *Quambalaria pitereka* damage in the Woondum population *of Corymbia citriodora* subsp. *variegata* (no. families = 125).

Pathogen and assessment[a]	h^2_{op}	SE	P[b]
A. psidii			
Inoculation - batch 1	0.63	0.10	<0.001
- batch 2	0.59	0.10	<0.001
Q. pitereka			
Inoculation - batch 1	0.08	0.07	0.113
- batch 2	0.08	0.09	0.196
- combined[b]	0.12	0.05	<0.001
Natural infection	0.42	0.06	<0.001

[a]Indicates screening batch for artificial inoculations, and natural infection versus artificial inoculation in the case of *Q. pitereka.*
[b]Significance of the additive genetic variance estimate from zero.
[b]Combined data with the difference between batches included in the modelled replicate effect.

Table 4 Genetic correlations of *Quambalaria pitereka* and *Austropuccinia psidii* damage in the Woondum population of *Corymbia citriodora* subsp. *variegata* (no. families = 125).

	Q. pitereka a.i.[a]	*A. psidii* batch1	*A. psidii* batch 2
A. psidii batch 1	0.21 (0.18)[ns]		
A. psidii batch 2	0.13(0.19)[ns]	0.77 (0.08)***	
Q. pitereka f.t.[b]	0.71 (0.15)***	-0.21 (0.11)[ns]	-0.20 (0.12)[ns]

[a]Artifical inoculation.
[b]Natural infection (crown damage) in field trials.
[ns] Not significantly different from zero at the 0.05 level; ***, P<0.001.