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Consequences of *Corymbia* (Myrtaceae) hybridisation on leaf-oil profiles

R. Andrew Hayes[A,D], *Helen F. Nahrung*[A,B] *and David J. Lee*[B,C]

[A]Agri-Science Queensland, Department of Agriculture, Fisheries and Forestry, Ecosciences Precinct, Dutton Park, Qld 4102, Australia.
[B]Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore DC, Qld 4558, Australia.
[C]Agri-Science Queensland, Department of Agriculture Fisheries and Forestry, Gympie, Qld 4570, Australia.
[D]Corresponding author. Email: andrew.hayes@daff.qld.gov.au

Abstract. The present study examines patterns of heritability of plant secondary metabolites following hybridisation among three genetically homogeneous taxa of spotted gum (*Corymbia henryi* (S.T.Blake) K.D.Hill & L.A.S.Johnson, *C. citriodora* subsp. *variegata* (F.Muell.) K.D.Hill & L.A.S.Johnson and *C. citriodora* (Hook.) K.D.Hill & L.A.S.Johnson subsp. *citriodora* (section *Maculatae*), and their congener *C. torelliana* (F.Muell.) K.D. Hill & L.A.S.Johnson (section *Torellianae*)). Hexane extracts of leaves of all four parent taxa were statistically distinguishable (ANOSIM: *global* $R = 0.976$, $P = 0.008$). Hybridisation patterns varied among the taxa studied, with the hybrid formed with *C. citriodora* subsp. *variegata* showing an intermediate extractive profile between its parents, whereas the profiles of the other two hybrids were dominated by that of *C. torelliana*. These different patterns in plant secondary-metabolite inheritance may have implications for a range of plant–insect interactions.

Additional keywords: eucalypt, foliar chemistry, gas chromatography–mass spectrometry.

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Introduction

Hybridisation is a widespread phenomenon in natural plant populations (e.g. Griffin *et al*. 1988; Arnold 1994; Floate and Whitham 1995; Ellstrand *et al*. 1996; Humphreys *et al*. 2008), with ~70% of all angiosperms believed to have been produced by natural hybridisation across species and genera (Whitham *et al*. 1991; Arnold 1994). Additionally, artificial hybridisation is a common procedure in agriculture and silviculture, because it has long been recognised that hybrids can combine desirable features of parental types, allow planting into environments that are unsuitable for a pure species, or even display novel phenotypes as a result of increases or changes in genetic composition (Strauss 1994).

Plant secondary metabolites are compounds produced by a plant that are not used for growth and development and whose expression differs among different plant groups (Pichersky and Gang 2000). These metabolites play a vital role in a plant's interaction with its biotic environment, such as protection from herbivores, pathogens and competitors and attraction of pollinators (Mitchell-Olds *et al*. 1998). Hybridisation may result in offspring that differ from the parental taxa both quantitatively and qualitatively with respect to plant secondary metabolites (Harbourne and Turner 1984; Kirk *et al*. 2005; Nahrung *et al*. 2009; Oberprieler *et al*. 2010, 2011). Hybrids may express a suite of secondary metabolites that are

(i) similar to one of the parental taxa,
(ii) intermediate between the parents,
(iii) overexpressed, in concentrations greater than in either parent,
(iv) underexpressed, in concentrations lower than in either parent,
(v) deficient, where the compound is absent from the hybrid, or
(vi) novel, where the hybrid produces metabolites not detected in either parent (Orians 2000; Kirk *et al*. 2005).

Eucalypts are well known for their hybridisation ability (Griffin *et al*. 1988; Humphreys *et al*. 2008; Dickinson *et al*. 2012). Across many eucalypt-growing regions in the world, silvicultural practice has often been to grow interspecific eucalypt hybrids for forest plantations, as a strategy to maximise tree performance by combining the desirable traits of different species (de Assis 2000). Eucalypt hybrids have shown improvement in a variety of traits, such as growth rate, coppicing and propagation ability, pulp yield, wood density (Lee 2007) and resistance to frost, drought, salinity (Dale and Dieters 2007; Lee *et al*. 2009) and pests and diseases (Potts and Dungey 2004). One of the most prominent characteristics of the eucalypts is the high essential-oil content of the leaves, and its substantial variability among taxa (Bignell *et al*. 1998; Dunlop *et al*. 1999; Asante *et al*. 2001; Brophy and Southwell 2002). These essential oils are plant secondary metabolites that effect resistance to herbivores and

pathogens (e.g. Rosenthal and Janzen 1979; Edwards *et al*. 1993; Steinbauer *et al*. 2004) and are an important component of hybrid fitness.

Corymbia (formerly *Eucalyptus*) *torelliana* (F.Muell.) K.D.Hill & L.A.S.Johnson (section *Torellianae* (Parra-O *et al*. 2009)) is an 'exceptional' Australian species because of its tropical habitat (Sutherland *et al*. 1960). It grows in rainforest (mesophyll vine forest) and its endemic range occupies coastal northern Queensland (Qld) from ~16 to 19 °S (Boland *et al*. 2006). *C. torelliana* hybridises readily with the spotted gums *C. henryi* (S.T.Blake) K.D.Hill & L.A.S.Johnson, *C. citriodora* subsp. *variegata* (F.Muell.) K.D.Hill & L.A.S.Johnson and *C. citriodora* (Hook) K.D.Hill & L.A.S.Johnson subsp. *citriodora* (section *Maculatae* (Parra-O *et al*. 2009)), which occur from New South Wales (NSW) (27°S) to northern Qld (17°S), respectively. *C. henryi*, *C. citriodora* subsp. *variegata* and *C. citriodora* subsp. *citriodora* are genetically homogeneous with respect to multiple microsatellite loci (Ochieng *et al*. 2008, 2010; Shepherd *et al*. 2008), but chemically distinct (Asante *et al*. 2001). Hybrids between *C. torelliana* and *C. citriodora* subsp. *citriodora* were reported to have superior growth traits compared with parent species (Kapoor and Sharma 1983, 1984) and have since become among the preferred plantation species in subtropical Qld and NSW (Lee 2007; Lee *et al*. 2009, 2010).

In plantations, *Corymbia* taxa are attacked by a range of herbivores from several feeding guilds (Nahrung *et al*. 2011, 2012). Foliar chemistry of host plants is known to influence plant–herbivore interactions, and variations in the leaf oils are cited as an explanation for differences in host susceptibility to herbivory (Zangerl and Berenbaum 1993).

To determine whether hybridisation increases (or changes) the variation of plant secondary metabolites in offspring, and how this varies among taxa, we examined the chemical profile of *C. torelliana* (CT), *C. henryi* (CH), *C. citriodora* subsp. *variegata* (CCV), *C. citriodora* subsp. *citriodora* (CCC), and the hybrids between *C. torelliana* and the three 'pure' taxa (CT × CH, CT × CCV and CT × CCC), comparing metabolites across all possible combinations of these taxa. We examined how patterns of hybridisation might differ among parental taxa to look at how the hybrids vary both from their parents, and also from each other.

Materials and methods

Foliar chemistry was analysed using plants grown under glasshouse conditions. Seedlings sampled for the pure taxa (CT, CH, CCV and CCC) were from broadly based bulk seedlots (Table 1). The mothers of the hybrids were landrace CT trees from the Gympie region. On the basis of microsatellite data (McVey 2004), these trees were considered to have originated from the same region (Cairns) as the pure CT trees sampled during the present study. The hybrids (CT × CH, CT × CCV and CT × CCC) were from full-sib families, using three CT mothers artificially pollinated with pollen from a single unpedigreed father of each parental taxon. Each mother and father was used only once in the hybrids tested. The seedlings tested in the present study were considered to be hybrids on the basis of their morphology, which is distinct from and often intermediate to that of their parental taxa (Nahrung *et al*. 2009; Abasolo *et al*. 2012). Given the narrow genetic base of both the female parents and the single father representing each taxon, the results reported here may be specific to the germplasm tested, and this should be considered when extrapolating these results to different *Corymbia* hybrids (*sensu* Lee *et al*. 2009). Seeds were sown in potting mix comprising 50% pine-bark fines, 25% peat (Aussie Peat, Rockhampton, Qld) composted and 25% perlite to which Osmocote (Scotts Australia, Bella Vista, NSW) and Ag lime were added, each at 4 kg m^{-3}, and gypsum, Micromax (Scotts Australia) (fertiliser) and Hydraflo2 (Scotts Australia) (wetting agent) were added each at 1 kg m^{-3}. Seedlings were raised in the glasshouse for the first 6 weeks under mist, and then put under shade cloth for 2 weeks and then put out into full sun. Plants were re-potted into 130-mm-diameter pots and housed in a glasshouse (24°C, ambient light), with random placement, for several months, before use in experiments. Only the first two to four fully expanded, apical leaves were used in the trials to standardise the age of foliage during testing.

Table 1. Provenance or origin data of trees sampled in the study

Taxon	Provenance or origin	No. of seedlings sampled
Corymbia henryi (CH)	Range-wide sample (4 provenances)	5
C. citriodora subsp. *variegata* (CCV)	Woondum (bulk of 58 trees)	5
C. citriodora subsp. *citriodora* (CCC)	Barron River (bulk of 10 trees)	5
C. torelliana (CT)	Cairns (bulk of 5 trees)	5
CT[A] × CH	Control cross full-sib family	5
CT[A] × CCV[B]	Control cross full-sib family	5
CT[A] × CCC	Control cross full-sib family	5

[A]CT is the female parent in these hybrids and is Gympie landrace CT, likely to be originally from the Cairns region, on the basis of microsatellite data (McVey 2004). The male parent (CH, CCC or CCV) is from unpedigreed trees of that taxon.
[B]CCV father is of Queensland origin.

Replicate samples ($n = 5$) of foliage (0.935 ± 0.038 g FW) from five randomly chosen plants of each taxon were collected, cut into squares (≤1 cm^2) and extracted with hexane (≥99%, Sigma-Aldrich) (~10 mL) for 50 min, stirring for 1 min, three times within this period. The extract was filtered through filter paper (Whatman, Mornington, Vic.) and stored in the freezer (−20°C) until analysis (method after Jones *et al*. 2002; Rapley *et al*. 2004; Nahrung *et al*. 2009).

Samples (1 µL) were analysed using a gas chromatograph (GC) (Agilent 6890 Series) coupled to a mass spectrometer (MS) (Agilent 5975) and fitted with a silica capillary column (Agilent, Model HP5-MS, 30 m × 250 µm ID × 0.25 µm film thickness). Data were acquired under the following GC conditions: inlet temperature 250°C, carrier gas helium at 51 cm s^{-1}, split ratio 13 : 1, transfer-line temperature 280°C, initial temperature 40°C, initial time 2 min, rate 10°C min^{-1}, final temperature 260°C, final time 6 min. The MS was held at 280°C in the ion source, with a scan rate of 4.45 scans s^{-1}.

Peaks that were present in blank hexane (control) samples were discarded from analysis in test samples. Tentative identities were assigned to peaks with respect to the National Institute of

Table 2. Retention times, tentative identities and mean ± s.e.m. relative area under the chromatogram of components detected in hexane extracts of *Corymbia* leaves

The number of replicates of each taxon in which the component was detected is indicated in parentheses (mean values are based on all replicates). Unidentified long-chain hydrocarbons and aldehydes are indicated as l. c. hydrocarbon and l. c. aldehyde respectively

Retention time (min)	Tentative identity	CH	CCV	CCC	CT	CT × CH	CT × CCV	CT × CCC
3.83	Unknown	0 (0)	0 (0)	0 (0)	0.61 ± 0.20 (4)	1.4 ± 0.40 (4)	0.11 ± 0.085 (2)	1.23 ± 0.38 (5)
4.31	α-Pinene	9.7 ± 1.1 (5)	38 ± 2.3 (5)	1.5 ± 0.62 (5)	11 ± 2.3 (5)	15 ± 3.2 (5)	26 ± 3.5 (5)	7.6 ± 1.9 (5)
5.10	β-Pinene	2.4 ± 0.64 (4)	1.0 ± 0.41 (5)	0.27 ± 0.15 (3)	1.9 ± 0.51 (5)	0.71 ± 0.21 (5)	3.1 ± 0.33 (5)	0.77 ± 0.26 (4)
5.95	Limonene	0 (0)	0.17 ± 0.045 (4)	0 (0)	0 (0)	0 (0)	0.45 ± 0.077 (5)	0 (0)
6.17	3-Carene	0.16 ± 0.10 (2)	0.14 ± 0.10 (2)	0 (0)	0.16 ± 0.16 (1)	0.25 ± 0.11 (3)	0.28 ± 0.10 (5)	0 (0)
6.40	1,8-Cineole	2.0 ± 0.82 (5)	0 (0)	0.39 ± 0.12 (4)	0 (0)	1.2 ± 0.22 (5)	0.66 ± 0.29 (3)	0 (0)
7.84	Citronellal	0 (0)	0 (0)	56 ± 4.8 (5)	0 (0)	0 (0)	0 (0)	0 (0)
9.11	Citronellol	0 (0)	0 (0)	12 ± 5.4 (5)	0 (0)	0 (0)	0 (0)	0 (0)
9.50	Monoterpene	0 (0)	0 (0)	0.12 ± 0.12 (1)	0 (0)	0 (0)	0 (0)	0 (0)
10.3	Monoterpene	0 (0)	0 (0)	0.066 ± 0.066 (1)	0 (0)	0 (0)	0 (0)	0 (0)
10.6	Eugenol	3.5 ± 0.62 (5)	0 (0)	0.72 ± 0.72 (1)	0 (0)	0 (0)	0.054 ± 0.054 (1)	0 (0)
11.1	Isoledene	0.19 ± 0.12 (2)	0.73 ± 0.060 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
11.2	β-Caryophyllene	0.87 ± 0.24 (4)	0.31 ± 0.076 (5)	0.48 ± 0.12 (5)	1.1 ± 0.45 (5)	0.50 ± 0.17 (5)	0.47 ± 0.16 (5)	0.61 ± 0.21 (4)
11.3	α-Himachalene	0.17 ± 0.17 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
11.4	Alloaromadendrene	0 (0)	2.3 ± 0.47 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
11.6	β-Cubebene	3.4 ± 0.95 (5)	0.84 ± 0.047 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
11.7	Sesquiterpene	0 (0)	0.42 ± 0.076 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
12.1	β-Patchoulene	0.13 ± 0.13 (1)	0.85 ± 0.19 (5)	0 (0)	0.14 ± 0.067 (4)	0.081 ± 0.050 (2)	0 (0)	0 (0)
12.1	Bicyclogermacrene	0 (0)	3.2 ± 0.68 (5)	0.055 ± 0.055 (1)	0 (0)	0 (0)	0.14 ± 0.11 (2)	0.033 ± 0.033 (1)
12.4	Elemol	0 (0)	0.49 ± 0.11 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
12.5	Elemene	5.4 ± 0.53 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
12.5	Cadina-1,4-diene	0.23 ± 0.17 (2)	1.0 ± 0.082 (5)	0.18 ± 0.022 (5)	0 (0)	0 (0)	0 (0)	0 (0)
12.7	Unknown	0 (0)	0 (0)	0 (0)	0.25 ± 0.030 (5)	0.31 ± 0.063 (5)	0 (0)	0.36 ± 0.052 (5)
13.4	Isolongifolen-8-ol	57 ± 1.5 (5)	31 ± 2.7 (5)	2.0 ± 0.83 (5)	0 (0)	0 (0)	15 ± 2.8 (5)	0 (0)
13.5	Unknown	0 (0)	0 (0)	0 (0)	1.1 ± 0.38 (5)	7.6 ± 2.6 (5)	0 (0)	2.3 ± 0.94 (5)
13.6	Unknown	0 (0)	0.20 ± 0.075 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
13.9	Sesquiterpene	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.15 ± 0.098 (2)	0 (0)
14.0	α-Eudesmol	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.44 ± 0.19 (3)	0 (0)
17.0	Unknown	0 (0)	0 (0)	0.094 ± 0.058 (2)	0.10 ± 0.065 (2)	0.11 ± 0.071 (2)	0.059 ± 0.040(2)	0 (0)
17.3	N-containing	0 (0)	0 (0)	0 (0)	1.05 ± 0.42 (5)	0.11 ± 0.11 (1)	0.12 ± 0.050 (4)	0.73 ± 0.28 (4)
18.5	Unknown	0 (0)	0 (0)	0 (0)	0.34 ± 0.11 (5)	0.42 ± 0.12 (5)	0.057 ± 0.025 (3)	0.28 ± 0.16 (3)
20.6	Unknown	0 (0)	0.21 ± 0.082 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
20.7	Unknown	0 (0)	1.4 ± 0.38 (5)	0 (0)	0.85 ± 0.30 (5)	1.3 ± 0.70 (4)	2.9 ± 0.58 (5)	1.1 ± 0.43 (4)
20.8	l.c. hydrocarbon	0 (0)	1.3 ± 0.38 (5)	0 (0)	1.2 ± 0.29 (5)	1.4 ± 0.72 (5)	3.2 ± 0.58 (5)	1.2 ± 0.45 (5)
21.8	l. c. hydrocarbon	0.60 ± 0.27 (3)	0 (0)	0 (0)	0.61 ± 0.14 (5)	0 (0)	0 (0)	0 (0)
21.9	Unknown	0.72 ± 0.30 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
22.0	Unknown	0.78 ± 0.33 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
22.2	l. c. aldehyde	0 (0)	0 (0)	0 (0)	0.39 ± 0.068 (5)	0 (0)	0.25 ± 0.051 (5)	0 (0)
22.2	Unknown	1.8 ± 0.51 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
22.3	Unknown	1.23 ± 0.53 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
23.1	l. c. aldehyde	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.067 ± 0.067 (1)	0 (0)
23.2	l. c. aldehyde	0.32 ± 0.20 (5)	5.1 ± 0.68 (5)	4.1 ± 0.42 (5)	9.9 ± 1.4 (5)	9.5 ± 0.94 (5)	7.7 ± 0.61 (5)	14 ± 1.4 (5)
25.9	Eicocene	3.3 ± 0.83 (2)	3.0 ± 1.3 (5)	5.0 ± 0.94 (5)	32 ± 5.3 (5)	27 ± 4.4 (5)	17 ± 2.7 (5)	30 ± 3.5 (5)

Standards and Technology (NIST) mass spectral library. Mass spectra of peaks from different samples with the same retention time were compared with ensure that the compounds were indeed the same.

The presence of peaks in the chromatograms, and their relative areas, were analysed by non-parametric methods (Bray–Curtis cluster analysis and multidimensional scaling (MDS) ordination) (Clarke 1993) to ascertain whether any differences could be detected among the samples.

To determine whether clusters of individual plants relating to the taxa investigated were significantly different from each other, an analysis of similarity (ANOSIM) was used. The ANOSIM tests are a range of Mantel-type permutations of randomisation procedures, which make no distributional assumptions. These tests depend only on rank similarities, and thus are appropriate for these types of data. We used a 'similarity percentages' (SIMPER) analysis to ascertain the relative contribution of each of the components to assign the leaves to *a priori* determined groups, to determine differences among groups and to assess similarity among individuals within each group. The software used for the multivariate analysis was Primer 5 for Windows (V 5.2.9, Clarke and Gorley 2001). These analytical procedures have been used successfully in previous studies to compare chromatographic data statistically (e.g. Hayes *et al*. 2006; Nahrung *et al*. 2009).

Results

The compounds identified in extracts from *Corymbia* leaves were primarily mono- and sesquiterpenes, as well as some waxes and long-chain hydrocarbons that could not be unambiguously identified (Table 2). Pairwise comparisons indicated that all plant types were significantly different from each other in oil profile, except CT × CCC from CT and CT × CH (ANOSIM: global $r = 0.976$, $P = 0.008$; see Table 3 for pairwise comparisons).

The Bray–Curtis cluster analysis dendrogram produced using the single linkage (nearest neighbour') algorithm (Fig. 1*a*) showed that (in order of increasing similarity) CCC, CH and CCV were the taxa most distinct from all the others. CT × CCV was separate from the remaining taxa, which, when grouped together with CT, formed a cluster at a similarity of ~80%.

The multidimensional scaling output (Fig. 1*b*) provided another visual representation of the data described by the ANOSIM. Each point on the figure represents an individual extract. The three parental taxa and the CT × CCV hybrid are distinct, whereas CT overlaps with the CT × CCC and CT × CH hybrids.

The SIMPER analysis is a measure of the similarities of samples within a defined grouping (in this case taxon), as well as a measure of the difference (percentage dissimilarity) among taxa (Table 3). All groups had high levels of similarity (mean similarity = 83.32 ± 1.68%). The levels of difference were high between the parental taxa (~61%), intermediate between CT × CCV and its parental taxa and the other hybrids (~39%), whereas the only overlapping groups (CT × CH with CT × CCC and CT with CT × CH and CT × CCC) had a distinctly lower level of difference than did the remaining groups (~23%; Table 3).

Table 3. Pairwise comparisons of the ANOSIM analysis and SIMPER analysis, showing mean difference (percentage dissimilarity) for hexane extracts from leaves of *Corymbia henryi* (CH), *C. citriodora* subsp. *citriodora* (CCC), *C. citriodora* subsp. *variegata* (CCV), *C. torelliana* (CT) and hybrids of CT with the other *Corymbia* taxa (CT × CH, CT × CCV, CT × CCC)

Comparison	*R*-statistic	*P*	Dissimilarity (%)
CH *v.* CCV	1	0.008	55.95[A]
CH *v.* CCC	1	0.008	62.03[A]
CH *v.* CT	1	0.008	68.41[A]
CH *v.* CT × CH	1	0.008	65.36[A]
CH *v.* CT × CCV	1	0.008	56.25[A]
CH *v.* CT × CCC	1	0.008	72.61[A]
CCV *v.* CCC	1	0.008	61.25[A]
CCV *v.* CT	1	0.008	55.45[A]
CCV *v.* CT × CH	1	0.008	55.73[A]
CCV *v.* CT × CCV	1	0.008	40.72[B]
CCV *v.* CT × CCC	1	0.008	58.33[A]
CCC *v.* CT	1	0.008	64.28[A]
CCC *v.* CT × CH	1	0.008	59.02[A]
CCC *v.* CT × CCV	1	0.008	56.19[A]
CC *v.* CT × CCC	1	0.008	62.76[A]
CT *v.* CT × CH	0.512	0.008	24.47[C]
CT *v.* CT × CCV	0.976	0.008	35.75[B]
CT *v.* CT × CCC	0.168	0.07	22.07[C]
CT × CH *v.* CT × CCV	0.952	0.008	37.10[B]
CT × CH *v.* CT × CCC	0.292	0.05	22.64[C]
CT × CCV *v.* CT × CCC	0.968	0.008	41.52[B]

[A]Groups with high levels of difference (~61%).
[B]Groups with intermediate levels of difference (~39%).
[C]Groups with lower levels of difference (~23%).

The suite of components in each hybrid that are derived from each parent and are different, and the number of components that are from both parents, from only one parent or novel to the hybrid are shown (Fig. 2).

Discussion

Variation in foliar plant secondary metabolites in Australian eucalypts is common (e.g. Dunlop *et al*. 1995, 1999; Bignell *et al.* 1998; Asante *et al*. 2001; Brophy and Southwell 2002; Keszei *et al*. 2008), and, among other things, is associated with changes in susceptibility to herbivory and pathogens (e.g. Rosenthal and Janzen 1979; Edwards *et al*. 1993; Steinbauer *et al*. 2004; Henery *et al*. 2008; Paine *et al.* 2011). Hybridisation has been shown to influence the variation of plant secondary metabolites (Oberprieler *et al*. 2010, 2011; Cheng *et al*. 2011), and understanding how they vary between species and their hybrids enables an understanding of the mechanisms of host choice by insect herbivores (Hallgren *et al*. 2003).

Hybrid susceptibility to herbivores is predicted in eucalypts (Dungey and Potts 2003; Potts and Dungey 2004), although the situation in field trials is not always so clear (e.g. Nahrung *et al*. 2009, 2012). Further field trials are required to examine how the variations in susceptibility match with metabolite variations identified in the present study.

Here, overall foliar chemical compositions were distinct among all four parental taxa, and CT × CCV. The CT × CH hybrid was statistically different from the four parental taxa,

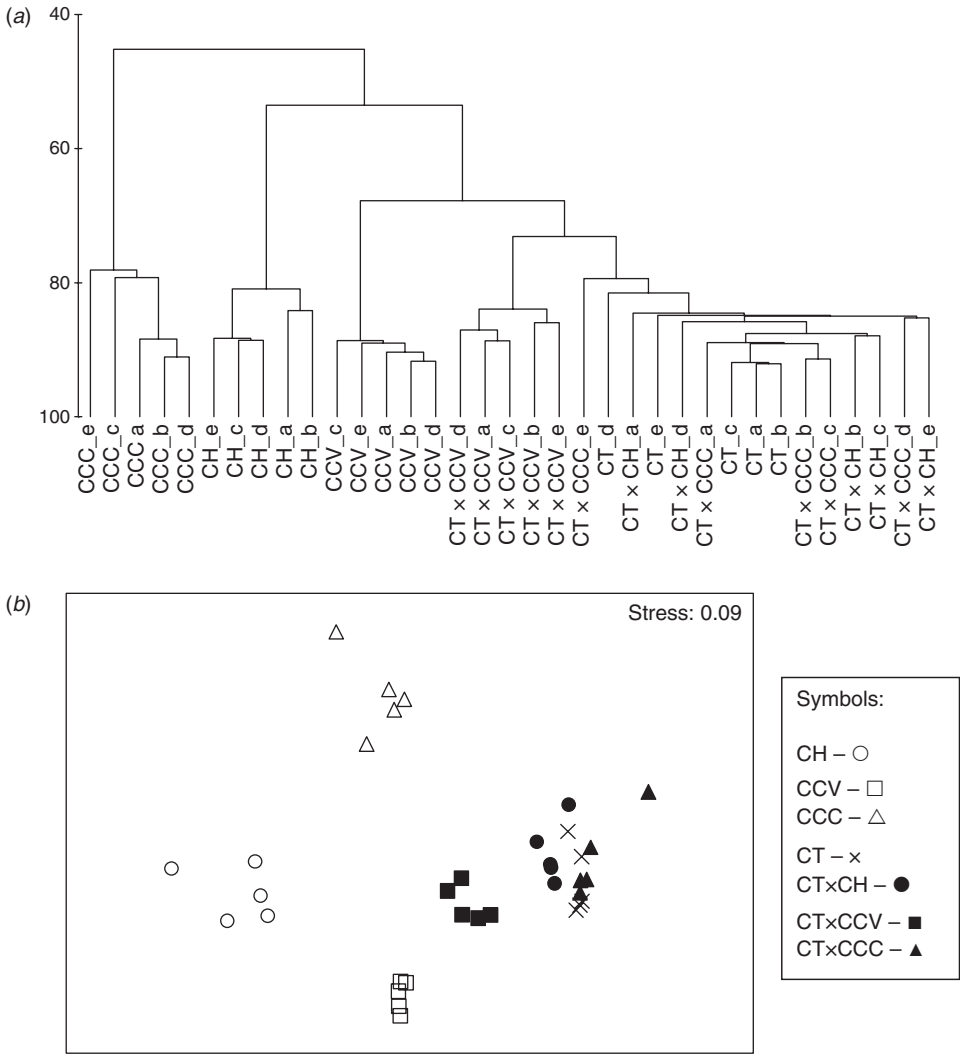

Fig. 1. (*a*) Bray–Curtis similarity single linkage cluster analysis dendrogram of extracts from *Corymbia* leaves. The three pure taxa (*C. henryi* (CH), *C. citriodora* subsp. *variegata* (CCV) and *C. citriodora* subsp. *citriodora* (CCC)) are distinct, whereas *C. torelliana* (CT) forms closer clusters with the hybrid taxa, although the intermediate nature of *C. torelliana* × *C. citriodora* subsp. *variegata* (CT × CCV) is clear. (*b*) Two-dimensional multidimensional scaling ordinations of the leaf extracts. The plot is based on square root-transformed relative abundances and a Bray–Curtis similarity matrix. Samples from the three parental taxa, *C. torelliana* × *C. henryi* (CT × CH) and *C. torelliana* × *C. citriodora* subsp. *variegata* (CT × CCV) cluster separately, whereas extracts from *C. torelliana* × *C. citriodora* subsp. *citriodora* (CT × CCC) are not separable from samples from *C. torelliana* × *C. henryi* (CT × CH) and *C. torelliana* (CT).

and the CT × CCV hybrid, but overlapped with the CT × CCC hybrid (Table 3). The CT × CCC hybrid was not statistically distinguishable from CT, although it was distinct from its CCC parental taxon. With the increasing use of hybrids in agriculture and silviculture to combine desirable features of parental types, or even display novel phenotypes (Strauss 1994), such changes in plant secondary metabolites have important implications. Attack by insect pests may increase (e.g. Dungey and Potts 2003) or decrease (e.g. Boecklen and Spellenberg 1990), or new pests may invade hybrids as a result of shifts in host-plant secondary metabolites. In some cases, previously host-specific pathogens have hybridised along with their hosts (Newcombe *et al*. 2000), leading to a novel host association.

The progeny from the cross CT × CCV appears to express the oils in a manner different from either the CT hybrid with CH or CCC. The suite of foliar chemicals of the CT × CCV hybrid was intermediate to the foliar chemicals of either parent taxon. This is the same pattern that we have reported earlier (Nahrung *et al*. 2009). The suite of foliar chemicals of the other two hybrids was dominated by the CT mother. Further, for components derived from only one parent, in the CT × CH and CT × CCC hybrids, the majority was from the CT parental taxon. Indeed, the two major and distinctive components of CCC, citronellal and citronellol (which gives the lemon scent), were present in the CCC father, but were missing from the full-sib family tested here. In a study of the morphology of similar *Corymbia* species and hybrids, Abasolo *et al*. (2012) found that crosses between more genetically divergent parent taxa (on the basis of geographic distance) were better resolved than were hybrids developed using taxa from similar geographic ranges. In the current study, this was not

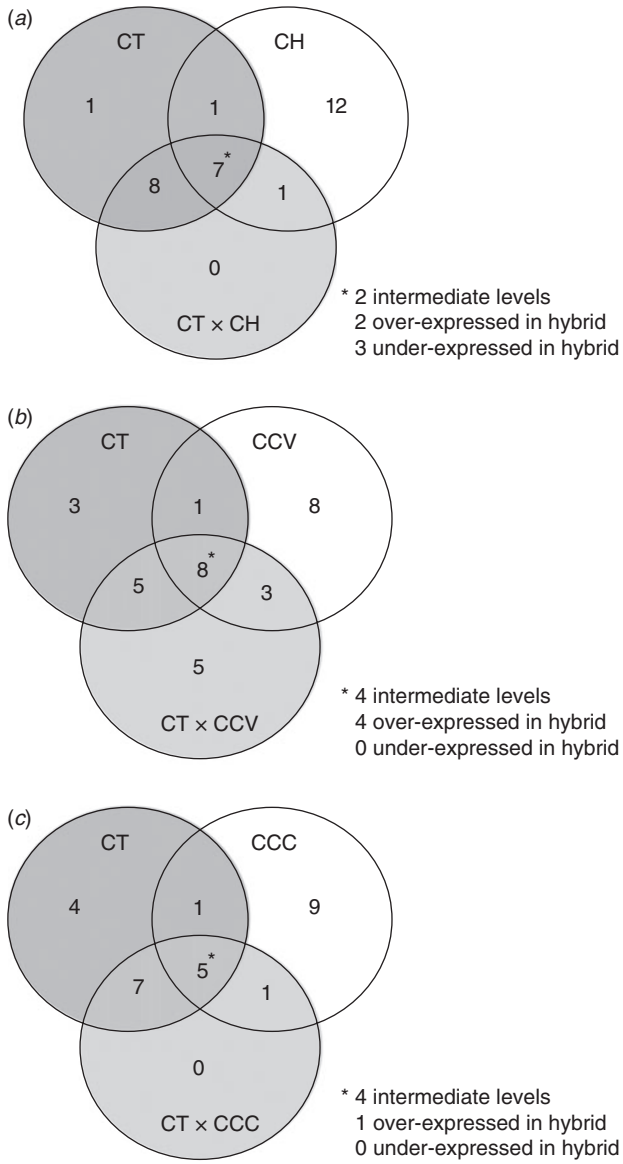

Fig. 2. The impact of hybridisation on components detected in *Corymbia* hybrid taxa that are derived from both parents, from only one parent or are novel to the hybrid. The hybrid taxa include (*a*) *C. torelliana* (CT) × *C. henryi* (CH), (*b*) CT × *C. citriodora* subsp. *variegata* (CCV) and (*c*) CT × *C. citriodora* subsp. *citriodora* (CCC).

the case, with the CT × CCV hybrids resolving well and the CT × CH and CT × CCC hybrids being poorly resolved relative to the CT female parental taxon, even though CT × CH was the cross between the genetically most divergent taxa. For example, isolongifolen-8-ol, which is found in all members of section *Maculatae* but not in CT, is expressed in the CT × CCV hybrid, but is not found in either of the other hybrids.

In addition, we detected novel compounds in the CT × CCV hybrid that were not detected in either parent taxon, a characteristic that we did not see in either of the other hybrids. Two sesquiterpenes, including α-eudesmol and an unidentified compound, were not detected in either parental taxon, but occurred in significant levels in CT × CCV hybrids. This may, however, be an artefact, because the fathers of the CCV hybrids were not sampled and variation in the CCV taxon may account for the novel compounds detected here.

The monoterpenes α-pinene and β-pinene were detected in all leaves sampled; however, the effect of hybridisation on their levels varied across taxa. Levels of α-pinene were intermediate in CT × CCC and CT × CCV, whereas this compound was overexpressed in CT × CH. This was the same pattern for α-pinene in the taxa in common to those in a previous study (Nahrung *et al*. 2012). β-Pinene levels showed three different patterns in the three hybrids, being under-expressed in the hybrid with CH, intermediate in the hybrid with CCC and overexpressed in CT × CCV. Abasolo *et al*. (2012) found that as geographic distance increased there was an increase in transgressive morphological traits. The oil profiles in our study also showed similar transgressive tendencies, with the over- and under-expression detailed. The sesquiterpene β-caryophyllene, which was also detected in all plants sampled, showed intermediate expression in all three hybrid taxa. There do not appear to be any general patterns in expression within a compound class, but variation is instead specific for individual components.

A limitation in the present study was that the hybrids were compared with the parental taxa but not with the parents of the full-sib crosses. The main reason for this was that the trees used as the source of pollen produced very little seed because of asynchronous flowering (Lee 2007). As a result, seeds of the parents were not available for the current study, even though the trees had light flowerings that allowed collection of the pollen used to make these crosses. This may have impacted the patterns of the secondary compounds observed in this study. Future examination of inheritance patterns of secondary metabolites in *Corymbia* hybrids should include seedlings from all parents used to validate the patterns observed.

Patterns of inheritance in the plant secondary metabolites varied among hybrids formed between CT and the members of section *Maculatae*. The hybrids with CCC and CH were dominated by the CT parent, whereas the hybrid formed from CCV showed an additive pattern (*sensu* Fritz *et al*. 1999) and was intermediate to the parents. These different patterns in plant secondary metabolites may have implications for a range of plant traits that will influence, for example, survival, including defence, nitrogen storage and UV potential. Understanding the biosynthesis, heritability and ecological significance of plant secondary metabolites, particularly terpenes, is vital in understanding plant–herbivore interactions.

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