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Intensive sampling identifies previously unknown chemotypes, population divergence and biosynthetic connections among terpenoids in *Eucalyptus tricarpa*

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ABSTRACT

Australian members of the Myrtaceae produce large quantities of ecologically and economically important terpenes and display abundant diversity in both yield and composition of their oils. In a survey of the concentrations of leaf terpenes in *Eucalyptus tricarpa* (L.A.S. Johnson) L.A.S. Johnson & K.D. Hill, which were previously known from few samples, exceptional variability was found in composition. The aim was to characterize the patterns of variation and covariation among terpene components in this species and to use this information to enhance our understanding of their biosynthesis. There were marked discontinuities in the distributions of numerous compounds, including the overall proportions of mono- and sesquiterpenes, leading us to delineate three distinct chemotypes. Overall, positive covariation predominated, but negative covariation suggested competitive interactions involved in monoterpene synthesis. Two groups of covarying monoterpenes were found, each of which was positively correlated with a group of sesquiterpenes and negatively correlated with the alternate sesquiterpene group. These results imply substantial cross-talk between mono- and sesquiterpene biosynthesis pathways. However, only those compounds hypothesized to share final carbocation intermediates or post-processing steps were strongly positively correlated within chemotypes. This suggests that the broader patterns of covariation among groups of compounds may result from co-regulation of multiple biosynthetic genes, controlling the complex terpene profiles of the chemotypes of *Eucalyptus*.

1. Introduction

The distribution of terpenoids among plants arguably represents a better identified, characterized and understood phytochemical data set than any other. Furthermore, their roles in allelopathy, herbivore defense and tritrophic interactions have been studied intensively in a range of systems (Bohlmann and Keeling, 2008; Gershenzon and Dudareva, 2007). Natural variation in terpenes is of great importance to ecology and evolution (Padovan et al., 2012), and has been a source for industrial and medicinal applications (Barton and Tjandra, 1989; Greay et al., 2010). However, there is little information on factors that determine chemical variability and the frequency of chemical forms within most species.

Monoterpenes and sesquiterpenes are the main groups of volatile terpenoids, and are both important in direct and indirect interactions with herbivores and other organisms. The biosynthesis of monoterpenes and sesquiterpenes is compartmentally separated in many plants. The 2-*C*-methyl-D-erythritol 4-phosphate (MEP) and mevalonic acid (MVA) pathways lead to the synthesis of monoterpenes and sesquiterpenes in the plastids and the cytosol, respectively (Cheng et al., 2007; Newman and Chappell, 1999). However, there is significant cross-talk between the two pathways in the form of isopentenyl pyrophosphate (IPP) transport across the chloroplast membrane, and it has been shown that both pathways are capable of using substrates from the alternate compartment (Bartram et al., 2006; Dudareva et al., 2005; Hemmerlin et al., 2003). Therefore, there may be scope for substrate competition and other forms of co-regulatory associations between mono- and sesquiterpenes. Yet few studies have focused on natural variation in allocation to these classes of compounds.

The patterns of natural variation are not only interesting from an ecological perspective; the relationships among terpene components can also help us to understand something deeper about their biosynthesis (Latta et al., 2003). For example, positive correlations may indicate that multiple compounds are synthesized by the same enzyme, whereas negative ones may result from competition by enzymes for a common substrate. In many cases, discontinuous variation in chemical composition (i.e. chemical variants or "chem-

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otypes") is likely to be due to the action of one or a few genes (Keszei et al., 2008). Differences between chemotypes can reflect the activity of terpene synthases, since these enzymes control the final products of terpene cyclisation. Numerous terpene synthase genes occur in the *Eucalyptus* genome (Külheim et al., 2011a) and several are known to be expressed in most species studied to date (Keszei et al., 2010).

The results presented here describe, the variation in leaf terpenes of *Eucalyptus tricarpa* (L.A.S.Johnson) L.A.S.Johnson & K.D.Hill an Australian species of the Myrtaceae family, whose members produce large quantities of ecologically and economically important terpenes (Boland et al., 1991; Brophy and Southwell, 2002). There is abundant diversity in both yield and composition of terpenoid-based oils in the Australian Myrtaceae. Chemotypes have been documented in a range of Australasian Myrtaceae, including *Melaleuca alternifolia* (Butcher et al., 1994), *Backhousia citriodora* (Doran et al., 2001), *Leptospermum scoparium* (Douglas et al., 2004) and numerous species of eucalypts (Asante et al., 2001; Moore et al., 2004; Penfold and Willis, 1953). Terpenoids also play important roles in the ecology of herbivory on *Eucalyptus* (Lawler et al., 1999; Marsh et al., 2006). In addition to several monoterpene chemotypes in *Eucalyptus* (Boland et al., 1991; Brophy and Southwell, 2002), chemotypes that are differentiated based on the predominance of aromadendrane and eudesmane type sesquiterpene compounds are also known from several eucalypts, e.g. *Eucalyptus globulus* (Keszei et al., 2010; Wallis et al., 2011). Certain chemotypes are typically excluded from breeding programs and commercial production of essential oils.

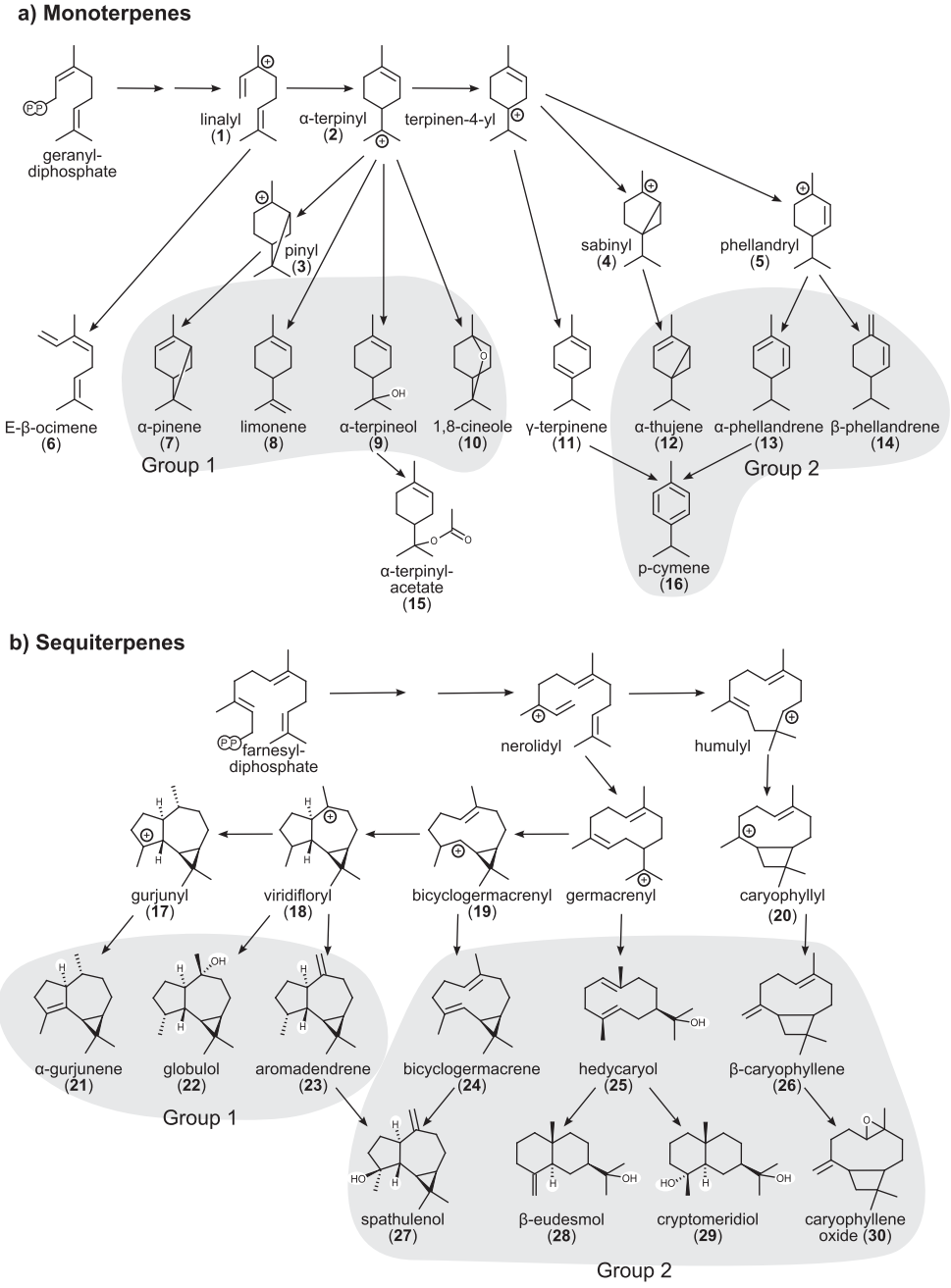

Fig. 1. Likely biosynthetic pathways of main sesquiterpenes and monoterpenes of *E. tricarpa* (Keszei et al., 2008). Carbocation intermediates and final products are labeled and groups of positively correlated products are identified by grey shading.