

Research Article

The odd one out or a hidden generalist: Hawaiian *Melicope* (Rutaceae) do not share traits associated with successful island colonizationClaudia Paetzold^{1*}, Michael Kiehn^{2,3}, Kenneth R. Wood³, Warren L. Wagner⁴, and Marc S. Appelhans^{1,4}¹Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), Goettingen University, Untere Karspuele 2, Goettingen, D-37073, Germany²Core Facility Botanical Garden, University of Vienna, Rennweg 14, A-1030, Austria³National Tropical Botanical Garden, 3530 Papalina Road, Kalaheo, HI 96741, USA⁴Department of Botany, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, MRC 166, Washington, DC 20013-7012, USA

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Abstract Oceanic islands are unique in their species composition, which is defined by arrival of colonizers via long distance dispersal followed by establishment of species followed in some cases by adaptive radiation. Evolutionary biologists identified traits facilitating successful colonization of islands as including polyploidy, self-compatibility, herbaceousness and ability for long-distance dispersal. Successful establishment and evolutionary diversification of lineages on islands often involves shifts to woodiness and shifts in methods of outcrossing as well as changes in dispersal ability. The genus *Melicope* colonized numerous archipelagos throughout the Pacific including the Hawaiian Islands, where the lineage comprises currently 54 endemic species and represents the largest radiation of woody plants on the islands. The wide distributional range of the genus illustrates its high dispersibility, most likely due to adaption to bird dispersal. Here we investigate ploidy in the genus using flow cytometry and chromosome counting. We find the genus to be paleopolyploid with $2n = 4x = 36$, a ploidy level characterizing the entire subfamily Amyridoideae and dating back to at least the Palaeocene. Therefore Hawaiian *Melicope* have not undergone recent polyploidization prior to colonization of the islands. Thus *Melicope* retained colonization success while exhibiting a combination of traits that typically characterize well established island specialists while lacking some traits associated to successful colonizers.

Key words: bird dispersal, colonization, establishment, long distance dispersal, Pacific, polyploidy.

1 Introduction

Ever since Charles Darwin wrote about his observations upon visiting the Galapagos Islands (Darwin, 1859), oceanic islands have been a focal point for biologists in their quest to unravel the process of evolution. The study of islands that have never been connected to a continental land mass, especially those that are greatly isolated and of volcanic origin, offer several unique advantages (Emerson, 2002). Islands are discrete systems with oceanic boundaries restricting gene flow between land masses. In spite of their small size (compared to continents), many oceanic islands offer a wealth of habitats and ecological niches, which are often in a constant flux due to influences of outside forces, e.g., plate tectonics, volcanic activity, erosion, flooding and tropical storms.

Yet, island floras are not merely ‘downscaled’ versions of the neighbouring continental ones. In contrast, islands possess unique species compositions differing remarkably from those of the continental land mass and typically with a high degree of endemism. For example, in the Canary Islands

about 40% of all angiosperm taxa are endemic (Francisco-Ortega et al., 2000) and about 90% in the Hawaiian Islands (Wagner et al., 1999; Keeley & Funk, 2011). The species composition of an island is dependent on three main factors: distance, geology (incl. altitudinal variation) and age. Distance refers to the distance between an island and other land-masses serving as a possible origin of colonizers. Increasing distance decreases the frequency of successful colonization events and restricts the diversity of possible colonizers to those with propagules ‘equipped’ to travel the distance. The geology and size of the island determines the quality and the quantity of ecological niches it provides. The age of an island represents the time frame available for colonization, establishment, adaptive radiation and even extinction of species (Carlquist, 1966a).

Successful colonizations of oceanic islands are rare, so that arrivals, especially to young islands, probably experience less selective pressure from other species than in their continental environment (Baldwin, 1998). When a viable seed reaches a given island and meets conditions allowing its establishment,

the colonizer may undergo extensive adaptive radiation giving rise to a lineage of diverse species (Carlquist, 1966a; Givnish et al., 1996).

The synergy of colonization by few founders, along with establishment in available ecological niches and adaptive radiation result in unique island floras that are vastly different from their source areas – both morphologically, ecologically and in terms of species richness (Carr, 1998). Yet, despite the individuality of each island system, after close to two centuries of island evolution research, several evolutionary trends have become apparent. In the past island biodiversity has been associated with multiple colonization events per lineage based on the presence of divergent morphological characters. However, more recent molecular phylogenetic and biogeographic studies revealed that this not the case and that, e.g., the 1192 species of vascular plants native to the Hawaiian Islands are the result of only 263-270 colonization events (Keeley & Funk, 2011). Most island lineages are monophyletic descending from one successful colonization event, e.g., the Hawaiian lobeliads (Campanulaceae; Givnish et al., 1996), *Dendroseris* D. Don (Asteraceae) on the Juan Fernandez Islands (Crawford et al., 1998) or the woody *Sonchus* L. (Asteraceae) alliance in Macaronesia (Kim et al., 1996). In many cases, island colonizers seem to be single, broadly adapted, often herbaceous, generalist species that radiated into several highly specialized, locally adapted and restricted species (Grant, 1998). Common traits of successful colonizers and the subsequent evolutionary shifts during establishment and radiation on oceanic islands include:

(1) Polyploidization. The advent of modern sequencing techniques has revealed that a whole genome duplication (WGD) event predated the diversification of all angiosperms, rendering all flowering plants ‘polyploid’ (Amborella Genome Project, 2013). For simplicity in this paper these most ancient events will be ignored and polyploidy will concern only chromosome number changes post-dating them. That being said, many oceanic island floras are characterized by a high number of polyploid plant taxa. Conventional estimations of polyploidy are often based on identifying the most likely base number of angiosperms by widespread comparison of numerous lineages combined with chromosome pairing analysis and postulating a threshold. Using this method Grant (1963) postulated that plants with a basic chromosome number of $n=14$ or higher are most likely polyploid. While detailed comparisons and genomic and cytological estimations are required to identify ploidy levels on a lineage-by-lineage basis, this approach serves as an adequate approximation.

Employing this approach, more than 80% of Hawaiian endemics (including *Melicope* J.R. Forst. & G. Forst.) are polyploid (Carr, 1998), as are 66% of all endemics on the Juan Fernandez Islands (Stuessy et al., 1992), while on the Canary Islands the fraction is only 24.5% (Bramwell, 1976). These numbers indicate that polyploidy has a different impact or prevalence on islands depending on island age and distance to continental land masses (Whitaker, 1998). High levels of polyploidy on many oceanic islands do not reflect high instances of in situ polyploidization, as island lineages often display chromosomal stasis during speciation (Stuessy & Crawford, 1998; Kiehn, 2005). Instead, the high percentage of polyploid endemics indicates the success of polyploid

immigrants (Stuessy & Crawford, 1998) in the competition for colonization and adaptive radiation. In the grass subfamily Danthonioideae, polyploidization events were shown to facilitate Long-Distance Dispersal (LDD; Linder & Barker, 2014). Polyploidy offers advantages that may be particularly potent for establishment on oceanic islands, including increased vigour through heterosis and gene redundancy (Comai, 2005). Although detailed molecular studies for many lineages are still lacking, the few radiations that have been investigated indicate an allo- or autopolyploidization event directly predates the colonization of oceanic islands. While the phenomenon is fairly well researched in Asteraceae (Crawford et al., 2009), it is perhaps most striking in the sandalwoods (*Santalum* L., Santalaceae). Members of *Santalum* colonized islands throughout the Pacific in a stepwise fashion, following at least six polyploidization events leading to three additional ploidy levels (Harbaugh & Baldwin, 2007; Harbaugh, 2008). Hawaiian examples include the silversword alliance originating from an allopolyploidization event in California ca. 15 million years ago (mya) (Baldwin et al., 1991; Baldwin & Sanderson, 1998), or the Hawaiian violets arriving as recently as ca. 1.2-2 mya (Havran et al., 2009). Following the classification of Ehrendorfer (1980) on those few investigated lineages, colonizers classify as neo- or mesopolyploids.

(2) Dispersibility. Immigrants to remote oceanic islands arrive by definition via LDD. While there is an element of chance to that, the likelihood of successful LDD event(s) increases with diaspores adapted to efficient dispersal, as evidenced by families or genera that colonized multiple islands. Adaptions of highly dispersible diaspores include smallness of spores or seeds for wind dispersed taxa (e.g., ferns, orchids), hooks, barbs and adhesive layers for exozoochory (e.g., *Bidens* L. (Asteraceae); *Peperomia* Ruiz & Pav. (Piperaceae)), or pulpy parts (often containing many tiny seeds) attracting feeders for endozoochory (e.g., *Rubus* L. (Rosaceae)). Regardless of vector, a small seed size is a common factor among efficient dispersers, both because this makes them easy to carry or swallow and because most immigrants are herbs (see 4). Weedy or herbaceous open habitat species tend to have small seeds as seedlings are exposed to sunlight shortly after germination. In contrast woody species tend to have larger seed sizes, as the seed contains stored nutrients, from which the seedling will grow until it reaches higher forest strata and exposure to sunlight (Carlquist, 1966a). Though detailed studies are scarce, trends for island species to drastically reduce their dispersal ability as pertaining to LDD and water barriers have been observed (e.g., Carlquist, 1966b, 1966c; Price & Wagner, 2004; Fresnillo & Ehlers, 2008). In several fern genera an increase in spore size has been observed as well as reduction or loss of pappus awns in *Bidens* (Carlquist, 1966b) or an increase in fruit size in, e.g., *Polyscias* J.R. Forst. & G. Forst. (Araliaceae; as *Tetraplasandra* A. Gray) or *Zanthoxylum* L. (Rutaceae; as *Fagara* L.) (Carlquist, 1966c). Reducing dispersibility is an advantageous adaptation in an island setting as it decreases the likelihood of seeds becoming ‘lost at sea’ and reflects the condition where the habitable area of most species is often much smaller than the total island size (Carlquist, 1966a; Price & Wagner, 2004).

(3) Self-Compatibility to Outbreeding. In 1955 Herbert Baker proposed the hypothesis (later widely known as Baker’s law) that self-compatibility is an advantageous trait for an island

colonizer to possess. Since colonization events are rare and typically involve only one or a small number of individual(s), being self-compatible allows establishment on an island in the absence of potential mates and/or pollinators or when potential mates are present but incompatible (Panell, 2015). However, high instances of outbreeding mechanisms observed on oceanic islands (Carlquist, 1966a) seem to point towards the development of said mechanisms following establishment to counter possible negative effects of small population sizes and gene pools. In New Zealand 12–13% of species are dioecious (Webb & Kelly, 1993) as are 14% of species on the Hawaiian Islands (Sakai et al., 1995), where the worldwide ratio is at 4% (Yampolsky & Yamplosky, 1922). On the Hawaiian archipelago approximately one third of all dimorphic species evolved from a monomorphic colonizer (Sakai et al., 1995).

(4) Herbaceousness to insular woodiness. Stuessy & Crawford (1998) argued that in many cases successful island colonizers are predominantly herbs. Decreased generation times of herbs, as compared to woody species, should enable them to adapt to a new environment more quickly. Upon establishment, however, a shift to a woody growth form can often be observed, which Carlquist (1974) termed ‘insular woodiness’. It has been observed in several Angiosperm families and islands, and evolved in numerous lineages independently. In Asteraceae this pattern is highly prominent with the woody *Sonchus* alliance on the Macaronesian islands (Kim et al., 1996), *Dendroseris* and *Robinsonia* DC. (Asteraceae) on the Juan Fernandez Islands (Crawford et al., 1998) the Hawaiian silversword alliance (Baldwin, 1998) or Hawaiian *Schiedea* Cham & Schltdl. (Caryophyllaceae, Wagner et al., 2005).

Of course, not all successful radiations exhibit all of these traits, and research is incomplete for a majority of lineages. While some species of Hawaiian mints are shrubby or herbs with a “somewhat a woody base”, others are herbaceous (Wagner et al., 1999), and as such the lineage as a whole does not exhibit insular woodiness (Lens et al., 2013). Since no detailed study exists regarding the woodiness in Hawaiian mints, and as the boundary between herbaceous and woody is considered fuzzy (Lens et al., 2013), evaluation of this trait is not final. On the other hand Hawaiian mints are of allopolyploid origin and share the same chromosome number ($2n = 64$) as their closest relatives in the genus *Stachys* L. (Lamiaceae). However, with chromosome numbers ranging from $2n = 10$ to 102 in the genus *Stachys* (Wagner et al., 1999; Lindqvist & Albert, 2002; Roy et al., 2015), the ancestor of Hawaiian mints may be classified as a mesopolyploid. And while we do know that the largest oceanic radiation in the world, Hawaiian lobeliads (Campanulaceae), is polyploid (Lammers, 1988; Carr, 1998), we do not know whether polyploidization occurred prior to colonization. We can surmise, however, that successful adaptive radiations on oceanic islands seem to show at least one or several, if not necessarily all of these traits.

Melicope J.R.Forst. & G.Forst. (Rutaceae) in its traditional circumscription comprises ca. 230 species of shrubs and trees distributed in east-west-extension from Madagascar to the Hawaiian Islands and in north-south-extension from Japan to New Zealand. Currently (Hartley, 2001) the genus is subdivided into four sections: *Lepta* (Lour.) T.G.Hartley, *Melicope*, *Pelea* (A.Gray) Hook. and *Vitiflorae* (F.Muell.) T.G.Hartley. Recent

molecular work has revealed that several genera are nested within *Melicope* and that the enlarged genus now contains about 300 species (Appelhans et al., 2014a). The Hawaiian genus *Platydesma* H.Mann was one of these genera and has recently been included in *Melicope* (Appelhans et al., 2017). *Melicope* has its origin in the Australasian region but has colonized numerous archipelagos throughout the Pacific and even Madagascar and the Mascarene Islands (Appelhans et al., accepted). The Hawaiian Island lineage of *Melicope* is monophyletic and nested deeply within the genus. The clade belongs to section *Pelea* and comprises 54 currently accepted species (Hartley, 2001; Appelhans et al., 2017; Wood et al., 2017). It represents the largest radiation of woody plants on the Hawaiian Islands (Wagner et al., 1999) and colonization predates the age of the current high islands (Appelhans et al., accepted). At first glance the lineage seems to match the pattern for insular specialist very nicely; the species are woody, mostly distributed in forests and about 80% of the species are endemic to a single island (when Maui Nui is treated as a single island) with only small distributional ranges on the islands. Also they are mostly dioecious and their capsular/follicular fruits display shiny black seeds in varying sizes with a spongy and nutritious sarcotesta and a thick sclerotesta, which have been interpreted as an adaption to bird dispersal (Hartley, 2001). However, all species of not only the genus *Melicope* but also all related genera (Appelhans et al., 2014b) are woody and bird dispersed and all species of *Melicope* section *Pelea* are dioecious. Therefore these traits are ancestral and not acquired following colonization of the archipelago. Whether the same is true regarding the ploidy is not yet clear. Up until now chromosome counts exist for 20 *Melicope* species, two species of *Acronychia* J.R.Forst. & G. Forst., which is nested in *Melicope* as well as one recorded count for *Comptonella* Baker f., which was revealed to be nested within *Melicope* sect. *Vitiflorae* (Appelhans et al., 2014b) (Table 1). Altogether these records span the entire distributional range of *Melicope* (except Madagascar and the Mascarene Islands) and all four sections of the genus. The 14 species representing non-Hawaiian lineages of *Melicope*, the two specimens of *Acronychia* as well as the record for *Comptonella* revealed a base chromosome number of $2n = 36$; with the exception of one count for *M. semecarpifolia* (Merr.) T.G.Hartley ($n = 12$; Hsu, 1968) and the result for *M. brassii* T.G. Hartley ($2n = 32$; Borgmann, 1964). Though an ancestral state of $n = 18$ has also been suggested (Stace et al., 1993), the ancestral haploid chromosome number in Rutaceae is most likely nine (Kubitzki et al., 2011), as the most closely related sister clades (Meliaceae, Simaroubaceae) also show a base chromosome number of $n = 9$ (Fig. 1). Within Rutaceae only the species-poor subfamilies Aurantioideae and Rutoideae (Morton & Telmer, 2014; ~300 species in 33 genera) possess $n = 9$ (or more rarely $n = 10$). The vast majority of Rutaceae (including *Melicope*) is represented by subfamily Amyridoideae (Morton & Telmer, 2014), a clade of 1800 species in 113 genera with $n = 18$ as base chromosomal number (Kubitzki et al., 2011). The shift from $n = 9$ to $n = 18$ likely happened in the Paleocene or even the Late Cretaceous (Appelhans et al., 2012; Fig. 1). Therefore the Amyridoideae genera including *Melicope* can be considered paleopolyploids.

The observation of a depauperate sisterclade to a highly diverse, species-rich, polyploid one with a delay between the

Table 1 Chromosome counts for 12 Hawaiian and 13 non-Hawaiian *Melicope* species, two species of *Acronychia* and one species of *Comptonella*, both of which are nested within *Melicope*

Species	n	2n	Section	Origin	Coll. No. (Herbarium of voucher deposition for new counts)	Reference
Hawaiian taxa						
<i>Melicope adscendens</i> (St.John & Hume) T.G. Hartley & B.C.Stone	36		<i>Pelea</i>	Maui	Oppenheimer #H20907 & Perlman (BISH, WU)	Kiehn, this paper
<i>M. anisata</i> (H.Mann) T.G. Hartley & B.C.Stone	34-36		<i>Pelea</i>	Kaua'i	Perlman & Kiehn SP 21325 (PTBG, WU)	Kiehn, this paper
<i>M. barbiger</i> A.Gray	36		<i>Pelea</i>	Kaua'i		Kiehn, 2005
<i>M. clusiifolia</i> (A.Gray) T.G. Hartley & B.C.Stone	36		<i>Pelea</i>	Kaua'i	Perlman & Kiehn SP 21328 (PTBG, WU)	Kiehn, this paper
<i>M. cornuta</i> (Hillebr.) Appelhans, K.R.Wood & W.L.Wagner	18		<i>Pelea</i>	O'ahu		Carr, 1978 (as <i>Platydesma</i> c.)
<i>M. elliptica</i> A.Gray	18		<i>Pelea</i>	O'ahu		Carr, 1978 [as <i>Pelea</i> e.]
<i>M. ovata</i> (St.John & Hume) T.G.Hartley & B.C.Stone	34-36		<i>Pelea</i>	Kaua'i	Perlman & Kiehn SP 21333 (PTBG [PTBG1000031090], WU)	Kiehn, this paper
<i>M. ovata</i> (St.John & Hume) T.G.Hartley & B.C.Stone	18, 36		<i>Pelea</i>	Kaua'i		Kiehn, 2005 (as <i>M. sp.</i>)
<i>M. puberula</i> (H.St.John) T.G.Hartley & B.C.Stone	18	36	<i>Pelea</i>	Kaua'i	Perlman & Kiehn SP 21327 (PTBG, WU)	Kiehn, this paper
<i>M. rostrata</i> (Hillebr.) Appelhans, K.R.Wood & W.L.Wagner	36		<i>Pelea</i>	Kaua'i		Guerra, 1984 (as <i>Platydesma rostratum</i> Hillebr.)
<i>M. sp. indet</i>	36		<i>Pelea</i>	O'ahu		Kiehn, 2005
<i>M. wawraeana</i> Rock	72		<i>Pelea</i>	Kaua'i		Guerra, 1984 (as <i>Pelea</i> w.)
<i>M. zahlbruckneri</i> (Rock) T.G.Hartley & B.C.Stone	36		<i>Pelea</i>	Hawai'i (Big Island)	Kiehn & Pratt MK-090211-4/4 (BISH, WU)	Kiehn, this paper
Non-Hawaiian taxa						
<i>Acronychia suberosa</i> C.T.White	36			Australia: Queensland		Guerra, 1984
<i>A. pubescens</i> (F.M.Bailey) C.T.White	34			Australia: Queensland		Guerra, 1984
<i>Comptonella</i> Baker f.	18			France: New Caledonia		Kubitzki et al., 2011
<i>M. brassii</i> T.G.Hartley	32		<i>Pelea</i>	Papua New Guinea		Borgmann, 1964
<i>M. bonwickii</i> (F.Muell.) T.G.Hartley	36		<i>Lepta</i>	Philippines: Luzon		Pancho, 1971 (as <i>Euodia villamillii</i> Merr.)
<i>M. frutescens</i> (Blanco) Appelhans & J.Wen	36 (38?)		<i>Lepta</i>	Philippines: Luzon		Pancho, 1971 (as <i>Euodia confusa</i> (Blco.) Merr.)
<i>M. grisea</i> (Planch.) T.G.Hartley	36		<i>Lepta</i>	Japan: Bonin Islands		Ono & Masuda, 1981 (as <i>Boninia grisea</i> Planch.)
<i>M. lunu-ankenda</i> (Gaertn.) T.G.Hartley	36		<i>Lepta</i>	Sri Lanka		Morawetz, 1986 (as <i>Evodia roxburghiana</i> Benth.)
<i>M. mantellii</i> Buchanan	18		<i>Melicope</i>	New Zealand: cult. Auckland University College		Rattenbury, 1957

Continued

Table 1 Continued

Species	n	2n	Section	Origin	Coll. No. (Herbarium of voucher deposition for new counts)	Reference
<i>M. micrococca</i> (F.Muell.) T.G.Hartley	18		<i>Lepta</i>	Australia		Smith-White, 1954 (as <i>Euodia micrococca</i> F.Muell.)
<i>M. quadrilocularis</i> (Hook. & Arn.) T.G.Hartley		36	<i>Lepta</i>	Japan: Bonin Islands		Ono & Masuda, 1981 (as <i>Boninia glabra</i> Planch.)
<i>M. retusa</i> (A.Gray) T.G.Hartley		36	<i>Pelea</i>	Philippines: Luzon		Pancho, 1971
<i>M. rubra</i> (Lauterb. & K. Schum.) T.G.Hartley		36	<i>Lepta</i>	Papua New Guinea		Borgmann, 1964
<i>M. semecarpifolia</i> (Merr.) T.G.Hartley	12		<i>Lepta</i>	China: Taiwan		Hsu, 1968 (as <i>Euodia confusa</i> (Blco.) Merr.)
<i>M. semecarpifolia</i> (Merr.) T.G.Hartley		36	<i>Lepta</i>	Philippines: Luzon		Pancho, 1971
<i>M. simplex</i> A.Cunn.		36	<i>Melicope</i>	New Zealand		Rattenbury, 1957
<i>M. ternata</i> J.R.Forst. & G.Forst.	18		<i>Melicope</i>	New Zealand: cult. Auckland University College		Rattenbury, 1957
<i>M. ternata</i> J.R.Forst. & G.Forst.		36	<i>Melicope</i>	cult. Botanical Garden University of Vienna (WU)		Guerra, 1984

Details on origin of specimens, collection numbers including deposition of Herbarium vouchers for new records and references are given. Herbarium acronyms are according to Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>).

polyploidization event and the onset of diversification fits the WGD radiation lag-time model (Schranz et al., 2012). One hypothesis for this lag phase is, that this time is required for diploidization to take place (Dodsworth et al., 2016). Diploidization is a post-genome-duplication process that includes operations between duplicated genes, e.g. neo-functionalization, subfunctionalization and non-functionalization as well as operations between duplicated genomes, e.g. genome downsizing (Ma & Gustafson, 2005; Dodsworth et al., 2016).

Comparing chromosome numbers and DNA content in Rutaceae (C-value database Kew, <http://data.kew.org/cvalues>; assessed on 01. 16. 2017) shows no linear relationship. *Ruta graveolens* L. for example shows a chromosome number of $n = 78$ at a DNA content of 0.75 pg and illustrates the effects of genome downsizing. So far the only *Melicope* species for which both a chromosome count as well as genome size have been measured is *Melicope ternata* J.R.Forst. & G.Forst. This species has a chromosome number of $n = 18$ and shows a genome size of 0.93 pg (Guerra, 1984) comparable to that of many diploid members of Rutaceae.

The seven *Melicope* species from Hawaii investigated previously do not show a consistent picture. Three different chromosome numbers were reported ($2n = 18, 36$ or 72 ; Table 1) indicating possible polyploidization or hybridization events within the lineage (Kiehn, 2005).

The main aim of this study is to investigate evolutionary trends characteristic for oceanic islands in the Hawaiian lineage of the genus *Melicope*. To that end we also investigate if specimens show traits specific for colonization of and/or establishment on islands, and if these traits are unique to the

Hawaiian radiation or characteristic for the genus as a whole. We have conducted a literature search regarding traits of insular woodiness, dispersibility, and reproductive systems. We further investigate ploidy levels in the Hawaiian radiation of the genus as well as representatives of the non-Hawaiian species to infer whether the colonizer of the archipelago was a neo- or mesopolyploid.

2 Material and Methods

2.1 Flow cytometry

DNA content was assessed for 61 samples representing 66% of the Hawaiian radiation of *Melicope* as well as nine samples of non-Hawaiian species via flow cytometry. Table 2 details geographic origins and collection details for the samples. Due to scarcity of material, only one measurement was taken per sample.

Leaf material was ground with a TissueLyzerII (Quiagen, Hilden, Germany) at 15 Hz for 45 s using a steel bead ($\varnothing$ 3 mm) in a 2 mL Eppendorf cap. Nuclei were isolated by 8 min incubation in 300 μ L Otto I buffer (Otto, 1990). After filtering the mix (30 μ m mesh, CellTrics[®] Partec GmbH, Münster, Germany), 800 μ L staining solution (Otto II buffer, Doležel & Göhde, 1995) was added and the solution again incubated for 8 min on ice. The solution was then measured on the flow cytometer (CyFlow[®] Ploidy Analyser, Sysmex Deutschland GmbH, Norderstedt, Germany) using the blue UV LED channel. Fluorescence intensity was measured and peaks medians were calculated using the program CyFlow Cube v 1.5.7.3 (Sysmex Deutschland GmbH, Norderstedt, Germany).

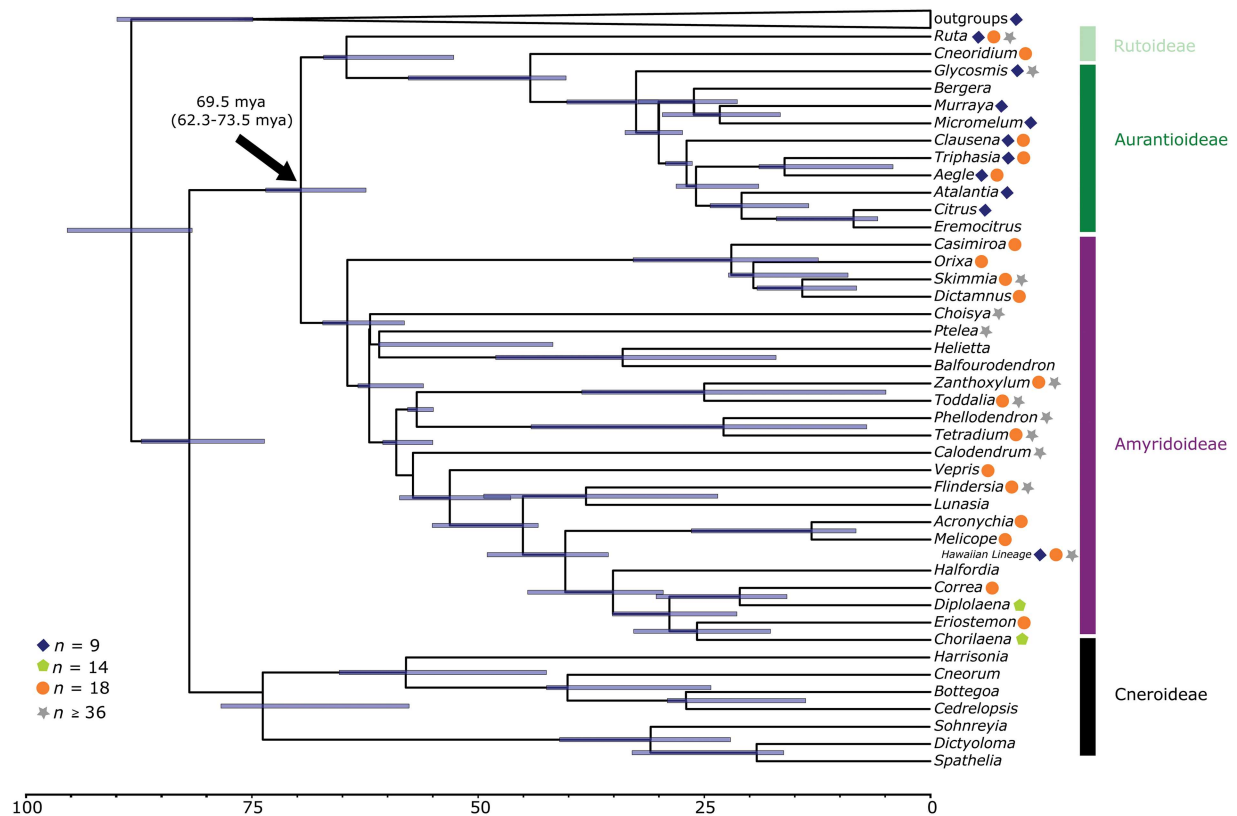


Fig. 1. Phylogenetic relationships of Rutaceae genera (modified from Appelhans et al., 2012) with known ploidy levels as inferred from known chromosome numbers (Kew C-value database (<http://data.kew.org/cvalues>; assessed on 01. 16. 2017), Kubitzki et al., 2011) plotted to each genus. Rutaceae subfamilies are indicated by black (Cneoroideae), violet (Amyridoideae), mint (Rutoideae) and green (Aurantioideae) bars. Outgroups refer to the most closely related families Meliaceae and Simaroubaceae (Appelhans et al., 2012). A black arrow marks the split of the Aurantioideae and Amyridoideae subfamilies in the Palaeocene and the coinciding polyploidization event.

Samples were measured at gain 450 with *Pisum sativum* L. (Fabaceae) as internal standard. Several samples failed to produce a peak due to inference of debris particles, and the measurement was repeated for those at gain 480 with *Paspalum notatum* Flugge (Poaceae) as external standard. The mean peak value of all reference measurements (>15) was used to calculate the DNA content of samples using the formula (sample mean peak * reference mean peak)/reference DNA content. Reference mean C1 values were obtained from the Kew C-value database (<http://data.kew.org/cvalues>; assessed on 16. 01. 2017) as $1C = 4.88$ pg for *Pisum sativum* and $1C = 0.89$ pg for *Paspalum notatum*. The software Past v 3.17 (Hammer et al., 2001) was used to test for normal distribution of measurements.

2.2 Chromosome counts

Chromosome counts are based on field fixations or fixations from plants cultivated at the Botanical Garden of the University of Vienna (Austria). Fixations of meristematic tissues (actively growing root tips, young flowers or apices for counts of mitotic numbers, young flower buds for meiotic investigations) were made in a freshly mixed 3:1 solution of ethanol (96%):glacial acetic acid or in a 4:3:1 mixture of chloroform:100% ethanol:glacial acetic acid. Some germinating seeds were pretreated

with 0.002 M 8-hydroxyquinoline solution for 6 h at 8–10 °C in the dark before fixations were made (see Table 1). Each fixation represents one individual in the case of field fixations, or individually distinguishable seedlings in the case of fixations of germinating seeds. Chromosome staining was performed with Feulgen reagent, Giemsa, or aceto-carmine (for details on staining procedures see Kiehn, 2005). Exact counts could not be achieved in some cases because of limited material. A range of chromosome numbers is given in such cases. Permanent slides for the counts are deposited in the personal collection of MK. Reference voucher specimens for each investigated collection have been deposited in at least one of the following herbaria: Bishop Museum, Honolulu, Hawaii (BISH), National Tropical Botanical Garden, Kalaheo (Kaua'i), Hawaii (PTBG), University of Hawaii (HAW), or University of Vienna (WU).

3 Results

Table 2 summarizes the genome sizes for 61 samples of *Melicope* as estimated by flow cytometry. With the exception of *M. ternata* (Guerra, 1984) none of these species have been assessed regarding their genome sizes before. The results are normally distributed ($p = 0.71$; Shapiro-Wilk = 0.988). The

Table 2 DNA content of 62 Hawaiian and 11 non-Hawaiian *Melicope* specimens as measured by flow cytometry using *Pisum sativum* (†) or *Paspalum notatum* (‡) as reference

Species	Herbarium voucher	Origin	C (pg) †	C (pg) ‡
Hawaiian taxa				
<i>Melicope adscendens</i> (St.John & Hume) T.G.Hartley & B.C.Stone	Appelhans MA628 (silica sample only, cultivated at Olinda Rare Plant Facility)	Maui		0.71
<i>Melicope anisata</i> (H.Mann) T.G.Hartley & B. C.Stone	Appelhans MA665 (GOET [GOET019849], PTBG [PTBG 1000057433])	Kaua'i	0.75	0.79
<i>Melicope anisata</i> (H.Mann) T.G.Hartley & B.C.Stone	Appelhans MA668 (GOET [GOET019850], PTBG [PTBG 1000057439], US)	Kaua'i		0.78
<i>Melicope barbigera</i> A.Gray	Appelhans MA664 (GOET [GOET019851], PTBG [PTBG 1000057432], US)	Kaua'i	0.72	0.69
<i>Melicope barbigera</i> A.Gray	Appelhans MA666 (BISH, GOET [GOET019852], PTBG [PTBG 1000057437], US)	Kaua'i		0.79
<i>Melicope barbigera</i> A.Gray	Wood 16718 (PTBG)	Kaua'i	0.78	0.73
<i>Melicope christophersenii</i> (St.John) T.G.Hartley & B.C.Stone	Appelhans MA617 (BISH, GOET [GOET019853], PTBG [PTBG 1000057596], US)	O'ahu		0.75
<i>Melicope christophersenii</i> (St.John) T.G.Hartley & B.C.Stone	Appelhans MA621 (silica sample only, cultivated at Pu'u Ka'ala)	O'ahu		0.73
<i>Melicope christophersenii</i> (St.John) T.G.Hartley & B.C.Stone	Takahama s.n. (silica sample only)	O'ahu	0.86	
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Appelhans MA615 (GOET [GOET019855], PTBG [PTBG 1000057517])	O'ahu		0.82
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Appelhans MA634 (PTBG [PTBG 1000057507])	Maui		0.78
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Appelhans MA650 (GOET [GOET019857], PTBG [PTBG 1000057504], US)	Maui	0.82	
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Appelhans MA651 (BISH, GOET [GOET019856], PTBG [PTBG 1000057511], US)	Maui	0.85	
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Appelhans MA655 (silica sample only)	Maui		0.76
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Appelhans MA657 (GOET [GOET019858], PTBG [PTBG 1000057572], US)	Maui	0.80	
<i>Melicope clusiifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Oppenheimer H91641 (US)	Lāna'i		0.67
<i>Melicope cruciata</i> (A.Heller) T.G.Hartley & B.C.Stone	Wood 16251 (PTBG)	Kaua'i		0.76
<i>Melicope feddei</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA688 (BISH, GOET [GOET019864], PTBG [PTBG 1000057431], US)	Kaua'i		0.74
<i>Melicope haleakalae</i> (B.C.Stone) T.G.Hartley & B.C.Stone	Appelhans MA637 (BISH, GOET [GOET019866], PTBG [PTBG 1000057497], US)	Maui		0.79
<i>Melicope haleakalae</i> (B.C.Stone) T.G.Hartley & B.C.Stone	Appelhans MA641 (BISH, GOET [GOET019865], PTBG [PTBG 1000057502])	Maui		0.74
<i>Melicope haleakalae</i> (B.C.Stone) T.G.Hartley & B.C.Stone	Appelhans MA645 (BISH, GOET [GOET019867], PTBG [PTBG 1000057495])	Maui		0.75
<i>Melicope haleakalae</i> (B.C.Stone) T.G.Hartley & B.C.Stone	Appelhans MA646 (BISH, GOET [GOET019868], PTBG [PTBG 1000057496], US)	Maui		0.76

Continued

Table 2 Continued

Species	Herbarium voucher	Origin	C (pg) †	C (pg) ‡
<i>Melicope haupeensis</i> (St.John) T.G.Hartley & B.C.Stone	Appelhans MA687 (BISH)	Kaua'i		0.73
<i>Melicope haupeensis</i> (St.John) T.G.Hartley & B.C.Stone	Wood 16794 (PTBG)	Kaua'i		0.65
<i>Melicope hawaiiensis</i> (Wawra) T.G.Hartley & B.C.Stone	Appelhans MA633 (BISH, GOET [GOET019869], PTBG [PTBG 1000057494], US)	Maui		0.78
<i>Melicope kavaensis</i> (H.Mann) T.G.Hartley & B.C.Stone	Appelhans MA679 (BISH, GOET [GOET019871], PTBG [PTBG 1000057501], US)	Kaua'i	0.77	
<i>Melicope knudsenii</i> (Hillebr.) T.G.Hartley & B.C.Stone	Appelhans MA629 (silica sample only, cultivated at Olinda Rare Plant Facility)	Maui		0.71
<i>Melicope knudsenii</i> (Hillebr.) T.G.Hartley & B.C.Stone	Oppenheimer H41610 (BISH)	Maui		0.66
<i>Melicope knudsenii</i> (Hillebr.) T.G.Hartley & B.C.Stone	Wood 17119 (PTBG)	Kaua'i		0.65
<i>Melicope lydgatei</i> (Hillebr.) T.G.Hartley & B.C.Stone	Ching s.n. (silica sample only)	O'ahu		0.71
<i>Melicope makahae</i> (B.C.Stone) T.G.Hartley & B.C.Stone	Takahama s.n. (silica sample only)	O'ahu		0.71
<i>Melicope makahae</i> (B.C.Stone) T.G.Hartley & B.C.Stone (cf.)	Appelhans MA609 (GOET [GOET019872], PTBG [PTBG 1000057509])	O'ahu		0.76
<i>Melicope molokaiensis</i> (Hillebr.) T.G.Hartley & B.C.Stone	Appelhans MA635 (BISH, GOET [GOET019875], PTBG [PTBG 1000057498])	Maui		0.74
<i>Melicope molokaiensis</i> (Hillebr.) T.G.Hartley & B.C.Stone	Appelhans MA643 (BISH, GOET [GOET019874], PTBG [PTBG 1000057560], US)	Maui		0.72
<i>Melicope mucronulata</i> (St.John) T.G.Hartley & B.C.Stone	Appelhans MA630 (silica sample only, cultivated at Olinda Rare Plant Facility)	Maui		0.71
<i>Melicope oahuensis</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA610 (BISH, GOET [GOET019876], PTBG [PTBG 1000057508], US)	O'ahu		0.82
<i>Melicope orbicularis</i> (Hillebr.) T.G.Hartley & B.C.Stone	Appelhans MA656 (BISH, GOET [GOET019877], PTBG [PTBG 1000057584], US)	Maui	0.80	
<i>Melicope orbicularis</i> (Hillebr.) T.G.Hartley & B.C.Stone	Appelhans MA659 (GOET [GOET019878], PTBG [PTBG 1000057578])	Maui	0.79	
<i>Melicope ovata</i> (St.John & Hume) T.G.Hartley & B.C.Stone	Appelhans MA662 (GOET [GOET019880], PTBG [PTBG 1000057460], US)	Kaua'i	0.75	
<i>Melicope ovata</i> (St.John & Hume) T.G.Hartley & B.C.Stone	Appelhans MA663 (BISH, GOET [GOET019879], PTBG [PTBG 1000057427], US)	Kaua'i	0.78	
<i>Melicope ovata</i> (St.John & Hume) T.G.Hartley & B.C.Stone	Appelhans MA684 (BISH, GOET [GOET019881])	Kaua'i		0.73
<i>Melicope ovata</i> (St.John & Hume) T.G.Hartley & B.C.Stone	Wood 17082 (PTBG)	Kaua'i		0.77
<i>Melicope pallida</i> (Hillebr.) T.G.Hartley & B.C.Stone	Appelhans MA689 (silica sample only)	Kaua'i		0.77
<i>Melicope pallida</i> (Hillebr.) T.G.Hartley & B.C.Stone	Wood 16789 (PTBG)	Kaua'i		0.75
<i>Melicope paniculata</i> (St. John) T.G.Hartley & B.C.Stone	Perlman 19387 (PTBG) = Appelhans MA660 (silica sample)	Kaua'i	0.85	
<i>Melicope peduncularis</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA613 (BISH, GOET [GOET019882], PTBG [PTBG 1000057524], US)	O'ahu		0.79
<i>Melicope peduncularis</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA652 (BISH, GOET [GOET019883], PTBG [PTBG 1000057547],	Maui	0.87	0.80

Continued

Table 2 Continued

Species	Herbarium voucher	Origin	C (pg) †	C (pg) ‡
	US)			
<i>Melicope peduncularis</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA653 (BISH, GOET [GOET019884], PTBG [PTBG 1000057513], US)	Maui		0.79
<i>Melicope pseudoanisata</i> (Rock) T.G.Hartley & B.C.Stone	Appelhans MA632 (silica sample only, cultivated at Olinda Rare Plant Facility)	Maui		0.70
<i>Melicope pseudoanisata</i> (Rock) T.G.Hartley & B.C.Stone	Appelhans MA636 (silica sample only)	Maui		0.71
<i>Melicope pseudoanisata</i> (Rock) T.G.Hartley & B.C.Stone	Appelhans MA642 (GOET [GOET019885], PTBG [PTBG 1000057554], US)	Maui		0.79
<i>Melicope puberula</i> (St.John) T.G.Hartley & B.C.Stone	Appelhans MA680 (GOET [GOET019886], PTBG [PTBG 1000057484], US)	Kaua'i		0.73
<i>Melicope radiata</i> (St.John) T.G.Hartley & B.C.Stone	Appelhans MA698 (BISH, GOET [GOET019888], PTBG [PTBG 1000057523], US)	Hawai'i (Big Island)		0.71
<i>Melicope rostrata</i> (Hillebr.) Appelhans, K.R.Wood & W.L.Wagner	Appelhans MA683 (BISH, GOET [GOET019889])	Kaua'i		0.85
<i>Melicope rotundifolia</i> (A.Gray) T.G.Hartley & B.C.Stone	Ching s.n. (silica sample only)	O'ahu	0.72	
<i>Melicope sandwicensis</i> (Hook. & Arn.) T.G.Hartley & B.C.Stone	Ching s.n. (silica sample only)	O'ahu		0.69
<i>Melicope sessilis</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA644 (BISH, GOET [GOET019890], PTBG [PTBG 1000057483], US)	Maui	0.79	
<i>Melicope sessilis</i> (H.Lév.) T.G.Hartley & B.C.Stone	Appelhans MA654 (BISH, GOET [GOET019891], PTBG [PTBG 1000057519], US)	Maui	0.77	
<i>Melicope spathulata</i> A.Gray	Wood 16836 (PTBG [PTBG 1000059483])	Kaua'i		0.77
<i>Melicope stonei</i> K.R.Wood, Appelhans & W.L.Wagner	Wood 17505 (PTBG)	Kaua'i	0.81	
<i>Melicope volcanica</i> (A.Gray) T.G.Hartley & B.C.Stone (cf.)	Oppenheimer s.n. (silica sample only)	Lāna'i		0.69
<i>Melicope wawreana</i> (Rock) T.G.Hartley & B.C.Stone	Wood 17478 (PTBG)	Kaua'i	0.86	
Non-Hawaiian taxa				
<i>Melicope elleryana</i> (F.Muell.) T.G.Hartley	Lorence 6602 (PTBG)	cultivated National Tropical Botanical Garden, Kalaheo, Kaua'i, Hawaii		0.70
<i>Melicope elleryana</i> (F.Muell.) T.G.Hartley	Appelhans MA404 (LAE, US)	New Guinea		0.71
<i>Melicope elleryana</i> (F.Muell.) T.G.Hartley	Appelhans MA413 (LAE, US)	New Guinea		0.74
<i>Melicope frutescens</i> (Blanco) Appelhans & J.Wen	Brambach 464 (GOET)	Indonesia: Sulawesi		0.74
<i>Melicope latifolia</i> (DC.) T.G.Hartley	Lorence 10298 (PTBG [PTBG 1000027858])	cultivated National Tropical Botanical Garden, Kalaheo, Kaua'i, Hawaii		0.77
<i>Melicope mantellii</i> Buchanan	Pelser 3122 (GOET)	New Zealand		0.81
<i>Melicope maxii</i> T.G.Hartley		Indonesia: Sulawesi		0.77
<i>Melicope ternata</i> J.R.Forst.		cultivated Botanical		0.81

Continued

Table 2 Continued

Species	Herbarium voucher	Origin	C (pg) †	C (pg) ‡
& G.Forst. <i>Melicope triphylla</i> (Lam.) Merr.		Garden Göttingen cultivated Hortus Botanicus Leiden		0.87

Details for placement of herbarium vouchers and origin of samples are given. Herbarium acronyms are according to Index Herbariorum (<http://sweetgum.nybg.org/science/ih/>).

mean 1C value of all samples is 0.76 pg with a standard deviation of 0.05. The lowest and highest genome sizes were estimated for the Hawaiian *M. haupuensis* (St.John) T.G. Hartley & B.C.Stone and *M. peduncularis* (H.Lév.) T.G.Hartley & B.C.Stone with 1C = 0.65 pg and 1C = 0.87 pg, respectively. In samples using *Pisum sativum* as reference, estimated genome sizes were slightly higher (mean 1C = 0.8 pg). Four samples (*M. anisata* (H.Mann) T.G.Hartley & B.C.Stone [Appelhans MA665], *M. barbiger* A.Gray [Appelhans MA664], *M. barbiger* [Wood KW 16718] and *M. peduncularis* [Appelhans MA652]), that could be measured successfully with both available references, show a slightly higher 1C value when measured with *P. sativum* as a reference, indicating that there seems to be a slight bias introduced due to the different genome sizes of the references (Fig. 2).

Chromosome numbers for six *Melicope* species were newly determined, increasing the total number of assessed species to 25 (including *Acronychia* and *Comptonella*), of which 12 represent the Hawaiian lineage (Table 1). All new reports reveal chromosome numbers of $n = 18$ or $2n = 36$, as did the majority of the previous counts for the genus. Altogether 12 species with known chromosome numbers are represented in the flow cytometry taxon sampling, including two of the four species showing varying chromosome numbers (*M. ovata* (St. John & Hume) T.G.Hartley & B.C.Stone and *M. wawraeana* (Rock) T.G.Hartley & B.C.Stone). DNA content measured in these species does not deviate (compare Tables 1, 2).

4 Discussion

All newly reported chromosome numbers of Hawaiian *Melicope* exhibit $n = 18$ or $2n = 36$. Most Amyridoideae (Morton & Telmer, 2014) show identical or similar chromosome numbers (Kubitzki et al., 2011), so that we confirm *Melicope* to be a Palaeocene paleopolyploid. The DNA content of the genus *Melicope* as measured by flow cytometry is also reasonably uniform. None of the estimated DNA amounts represents one and a half times ($3n$) or twice ($4n$) that of any other. That includes the assessed specimens of *M. wawraeana* and *M. ovata*, of which earlier studies had indicated a shift in chromosome numbers (Guerra, 1984; Kiehn, 2005). Therefore we conclude that *Melicope* is characterized by a mean DNA amount of $2C = 0.76$ pg, which corresponds to the chromosome number $2n = 36$ (Fig. 3).

Guerra (1984) reported $2n = 72$ for *M. wawraeana*, which might indicate a polyploidization event on the Hawaiian Islands. Since our measurements did not support this result, we conclude that the species as a whole likely did not experience a shift in ploidy level. Instead, our result could indicate that there is an

individual or a population of *M. wawraeana* originating from a recent polyploidization event resulting in $2n = 72$ chromosomes. At least 11 genera in Rutaceae are facultative apomicts (Carman, 1997), a reproductive strategy highly associated with polyploidy (Asker & Jerling, 1992). As of yet reproduction in *Melicope* has not been researched, but *Zanthoxylum*, a distantly related genus within the same subfamily (Bayly et al., 2013) reproduces both by facultative apomixis and adventitious embryony, a strategy strongly associated with paleopolyploidy (Carman, 1997). With $2n = 136$ –144 several species of *Zanthoxylum* have the highest chromosome number known in the family (Kiehn & Lorence, 1996). The observed chromosome number of $2n = 72$ in an individual of *M. wawraeana* (Guerra, 1984) might therefore indicate the influence of apomixis or a recent hybridization event. However, since the species is a member of the youngest clade within Hawaiian *Melicope* (Appelhans et al., 2014b), this putative polyploidization event is not basal in the lineage but would have occurred on the Islands.

The only report of a lower ploidy level in a seedling of *M. ovata* (Kiehn, 2005; as *M. spec.*: $2n = 18$ for one seedling with three other seedlings from the same fruit exhibiting $2n = 36$) cannot be explained with certainty, but might be an effect of irregularities in embryogenesis.

There are only two other reports for *Melicope* of chromosome numbers deviating from $n = 2x = 18$. One is for *M. semecarpifolia*, which was assessed by Pancho (1971) with $n = 18$, but with $n = 12$ by Hsu (1968; as *Euodia confusa* Merr.). Figure 37 of this latter publication shows a drawing of an anaphase I stadium of pollen mother cell meiosis. While it cannot be excluded that the count is correct, the drawing could also be interpreted to show a higher number of chromosomes (personal observation M. Kiehn). All other accounts within section *Lepta* (Table 1) revealed $n = 18$ and $2n = 36$, respectively, so this seems to be an isolated deviation, as does the second deviating count of $2n = 32$ in *M. brassii* (Borgmann, 1964).

In summary it can be stated that Hawaiian *Melicope* are uniform in terms of chromosome numbers and 1C values (Fig. 3). Aberrations likely represent local events, e.g., disruptions in embryogenesis, possible hybridization events, chromosome loss or putative effects of apomixis. Also, there is no indication for a difference between Hawaiian representatives of the genus and the remainder of the genus indicating there was no polyploidization event prior to the colonization of the islands.

In terms of the traits for successful island colonization and adaptive radiation, it seems that at least Hawaiian *Melicope* do not exhibit features characteristic for many examples of lineages that colonized distant islands. While sampling herein is not sufficient to exclude polyploidy in all island radiations of the genus, we have shown that the Hawaiian colonizer was not a

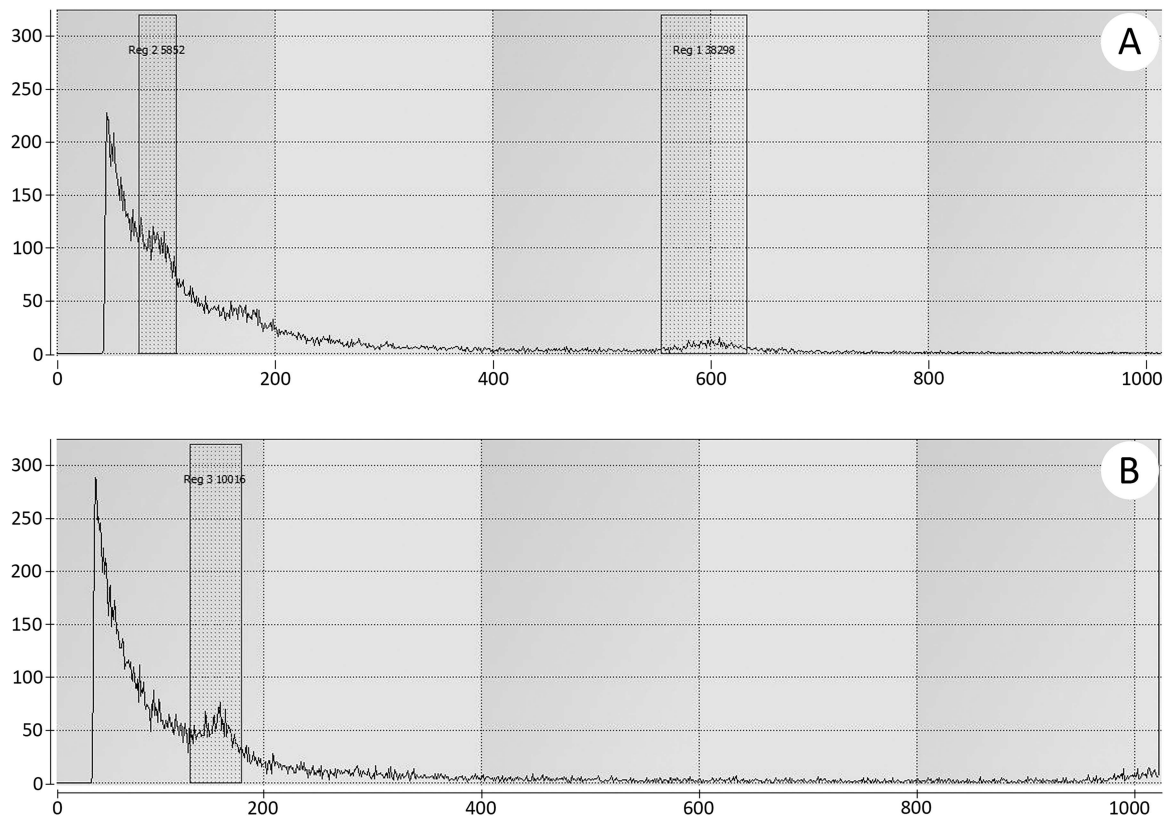


Fig. 2. Flow Cytometry measurements of *Melicope barbiger* A.Gray [Appelhans MA664] at gains 450 (A) and 480 (B). X-axis shows amount of particles at a given fluorescence intensity. Intensity peaks are marked (Reg 2 & 3, *M. barbiger*; Reg 1, reference *Pisum sativum*).

recently formed neo- or mesopolyploid. Woodiness is a pervading character of the whole genus (Hartley, 2001). Dioecy is present in two subsections of *Melicope*, *Pelea* and *Lepta* with the latter also containing monoecious species (Hartley, 2001). While the genus as a whole seems to show several shifts in breeding system (compare Hartley, 2001; Appelhans et al., 2014a), the dioecy of the Hawaiian lineage seems to be a trait acquired before the colonization. Up to now, a detailed study on the dispersibility of *Melicope* species has not been undertaken. However, the whole genus displays dehiscent fruits (follicles or capsules), with shiny black seeds, which remain attached upon dehiscence (Hartley, 2001). This, together with the spongy sarcotesta and the thick sclerotesta, likely represents an adaption to bird dispersal (Carlquist, 1966c; Hartley, 2001). This hypothesis is supported by field observations (Frith et al., 1976; Floyd, 1989; Innis, 1989; Hartley, 2001; Medeiros, 2004). Seed size varies in the genus – and indeed within the Hawaiian lineage ranging from relatively small ($\varnothing$ 2.5 mm) to several times that size (Wagner et al., 1999) showing no clear trend for reduction of spatial dispersibility by seed size on the island (Carlquist, 1966a).

There are three possible explanations for the apparent deviation of the genus from the generalist-colonizer-to-specialist-island-endemic pattern.

(1) The-odd-one-out. LDD events are very rare and therefore not governed by regular migration patterns (e.g., Carlquist, 1966a; Appelhans et al., accepted). Unusual behavior of

vectors, catastrophic events or uncommon vectors are suspected causes (Higgins et al., 2003; Nathan et al., 2008). Thus there is a significant element of chance to migration and establishment of a lineage on an island. While certain prerequisites increasing the likelihood of an establishment followed by adaptive radiation exist and researchers seem to have made strides in identifying them, chance might also be an influencing factor here. On the Juan Fernandez Islands 35.6% of the endemic flora is represented by species directly derived from their continental relatives (Stuessy et al., 1998) without any apparent radiation, despite some of them being a member of families renowned for successful island adaptive radiations. Chance may prevent an adaptive radiation in a lineage despite it meeting all identified predispositions or it may allow an ‘unexpected’ radiation in a lineage not exhibiting any of the facilitating factors. However, that is unlikely the case in *Melicope*, as the Hawaiian radiation is not an exception in an otherwise poorly distributed group. The genus has colonized numerous islands throughout the Pacific, and even colonized Madagascar and the Mascarene Islands radiating into ca. 20 spp. there (Appelhans et al., accepted). That many successful colonization events followed by adaptive radiation seem unlikely without the genus exhibiting predisposing traits. Due to the rarity of LDD events, exceptional occurrences (Higgins et al., 2003; Nathan et al., 2008), or vectors (Wenny et al., 2016) cannot be ruled out as causes for colonization of an island. However, the adaptations of

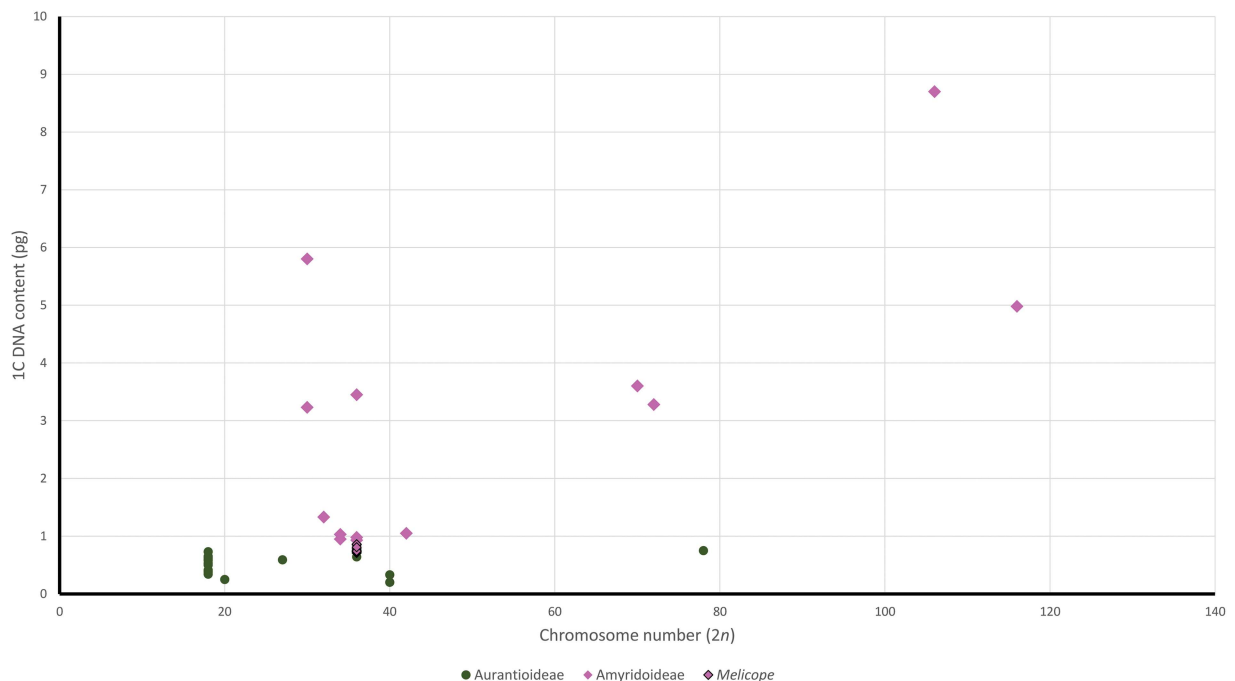


Fig. 3. Comparison of chromosome numbers and DNA content in 49 species of Rutaceae including newly assessed Hawaiian *Melicope* specimens. Values were extracted from the Kew C-value database (<http://data.kew.org/cvalues>; assessed on 01. 16. 2017). Green circles represent species in Aurantioideae and Rutoidae (base chromosome number $n=9$). Violet diamonds represent species in Amyridoideae (base chromosome number $n=18$). *Melicope* species are indicated by a black frame around the violet diamonds. There is no linear increase of DNA content with increasing ploidy levels. Instead the effects of diploidization can be observed in paleopolyploids with C-values comparable to diploids.

Melicope to bird dispersal (Hartley, 2001) seem to be the key feature facilitating high dispersibility as evidenced by the high number of successful island colonizations (Appelhans et al., 2014b).

(2) The hidden generalist. The vast majority of Hawaiian *Melicope* species are highly endemic (about 80% single-island endemics), with only a small number of species being more widespread (Hartley, 2001; Appelhans et al., 2014a). The relatively small distributional niches most of these species occupy certainly fit the picture of the island specialist with a very narrow distributional range. Carlquist (1966a) also observed a loss of dispersibility manifested as an increase in seed size in some species of *Melicope*. On the other hand these specialist Hawaiian lineages spawned two successful independent colonizations of the remote Marquesas Islands (distance > 3500 km) resulting in a local radiation of seven species (Appelhans et al., 2014a; accepted). Successful colonizations of oceanic islands with subsequent adaptive radiations originating from an insular lineage is a repeated occurrence in the genus (Appelhans et al., 2014a; accepted). This indicates the possibility of some species having a broader ecological capacity than suggested by the niches they are observed to occupy. The comparatively small distributional ranges of these species would then likely be due to competition. If this pressure is removed by transmission to another island system with a different species composition, the colonizer may occupy any fraction in a comparatively wide range of ecological conditions. This is corroborated by the fact that both colonizers of the Marquesas Islands are from clades

comprising narrowly distributed species (Appelhans et al., 2014a).

(3) The incomplete picture. Although evolutionary patterns on oceanic islands has been a research focus of biologists for more than 200 years, the application of molecular methods has been comparatively recent. Applying these methods to insular radiations and their continental relatives has helped confirm some and rescind other long standing theories. The high morphological diversity in island lineages has often lead to overestimation of the frequency of colonization events, e.g., in the Hawaiian lobeliads (Givnish et al., 2009) or Hawaiian *Cyrtandra* J.R.Forst. & G.Forst. (Cronk et al., 2005; Johnson et al., 2017), or of phylogenetic affiliations as in *Melicope* (Appelhans et al., 2014a), which are rectified by results of molecular investigations. However, most studies focus on resolving phylogenetic relationships (e.g., Givnish et al., 2009) or one specific trait of the island pattern, e.g., dispersal routes (e.g., Appelhans et al., accepted) or ploidy levels in lineages (e.g., Harbaugh, 2008) or archipelagos (e.g., Carr, 1998). Attempts of identifying underlying patterns are then made by synergy of these studies. Continued research into adaptive island radiations, especially comparison of displayed traits between species rich lineages and colonizers not undergoing radiation, could help to ultimately identify traits facilitating island adaptive radiations. As of now, the picture is most likely incomplete. For instance, the high proportion of polyploid lineages on islands (e.g., Stuessy et al., 1992; Carr, 1998) indicates polyploidy to be a positive trait. However, we do not have a clear picture here, yet. *Melicope*

are paleopolyploid having likely undergone extensive diploidization already as indicated by comparing chromosome counts and genome sizes in Rutaceae (Fig. 3). All investigated species of *Melicope* including all Hawaiian representatives show genome sizes highly similar to diploid Rutaceae. Therefore the genus has most likely undergone profound post-ploidization diploidization and may be regarded as genetically and cytologically diploidized. However as of yet there are no studies on the formation of bivalents during meiosis; so whether the species' are functionally diploid remains unclear. Research of the Hawaiian silversword alliance (Sakai et al., 1995), the Canarian *Argyranthemum* Webb (Asteraceae; Francisco-Ortega et al., 2000) or Pacific sandalwoods (Harbaugh, 2008) suggest a recent polyploidization prior to colonization. However, it is entirely unknown whether the colonizer spawning the polyploid Hawaiian lobeliads (Lammers, 1988; Kiehn, 2005) should be considered a neo-, meso- or paleopolyploid. Long term effects of polyploidization and the cytological mechanisms responsible for them are poorly understood (Wendel, 2015). While neopolyploids may exploit the effects of heterosis and gene redundancy (Comai, 2005), meso- and paleopolyploids may exploit ongoing diploidization to maintain genetic diversity over long periods of time (Hohmann et al., 2015). In fact there seems to be a correlation between increased genome downsizing, even beyond the size of the diploid ancestor, and increased diversification rates (Hohmann et al., 2015; Dodsworth et al., 2016). In *Arabidopsis thaliana* (L.) Heybh. ($n = 5$) and several other angiosperm species' genome reduction during post-polyploidization diploidization has led to a small number of chromosomes and obscured several WGD events (Leitch & Bennet, 2004; Hohmann et al., 2015). The same might be the case in several Hawaiian lineages, possibly even including *Melicope*. Applying genomic methods to Hawaiian plant lineages is required to reliably identify polyploids, their origin and diversity. In addition, even identifying the trait as 'polyploidy' might be misleading. It is entirely plausible, that polyploidy is merely a 'casualty' of the actual trait: hybrid origin. All of the aforementioned neo- and mesopolyploid lineages are allopolyploid and hybridization is suspected to facilitate adaptive radiations (Seehausen, 2013). Seemingly non-polyploid colonizers spawning successful lineages may still be the result of a homoploid hybridization. It has been shown that homoploid hybrid speciation can rapidly reach stability, especially when spatially separated from the parents (Seehausen, 2014). While there are no investigations yet regarding hybridization within Hawaiian *Melicope*, *M. mantellii* Buchanan on New Zealand was suggested to be a hybrid of the closely related *M. simplex* A.Cunn. and *M. ternata* (Cokayne & Allen, 1934; Wagner et al., 1999). If this is indeed true, it would constitute a case of homoploid hybrid speciation within the genus. Further investigations are needed to reach definitive conclusions regarding not just the trait polyploidy, but the entire pattern. Once we have clearly identified the pattern, we might find Hawaiian *Melicope* to meet it very well.

5 Conclusion

With successful colonizations of nearly all Pacific archipelagos, including the remote Hawaiian Islands in the East and

Madagascar and the Mascarene Islands in the West, as well as the only known instance of two independent colonizations of the Marquesas Islands within a single genus, *Melicope* shows a very high dispersal ability. Characteristics of successful colonizers were identified as the genomic flexibility a polyploidization event facilitates, herbaceousness, self-compatibility and high dispersal ability. Successful establishments are characterized by shifts to reduced dispersibility, outcrossing and secondary woodiness. In the case of *Melicope* the main driving factor for successful colonizations seems to be the adaption to bird dispersal. We have shown that the Hawaiian radiation of *Melicope* did not experience a recent polyploidization event prior to colonization of the islands. As the genus is woody and several lines show adaptations to outcrossing (i.e., dioecy), including the clade spawning the Hawaiian lineage, evolutionary shifts characteristic to establishment are observed in the entire genus, not merely in oceanic island lineages. In terms of reduced dispersibility on islands, the picture is not yet clear. Both an increase and a decrease in seed size have been observed, the latter being attributed to an adaption to bog habitats by Carlquist (1966c), but as to how this might affect dispersibility on a case by case basis is unclear. Future research of oceanic lineages will reveal, whether *Melicope* represent a lineage thriving on islands despite not expressing most traits associated with successful colonizations or if we have not yet identified important parts of the island evolution picture.

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