ORIGINAL ARTICLE

The Hawaiian Archipelago is a stepping stone for dispersal in the Pacific: an example from the plant genus *Melicope* (Rutaceae)

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ABSTRACT

Aim Pacific biogeographical patterns in the widespread plant genus *Melicope* J.R. Forst. & G. Forst. (Rutaceae) were examined by generating phylogenetic hypotheses based on chloroplast and nuclear ribosomal sequence data. The aims of the study were to identify the number of colonization events of *Melicope* to the Hawaiian Islands and to reveal the relationship of Hawaiian *Melicope* to the Hawaiian endemic genus *Platydesma* H. Mann. The ultimate goal was to determine if the Hawaiian Islands served as a source area for the colonization of Polynesia.

Location Nineteen accessions were sampled in this study, namely eight *Melicope* species from the Hawaiian Islands, four from the Marquesas Islands, one species each from Tahiti, Australia and Lord Howe Island, two Australian outgroups and two species of the Hawaiian endemic genus *Platydesma*. To place our results in a broader context, 19 sequences obtained from GenBank were included in an additional analysis, including samples from Australia, Papua New Guinea, New Zealand, Southeast Polynesia and Asia.

Methods DNA sequences were generated across 19 accessions for one nuclear ribosomal and three chloroplast gene regions. Maximum parsimony analyses were conducted on separate and combined data sets, and a maximum likelihood analysis was conducted on the combined nuclear ribosomal and chloroplast data set. A broader nuclear ribosomal maximum parsimony analysis using sequences obtained from GenBank was also performed. Geographic areas were mapped onto the combined chloroplast and nuclear ribosomal tree, as well as onto the broader tree, using the parsimony criterion to determine the dispersal patterns.

Results Phylogenetic analyses revealed that *Platydesma* is nested within *Melicope* and is sister to the Hawaiian members of *Melicope*. The Hawaiian *Melicope* + *Platydesma* lineage was a result of a single colonization event, probably from the Austral region. Finally, Marquesan *Melicope* descended from at least one, and possibly two, colonization events from the Hawaiian Islands.

Main conclusions These data demonstrate a shifting paradigm of Pacific oceanic island biogeography, in which the patterns of long-distance dispersal and colonization in the Pacific are more dynamic than previously thought, and suggest that the Hawaiian Islands may act as a stepping stone for dispersal throughout the Pacific.

Keywords

Hawaiian Islands, island biogeography, long-distance dispersal, *Melicope*, molecular phylogeny, Pacific biogeography, Rutaceae, stepping stone.

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INTRODUCTION

In the four decades since MacArthur & Wilson's (1967) seminal work *The Theory of Island Biogeography* much progress has been made in our understanding of the derivation of island flora and fauna. One fundamental principle of traditional island biogeographical theory is that plants and animals on isolated archipelagos stem from a one-way colonization event from more diverse regions such as continents, with attenuation of diversity to the most remote islands, sometimes dispersing in stepping-stone fashion across regions with smaller inter-archipelago distances (MacArthur & Wilson, 1967; Emerson, 2002). The advent of cladistic approaches to phylogenetics and the incorporation of molecular data have launched a new and exciting era of island biogeography, by revealing often unexpected long-distance dispersal patterns in both plants and animals (Funk & Wagner, 1995; Heaney, 2007), including dispersal between distant oceanic islands, between continents, and from oceanic islands to continents (e.g. Raxworthy *et al.*, 2002; Allan *et al.*, 2004; Filardi & Moyle, 2005; Glor *et al.*, 2005; Dávalos, 2007; Harbaugh & Baldwin, 2007).

The Hawaiian Archipelago is one of the most isolated island chains on Earth, more than 2600 km from its nearest continent to the east (Fig. 1). A majority of its diversity previously has been presumed to have been derived from an attenuation of palaeotropical biota from the western Pacific, through stepping-stone island chains, such as the Society and Marquesas Islands (Thorne, 1963; Carlquist, 1974; Wagner *et al.*, 1990; Mueller-Dombois & Fosberg, 1998; Gillespie, 2002). However, molecular phylogenetic hypotheses are revealing a much more complex pattern of long-distance dispersal throughout the Pacific, and have demonstrated that the Hawaiian biota is derived not only from the south and western Pacific (Howarth *et al.*, 1997; Wright *et al.*, 2000, 2001; Gemmill *et al.*, 2002; Nepokroeff *et al.*, 2003), Australia (Howarth *et al.*, 2003) and Southeast Asia (Hao *et al.*, 2004), but also from the Americas (Baldwin *et al.*, 1991; DeJoode & Wendel, 1992; Baldwin & Robichaux, 1995; Howarth *et al.*, 1997; Pax *et al.*, 1997; Vargas *et al.*, 1998; Costello & Motley, 2001; Lindqvist & Albert, 2002; Wanntorp & Wanntorp, 2003), the subarctic (Ballard & Sytsma, 2000; Wagner *et al.*, 2005) and even Africa (Seelanan *et al.*, 1997; Kim *et al.*, 1998).

As the last island archipelago in the chain of stepping-stone islands in the Pacific, and given its extreme isolation from any continental landmass, the Hawaiian Archipelago has been assumed to be a sink for dispersals, not a source (Carlquist, 1974). However, molecular phylogenetic analyses are revealing an emerging trend that the Hawaiian Islands can act as a stepping stone for the dispersal of plants and animals in the Pacific, and even as a source for continental colonization. Results from nuclear ribosomal, chloroplast, and low-copy nuclear sequence data have provided unequivocal evidence for two dispersals of the sandalwoods (*Santalum*, Santalaceae) from the Hawaiian Islands: (1) to the Bonin Islands, and (2) to Southeast Polynesia (Harbaugh & Baldwin, 2007; Harbaugh, 2008). In addition, molecular phylogenies have elucidated a pattern of dispersal from the Hawaiian Islands to Polynesia in the plant genera *Kadua* (Rubiaceae) (Motley, 2003) and *Plantago* (Plantaginaceae) (Hoggard *et al.*, 2003). The Hawaiian Islands also have been demonstrated to be a source area for a continental colonization in the fly genus *Scaptomyza* (Drosophilidae) (O'Grady & DeSalle, 2008).
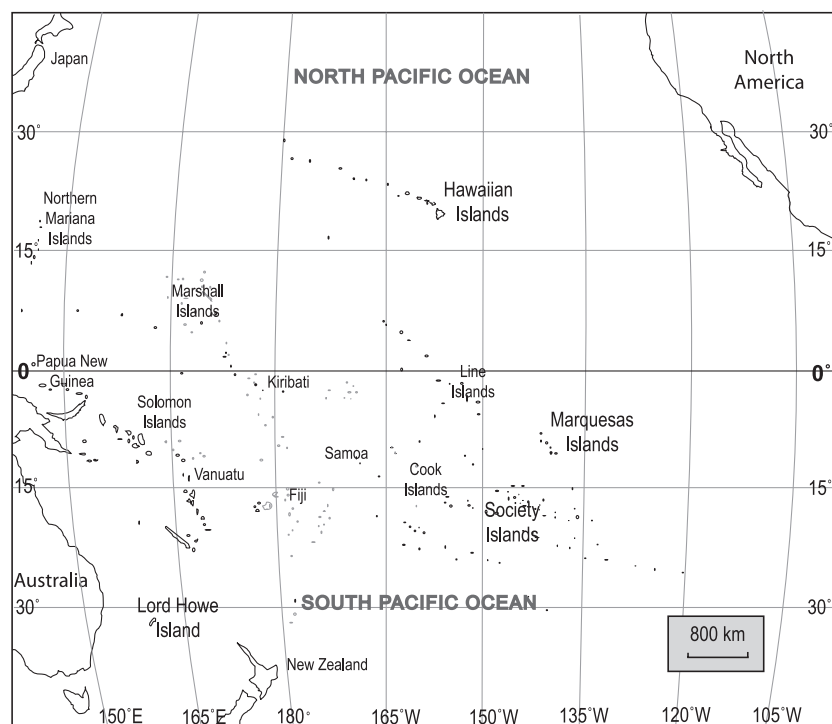

Figure 1 Map of the Pacific region showing locations of major island archipelagos and continental landmasses, some of which are areas where specimens were collected for this study.

Several authors have hypothesized that the two Marquesas Islands' endemic species (known at that time) in the plant genus *Melicope* J.R. Forst. & G. Forst. (Rutaceae) were derived from long-distance dispersal from the Hawaiian Islands (Stone, 1969; Carlquist, 1974; Hartley, 2000). *Melicope* is a moderate-sized genus with *c.* 230 species of shrubs and trees with a distribution ranging from the Malagasy and Indo-Himalayan regions, south to Australia and New Zealand, and east to eastern Polynesia and the Hawaiian Islands (Hartley, 2000) (Fig. 1). In the Hawaiian Islands, *Melicope* has had an explosive radiation, making it one of the largest flowering plant genera, with 47 species, in the islands (Stone *et al.*, 1999). Widespread habitat alteration and introduced mammals have led to the extinction of at least four Hawaiian *Melicope* taxa, leaving most of the other 43 taxa rare, vulnerable or endangered (Wagner *et al.*, 1999). Furthermore, all four species in a closely related endemic genus, *Platydesma* H. Mann, are also considered to be rare or vulnerable to extinction (Wagner *et al.*, 1999).

The Hawaiian *Melicope* lineage is hypothesized to trace back to a single colonization event from the Indo-Pacific region (Fosberg, 1948; Hartley, 2000). As in other Hawaiian adaptive radiations such as those of the silversword alliance (Baldwin *et al.*, 1990; Baldwin & Sanderson, 1998), the lobelioids (Givnish *et al.*, 1995, 1996) and the genus *Schiedea* (Wagner *et al.*, 1995, 2005), the Hawaiian *Melicope* have undergone spectacular morphological and ecological diversification (Stone, 1966). Species range from scandent shrubs to trees, up to 16 m in height (Carlquist, 1980; Stone *et al.*, 1999), and occur on recent lava flows, in bogs, or in upper dry forests and wet forests (Stone, 1969; Carlquist, 1974, 1980; Stone *et al.*, 1999) from 300 to 2000 m in elevation (Stone, 1969; Stone *et al.*, 1999). Although all species are hypothesized to be bird-dispersed, they have wide-ranging fruit and seed morphologies (Carlquist, 1974; Hartley, 2000). In addition, the breeding systems in Hawaiian *Melicope* are variable (Hartley, 2000). Delimitation of taxa in Hawaiian *Melicope* has been problematic, based as it is on many continuously varying morphological characters (Rock, 1918; Stone *et al.*, 1999); hybridization may have been involved in some cases (Stone *et al.*, 1999).

The Hawaiian and Marquesan species of *Melicope* all belong to section *Pelea* (Hartley & Stone, 1989; Stone *et al.*, 1999; Hartley, 2000). These members of *Melicope* are thought to be closely related to other Rutaceae of the South Pacific, including *Melicope*, *Euodia* J.R. Forst. & G. Forst. and *Acronychia* J.R. Forst. & G. Forst. (Stone, 1961, 1969; Carlquist, 1974; Hartley & Stone, 1989). Relationships between Hawaiian *Melicope* and another Hawaiian endemic genus in the family Rutaceae, *Platydesma* (four species), have puzzled taxonomists (Funk & Wagner, 1995). Earlier workers (Stone, 1962a; Stone *et al.*, 1999) suggested that *Platydesma* represents an introduction independent of the other Hawaiian Rutaceae. Various potential origins have been suggested, ranging from North America to Australia and New Caledonia (Stone, 1962a). However, Carlquist (1974) conjectured that the genus may have originated within the islands and represents a highly derived offshoot of *Melicope*. Whereas most members of Hawaiian *Melicope* are dioecious, flowers of *Platydesma* are hermaphroditic. Thus, if Carlquist's hypothesis is correct, this would represent a rare reversal from dioecy to bisexuality in the Hawaiian Islands. Examples of Hawaiian and Marquesan *Melicope* and *Platydesma* can be seen in Fig. 2.

In this study, sequence data from four commonly used non-coding DNA regions, including one nuclear ribosomal [internal transcribed spacer (ITS)] and three chloroplast (*trnL-F*, *trnE-T* and *trnH-psbA*) regions, were combined to produce a phylogenetic framework in which to explore the biogeographical patterns and relationships in *Melicope* and closely related genera. This phylogenetic hypothesis was used to investigate the following questions. (1) Did the Hawaiian members of *Melicope* result from a single colonization event? (2) Did the Marquesan *Melicope* arise from long-distance dispersal from the Hawaiian Islands? (3) Did the morphologically divergent genus *Platydesma* originate from Hawaiian *Melicope*? Finally, the importance of the Hawaiian Islands as a source area, or stepping stone, for dispersals will be discussed, focusing on flowering plant groups.

MATERIALS AND METHODS

Taxonomic sampling

We initially included in our study eight Hawaiian *Melicope* species, four *Melicope* species from the Marquesas Islands, and one species each of *Melicope* from Tahiti (Society Islands), Australia and Lord Howe Island (see Fig. 1 for a map of the Pacific region). Two Australian outgroups, *Acronychia oblongifolia* (Hook.) Endl. and *Euodia pubifolia* T.G. Hartley, were chosen to root the phylogenies after a number of potential outgroups had been tried and only these were alignable with *Melicope*. All taxonomic subgroups of Hawaiian *Melicope* were sampled, covering the breadth of morphological variation. Two *Platydesma* species from the Hawaiian Islands were also included in the study. A complete list of taxa, collection localities, voucher information, and GenBank accession numbers are provided in Appendix 1. The majority of the plant samples were collected and stored in silica gel prior to extraction. Two samples included in the analyses, *Melicope polybotrya* (C. Moore & F. Muell.) T.G. Hartley and *Acronychia oblongifolia*, were collected into CTAB (cetyl trimethyl ammonium bromide) solution from plants cultivated in the Canberra Botanical Garden and then shipped to the USA.

DNA extractions, amplification and sequencing

DNA was isolated from the first 19 accessions listed in Appendix 1 using the Qiagen DNeasy DNA isolation kit (Qiagen, Valencia, CA). Templates of the nuclear ribosomal DNA ITS region were amplified using the primers ITS5HP (Suh *et al.*, 1993) and ITS4 (White *et al.*, 1990). Three chloroplast spacer regions were amplified: *trnL-F* (using primers trnLc and trnLf; Taberlet *et al.*, 1991), *trnE-T* (using

Figure 2 Examples of Hawaiian (a, b) and Marquesan (c) *Melicope* and of the Hawaiian genus *Platydesma* (d–f). (a) Functionally female flowers of *Melicope clusiifolia* with calyx lobe and petal removed to view non-functional stamens and ovary. (b) Fruiting stem of *Melicope molokaiensis*. (c) Fruiting stem of *Melicope revoluta*. (d) Fruiting stem of *Platydesma spathulata*. (e, f) Flowers of *P. spathulata* showing connate stamens into a tube. Photographs by G. D. Carr (a, e, f), by W. L. Wagner (b, d) and by K. R. Wood (c).

primers trnE and trnTr; Zimmer *et al.*, 2002) and *trnH-psbA* [using primers psbA3′f (Sang *et al.*, 1997) and trnHf (Tate & Simpson, 2003)]. Standard protocols were used for the amplifications and sequencing.

Sequence alignments and phylogenetic analyses

Sequences were aligned manually with the aid of the sequence alignment editor Se-Al ver. 2.0a9 (Rambaut, 2002; Oxford, UK). Maximum parsimony (MP) analyses were performed in PAUP* ver. 4.0b10 (Swofford, 2002) on separate ITS, *trnL-F, trnE-T* and *trnH-psbA* data sets, on the combined chloroplast data set and on the combined data set for all four spacer regions. Characters were unordered and equally weighted. For the ITS and *trnL-F* data sets, a branch and bound search was performed, with default PAUP* settings. For the *trnE-T* and *trnH-psbA* data sets, a heuristic search with TBR (tree bisection–reconnection) branch-swapping, MulTrees on, and random addition with 10,000 replicates was performed.

Congruence between all of the separate gene data sets was tested before they were combined using the partition homogeneity test as implemented in PAUP*, with 1000 replicates, 10,000 random additional sequences per replicate, TBR branch swapping, and MulTrees off. Results indicated that there were no significant differences between the separate gene regions ($P = 0.88$), so they were combined for MP and maximum likelihood (ML) analyses. A MP analysis was performed on the combined chloroplast data set, and on the total data set (cp + nr DNA), using a heuristic search with TBR branch-swapping, MulTrees on, and random addition with 10,000 replicates. MP bootstrap values were computed in PAUP* on all of the individual data sets, as well as on the combined chloroplast and total data sets, by running 1000 replicates with a heuristic search, 10,000 random-addition sequences, TBR branch-swapping, and MulTrees off.

A ML analysis was run on the total data set (cp + nr DNA) using the best-fit model TIM + I + G selected by Akaike's information criterion (AIC) in MODELTEST ver. 3.6 (Posada & Crandall, 1998). A heuristic search was performed with 10 random-addition sequence replicates and TBR branch-swapping. ML bootstrap values were computed in PAUP* by running 100 replicates with a full heuristic search using 10 random-addition sequences, nearest-neighbour-interchange (NNI) branch-swapping, and MulTrees on.

To provide a broader context for our study, we conducted an additional MP analysis using 19 ITS sequences obtained from GenBank, which are listed in Appendix 1. All characters were unordered and equally weighted. A MP analysis was performed in PAUP* using a heuristic search with MulTrees on, TBR branch-swapping, 1000 random-addition sequences, with the search stopping after 1,000,000 rearrangements per replicate. MP bootstrap values were computed in PAUP* by running 1000 replicates with a heuristic search, 10,000 random-addition sequences, TBR branch-swapping, and MulTrees off.

Biogeographical analyses

Geographic areas were mapped onto the combined chloroplast and nuclear ribosomal tree (MP = ML), as well as onto the ITS MP tree with included GenBank sequences, using the parsimony criterion to determine the dispersal patterns. The geographic areas were converted into five unordered characters (1, Austral: Australia, Papua New Guinea; 2, Hawaiian Islands; 3, Polynesia: Marquesas Islands, Society Islands; 4, New Zealand: New Zealand and Lord Howe Island; 5, Asia) and traced onto the tree using MacClade ver. 4.06 (Maddison & Maddison, 2000). For all GenBank accessions, geographic areas were designated (see Appendix 1) based on the distribution for that taxon, as many of the exact localities of the actual samples used were unknown. To conservatively test for dispersal out of the Hawaiian Islands to the Marquesas, all taxa occurring in the Marquesas Islands and the Society Islands, and *Euodia hortensis* J.R. Forst. & G. Forst (which occurs from Papua New Guinea to the Society Islands) were scored as Polynesia. For the broader ITS tree with added GenBank sequences, one of the most parsimonious trees was randomly chosen, and any polytomies were randomly resolved (however, they did not affect the biogeographical patterns). Biogeographical areas were mapped onto the branches using both ACCTRAN (accelerated transformation) and DELTRAN (delayed transformation) optimization.
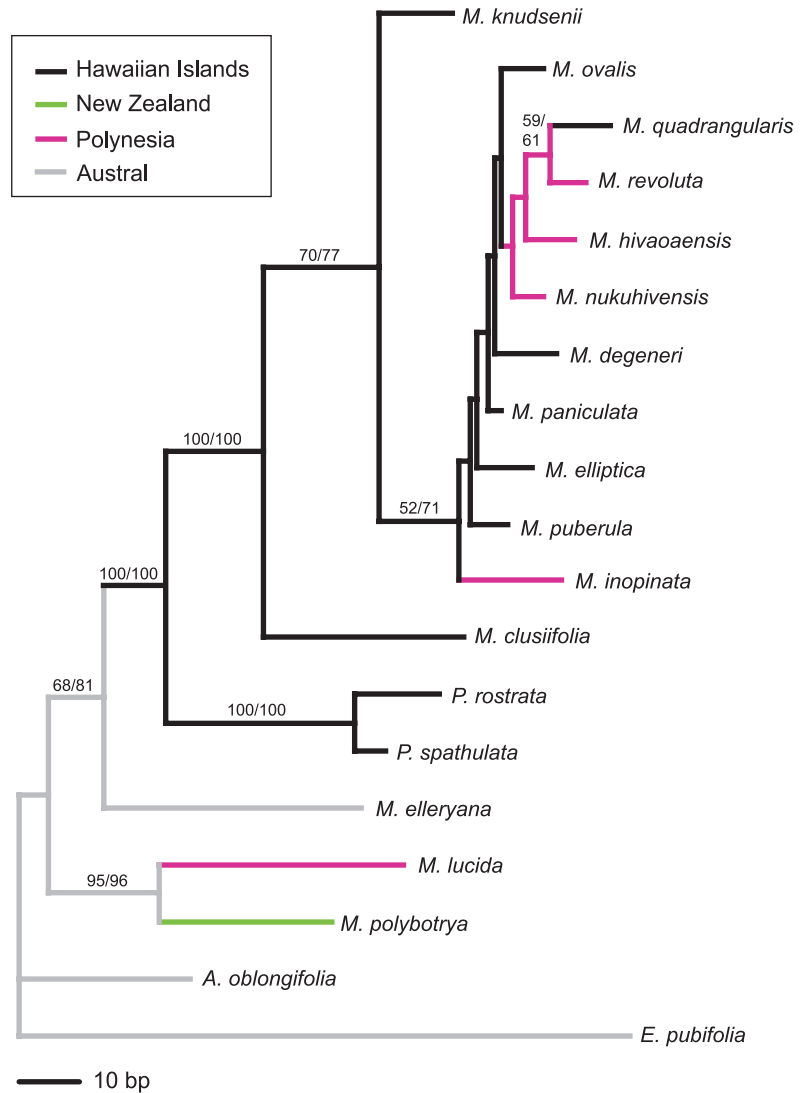
RESULTS

Maximum parsimony and maximum likelihood analyses

The total aligned length, number of parsimony-informative characters, shortest tree length, consistency and retention index values for separate, combined chloroplast, and total data set MP analyses are listed in Table 1, as are those for the broader ITS data set with added GenBank accessions. The aligned matrix contained a number of indels (insertions/deletions); however, no indels were phylogenetically-informative, either being autapomorphic or outgroup-specific. Each chloroplast spacer region analysed separately resulted in fairly unresolved topologies, but they were all consistent with one another (not shown). When the three chloroplast regions were combined, they resulted in an even better resolved phylogeny (not shown), which was also consistent with the restricted ITS phylogeny alone (not shown), except for the relationships between several of the Hawaiian *Melicope* and the Marquesan *Melicope*, which were poorly supported. When all of the chloroplast and the nuclear ribosomal genes were combined, they resulted in a single, completely resolved phylogeny (Fig. 3). The non-significant result of the partition homogeneity test ($P = 0.88$) indicates that all of the gene regions share a common history, and there is no evidence for hybridization. The results of the ML analysis of the combined data set resulted in a single tree with a topology identical to that from the MP analysis (Fig. 3; $-\ln L = -7047.71435$). The MP

Table 1 The statistics from the maximum parsimony analyses. The total aligned length, number of parsimony-informative characters, shortest tree length, consistency index (CI) and retention index (RI) values for all separate, combined chloroplast and total data sets using sequences generated in this study are listed, as are those for the broader internal transcribed spacer (ITS) data set that includes sequences obtained from GenBank.

Spacer/combination	Total number of characters	No. parsimony-informative characters	Total no. trees	Length	CI	RI
ITS	630	60	60	216	0.8148	0.7740
trnL-F	1027	28	876	127	0.7638	0.5833
trnE-T	909	11	1144	46	0.9565	0.9167
trnH-psbA	492	11	16,230	55	0.9636	0.9444
cpDNA	2428	50	542	231	0.8398	0.7197
cp + nr DNA	3058	110	1	453	0.8168	0.7314
ITS w/GenBank	755	128	640	316	0.7310	0.8552

Figure 3 The combined chloroplast and nuclear ribosomal phylogram from the maximum parsimony (MP) analysis using sequences obtained in this study. The topologies of the MP and maximum likelihood (ML) phylogenies are identical. The genera are abbreviated with the following letters: *A. Acronychia*; *E. Euodia*; *M. Melicope* and *P. Platydesma*. Numbers above branches represent MP bootstrap values (first) and ML bootstrap values (second). Geographical areas are mapped onto the phylogeny as described in the Materials and Methods section.

analysis of the broader ITS phylogeny with added GenBank accessions also resulted in a well-resolved and fairly well-supported phylogeny (Fig. 4), consistent with the more restricted sampling in the combined phylogeny (Fig. 3), although the Marquesan *Melicope revoluta* J. Florence, *Melicope hivaoaensis* J. Florence, and *Melicope nukuhivensis* (F. Brown) T.G. Hartley & B.C. Stone are resolved in a weakly supported polytomy.

Taxonomy

Both the restricted combined analysis in Fig. 3 and the broader ITS phylogeny in Fig. 4 clearly demonstrate that the genus *Platydesma* is nested within the genus *Melicope* and is sister to the clade of *Melicope* section *Pelea* from the Hawaiian and Marquesan Islands. Fig. 4 also shows that Hartley's (2000) *Melicope* sections *Pelea, Lepta* and *Melicope* are monophyletic,
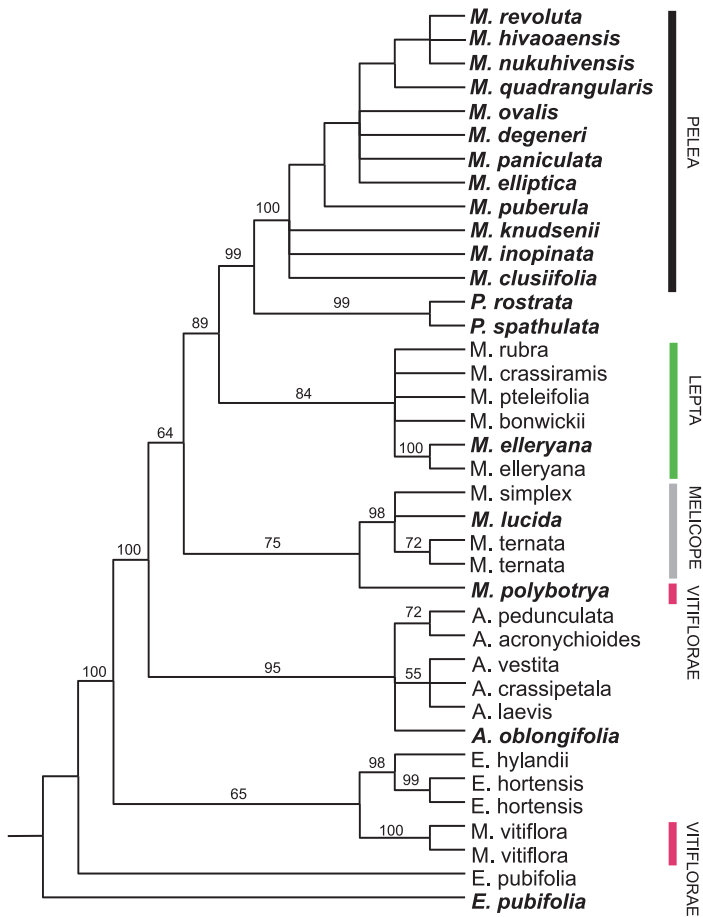
Figure 4 The internal transcribed spacer (ITS) strict-consensus maximum parsimony (MP) phylogeny using sequences generated in this study and obtained from GenBank. The genera are abbreviated with the following letters: *A. Acronychia*; *E. Euodia*; *M. Melicope* and *P. Platydesma*. Numbers above branches represent bootstrap values. Taxon names in bold italics represent samples sequenced in this study, whereas the others are samples obtained from GenBank. Along the right side are *Melicope* section names from Hartley (2000).

whereas section *Vitiflorae* is polyphyletic, with *Melicope vitiflora* nested within the genus *Euodia*.

Biogeography

When the biogeography was mapped onto the combined phylogeny (Fig. 3), the ACCTRAN and DELTRAN optimization resulted in the same biogeographical pattern, except that the branch leading to *M. polybotrya* (C. Moore & F. Muell.) T.G. Hartley and *Melicope lucida* (A. Gray) A.C. Sm. is unresolved as originating either in Lord Howe Island (New Zealand) or in Tahiti (Polynesia). When the biogeographical areas were mapped onto the broader ITS phylogeny (Fig. 5) both ACCTRAN and DELTRAN optimization resulted in identical patterns of dispersal.

Both the more restricted combined analysis (Fig. 3) and the broader ITS phylogeny (Fig. 5) provide unequivocal evidence for a single dispersal event to the Hawaiian Islands, probably from the Austral region, leading to both *Platydesma* and *Melicope* section *Pelea*. The most parsimonious reconstruction of biogeography using the combined analysis (Fig. 3) shows a second dispersal from the Marquesas Islands to the Hawaiian Islands; however, there is no support for this node, and this pattern is not evident in the broader ITS analysis (Figs 4 & 5). Both analyses also show two separate dispersal events out of the Hawaiian Islands to the Marquesas (Figs 3 & 5) although

the nodes separating the two Marquesan lineages are not well supported. However, the data show unequivocally that there was at least one dispersal event out of the Hawaiian Islands to the Marquesas Islands.

DISCUSSION

Taxonomy

The phylogenetic analyses in this study clearly identify a Hawaiian origin of the genus *Platydesma*, as Carlquist (1974) had hypothesized, which is consistent with botanist Asa Gray's classification of *Platydesma* as species of *Melicope* (Stone, 1962b). *Platydesma* is morphologically distinctive from the Hawaiian *Melicope*; therefore, this is an example of the divergent evolution that often arises in an insular setting. Whereas most members of Hawaiian *Melicope* are dioecious, flowers of *Platydesma* are hermaphroditic, representing a rare reversal from dioecy to bisexuality.

A comparison of the MP topology of the ITS analysis shown in Fig. 3 with the ITS jackknife tree of Poon *et al.* (2007, their figure 1) shows that they are consistent. Although our analysis sampled more sections of *Melicope* than were sampled in Poon *et al.* (2007), both analyses recovered a close relationship between sections *Lepta* and *Melicope*, as well as the sister relationship between the genus *Acronychia* and the main
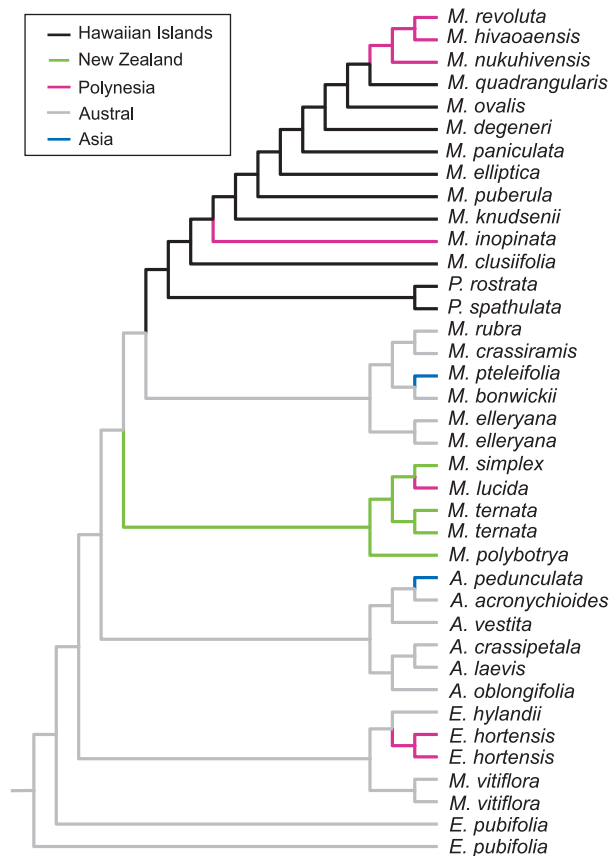
Figure 5 One of the 640 most parsimonious internal transcribed spacer (ITS) phylogenies with the polytomies randomly resolved. The genera are abbreviated with the following letters: *A. Acronychia*; *E. Euodia*; *M. Melicope* and *P. Platydesma*. Biogeographical areas are mapped onto the branches of the phylogeny. ACCTRAN (accelerated transformation) and DELTRAN (delayed transformation) optimization of geographical areas are identical. See text for explanation of geographical areas.

lineage of *Melicope*, followed by a more distant sister relationship with the genus *Euodia*, with *M. vitiflora* (F. Muell.) T.G. Hartley nested within *Euodia*. These relationships are consistent with those of Hartley (1974, 1981), who suggested that *Melicope* is most closely related to *Acronychia*. Our analysis added samples of section *Pelea* that were not included in Poon *et al.* (2007), confirming the sister relationship of sections *Pelea* (global) and *Lepta*, as hypothesized by Hartley (2000). The relationships of sections *Vitiflorae* and *Melicope* hypothesized by Hartley (2000) were not recovered in these analyses.

Biogeography

The main biogeographical conclusions from this study are the single origin for the Hawaiian *Melicope* + *Platydesma* and the dispersal of *Melicope* out of the Hawaiian Islands to the Marquesas. Although the nodes separating the two lineages of Marquesan *Melicope* (*Melicope inopinata* and *M. revoluta* + *M. nukuhivensis* + *M. hivaoaensis*) are not well supported, the two Marquesan lineages are widely separated, with at least six

nodes between them. In Hartley's (2000) diagnostic key he clearly isolates the Marquesan *M. inopinata* from the other Marquesan species based on carpel connation and petiole length. Therefore, based on molecular and morphological evidence, we conclude that there were probably two dispersal events out of the Hawaiian Islands. Further taxonomic sampling and additional DNA sequence regions are needed before we can come to an understanding of the patterns of dispersal within the Hawaiian Islands.

The results from *Melicope* join mounting evidence suggesting that the Hawaiian Islands are *not* the last-stop in dispersal but can act as a source for colonization of other areas across the Pacific. In 1992, Fosberg asserted that only one or two plant species in Polynesia originated in the Hawaiian Islands; since then, molecular phylogenetic evidence has identified Hawaiian origins for many more flowering plant groups distributed elsewhere in the Pacific, including *Melicope* (this study), *Santalum* (Harbaugh & Baldwin, 2007; Harbaugh, 2008), *Kadua* (I. Groeninckx, personal communications) and *Plantago* (Plantaginaceae) (Hoggard *et al.*, 2003). Several other flowering plant groups, including *Tetramolopium* (Okada *et al.*, 1997; Lowrey *et al.*, 2005), *Bidens* (Ganders *et al.*, 2000), *Ilex* (Cuénoud *et al.*, 2000; Manen *et al.*, 2002) and *Cyrtandra* (Cronk *et al.*, 2005) have sister clades in the South Pacific, leaving open the possibility of dispersal out of the Hawaiian Islands to the South Pacific. These trees join a number of other molecular phylogenies that have revealed single colonizations to the Hawaiian Islands of unresolved origin that should be re-examined for the possibility of dispersal out of the Hawaiian Islands (lobelioids: Givnish *et al.*, 1996, 2006; *Korthalsella*: Molvray *et al.*, 1999; *Vaccinium*: Powell & Kron, 2002; *Euphorbia*: Steinmann & Porter, 2002; *Leucaena*: Hughes *et al.*, 2003; *Chamaesyce*: Morden & Gregoritza, 2005). Molecular phylogenetic data will be necessary to elucidate the exact dispersal patterns, but, based on morphology, distribution and the centres of diversity, several other flowering plant groups are hypothesized by us to have dispersed out of the Hawaiian Islands, including *Pritchardia* (Arecaceae) to Southeast Polynesia and Fiji, *Charpentiera* (Amaranthaceae) to the Austral Islands, *Cheirodendron* (Araliaceae) to the Marquesas Islands, *Phyllostegia* (Lamiaceae) to Tahiti and *Leptecophylla* (Epacridaceae) to Polynesia.

The previous assumption that the Hawaiian Islands were a sink rather than a source for dispersal was based on the extreme isolation of the islands from any continental source and on their position at the end of a chain of intermediate stepping-stone archipelagos in the south and west Pacific. However, as molecular phylogenies are revealing, the Hawaiian Islands have received biota not only from the south and western Pacific, but also from nearly all corners of the Earth. Therefore, the discovery of the Hawaiian Islands as a stepping stone for dispersal to other parts of the Pacific, and to continents, should not be surprising. The varied, and often surprising, patterns of Pacific Island biogeography suggest that dispersal patterns are much more complex than simple attenuation of diversity from regions of high diversity, through

stepping-stone islands, and that ocean currents, wind and movement of birds may be more important.

Including *Melicope*, all flowering plant genera exhibiting the pattern of dispersal out of the Hawaiian Islands are bird-dispersed, either internally or externally (Carlquist, 1967). Ballard & Sytsma (2000) reviewed the current bird migration routes to the Hawaiian Islands and determined that a much higher percentage of birds move southwards from Eastern Asia and North America than northwards from the Austral region and Polynesia. Shorebirds, such as the Pacific golden plover (*Pluvialis fulva*), which migrate from Alaska, through the Hawaiian Islands, to Polynesia, are likely vectors for the dispersal of these fleshy-fruited species (Henshaw, 1910; Carlquist, 1967; Johnstone & McFarlane, 1967) such as *Melicope*. These birds may have used the equatorial Line Islands, guyots, or now vanished islands as stepping stones for long-distance dispersal from the Hawaiian Islands to other areas of the Pacific (Hartley, 2000).

ACKNOWLEDGEMENTS

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BIOSKETCH

Danica T. Harbaugh is currently a postdoctoral fellow in the Department of Botany at the National Museum of Natural History and the Laboratory of Conservation and Evolutionary Genetics at the Smithsonian Institution, Washington, DC. Her research focuses on reconstructing the phylogenetic and biogeographical patterns of a number of Pacific Island plant groups, including *Santalum* (Santalaceae) and *Metrosideros* (Myrtaceae) using DNA sequence data and morphology, as well as the genetic diversity and phylogeographic patterns of Hawaiian populations using microsatellite data. In addition, she is interested in examining the modes and mechanisms of long-distance dispersal to and colonization of oceanic islands, particularly the roles of hybridization and polyploidy.

Editor: David Bellwood

APPENDIX 1

Specimens of *Acronychia, Euodia, Melicope* and *Platydesma* sampled in this study, including their collection locality, voucher information and GenBank accession numbers. Herbarium abbreviations follow standard herbarium acronyms from the Index Herbariorum. The first 19 specimens listed are those sequenced in this study. These are followed by the 19 ITS sequences of *Acronychia*, *Euodia* and *Melicope* obtained from GenBank, including their collection locality and GenBank accession numbers.

Taxon Authority–Locality; *Voucher specimen* (Herbarium); GenBank Accessions ITS, *trnL-F*, *trnE-T*, *trnH-psbA*: **Acronychia oblongifolia** (Hook) Endl.–New South Wales, Australia; *Winsbury et al. 97* (CBG); EU493185, EU493242, EU493223, EU493204. **Euodia pubifolia** T.G. Hartley–Australia; *Sankowsky 1711*; EU493186, EU493243, EU493224, EU493205. **Melicope clusiifolia** (A. Gray) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wagner & Wood 6900* (PTBG); EU493178, EU493235, EU493216, EU493197. **Melicope degeneri** (B.C. Stone) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wood 7445* (PTBG); EU493179, EU493236, EU493217, EU493198. **Melicope elleryana** (F. Muell.) T.G. Hartley–Queensland, Australia; *Lorence 6602* (PTBG), EU493184, EU493241, EU493222, EU493203. **Melicope elliptica** A. Gray–O'ahu, Hawaiian Islands, USA; *Wagner & Lau 6905* (US); EU493180, EU493237, EU493218, EU493199. **Melicope hivaoaensis** J. Florence–Hiva Oa, Marquesas Islands, French Polynesia; *Meyer 826*; EU493173, EU493230, EU493211, EU493192. **Melicope inopinata** J. Florence–Nuku Hiva, Marquesas Islands, French Polynesia; *Meyer 887*; EU493176, EU493233, EU493214, EU493195. **Melicope knudsenii** (Hillebr.) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wagner & Wood 6891* (US); EU493168, EU493225, EU493206, EU493187. **Melicope lucida** (A. Gray) A.C. Sm.–Tahiti, Society Islands, French Polynesia, *Meyer 808*; EU493177, EU493234, EU493215, EU493196. **Melicope nukuhivensis** (F. Brown) T.G. Hartley & B.C. Stone–Nuku Hiva, Marquesas Islands, French Polynesia; *Meyer 889*; EU493175, EU493232, EU493213, EU493194. **Melicope ovalis** (St. John) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wood 7682* (PTBG), EU493169, EU493226, EU493207, EU493188. **Melicope paniculata** (St. John) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wood 7340* (PTBG); EU493171, EU493228, EU493209, EU493190. **Melicope polybotrya** (C. Moore & F. Muell.) T.G. Hartley–Lord Howe Island; *Hutton 284* (CBG); EU493183, EU493240, EU493221, EU493202. **Melicope puberula** (St. John) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wagner & Wood 6895* (US); EU493172, EU493229, EU493210, EU493191. **Melicope quadrangularis** (St. John & Hume) T.G. Hartley & B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wood et al. 0859* (PTBG); EU493170, EU493227, EU493208, EU493189. **Melicope revoluta** J. Florence–Nuku Hiva, Marquesas Islands, French Polynesia; *Meyer 888*; EU493174, EU493231, EU493212, EU493193. **Platydesma rostrata** Hillebr.–Kaua'i, Hawaiian Islands, USA; *Wood 8223* (PTBG); EU493181, EU493238, EU493219, EU493200. **Platydesma spathulata** (Gray) B.C. Stone–Kaua'i, Hawaiian Islands, USA; *Wagner & Wood 6893* (US); EU493182, EU493239, EU493220, EU493201.

Taxon Authority–Locality; GenBank Accession ITS: **Acronychia arcronychiodes** (F. Muell.) T.G. Hartley–Australia; DQ225819. **Acronychia crassipetala** T.G. Hartley–Australia; DQ225818. **Acronychia laevis** J.R. Forst. & G. Forst.–Australia; DQ225817. **Acronychia pedunculata** Miq.–Hong Kong; DQ225816. **Acronychia vestita** F. Muell.–Australia; DQ225820. **Euodia hortensis** J.R. Forst. & G. Forst.–Papua New Guinea, Tonga, Samoa, Cook Islands, or Society Islands, French Polynesia; DQ225812. **Euodia hortensis** J.R. Forst. & G. Forst.–Papua New Guinea, Tonga, Samoa, Cook Islands, or Society Islands, French Polynesia; DQ225813. **Euodia hylandii** T.G. Hartley–Australia; DQ225814. **Euodia pubifolia** T.G. Hartley–Australia; DQ225815. **Melicope bonwickii** (F. Muell.) T.G. Hartley–Australia or Papua New Guinea; DQ225809. **Melicope crassiramis** (K. Schum.) T.G. Hartley–Papua New Guinea; DQ499138. **Melicope elleryana** (F. Muell.) T.G. Hartley–Australia; DQ225808. **Melicope pteleifolia** (Champ. ex Benth.) T.G. Hartley–Hong Kong, Vietnam, or Taiwan; DQ225806. **Melicope rubra** (Lauterb. & K. Schum.) T.G. Hartley–Australia; DQ225807. **Melicope simplex** A. Cunn.–New Zealand; DQ499137. **Melicope ternata** J.R. Forst. & G. Forst.–New Zealand; DQ225805. **Melicope ternata** J.R. Forst. & G. Forst.–New Zealand; DQ225805. **Melicope vitiflora** (F. Muell.) T.G. Hartley–Australia; DQ225810. **Melicope vitiflora** (F. Muell.) T.G. Hartley–Australia; DQ225811.