



ORIGINAL
ARTICLE



Area and the rapid radiation of Hawaiian *Bidens* (Asteraceae)

Matthew L. Knope^{1*}, Clifford W. Morden², Vicki A. Funk³
and Tadashi Fukami¹

¹Department of Biology, Stanford University,
371 Serra Mall, Stanford, CA 94305, USA,

²Department of Botany and PCSU, University
of Hawaii, Manoa, 3190 Maile Way,

Honolulu, HI 96822, USA, ³Department of
Botany, Smithsonian Institution, PO Box
37012, Washington, DC 20013-7012, USA

ABSTRACT

Aim To estimate the rate of adaptive radiation of endemic Hawaiian *Bidens* and to compare their diversification rates with those of other plants in Hawaii and elsewhere with rapid rates of radiation.

Location Hawaii.

Methods Fifty-nine samples representing all 19 Hawaiian species, six Hawaiian subspecies, two Hawaiian hybrids and an additional two Central American and two African *Bidens* species had their DNA extracted, amplified by polymerase chain reaction and sequenced for four chloroplast and two nuclear loci, resulting in a total of approximately 5400 base pairs per individual. Internal transcribed spacer sequences for additional outgroup taxa, including 13 non-Hawaiian *Bidens*, were obtained from GenBank. Phylogenetic relationships were assessed by maximum likelihood and Bayesian inference. The age of the most recent common ancestor and diversification rates of Hawaiian *Bidens* were estimated using the methods of previously published studies to allow for direct comparison with other studies. Calculations were made on a per-unit-area basis.

Results We estimate the age of the Hawaiian clade to be 1.3–3.1 million years old, with an estimated diversification rate of 0.3–2.3 species/million years and 4.8×10^{-5} to 1.3×10^{-4} species Myr⁻¹ km⁻². *Bidens* species are found in Europe, Africa, Asia and North and South America, but the Hawaiian species have greater diversity of growth form, floral morphology, dispersal mode and habitat type than observed in the rest of the genus world-wide. Despite this diversity, we found little genetic differentiation among the Hawaiian species. This is similar to the results from other molecular studies on Hawaiian plant taxa, including others with great morphological variability (e.g. silverswords, lobeliads and mints).

Main conclusions On a per-unit-area basis, Hawaiian *Bidens* have among the highest rates of speciation for plant radiations documented to date. The rapid diversification within such a small area was probably facilitated by the habitat diversity of the Hawaiian Islands and the adaptive loss of dispersal potential. Our findings point to the need to consider the spatial context of diversification – specifically, the relative scale of habitable area, environmental heterogeneity and dispersal ability – to understand the rate and extent of adaptive radiation.

Keywords

Adaptive radiation, Asteraceae, carrying capacity, Compositae, diversification rate, endemism, extinction, island evolution, islands, speciation.

*Correspondence: Matthew Knope, Department of Biology, 371 Serra Mall, Stanford University, Stanford, CA 94305, USA.
E-mail: knope@stanford.edu

INTRODUCTION

Remote islands have played a central role in the development of evolutionary theory, particularly with regard to the factors

responsible for speciation and adaptive radiation (Darwin, 1859; Lack, 1947; Carlquist, 1974; Schluter, 2000). The Hawaiian Islands (about 4000 km from the nearest land mass) are noteworthy in this regard, as adaptive radiation is

prominent in numerous and phylogenetically diverse plant families (Wagner & Funk, 1995; Wagner *et al.*, 1999; Ziegler, 2002; Keeley & Funk, 2011). One of the best known and most well documented of these is the silversword alliance (Asteraceae or Compositae), with at least 30 species found in a vast array of habitats and all descended from a single recent common ancestor (Baldwin & Sanderson, 1998; Carlquist *et al.*, 2003). Similarly, Hawaiian *Bidens* (Asteraceae), lobeliads (Campanulaceae), mints (Lamiaceae), *Cyrtandra* (Gesneriaceae) and *Schideia* (Caryophyllaceae), among others, have many morphologically diverse species, now found in a wide range of habitats, and each lineage is thought to be derived from a single ancestor that colonized Hawaii (Wagner & Funk, 1995; Price & Wagner, 2004).

One of the most striking features of these spectacular Hawaiian plant radiations is the small geographical area in which they have occurred and the short time period over which speciation has taken place (Price & Wagner, 2004; Baldwin & Wagner, 2010; Keeley & Funk, 2011). The total area of the main Hawaiian Islands is 16,644 km² (about half the size of Belgium), and the oldest among the current high islands is Kauai, which formed *c.* 4.7 million years ago (Ma) (Price & Clague, 2002). Consequently, Hawaiian radiations are generally relatively young, occupying a limited but highly diverse geographical area. Dispersal mechanisms have also changed from those that facilitated long-distance dispersal in their ancestors to ones favouring more limited, local dispersal (Carlquist, 1974). In contrast, continental radiations often involve geographical areas that are orders of magnitude larger, longer time spans and multiple mechanisms to promote dispersal away from the parental populations (e.g. Hughes & Eastwood, 2006; Valente *et al.*, 2010).

In understanding the determinants of the rate and extent of adaptive radiation, the effect of area has recently received increasing attention (e.g. Rosenzweig, 1995; Losos & Schluter, 2000; Gavrillets & Vose, 2005; Seehausen, 2006; Whittaker *et al.*, 2008; Gillespie & Baldwin, 2010; Losos & Parent, 2010). There are several reasons proposed for the effect of area, with the following appearing most often in the literature. First, the diversity of habitats within an area tends to increase with area, providing more opportunities for speciation by divergent natural selection (Ricklefs & Lovette, 1999; Losos & Schluter, 2000; Gavrillets & Vose, 2005; Losos & Parent, 2010). Second, the opportunity for geographical isolation within an area (allopatric speciation) increases with area (MacArthur & Wilson, 1967; Gavrillets & Vose, 2005). Third, population size also generally increases with area (MacArthur & Wilson, 1967; Gavrillets & Vose, 2005), providing more mutations upon which selection can act (Lenski *et al.*, 1991; Hall *et al.*, 2010). Additionally, in large populations there is a greater probability of propagules dispersing to suitable habitats, producing, in some cases, species-rich genera that range over large areas [e.g. *Lupinus* (Fabaceae), *Astragalus* (Fabaceae), *Senecio* (Asteraceae), *Poa* (Poaceae) and others].

Despite much theoretical development, relatively few empirical studies of adaptive radiation have considered the

effect of area explicitly. The adaptive radiation of the 19 species and eight subspecies of endemic Hawaiian *Bidens* offers a particularly good system in which to examine the effect of area on diversification rate because they are a young lineage that has radiated within a confined area with a well-known geological history. In this paper, we present molecular phylogenetic evidence that, on a per-unit-area basis, the speciation rates of Hawaiian *Bidens* are among the highest of plant adaptive radiations documented to date. We propose that two main drivers of allopatric speciation caused this rapid radiation: the great diversity of ecological opportunities in Hawaii and the adaptive loss of long-distance dispersal potential by achenes (dry fruits). Based on our findings, we propose that young clades can be used to provide detailed explanations for rates of speciation using the spatial scale of habitat availability in combination with the dispersal potential of the organisms under study.

MATERIALS AND METHODS

Study system

The 19 species of *Bidens* endemic to the Hawaiian Islands display greater morphological and ecological diversity than the other *c.* 320 currently described species in the genus, even though the genus is found on five continents (Ganders & Nagata, 1984). *Bidens* occur on all six of the main Hawaiian Islands (Kauai, Oahu, Maui, Lanai, Molokai, Hawaii) and can be found from sea level to 2200 m elevation in bogs, woodlands, scrublands, rain forests, cinder deserts, sand dunes and lava flows (Fig. 1). It appears that adaptive shifts have occurred in seed (achene) dispersal mode (Carlquist, 1980, see plate on p. 164), pollination syndrome and growth form (Carlquist, 1974; Ganders & Nagata, 1984; Carr, 1987).

There has been no previous attempt to estimate the age or rate of the Hawaiian *Bidens* diversification, although it is probably relatively recent. Of the five Hawaiian *Bidens* species and two Hawaiian hybrids previously surveyed, all had identical sequences of nuclear ribosomal internal transcribed spacer (ITS) DNA (Ganders *et al.*, 2000), suggesting that the radiation has occurred within the time span provided by the present high islands (< 5 Myr). However, due to low taxonomic sampling of Hawaiian *Bidens*, this study was limited in its ability to address how recently the radiation may have occurred. Additionally, Helenurm & Ganders (1985) found little divergence at isozyme loci amongst 15 Hawaiian *Bidens* species surveyed, also suggesting recent colonization and radiation. In contrast, Gillett (1975) proposed that the great morphological diversity of this clade pointed to a long history in the Hawaiian Islands, extending back to colonization of the north-western Hawaiian Islands prior to the formation of Kauai (*c.* 4.7 Ma; Price & Clague, 2002). Further, without the inclusion of all Hawaiian *Bidens* species and their putative sister clades, the hypothesized monophyly of the Hawaiian radiation has not been

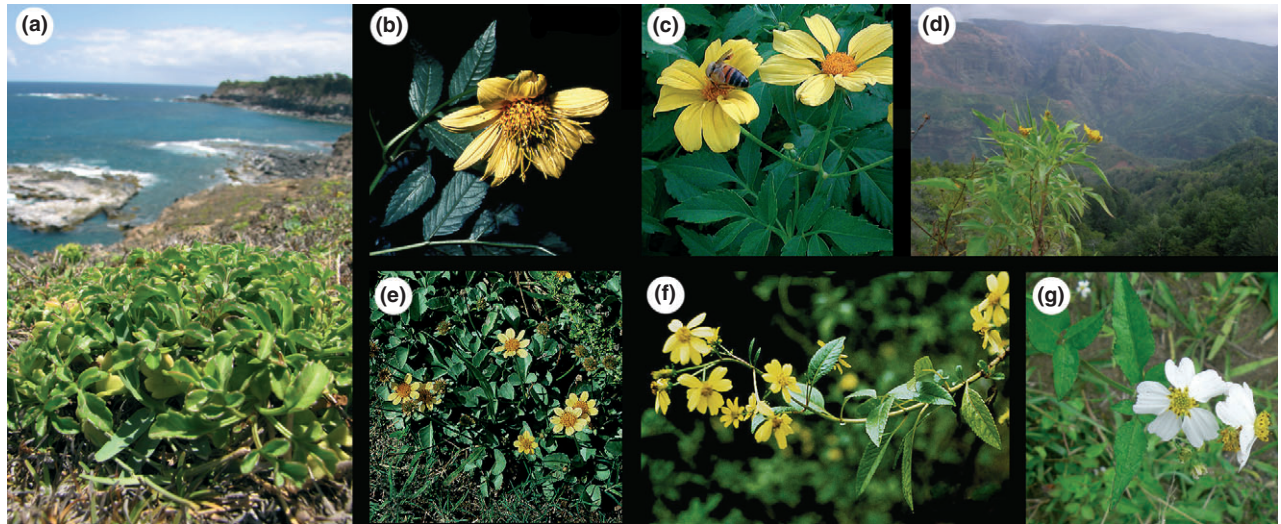


Figure 1 Representative Hawaiian *Bidens* species showing a sample of the morphological and ecological variation found among species. Clockwise from left: (a) *B. hillebrandia* (photo credit: Forest and Kim Starr); (b) *B. cosmoides* (photo credit: C.H. Lamoureux); (c) *B. amplexans* (photo credit: C.H. Lamoureux); (d) *B. sandvicensis confusa* (photo credit: M.L. Knope); (e) *B. mauiensis* (photo credit: G.D. Carr); (f) *B. hawaiiensis* (photo credit: C.H. Lamoureux); and (g) *B. alba radiata* (Central American species in basal sister clade to Hawaiian species) (photo credit: J.C. Knope).

adequately established, which could affect estimates of the rate of diversification.

Taxon sampling and molecular methods

Material was obtained from field collection, botanical gardens and the University of British Columbia herbarium (see Appendix S1 in Supporting Information). Voucher specimens of newly collected samples were deposited at the Bernice Pauahi Bishop Museum in Honolulu, HI, USA (BISH; accession 2009.104). All DNA extracts were deposited at the Hawaiian Plant DNA Library at the University of Hawaii, Manoa, USA. DNA was obtained from leaf material from 59 specimens representing all 19 Hawaiian species, six Hawaiian subspecies, two Hawaiian hybrids and four non-Hawaiian outgroup species (Appendix S1). DNA amplification and sequencing were performed using standard methodology (Appendix S2). Inclusion of nuclear ITS data from GenBank resulted in 13 additional non-Hawaiian *Bidens* species (accession numbers given in Fig. 2). Molecular markers were chosen that are likely to have high rates of molecular evolution. The ITS and external transcribed spacer (ETS) are both nuclear non-coding markers with generally rapid rates of evolution. They have been used extensively for plant molecular systematics, particularly at lower taxonomic levels, and have been shown to be useful in Hawaiian Asteraceae (Baldwin & Markos, 1998; Baldwin & Carr, 2005; Mort *et al.*, 2007). The chloroplast loci used in this study (*trnV-ndhC*, *rpl32f-trnL*, *trnQ-rps16* and *rpl32r-ndhF*) are non-coding introns and spacers and are (on average across a wide variety of plant families) the four most rapidly evolving markers known from this genome (Shaw *et al.*, 2007),

although substitution rate heterogeneity across taxa is commonly observed.

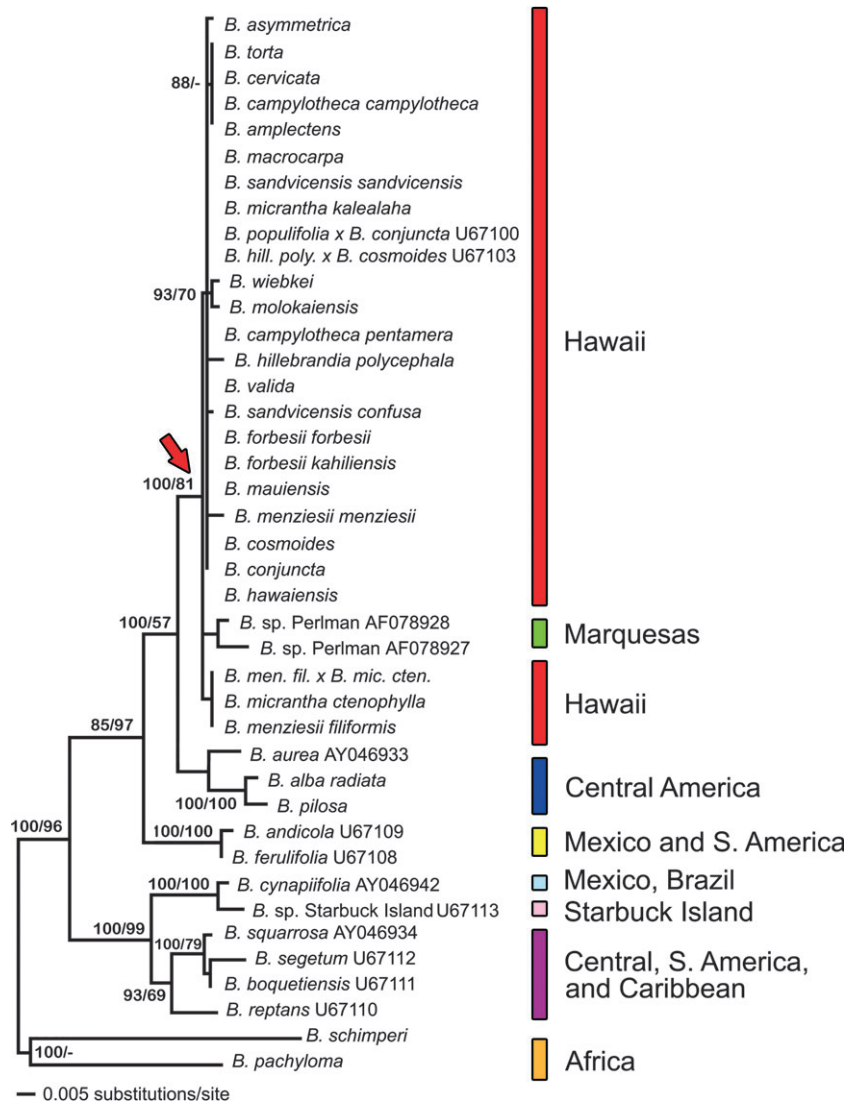
DNA sequences

Approximately 5400 base pairs (bp) were sequenced (1700 bp of nuclear and 3700 bp of plastid DNA) for the 19 species and six subspecies of Hawaiian *Bidens* (see Wagner *et al.*, 1999, for species authorities), the two Central American (*Bidens pilosa* and *Bidens alba* var. *radiata*) and two African species (*Bidens shimperi* and *Bidens pachyloma*). See Appendix S1 for the length of the aligned sequences for each marker used in the phylogenetic analysis (regions that were not successfully sequenced in some taxa are also indicated in this table). All sequences were deposited in GenBank (accession numbers GU736412–GU736579). Multiple alignments were performed (see Appendix S1) and all alignments are available in TreeBASE (<http://www.treebase.org>).

Phylogenetic analysis

The African species, *B. shimperi* and *B. pachyloma*, were chosen as the most appropriate outgroup for this phylogenetic analysis (Kim *et al.*, 1999). To assess recent common ancestry, putative sister taxa (the Central American species *B. pilosa* and *B. alba*, and two undescribed species from the Marquesas) were included in the ITS phylogeny to test for multiple origins of the Hawaiian clade. North American temperate *Bidens* are more closely related to North American temperate *Coreopsis* (Asteraceae) and *Thelesperma* (Asteraceae) (Crawford *et al.*, 2009). Therefore, we excluded all North American temperate *Bidens* species from this study. However, including them in a

Figure 2 Maximum likelihood phylogram based on DNA sequences of the nuclear internal transcribed spacer (ITS) marker for Hawaiian ingroup and non-Hawaiian out-group *Bidens* species. Accession numbers follow those taxa where the ITS data were downloaded from GenBank. Branch lengths represent genetic distances. Numbers above lines are maximum likelihood bootstrap support values (1000 replicates) to the left and Bayesian posterior probabilities to the right. The red arrow depicts the basal node of the Hawaiian radiation.



separate analysis made no difference in terms of the monophyly of the Hawaiian clade and the general conclusions of this work. For the 13 additional species for which we included ITS data from GenBank, the corresponding data for these species were not available in GenBank for the other five loci we examined. Therefore, for these five loci, we limited our analyses to the 19 Hawaiian *Bidens* species, their subspecies, and the Central American and African species. Phylogenetic relationships were assessed by maximum likelihood (ML) in PAUP* 4.0 (Swofford, 2002), and by Bayesian inference (BI) implemented in MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003). The total number of characters, number of variable characters, number of parsimony informative characters and ML-corrected pairwise sequence divergences were calculated in PAUP*. Pairwise ML-corrected genetic distances were calculated for Hawaiian and non-Hawaiian species, respectively.

Statistical confidence in nodes was assessed by bootstrapping with 1000 pseudoreplicates and ‘FAST’ stepwise-addition (Felsenstein, 1985) for ML analyses and by Bayesian posterior

probability values for BI analyses (Ronquist & Huelsenbeck, 2003). For the ML and BI analyses, MODELTEST 3.8 (Posada & Crandall, 1998), with the default parameters, was used to determine the appropriate model of sequence evolution for each locus independently. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) were used to discriminate among the 56 progressively more complex models of nucleotide evolution. The best-fit model for each locus is as follows: ITS = SYM + Γ + I; ETS = TVM + Γ ; *trnV*-*ndhC* = K81uf; *rpl32f*-*trnL* = K81uf + Γ ; *trnQ*-*rps16* = K81uf; and *rpl32r*-*ndhF* = K81uf + Γ . ML analysis was conducted with a heuristic search option with random, stepwise additions and tree bisection–reconnection (TBR) branch swapping. When searches converged on more than one tree, a single strict consensus tree was generated. Using the model of substitution determined by the BIC, the BI analysis was implemented with starting trees chosen by random selection and the analysis was run for 10,000,000 generations, sampling every 100 generations.

Estimation of age of the most recent common ancestor

The age of the most recent common ancestor (MRCA) was calculated by BI in BEAST 1.4.8 (Drummond & Rambaut, 2007) using the ITS sequence database with biogeographical calibration (Ho & Phillips, 2009). Biogeographical calibration was based on data from Clague *et al.* (2010), which indicate that after the submergence of Kōko Seamount at *c.* 33 Ma there was a period of *c.* 4 Myr when there were no emergent islands and thus no opportunity for colonization. Thus a new cycle of colonization began between *c.* 29 Ma and *c.* 23 Ma (Clague *et al.*, 2010), making 29 Ma, the most conservative value for our calculations. Note, however, that alternative views exist (Heads, 2011). The approximate age of the MRCA of the Hawaiian clade was inferred by using the mean rate of nucleotide substitution (0.00413 substitutions/site Myr⁻¹) for ITS in herbaceous angiosperms (Kay *et al.*, 2006) with the 29 Ma maximum and a uniform distribution prior. Kay *et al.* (2006) report that while ITS substitution rates varied by approximately an order of magnitude across 28 angiosperm lineages, there was much less variation within life-history categories. Consequently, we restricted our estimates of the rate of molecular substitution in ITS to values given for herbaceous plants, as the vast majority of *Bidens* species are herbaceous (with some secondary woodiness among the Hawaiian taxa; Carlquist, 1974). The GTR + Γ + I nucleotide substitution model with empirical base frequencies and eight rate categories was used in BEAST to best match the ML model of the ITS data. The age of MRCA of the Hawaiian *Bidens* was estimated in BEAST. Three independent chains of 10,000,000 iterations were run and combined in LOGCOMBINER 1.4.8 (Drummond & Rambaut, 2007). The effective sample size (ESS; the number of independent samples in the trace) for the age of MRCA of Hawaiian *Bidens* was 11,440, considerably higher than the recommended ESS cut-off value of 100 (Drummond & Rambaut, 2007). A molecular clock likelihood ratio test (Felsenstein, 1981) showed uniformity in substitution rate across the entire phylogeny.

Estimation of diversification rates

To estimate diversification rates, we used Magallón & Sanderson's (2001) method, which allows for direct comparison among clades that underwent diversification recently (as compiled in Table 1 of Valente *et al.*, 2010; see also Appendix S2 for discussion of alternative diversification rate equations). Thus, the diversification rate (\hat{r}_ϵ) was calculated as follows:

$$\hat{r}_\epsilon = 1/t \left\{ \log \left[\frac{1}{2} n(1 - \epsilon^2) + 2\epsilon \right. \right. \\ \left. \left. + \frac{1}{2} (1 - \epsilon) \sqrt{n(n\epsilon^2 - 8\epsilon + 2n\epsilon + n)} \right] - \log 2 \right\}$$

where ϵ is defined as $\epsilon = \mu/\lambda$, with μ being the extinction rate and λ the speciation rate; the variable t corresponds to the time

after the origin of the clade, here the present; n is the standing species diversity of the clade at time t . We implemented this estimation in the R package GEIGER (Harmon *et al.*, 2008). Following Magallón & Sanderson (2001), we calculated diversification rates for crown groups at two extremes of the relative extinction rate ($\epsilon = 0$, no extinction, and $\epsilon = 0.9$, high rate of extinction), and used the age estimates for MRCA based on the Bayesian 95% highest posterior density intervals of the BEAST analysis for the mean angiosperm ITS substitution rate.

RESULTS

Phylogeny and genetic distances

The monophyly of Hawaiian *Bidens* is well supported with high ML bootstrap and BI posterior probability values (Fig. 2, Appendix S3). Tree topologies were nearly identical in both analyses, therefore only the ML trees are shown. However, both bootstrap values and posterior probabilities are given at each node (Fig. 2, Appendix S3). Of the 5400 bp examined per individual across all the Pacific, African and American species we analysed, 475 bp were variable and 277 of those were parsimony informative. In contrast, within the Hawaiian species alone, there were only 143 bp differences, of which only 55 bp were parsimony informative. Similarly, the maximum ITS pairwise genetic distance (ML corrected) within the 19 Hawaiian species was 0.8%, whereas across all 35 *Bidens* species in this study the maximum genetic distance was 15.6%. Other loci examined show a similar pattern, with extremely small genetic distances between Hawaiian species and much greater genetic distances across all species (Appendix S3). In the ITS phylogeny (Fig. 2), the ML bootstrap values for nodes uniting taxa outside Hawaii are generally high, including the node connecting all taxa in the Hawaiian radiation and the two Marquesan species included in this study (see Appendix S2 for a supplementary discussion of Marquesan species and the direction of colonization).

Age and rate of diversification

Enforcing the molecular clock does not significantly add length to the ITS tree (chi-square test; $P > 0.05$). Using the mean ITS substitution rate for herbaceous angiosperms (Kay *et al.*, 2006) and a 29 Ma maximum (Clague *et al.*, 2010), the Bayesian 95% highest posterior density intervals estimate the MRCA of the Hawaiian clade to be 1.3–3.1 Ma, with a mean age of 2.1 Ma (Table 1). Based on these ages and assuming no extinction, the diversification rate is 0.9–2.3 species Myr⁻¹. Accounting for extinction by setting the speciation/extinction rate to 0.9, which is considered a relatively high value (Magallón & Sanderson, 2001), we estimate the net diversification rate to be 0.3–0.8 species Myr⁻¹. On a per-unit-area basis, we estimate the diversification rate to be 4.8×10^{-5} to 1.3×10^{-4} species Myr⁻¹ km⁻², or 0.7×10^{-2} to 5.4×10^{-1} species Myr⁻¹ log(km⁻²), incorporating the range of both estimates above; with and without extinction.

Table 1 Rates of diversification of Hawaiian *Bidens* in comparison with the other taxa documented as the most rapid plant radiations to date. Estimates are based on 95% highest posterior density intervals of the age estimates reported for each taxon.

Taxon and geographical region	No. of species in clade	Clade age (Ma)	Area (km ²)	Diversification rate (species Myr ⁻¹)	Diversification rate per unit area (species year ⁻¹ km ⁻²)	Diversification rate per unit (log) area (species Myr ⁻¹ (log)km ⁻²)	Citation
Hawaiian <i>Bidens</i>	19	1.3–3.1	16,644	0.3–2.3	4.8×10^{-5} to 1.3×10^{-4}	0.7^{-2} – 5.4^{-1}	This study
Eurasian <i>Dianthus</i>	200	1.9–7.0	45,310,246	2.2–7.6	4.9×10^{-8} to 1.7×10^{-7}	2.9 – 9.9^{-1}	Valente <i>et al.</i> (2010)
Andean <i>Lupinus</i>	81	1.2–1.8	970,950	1.3–3.8	$(1.3$ – $3.9) \times 10^{-6}$	2.2 – 6.3^{-1}	Hughes & Eastwood (2006)
Macaronesian <i>Echium</i>	19	2.7–3.9	10,372	0.4–1.5	3.9×10^{-5} to 1.4×10^{-4}	0.1 – 3.8^{-1}	García-Maroto <i>et al.</i> (2009)
Cape Floral Region Ruschioideae	1563	3.8–8.7	90,000	0.8–1.8	8.9×10^{-6} to 2.0×10^{-5}	1.6 – 3.6^{-1}	Klak <i>et al.</i> (2004)

DISCUSSION

Hawaiian *Bidens* show a great deal of divergence in morphological and ecological characters (Gillett, 1975; Ganders & Nagata, 1984; Carr, 1987; Ganders *et al.*, 2000). In contrast, we demonstrate here that rapidly evolving nuclear and plastid loci show little divergence among these species (Fig. 2, Appendix S1). Our results indicate that Hawaiian *Bidens* have undergone, on a per-unit-area basis, one of the most rapid adaptive radiations yet documented for flowering plants (Table 1).

Comparison of diversification rates

Estimated diversification rates for angiosperms as a whole range from 0.078 to 0.091 net speciation events Myr⁻¹ (Magallón & Castillo, 2009). In this context, the diversification rate we estimate for Hawaiian *Bidens* (0.3–2.3 species Myr⁻¹) is exceptionally high, approaching that of Andean *Lupinus* (1.3–3.8 species Myr⁻¹; Hughes & Eastwood, 2006, as recalculated by Valente *et al.*, 2010) and Eurasian *Dianthus* (Caryophyllaceae) (2.2–7.6 species Myr⁻¹; Valente *et al.*, 2010). However, both of these radiations were continental and occurred over much larger geographical areas. To compare diversification rates on a per-unit-area basis, we calculated the area of the Andean *Lupinus* and Eurasian *Dianthus* radiations based on the area of the minimum convex polygon of the distributions given in Hughes & Eastwood (2006) and Valente *et al.* (2010), respectively, using ArcGIS 9.2 (Table 1). For *Bidens*, we used the area of the Hawaiian Archipelago (16,644 km²) given in Price (2004). For *Lupinus*, only the main southern portion of their Andean distribution was used, giving a conservative estimate of 970,950 km² (see Figure 1 in Hughes & Eastwood, 2006). For *Dianthus*, area was calculated using their Eurasian distribution, estimated at 45,310,246 km² (see Figure S2 in the Data Supplement in Valente *et al.*, 2010). When considered on a per-unit-area basis, the radiation of Hawaiian *Bidens* (4.8×10^{-5} to 1.3×10^{-4} species Myr⁻¹ km⁻²) is one to four orders of magnitude faster than that of Andean *Lupinus* [(1.3–

$3.9) \times 10^{-6}$ species Myr⁻¹ km⁻²] and of Eurasian *Dianthus* (4.9×10^{-8} to 1.7×10^{-7}), although the three radiations have similar per-unit-log(area) rates [Hawaiian *Bidens*: 0.7×10^{-2} to 5.4×10^{-1} species Myr⁻¹ log(km⁻²); Andean *Lupinus*: $(2.2$ – $6.3) \times 10^{-1}$ species Myr⁻¹ log(km⁻²); and Eurasian *Dianthus*: $(2.9$ – $9.9) \times 10^{-1}$ species Myr⁻¹ log(km⁻²)].

When area is not considered, the diversification rates of the Macaronesian *Echium* (Boraginaceae) (García-Maroto *et al.*, 2009) and South African Cape Floral Region Ruschioideae (stone plants) (Klak *et al.*, 2004) radiations are not as rapid as the Hawaiian *Bidens*, Andean *Lupinus* or Eurasian *Dianthus* radiations (Table 1). However, the Macaronesian and South African radiations occurred over small areas, comparable in scale to Hawaii. Using the areas of the Macaronesian Archipelago and the Cape Floral Region of South Africa taken from the literature (Sundseth, 2000; McGinley, 2008), we find that, on a per-unit-area basis, the Hawaiian *Bidens* and Macaronesian *Echium* radiations have similar per-unit-area diversification rates [Macaronesian *Echium*: 3.9×10^{-5} to 1.4×10^{-4} species Myr⁻¹ km⁻² and $(0.1$ – $3.8) \times 10^{-1}$ species Myr⁻¹ log(km⁻²)]. The other plant adaptive radiations listed in Valente *et al.* (2010, Table 1 therein) are less rapid and are found over larger areas than Hawaiian *Bidens*.

Thus far, relatively few plant groups have been subjected to rigorous analyses of diversification rates. Many other groups warrant detailed examinations. For example, Hawaiian *Cyrtandra* (Gesneriaceae) has 58 species, which evolved from a single colonist and have high morphological diversity (Cronk *et al.*, 2005). Hawaiian *Tetramolopium* has 11 species and little divergence at ITS (Lowrey *et al.*, 2001). Hawaiian lobeliads display greater antiquity (*c.* 13 Myr) than Hawaiian *Bidens*, but they are composed of 126 extant species in six genera (Givnish *et al.*, 2008). *Tolpis* (Asteraceae) in the Canary Islands comprises nine species and show no ITS divergence (Mort *et al.*, 2007). Finally, *Scalesia* (Asteraceae) in the Galápagos Islands are composed of 15 morphologically diverse species. Although Schilling *et al.* (1994) reported age estimates for the MRCA of the *Scalesia* radiation, their estimates are based on chloroplast restriction site data, which are no longer

considered reliable for age estimation (Rutschmann, 2006). Future research on diversification rates of these and other clades should help in understanding how rapidly plant radiations may occur.

Ecological limits of species numbers

It is important to note that calculation of diversification rates depends on several assumptions. For example, Rabosky (2009) argued that inferences about diversification rates are compromised by the often erroneous assumption that species richness has increased unbounded through time. Ecological limits on diversity may be common (Rabosky, 2009; Ricklefs, 2009), and current species richness may therefore not be correlated with diversification rates. Biogeographical evidence suggests that Hawaiian *Bidens* may have already reached ecological limits on some of the islands. Specifically, the drop in species richness from Maui Nui to Hawaii may indicate inadequate time for species richness to reach 'carrying capacity' on Hawaii, as argued for *Tetragnatha* (Tetragnathidae) spiders by Gillespie (2004) and for *Cyanea* lobeliads by Givnish *et al.* (2008). If so, this drop indicates ecological saturation on the older islands (Whittaker *et al.*, 2008). In addition, unrecorded extinction events may further complicate estimation of diversification rates. Even so, *relative* diversification rates should be comparable among clades of the same or similar age, such as those we discussed above. Furthermore, even if one cannot be certain about true diversification rates, estimated rates for young clades such as Hawaiian *Bidens* can be informative in showing how rapid diversification may have been.

Temporal changes in area

The size of habitable areas has changed over time due to the rise and fall of sea level, shifts in climate, tectonic uplift and erosion and, in the case of Hawaii and Macaronesia, the geological dynamics of volcanic islands (Whittaker *et al.*, 2008). It is therefore important to consider temporal changes in habitable area in assessing the effect of area on speciation rates. For example, the island of Hawaii, which accounts for 62% (10,433 km²) of the present-day area of the entire Hawaiian Archipelago, is the youngest of the islands at c. 0.5 Ma (Price & Clague, 2002). Additionally, Maui Nui (c. 2 Myr old) as a single island obtained its maximum area c. 1.2 Ma (Price & Elliot-Fisk, 2004). Yet at no point in the history of the Hawaiian Islands relevant to the *Bidens* radiation (1.3–3.1 Ma to present) has the total habitable area been significantly greater than today (Carson & Clague, 1995; Price & Clague, 2002; Price & Elliot-Fisk, 2004). Therefore, the diversification rate per-unit-area that we calculate for the Hawaiian *Bidens* radiation may err on the conservative side. The only other plant radiation whose estimated diversification rate is similar to that of the Hawaiian *Bidens* on a per-unit-area basis is the Macaronesian *Echium* (Table 1). We therefore focus here on the changes in historically available area in Macaronesia. Similar to the Hawaiian Islands, the Canary

Islands (which comprise the majority of the land area in the Macaronesian island chain) are a group of intra-plate oceanic-island volcanoes and are considered to be the result of an upwelling mantle plume or hotspot (Carracedo & Day, 2002; Fernández-Palacios *et al.*, 2011). Also, similar to Hawaii they display a general age progression from east to west (Carracedo & Day, 2002). However, in contrast to the Hawaiian Islands, in the time frame relevant to the *Echium* radiation (García-Maroto *et al.*, 2009), the present-day Macaronesian islands are smaller than their maximum historical extent (Fernández-Palacios *et al.*, 2011), supporting the conclusion that Hawaiian *Bidens* had similar or higher per-unit-area rates of diversification relative to Macaronesian *Echium*. Lastly, while geological and climatic changes in Eurasia and the Andes Mountains undoubtedly resulted in dramatic changes in the total habitable area of the *Dianthus* and *Lupinus* adaptive radiations, respectively, these changes are unlikely to have been large enough to affect our general conclusions regarding relative per-unit-area rates of adaptive radiation, as total habitable area would have had to change (on average) by orders of magnitude from present-day conditions and relative to the other radiations under consideration (Table 1).

Mode and mechanism of speciation

What has made the rapid radiation of Hawaiian *Bidens* possible within such a small area? Three inter-related factors may be of particular importance. First, the majority (about 85%) of all possible inter-specific hybrid combinations, which can be easily formed in cultivation, are prevented by geographical isolation among natural populations (Ganders & Nagata, 1984). This high level of geographical isolation may have also been caused or maintained by the relative paucity of animal seed-dispersers in Hawaii. This second factor, the lack of these dispersal agents (particularly mammals), may have eliminated the adaptive potential of achene awns (slender, bristle-like appendages) found in mainland relatives, thus catalysing the loss of long-distance dispersal potential in achenes (Carlquist, 1974). The loss of dispersal potential is known in many island lineages of plants, insects and birds (Carlquist, 1974), and would correspondingly result in a further reduction in gene flow. Loss of dispersability may be favoured by selection, as dispersal off the island is likely to result in the loss of the organism and/or propagules in the large area of surrounding ocean. The ancestor of the Hawaiian *Bidens* most probably arrived attached to a sea bird, but this kind of transit is unpredictable and prone to failure as far as seed dispersal is concerned. Therefore, there would be little adaptive advantage to possessing mechanisms for seed dispersal away from the place where the parents have survived and reproduced successfully (Carlquist, 1980). Third, the Hawaiian Islands are characterized by high habitat heterogeneity (Carlquist, 1980; Ziegler, 2002), which may result in strong diversifying selection.

These factors may provide reasons to expect radiation in Hawaii, but they may not fully explain why, among the

Hawaiian clades, the rate of *Bidens* evolution has been especially rapid. One potential explanation concerns high variation in dispersal ability among *Bidens* species world-wide. Dispersal by the species ancestral to the Hawaiian radiation must have been quite efficient, because the genus has reached (by natural means) many of the smaller islands of Polynesia, as well as the Hawaiian Archipelago. However, all extant Pacific island species now have reduced dispersal ability (Carlquist, 1974). Additionally, while most species of Hawaiian *Bidens* are single-island endemics and do not disperse beyond a small area, a few are widespread within the archipelago and appear to have retained a moderate level of dispersal ability, based on achene morphology (Carlquist, 1974). Thus, it may be that the rapid radiation was facilitated by those ancestral species that retained inter-island dispersal potential, which, after coastal colonization, gave rise to poorly dispersed species adapted to the interior of islands. We suggest that new studies of diversification in Hawaiian *Bidens* should include evolutionary genetic studies of morphology and physiology such as those undertaken for Hawaiian *Tetramolopium* (Whitkus *et al.*, 2000). Such a study could elucidate the genetic basis for achene diversification, including the loss of dispersal ability.

Alternative explanations

We interpret the high morphological diversity combined with lack of genetic divergence in the Hawaiian *Bidens* as evidence for recent, rapid diversification. There are three alternative interpretations for the rapid rate of diversification in *Bidens* beyond those we have suggested, although none of them fully explains our results. First, hybridization and ongoing gene flow across all species could potentially occur, as all taxa are fully allogamous (cross-fertile) in the laboratory (Ganders & Nagata, 1984). As mentioned above this is unlikely as most species are allopatric and 19 of the 27 taxa (70%) are single-island endemics. Further, Ganders & Nagata (1984) estimated that allopatry due to habitat and ecological isolation (differences in flowering phenology or pollination mode) prevents natural hybridization in 93% of all possible inter-specific combinations of Hawaiian *Bidens*. Another factor that may have an effect is stabilizing selection, which theoretically could be operating on all of the regions sequenced in this study to prevent differentiation. Again, this does not seem likely as all regions used are non-protein coding, presumed neutral and rapidly evolving across all plant families studied (Baldwin & Markos, 1998; Kay *et al.*, 2006; Mort *et al.*, 2007; Shaw *et al.*, 2007). Finally, the rates of molecular evolution for ITS may have been unusually slow in the Hawaiian clade. We reject this hypothesis based on the results of our molecular clock test, which demonstrated a uniform rate of nucleotide substitution across *all* taxa, including 16 American and African *Bidens* species. Our study results are also consistent with those of Bromham & Woolfit (2004), who reviewed changes in substitution rate for island species radiations compared to their continental relatives and found no evidence for change in substitution rates in island lineages.

Species delineation

While taxonomic assignments are not the focus of this study, the close molecular relationships among Hawaiian taxa raise questions about the proper treatment of these species. For example, is it possible that all Hawaiian *Bidens* should be considered just one species? Sherff (1937) recognized 43 species and more than 20 varieties and forms of endemic Hawaiian *Bidens* based largely on leaf characters. These traits are now considered unreliable taxonomic characters in this group (Gillett & Lim, 1970; Ganders & Nagata, 1984). At the opposite end of the spectrum, Gillett (1975) suggested that all Hawaiian *Bidens* should be included in just two species, based on their genetic compatibility in crossing experiments and their ability to hybridize. However the lack of inter-specific crossing barriers is well known in insular taxa, particularly in the Asteraceae (Lowrey, 1995; Crawford *et al.*, 2009), and current species delineation, which is based on morphology, ecology and geographical data (Ganders & Nagata, 1984), proposes that there are 19 species and 8 subspecies of Hawaiian *Bidens*. Although all species are capable of hybridization in the laboratory (Ganders & Nagata, 1984), cross-compatibility is common in congeneric plants, as some species can remain compatible for at least 10 Myr (Parks & Wendel, 1990). In addition, all Hawaiian species display fixed heritable genetic differences and grow true to form in common garden experiments, demonstrating that phenotypic plasticity is not responsible for the diversity of the Hawaiian clade (Gillett & Lim, 1970). Therefore, we concur with Ganders & Nagata (1984) that there are 19 species and eight subspecies of Hawaiian *Bidens* despite the lack of genetic divergence at the loci we examined.

CONCLUSION

The Hawaiian *Bidens* radiation is, on a per-unit-area basis, among the most rapid radiations documented to date among flowering plants on islands and continental areas. Further investigation into the role of the habitat heterogeneity of the Hawaiian Islands, the loss of long-distance dispersal ability, and other potential drivers of this diversification should provide new insights into this radiation. Further, as more data become available, diversification rates of taxa in young clades, such as Hawaiian *Bidens*, that have radiated within areas of well-described palaeoclimatic and geological history should be instructive to evaluate assumptions about the factors that promote speciation and adaptive radiation.

ACKNOWLEDGEMENTS

We thank Dan Crawford (University of Kansas), Fred Ganders (UBC), Gabriel Johnson (Smithsonian), Karen Shigematsu and Alvin Yoshinaga (Lyon Arboretum), Ken Wood and Mike DeMotta (Allerton NTBG) and Kawika Winters (Limahuli NTBG) for DNA or tissue donation; Dave Carlon, Ken Hayes, Shaobin Hou and Gabriel Johnson for assistance with DNA

sequencing; Yi ‘Apollo’ Qi for assistance with ArcGIS; Gerry Carr and Pasha Feinberg for image preparation; Pat Aldrich, Stephanie Dunbar-Co, Shama Hinard, Jeffrey Knope, Tatiana Kutynina, Kathy McMillen, Kurtis McMillen, Colin Olito, Hank Oppenheimer and Maggie Sporck for assistance in the field; and Melinda Belisle, Rodolfo Dirzo, Elizabeth Hadly, Shama Hinard, Olivia Isaac, Sterling Keeley, Jeffrey Knope, Jonathan Payne, Cindy Sayre, Peter Vitousek, Ward Watt, Belinda Wheeler and Robert J. Whittaker for comments. Funding was provided in part by a University of Hawaii Ecology, Evolution, and Conservation Biology Research Grant Award to M.L.K. (National Science Foundation GK12 Fellowship grant no. DGE02-32016 to K. Y. Kaneshiro) and support from the Department of Zoology, University of Hawaii, Manoa and the Department of Biology, Stanford University.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Taxa sampled and molecular markers used in this study.

Appendix S2 Molecular methods and estimation of diversification rates.

Appendix S3 Maximum likelihood phylograms of external transcribed spacer and chloroplast DNA markers.

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BIOSKETCH

Matthew L. Knope is a PhD candidate in the Department of Biology at Stanford University specializing in ecology, evolution and population biology. His primary research interests are in the patterns and drivers of adaptive radiation.

Author contributions: M.L.K. and T.F. designed the research; M.L.K., C.W.M. and V.A.F. performed the research; M.L.K. analysed the data; M.L.K. and T.F. wrote the paper.

Editor: Jorge Crisci