

**INVASIVE CONGENERS ARE UNLIKELY TO HYBRIDIZE WITH
NATIVE HAWAIIAN *BIDENS* (ASTERACEAE)¹**

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- *Premise of the study:* Invasive plant species threaten native plants in multiple ways, one of which is genetic assimilation through hybridization. However, information regarding hybridization between related alien and native plant species is generally lacking. In Hawaii, the invasive Central American species *Bidens pilosa* and *Bidens alba* have colonized natural areas and often grow alongside the native Hawaiian *Bidens* species, a clade representing an adaptive radiation of 27 endemic taxa, many of which are threatened or endangered.
- *Methods:* To assess the risk of hybridization between introduced and native Hawaiian *Bidens* (which will readily hybridize with one another), we undertook crosses in cultivation between the invasive species and nine native *Bidens* taxa.
- *Key results:* The majority of the crosses formed no viable seed. Although seed did mature in several of the crosses, morphological screening of the resulting seedlings indicated that they were the result of self-pollination.
- *Conclusions:* This result suggests that *B. alba* and *B. pilosa* are incapable of hybridizing with these Hawaiian *Bidens* taxa. Further, we found that *B. alba* in Hawaii was self-compatible, despite self-incompatibility throughout its native range, and that the tetraploid species *B. alba* and the hexaploid species *B. pilosa* were cross-compatible, although pollen fertility was low.

Key words: adaptive radiation; *Bidens*; conservation; endangered species; endemic species; gene flow; introgression; invasive species; islands; threatened species.

Biotic invasions are a major threat to native biodiversity throughout the world (Mack et al., 2000; Cadotte et al., 2006; Lövei et al., 2012), and invasive plants are commonly cited as examples of alien species threatening native species (e.g., Kueffer et al., 2010; Young et al., 2010). There have recently been extensive efforts to identify the traits (e.g., life history, breeding system, prior evolutionary history) that predict invasiveness (e.g., Lloret et al., 2005; Abe et al., 2011; Castro-Díez et al., 2011; Kaiser-Bunbury et al., 2011) and the factors that account for differences in the invasiveness of species (Kueffer et al., 2010). One of the many processes by which invasive species pose threats to native species is interspecific hybridization (Levin et al., 1996; Daehler and Carino, 2001). If native species are cross-compatible and interfertile with alien species, and the fitness of the resulting hybrids is equal to or exceeds their parents in some habitats, genetic assimilation and competition

could lead to extinction of the native species. Assimilation occurs when highly fertile hybrids backcross to parental species, potentially resulting in the loss of one or both of the parental species through time. This risk is potentially heightened if the native populations are small relative to the alien populations (Wendel and Percy, 1990; Haber, 1998; Daehler and Carino, 2001). In addition, hybrid offspring that suffer lower fitness than either parent, through reduced vigor, fertility, or abortion of embryos, may adversely affect the native species, particularly in small populations due to gametic wastage and reduced seed set (Daehler and Carino, 2001).

The Hawaiian Islands flora may be particularly susceptible to the adverse affects of hybridization with invasive species. Approximately 89% of the 956 flowering plant species native to the Hawaiian Islands are endemic (Wagner et al., 1999) and hybridization with alien congeners is known to occur. For example, naturally occurring hybrids between native-alien *Rubus* (Randell et al., 1998) and native-alien *Portulaca* (Kim and Carr, 1990) and both natural and greenhouse hybrids between native-alien *Gossypium* species (Stephens, 1964; Dejoode and Wendel, 1992) have been documented in Hawaii. Daehler and Carino (2001) estimate that a total of 59 genera, consisting of 176 native flowering plant species in Hawaii, are potentially threatened with hybridization by alien congeners.

One of the largest lineages of Hawaiian flowering plants is in the genus *Bidens* (family Asteraceae or Compositae, tribe Coreopsidae), including 19 species and eight subspecies endemic to Hawaii, resulting from a single colonization event (Ganders et al., 2000; Knope et al., 2012). Despite their colonization of the archipelago within the last ~3 million years (Knope et al., 2012), more morphological and ecological diversity exists in the Hawaiian lineage than in the remaining ~300 species of *Bidens* distributed over five continents (Ganders and Nagata,

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1984; Crawford et al., 2009; and see Fig. 1). The Hawaiian taxa are considered to be one of the best examples of adaptive radiation in the Hawaiian flora (Carr, 1987, and nine taxa are currently of conservation concern, including both threatened and endangered taxa (Wagner et al., 1999). Given the great ecological diversity of the Hawaiian *Bidens* (Ganders and Nagata, 1984; Crawford et al., 2009), any threat to the Hawaiian taxa would impact a disproportionately high level of biological diversity relative to the overall number of taxa in the genus. Two widely distributed species, *Bidens pilosa* and *Bidens alba*, have been introduced into Hawaii, and they occur as the sister group in the same large clade as the native Hawaiian *Bidens* species (Ganders et al., 2000; Kimball and Crawford, 2004; Knope et al., 2012). The close phylogenetic relationship between the native and alien *Bidens* species suggests the potential for hybridization in Hawaii.

The native species grow from sea level to over 2200 m in elevation and occur in habitats ranging from semideserts to rainforests (Ganders and Nagata, 1984). About half of the Hawaiian native taxa are gynodioecious (dimorphic breeding system in which female individuals coexist with hermaphroditic individuals in populations), a form of sex expression not known elsewhere in *Bidens* (Sun and Ganders, 1986). The Hawaiian species are self-compatible and those that have been investigated have a mixed mating system (Ritland and Ganders 1985; Sun and Ganders, 1986). All of Hawaii's native *Bidens* species are hexaploid with a gametic chromosome number of 36 (Gillett

and Lim, 1970) and will readily hybridize with one another (Ganders and Nagata, 1984). The *Bidens pilosa* species complex, which has a center of diversity in its native range in Mexico (Ballard, 1986), is an invasive species now common in Hawaii, as it is in subtropical and tropical regions worldwide (IUCN ISSG, 2011). This species has a high score on the Hawaiian Weed Risk Assessment (HI-WRA, 2011), which is based on approximately 50 attributes that characterize invasive species. *Bidens pilosa* is morphologically variable, but the common weedy form, like the native Hawaiian *Bidens* species, is hexaploid with a chromosome number of $n = 36$ (Ballard, 1986). The species is self-compatible and exhibits autonomous autogamy with high seed set (Sun and Ganders, 1990; Grombone-Guaratini et al., 2004). *Bidens alba*, a species that is closely related to *B. pilosa* (Ballard, 1986; Ganders et al., 2000; Knope et al., 2012), is also invasive in Hawaii, and like *B. pilosa*, can be found growing side-by-side with native Hawaiian *Bidens* species (Gillett, 1972). We have also encountered five native *Bidens* taxa growing immediately adjacent to one or the other of the introduced *Bidens* species in various locations throughout Hawaii (M. L. Knope and R. J. Pender, personal observations). However, *B. alba* differs from *B. pilosa* in being tetraploid ($n = 24$) and has previously been considered self-incompatible (Ballard, 1986).

The purpose of the current study was to determine whether experimental hybrids between native Hawaiian *Bidens* species and *B. pilosa* or *B. alba* could be synthesized and thus provide

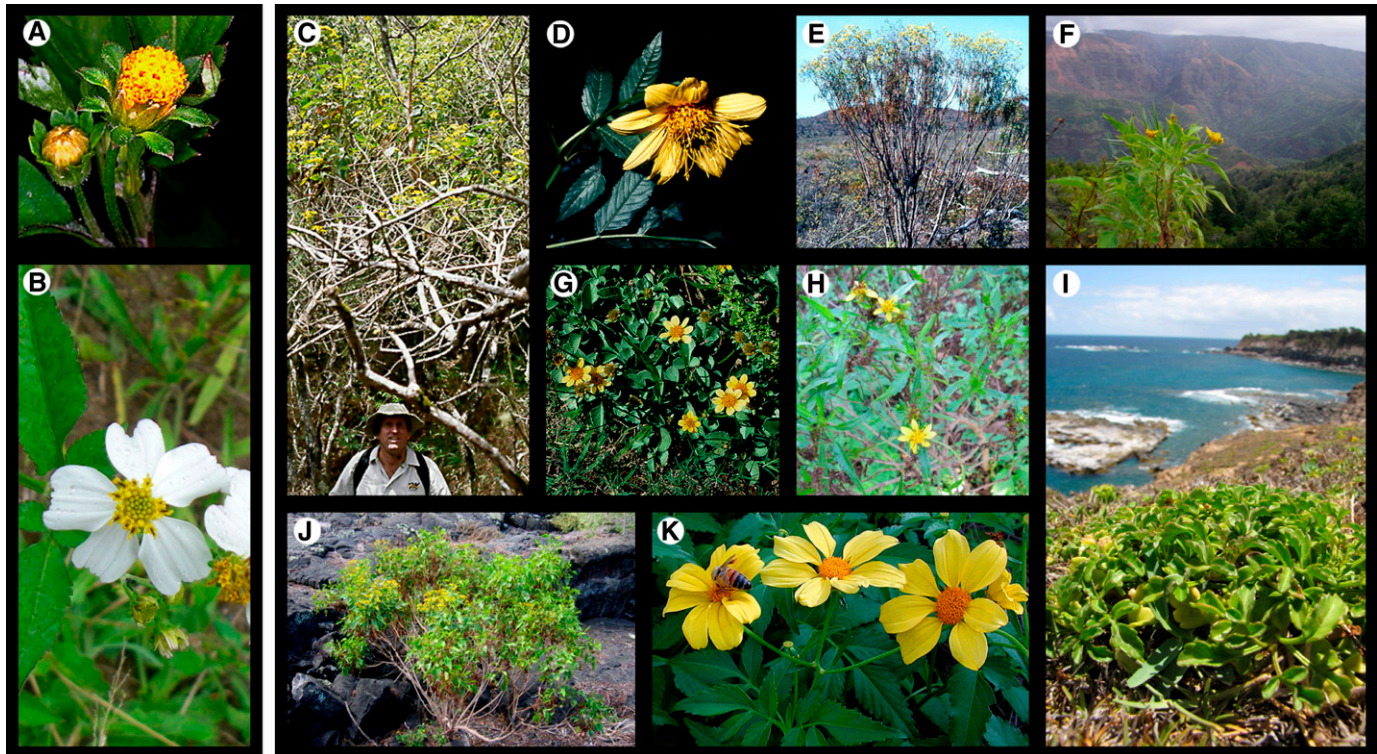


Fig. 1. Representative invasive and native Hawaiian *Bidens* taxa used in experimental crosses: (A) *B. pilosa* (Central American species now invasive in Hawaii) (photo credit: G. D. Carr); (B) *B. alba* (Central American species now invasive in Hawaii) (photo credit: J. C. Knope); (C) Hawaiian native *B. torta* with C. W. Morden in foreground for scale (photo credit: G. D. Carr); (D) Hawaiian native *B. cosmoides* (photo credit: C. H. Lamoureux); (E) Hawaiian native *B. menziesii* (photo credit: C. H. Lamoureux); (F) Hawaiian native *B. sandvicensis confusa* (photo credit: M. L. Knope); (G) Hawaiian native *B. maiensis* (photo credit: G. D. Carr); (H) Hawaiian native *B. sandvicensis sandvicensis* (photo credit: M. L. Knope); (I) Hawaiian native *B. hillebrandiana* (photo credit: Forest and Kim Starr); (J) Hawaiian native *B. micrantha* (photo credit: K. Magnacca); and (K) Hawaiian native *B. amplexens* (photo credit: G. D. Carr).

an assessment of the potential threat that these alien species pose to native *Bidens* species. Although there are prior reports on potential hybridization between native *Bidens* species and *B. pilosa*, the evidence is ambiguous and conflicting (Gillett, 1972; Sun and Ganders, 1990; Daehler and Carino, 2001). Further, given the rarity and vulnerability of the native species and the highly invasive nature of the alien species, a more definitive assessment of the potential for hybridization is warranted. Gillett (1972, p. 481), in a study on cross compatibility between Hawaiian and a Marquesan species of *Bidens*, commented that “experimental crosses between *B. pilosa* and Hawaiian species have all failed.” Gillett (1972) further stated that while *B. pilosa* and several Hawaiian species occur together in nature, there is no evidence of hybridization between them. Yet, Gillett (1972) did not indicate which Hawaiian species he attempted to cross with *B. pilosa*, nor present any other data. Sun and Ganders (1990, p. 140) stated that all crosses between *B. sandvicensis* and *B. pilosa* failed to set seed and further commented that “there is no evidence that any introduced species of *Bidens* in the Hawaiian Islands ever hybridize with the endemic species.” However, Daehler and Carino (2001) listed *Bidens* as one of the Hawaiian groups worthy of study for possible hybridization with alien congeners. This listing was based on observations by local botanists that natural hybrids between the two may occur, but more recently C. Daehler (University of Hawaii, Manoa, personal communication) expressed some uncertainty about whether the possible hybrids were between native and alien *Bidens* species. Additionally, as far as we are aware, there are no prior reports on attempted crosses between native *Bidens* species and *B. alba*.

MATERIALS AND METHODS

Achenes (dry fruits) were collected in the field or from botanical gardens in Hawaii from August 2008 through May 2009. Species identifications of adult plants bearing achenes were made by morphological examination of species diagnostic characters (Wagner et al., 1999) in the field and were subsequently confirmed by growing each species in cultivation. Achenes were collected from multiple individuals from each population. Seeds were placed in plastic bags in the field and then stored at 4°C in the laboratory until they were germinated.

Seeds from populations shown in Table 1 were germinated and the resulting progeny grown in greenhouses at the University of Hawaii (hereafter UH) or at the University of Kansas (hereafter KU), and used in crossing trials. Because *B. pilosa* has been reported as highly self-compatible and self-pollinating (Sun and Ganders, 1990), it was used as the pollen parent in crosses involving this species. *Bidens alba* was used as both egg and pollen parent in crosses. For the crosses undertaken at UH, a single individual of each species was used as the egg donor for each cross. For the pollen donor, multiple flowers, collected from a single plant per cross, were used to ensure that there were adequate pollen loads on the stigmas of the recipient flowers. For the crosses made at KU, again one plant was used per cross as the egg donor (with the exception of the gynodioecious *Bidens menziesii*, where two female plants were used) and florets from multiple capitula of individual plants were used as the pollen donor in all crosses.

The native Hawaiian *Bidens* species have previously been shown to be self compatible (via geitonogamous hand pollination), but autogamous pollination appears rare (Ritland and Ganders, 1985; Sun and Ganders, 1986). For this reason, we did not undertake intentional self-pollination treatments for the Hawaiian *Bidens* taxa. For the introduced species, we performed self-compatibility tests both by bagging flowers (at UH) and by isolating individual plants in different ranges of the greenhouses and manually self-pollinating (at KU). Mature fruits were then harvested from the open, dry, capitula. For *Bidens alba*, seed was collected from four plants (Iliou Loop Trail Population, Kokee, Kauai; Table 1). One seedling from each of these parent plants was grown to maturity to assess selfed seed set. The same method was used to assess selfed seed set in *B. pilosa*, except that seeds were collected from parent plants from two separate populations (1630 Kanalui St., Honolulu [10 plants] and from the UH Manoa

old BioMedical building [four plants]). To preclude the possibility of low pollen fertility limiting self seed set, we checked pollen viability of all selfed plants and found it to exceed 90%.

In crosses undertaken at UH, once the majority of florets in a capitulum had passed to the female phase (all Hawaiian species are protandrous), they were supplemented with copious pollen from one of the two alien species. To achieve this, we rubbed capitula of the male alien donor species onto the capitula of the native species. The pollen of the alien species conspicuously colors the style branches of native species, which ensured the application of adequate pollen loads. Crosses undertaken at KU used the same technique as that at UH for making some crosses and, in addition, employed an approach in which the capitula of the alien species were rubbed with the native species for several consecutive days. This presumably would result in pollen competition between the native and alien species, assuming some cross compatibility between them. In *Bidens cosmoides*, the styles are elongated and foreign pollen is easily placed on the style branches without pollen contamination from the female parent.

To assess each potential alien-native *Bidens* parental cross, we harvested mature achenes resulting from crosses and sowed achenes from one (randomly chosen) capitulum. Seed viability was determined by germination tests at KU by placing all the achenes from each cross (if fewer than 50 achenes), or a subsample of 50 achenes from crosses that produced more than 50 achenes, on moistened filter paper in Petri dishes. Seedlings were transplanted to soil and grown to flowering in the greenhouse. However, differences between dark, well-filled fruits (indicating presence of an embryo) and those that were shriveled and lighter colored (indicating no embryo present) were immediately apparent. Seeds that did not germinate were dissected and proved to lack embryos. Due to limited greenhouse space, 25 or fewer seedlings were grown to flowering to determine their parentage. However, it was determined that seedlings of the parental species are clearly distinguishable, based on vegetative characters, at an early stage. Pollen viability of the parental and of the first generation offspring was inferred by observing at least 200 grains stained with lactophenol aniline blue (Kearns and Inouye, 1993) for *B. alba* and *B. pilosa*. The large, plump, darkly staining viable pollen grains were readily distinguishable from the inviable shriveled, lightly to negligibly stained pollen grains.

RESULTS

With only two exceptions, no viable seed set occurred in crosses made at UH in which alien pollen was placed on style branches of the native species while florets were in the female phase. In the two instances of seed set in native-alien crosses (Table 2), the seeds were germinated and grown to maturity (flowering and fruiting), and they proved to be the result of selfing of the native *Bidens* taxa. Similarly, only selfed seeds were produced when pollen was supplemented on the capitula over multiple days for crosses undertaken at KU (Table 2). Generally, 25 progeny from these crosses were grown to fruiting to document that they were the result of selfing and additional seeds (55–85, depending on the number of seeds produced) were germinated, and morphological comparison with seedlings of the two parental species allowed definitive identification of all progeny as selfs. In addition, we noted that all the invasive and Hawaiian *Bidens* taxa grown in the greenhouses at both UH and KU had growth phenotypes similar to how they occur in nature, as found previously by Gillett and Lim (1970) for some of the Hawaiian taxa. In those crosses made at KU in which the same method used at UH was employed, no viable seed was produced. In the two crosses using female plants of the gynodioecious *B. menziesii*, no seed set occurred (Table 2).

While *B. alba* has been reported as self-incompatible throughout its native range (Ballard, 1986), our data indicated variable levels of self seed set (5–47%; mean of 26%; $n = 4$). Selfed seed set in all 14 plants of *B. pilosa* was higher than 90%. Additionally, we found evidence that the tetraploid *B. alba* will cross with the hexaploid *B. pilosa*, but the pollen fertility of the offspring of the four crosses between the two ranged from 31 to 49%. In comparison, the pollen fertility of the parent plants

TABLE 1. *Bidens* taxa used (followed by authorities) and collection information.

Taxon	Collection locality	Collector	Date
<i>Bidens alba</i> (L.) DC	Iliou Loop Trail, Kokee, Kauai	J. Knope, T. Kutynina, K. McMillen, and K. A. McMillen	15-Mar-09
<i>Bidens alba</i> (L.) DC	Manoa Valley next to Tropical Plant and Soil Sciences greenhouse complex	R. Pender	10-Nov-09
<i>Bidens amplexans</i> Sherff	Lyon Arboretum, grown from seed collected on Kealia Trail, behind Dillingham Air Force Base, Oahu	K. Kawakame	16-Mar-09
<i>Bidens conjuncta</i> Sherff	West Maui, Wailuku District, Waihe'e Valley, back valley south side, above main stream, below "Wall of Tears"	H. Oppenheimer	8-Aug-08
<i>Bidens cosmoides</i> (A. Gray) Sherff	Hui Ku Maoli Ola Native Plant nursery, Oahu	M. Knope and S. Hinard	24-Mar-09
<i>Bidens hawaiiensis</i> Gray	Amy Greenwall Botanical Garden, Hawaii	A. Yoshinaga	6-Jul-08
<i>Bidens hillebrandiana</i> (Drake) Degener	Hui Ku Maoli Ola Native Plant Nursery, Oahu	M. Knope and S. Hinard	24-Mar-09
<i>Bidens hillebrandiana</i> (Drake) Degener	Hui Ku Maoli Ola Native Plant Nursery, Oahu	R. Pender	15-Sept-09
<i>Bidens mauiensis</i> (A. Gray) Sherff	Maui Nui Botanical Garden	R. Pender	19-Jan-10
<i>Bidens menziesii</i> (A. Gray) Sherff	Amy Greenwall Botanical Garden, Hawaii	A. Yoshinaga	Unknown
<i>Bidens micrantha kalealaha</i> Nagata and Ganders	Kula Forest Reserve, Maui	A. Yoshinaga	25-Mar-09
<i>Bidens pilosa</i> L.	1629 Kanalui St. and Kaminaka Dr., Honolulu, Oahu	W. Haines	11-Mar-09
<i>Bidens pilosa</i> L.	Behind old BioMedical building on UH Manoa campus, Oahu	M. Knope	13-May-09
<i>Bidens pilosa</i> L.	Manoa Cliff Trail, Oahu	M. Waite	1-Nov-09
<i>Bidens torta</i> Sherff	Ridge trail between Pahole Gulch and Makua Valley, Wainae, Oahu	D. Okamoto	25-Mar-09
<i>Bidens sandvicensis confusa</i> Nagata and Ganders	Iliou Loop Trail, Kokee, Kauai	J. Knope, T. Kutynina, K. McMillen, and K. A. McMillen	3-Mar-09
<i>Bidens sandvicensis sandvicensis</i> Nagata and Ganders	Waahila Ridge, Oahu	W. Haines	17-Mar-09

used in these crosses was 95–100% for *B. alba* and 92–100% for *B. pilosa*.

DISCUSSION

Although it is difficult to discount rare hybridization events between otherwise cross-incompatible species, our results suggest that *B. alba* and *B. pilosa* are incapable of hybridizing with the Hawaiian *Bidens* taxa we examined (Table 1). While there have been reports by previous workers suggesting the inability of the Hawaiian and alien taxa to cross, the reports were presented as asides in papers focused on other topics and other

Bidens taxa (Gillett, 1972; Sun and Ganders, 1990). Other limitations of prior studies were lack of quantitative crossing data, the Hawaiian taxa were crossed with *B. pilosa* alone (and not *B. alba*), and the identity of the Hawaiian taxa used in those crosses was not provided. Thus, the present study confirms earlier reports for *B. pilosa* and provides the first evidence that the Hawaiian taxa will likely not cross with *B. alba*. However, because this study did not include all of the Hawaiian *Bidens* taxa, we cannot rule out the possibility that the introduced *Bidens* could hybridize with the Hawaiian taxa not included here. However, Ganders and Nagata (1984) obtained experimental hybrids involving combinations of all recognized endemic species of Hawaiian *Bidens* and found high pollen fertility in all the F1

TABLE 2. Results of crosses between native and alien species of *Bidens* in Hawaii. The first species in each cross was the egg parent and the second species in each cross was the pollen parent.

Taxa used in cross	No. viable seeds (total no. seeds in parentheses)	% viable seeds	Location of cross
<i>B. amplexans</i> × <i>B. pilosa</i>	0 (85)	0	KU
<i>B. cosmoides</i> × <i>B. pilosa</i>	0 (80)	0	KU
<i>B. hillebrandiana</i> × <i>B. alba</i>	1 (47)	2% (1 selfed seedling)	UH
<i>B. hillebrandiana</i> × <i>B. pilosa</i>	0 (33)	0	UH
<i>B. mauiensis</i> × <i>B. alba</i>	0 (21)	0	UH
<i>B. mauiensis</i> × <i>B. pilosa</i>	0 (36)	0	UH
<i>B. menziesii</i> × <i>B. alba</i>	0 (46)	0	UH
<i>B. menziesii</i> × <i>B. pilosa</i>	0 (53)	0	UH
<i>B. menziesii</i> (bisexual) × <i>B. pilosa</i>	30 (30) grown to flowering	100% (30 seeds grown to flowering, all selfs)	KU
<i>B. menziesii</i> (female) × <i>B. pilosa</i>	0 (201)	0	KU
<i>B. micrantha kalealaha</i> × <i>B. alba</i>	12 (12)	100% (12 seeds germinated, all selfs)	KU
<i>B. micrantha kalealaha</i> × <i>B. alba</i>	0 (42)	0	UH
<i>B. micrantha kalealaha</i> × <i>B. pilosa</i>	16 (140) grown to flowering	100% (50 of 140 total seeds germinated, all selfs)	KU
<i>B. sandvicensis</i> subsp. <i>confusa</i> × <i>B. alba</i>	1 (45)	2% (1 selfed seedling)	UH
<i>B. sandvicensis</i> subsp. <i>confusa</i> × <i>B. pilosa</i>	0 (40)	0	UH
<i>B. sandvicensis</i> subsp. <i>sandvicensis</i> × <i>B. alba</i>	0 (32)	0	UH
<i>B. sandvicensis</i> subsp. <i>sandvicensis</i> × <i>B. pilosa</i>	0 (120)	0	KU
<i>B. torta</i> × <i>B. pilosa</i>	0 (145)	0	KU

hybrids. These data, in combination with the crosses attempted in this study, suggest that all the endemic Hawaiian species are unlikely to be cross-compatible with the two alien species. Similarly, Daehler and Carino (2001) argue, using *Bidens* as an example of a Hawaiian lineage in which all species are cross-compatible, if an alien congener could hybridize with any one species in a lineage, then it would likely be able to hybridize with all endemics in that lineage. Further, this work suggests that the Marquesan *Bidens* species, which nest within the Hawaiian clade (Knope et al., 2012), may also be unable to hybridize with either *B. alba* or *B. pilosa*, which are also invasive in the Marquesas. This may be true for other native *Bidens* species that co-occur with these two invasive species in other areas of the world, as they may not be phylogenetically closer to *B. alba* and *B. pilosa* than the Hawaiian and Marquesan clade (Ganders et al., 2000; Knope et al., 2012). However, without a fully resolved phylogenetic hypothesis or crossing studies that include all species, this evaluation of breeding relationships remains speculative.

In addition to the crossing results between native Hawaiian and alien *Bidens*, we found that the recently introduced *B. alba* is self-compatible in Hawaii, despite self-incompatibility throughout its native range in North and Central America (Ballard, 1986). Grombone-Guaratini et al. (2004, 2005) similarly found that although *B. alba* had only been established in Brazil since 1980, the populations studied there were also self-compatible. The evolution of self-compatibility in plants has long received attention and colonization of new areas is thought to lead to the evolution of selfing or for selection of self-compatible variants (e.g., Baker, 1955; Stebbins, 1957; Ward et al., 2012; Petanidou et al., 2012). The evidence presented here and by Grombone-Guaratini et al. (2004, 2005) suggests that *B. alba* may have either latent genetic variation for self-compatibility not expressed in its native range or the polymorphism exists at such low levels (Barrett and Schluter, 2008) it has not been previously detected (Ballard, 1986). Additionally, we found that the tetraploid species *B. alba* and the hexaploid species *B. pilosa* were cross-compatible, but with low pollen fertility. While these two species have different ploidy levels, they are phylogenetically closer to one another than to the Hawaiian species (Ganders et al., 2000; Knope et al., 2012).

Members of the family Asteraceae commonly have generalist pollinators and congeneric species often share the same pollinators or suite of floral visitors (Ellis and Johnson, 2009; Horsburgh et al., 2011), and in some instances, it has been documented that native and alien species of Asteraceae have the same pollinators (Brock, 2009; Powell et al., 2011; Vanparys et al., 2011). Although no pollination studies of alien or native *Bidens* in Hawaii have been published, it is likely that the same insect pollinators visit sympatric alien and native *Bidens* species. However, as with almost all other Hawaiian plant taxa, studies of pollinator interactions with native and alien *Bidens* is an important and understudied issue in need of further investigation. In spite of this shortcoming, our results indicate that even if pollinators are shared between alien and native *Bidens*, other barriers prevent alien–native hybridization from occurring.

While we found no evidence for successful hybridization, even without fertilizing ovules, alien pollen could potentially decrease seed production in natives. This can occur by alien pollen obstructing native pollen, either at the stigma surface or in the style, and preventing conspecific pollen from accessing unfertilized ovules (Daehler and Carino, 2001). Given that

Bidens (like all known Asteraceae) likely has a sporophytic incompatibility system, any interference would likely occur at the receptive style branches because cross-incompatible pollen would not germinate (e.g., Allen et al., 2011). Also, even though the native species are self-compatible, they require floral visitors for seed set, and increased visitation of alien species by pollinators could reduce seed set in the native species (Dietzsch et al., 2011). Therefore, even without gene flow between native and alien *Bidens*, there still exists the possibility for reduced seed set in the native species due to the sympatric presence of the alien species.

Crossing data for native and alien congeners are valuable even when there is no evidence of the occurrence of natural hybrids. Lack of natural hybrids could be due to the recent introduction of the aliens or occurrence of native and alien species in different habitats. However, eventual spread of alien populations could bring native and alien taxa into contact (as is the case for *Bidens* in Hawaii), and knowing whether they are cross-compatible is important to formulating conservation strategies for native plants.

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