Research Article

The potential role of hybridization in diversification and speciation in an insular plant lineage: insights from synthetic interspecific hybrids

Benjamin Kerbs[1], Jacob Ressler[2], John K. Kelly[3], Mark E. Mort[3], Arnoldo Santos-Guerra[4], Matthew J. S. Gibson[5], Juli Caujapé-Castells[6] and Daniel J. Crawford[7]

[1]Department of Biological Sciences, Emporia State University, Emporia, KS 66801, USA
[2]Department of Biology, Indiana University, Bloomington, IN 47405, USA
[3]Department of Ecology & Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA
[4]Calle Guaidil 16, Urbanización Tamarco, 38280 Tegueste, Tenerife, Canary Islands, Spain
[5]Department of Biology, Indiana University, Bloomington, IN 47405, USA
[6]Jardín Botánico 13 Canario "Viera y Clavijo"-Unidad Asociada al CSIC (Cabildo de Gran Canaria), Camino del palmeral 14 15 (Tafira Alta), 35017 Las Palmas de Gran Canaria, Spain
[7]Department of Ecology & Evolutionary Biology, and the Biodiversity Institute, University of Kansas, Lawrence, KS 66045-7534, USA

Abstract. Hybridization is recognized as an important process in plant evolution, and this may be particularly true for island plants where several biotic and abiotic factors facilitate interspecific hybridization. Although rarely done, experimental studies could provide insights into the potential of natural hybridization to generate diversity when species come into contact in the dynamic island setting. The potential of hybridization to generate morphological variation was analysed within and among 12 families (inbred lines) of an F_4 hybrid generation between two species of *Tolpis* endemic to the Canary Islands. Combinations of characters not seen in the parents were present in hybrids. Several floral and vegetative characters were transgressive relative to their parents. Morphometric studies of floral, vegetative and fruit characters revealed that several F_4 families were phenotypically distinct from other families, and from their parents. The study demonstrates that morphologically distinct pollen-fertile lines, potentially worthy of taxonomic recognition if occurring in nature, can be generated in four generations. The ability of the hybrid lines to set self-seed would reduce gene flow among the lines, and among the hybrids and their parental species. Selfing would also facilitate the fixation of characters within each of the lines. Overall, the results show the considerable potential of hybridization for generating diversity and distinct phenotypes in island lineages.

Keywords: Canary Islands; phenotypic groups; synthetic hybrids; transgressive traits.

Corresponding author's e-mail address: dcrawfor@ku.edu

Introduction

The prevalence and significance of hybridization in evolution have been debated over the past decades with zoologists tending to minimize its impact (e.g. Mayr 1942; Dobzhansky 1970) and botanists, with rare exceptions (Wagner 1970), seeing a more important role for hybridization (Anderson and Stebbins 1954; Stebbins 1959; Raven 1976; Arnold 2016). However, it is now widely accepted that hybridization is an important factor in plant evolution and indeed in the evolution of many other groups of organisms (Arnold 2015, 2016; Mallet *et al.* 2016). Hybrids may display traits exceeding those found in their parents, i.e. transgressive traits (Rieseberg *et al.* 1999, 2003b; Stelkens and Seehausen 2009; Yakimowski and Rieseberg 2014), as well as exhibit novel combinations of traits from the two parents (Rieseberg and Ellstrand 1993; García-Verdugo *et al.* 2013). Both transgressive traits and new character combinations can facilitate evolutionary change in plant lineages (Stebbins 1959; Rieseberg *et al.* 2003a; Arnold *et al.* 2012; Arnold 2016), including the establishment and evolution of independent homoploid lineages that may be recognized as species (Abbott *et al.* 2010; Schumer *et al.* 2014; Yakimowski and Rieseberg 2014). Speciation associated with hybridization and increase in ploidy level is especially prevalent in flowering plants (Soltis *et al.* 2016).

Several factors make natural interspecific hybridization feasible in plant lineages on oceanic islands, most notably the combination of few intrinsic reproductive barriers with a dynamic ecological landscape. Species are typically isolated by ecological and spatial factors rather than intrinsic barriers (Crawford and Stuessy 1997; Crawford and Archibald 2016). The lack of intrinsic barriers has been confirmed in multiple lineages where fertile interspecific hybrids have been synthesized (Gillett and Lim 1970; Lowrey 1986; Mayer 1991; Brochmann *et al.* 2000; Carr 2003). Congeneric species often occupy distinct habitats but produce vigorous, fertile hybrids when they come into contact (e.g. Francisco-Ortega *et al.* 1997; Brochmann *et al.* 2000; Carr 2003). The insular landscape is dynamic, where natural and anthropogenic disturbances can bring species into contact and provide potential habitats for hybrids throughout different stages of island ontogeny. For example, It has been estimated that 10 % of the Hawaiian flora has been involved in natural hybridization (Whitney *et al.* 2010). Anderson and Stebbins (1954), in a classic paper on the impacts of hybridization on evolution, highlighted oceanic islands as places with changing environmental conditions that facilitate rapid bursts of hybridization.

In the present study, the genus *Tolpis* (Asteraceae) was used to examine the diversity generated by interspecific hybridization. This is a small (10–15 species) monophyletic group occurring in the Mediterranean and North Africa with its centre of diversity in the Canary Islands (Jarvis 1980; Gruenstaeudl *et al.* 2013; Mort *et al.* 2015). Despite being a small radiation Canarian *Tolpis* have breeding systems that range from self-incompatible (SI), through pseudo-self-compatible (PSC), to fully self-compatible (SC; Crawford *et al.* 2008, 2015). There is also variation in habit (perennials and an annual), floral and vegetative morphology, ploidy level and habitat preference (Jarvis 1980; Crawford *et al.* 2008). First generation hybrids have been obtained between Canary Island *Tolpis*, with reduced pollen fertility in some hybrids (Jarvis 1980; Crawford *et al.* 2009, 2015). In addition to the variation, Canarian *Tolpis* is an ideal subject for experimental hybridization because plants are easily cultivated in large numbers, are easily manipulated and have relatively short generation times, typically about 2 months.

We produced inbred lines starting with a cross between an annual, SC species and a perennial SI/PSC species. The SC annual *Tolpis coronopifolia*. It is the only Canarian species with the 'selfing syndrome' (Ornduff 1969; Slotte *et al.* 2012) including fewer florets per capitulum and smaller florets compared to outcrossing members of the genus (Fig. 1A). The species has dissected leaves (Fig. 1B) and occurs only on Tenerife Island where it grows in open habitats from 150 to 1400 m above sea level. The other parental species is the recently described *T. santosii* (Crawford *et al.* 2013), which is a SI to PSC perennial with large capitula (Fig. 1A). This species was chosen because it is a strong perennial that persists for a decade or longer in the greenhouse, and it grows and flowers profusely under cultivation. In contrast to *T. coronopifolia*, the leaves of *T. santosii* are nearly entire (Fig. 1B). The species is distributed only along the north and northeast coast of the island of La Palma.

Carr (2003) suggested that the ability to hybridize over time is more important in an evolutionary context than the hybrids present at any one time because the natural hybrids occurring at any given time will depend on the distribution of the parents. This means that the past and future roles of hybridization may be grossly underestimated if based only on the occurrence of natural hybrids, and that the results of artificial hybridization must be incorporated into estimates of the potential evolutionary significance of hybridization within a lineage. In oceanic islands, many factors may change the distributions of species over time. For example, human activities have resulted in the purposeful movement of endemic species among islands for use as ornamentals in gardens and roadsides (Francisco-Ortega *et al.* 2000), creating the potential for hybridization among species with weak post-zygotic isolating barriers. In addition,

Figure 1. (A) Capitula of *Tolpis coronopifolia* (left), *T. santosii* (right) and an F_1 hybrid (middle). (B) Leaf silhouettes of *T. coronopifolia* (left), *T. santosii* (right) and representative F_4 hybrids (middle three). Photo J. K. Archibald.

disturbances from human activities, such as road construction, facilitate the formation and establishment of interspecific hybrids when formerly isolated species come into contact (Brochmann *et al.* 2000). Over a larger temporal scale, Brennan *et al.* (2014) discuss climate change and hybridization, and Vallejo-Marín and Hiscock (2016) argue that species isolated by pre-zygotic barriers will likely be more affected by climate change than those with strong intrinsic post-zygotic barriers. As indicated earlier, most island plants, including *Tolpis*, fall into the former category. The parental species of *Tolpis* used in the present study occur on different islands and are not known to hybridize in nature. However, one or more of the factors mentioned above could affect species distribution in the future, especially human activities. While interspecific hybridization is apparently rare in Canarian *Tolpis*, examples are known. One example involves hybridization on Tenerife between sympatric populations of *T. coronopifolia* and the perennial *T. webbii* (J. K. Archibald and D. J. Crawford, University of Kansas, unpubl. data). These considerations suggest that results of the current study can provide some appreciation of the potential of hybridization to generate novelty and diversity when various factors bring species of *Tolpis* come into contact in the Canary Islands.

The major purpose of this study was to provide direct experimental evidence of the phenotypic diversity generated from hybridization between two species endemic to an oceanic archipelago. More specifically, we used inbred hybrid lines in *Tolpis* to: (1) determine whether any of the hybrid inbred lines form fertile, distinct phenotypic groups and (2) ascertain whether there are transgressive traits or novel combinations of traits were generated by in the hybridization.

Methods

One plant of *T. coronopifolia* (Crawford *et al.* 2008) was used as the pollen parent in a cross with an individual of *T. santosii* (Crawford *et al.* 2013); all hybrids were grown in the greenhouses at the University of Kansas. Vouchers of the parental species used to generate F_4 progeny are deposited in KANU under accession numbers: *Tolpis santosii* 397175 and *T. coronopifolia* 397158. High-quality digital images of the leaves of F_4 progeny are available upon request. Seed from one self-pollinated F_1 hybrid plant produced an F_2 generation (Soto-Trejo *et al.* 2013) from which one plant with fruit lacking the typical pappus of setaceous hairs (Fig. 2B) was selfed to produce the F_3 generation. Twelve F_3 plants that represented much of
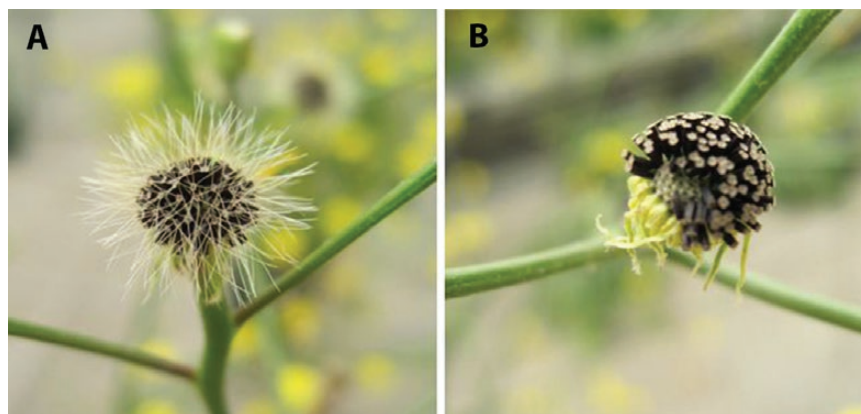
Figure 2. Fruits with (A) and lacking (B) a pappus of setaceous hairs.

the phenotypic variation seen in that generation were selfed to produce the F_4 families for study. The F_3 and F_4 generations allow the hybrid heterozygosity to segregate out into the three genotypic classes. Mendelian segregation predicts that on average half of the loci heterozygous in the inter-species hybrid (F_1) plant will be heterozygous in any one F_2 plant, while F_4 plants should be heterozygous at only about 12.5 % (1/8) of the loci that were heterozygous in the original F_1. Whether a particular F_4 plant is homozygous for the *T. coronopifolia* or *T. santosii* allele will vary, but should be positively correlated within F_4 families (they will be identical at all loci that were homozygous in their particular F_3 parent). Nearly all loci that were heterozygous in the founding F_2 plant should be polymorphic in the F_4 population (as a whole) with a predicted segregation ratio of 3:2:3 ratio (AA:AB:BB). A total of 188 F_4 plants were established from 12 families, with the number of individuals limited primarily by the number of viable fruits produced in the F_3 generation. Voucher specimens are deposited in the McGregor Herbarium (KANU) of the University of Kansas.

Morphometric methods, the quantitative analysis of quantitative and qualitative variables (Henderson 2006), were used to assess variation in the 12 F_4 generation lines. A total of 15 characters (4 vegetative, 6 floral, 4 fruit and 1 pollen) were measured across individuals from each inbred line (Table 1). In addition, a leaf dissection index was calculated using the method of Kincaid and Schneider (1983). These inbred lines contained 5 to 25 individuals (mean 15.4), and 3 to 5 leaves were measured per individual. Pollen viability and self-seed set were measured as percentages. The seed (technically the fruit) mass (μg) was the sum value of 20 achenes.

Per cent pollen fertility was determined for all plants by staining a minimum of 200 grains in lactophenol aniline blue (Kearns and Inouye 1993). The relatively large, darkly stained pollen grains were easily distinguished

from the shrivelled, very lightly stained grains. The percentage of self-seed set $\left(\dfrac{\text{number of seeds}}{\text{total number of florets}} \right)$ was estimated for each plant. The large, plump, dark (dark brown to black) fruits contain embryos and are easily distinguishable from the light tan, shrunken fruits lacking embryos. The presence/absence of a pappus was scored for each of the inbred lines.

Vegetative characters were measured using herbarium digitizing techniques. A Canon 5D Mark III (Melville, NY, USA) and a Photo-eBox lighting system were used to image 350 pressed and dried leaves from F_4 plants. Leaf measurements were taken using ImageJ (Schneider *et al.* 2012). Leaf length was measured from the base of the petiole to the apex of the leaf, and width was measured as the widest distance between lobes, typically half way up the midrib of the leaf. Leaf area and perimeter were calculated by increasing the image threshold and examining the number and boundaries of vegetative

Table 1. Characters measured in phenotypic study of *Tolpis* hybrids. Units in mm unless otherwise stated in parentheses.

Leaf	Floral	Fruit	Other
Leaf area	Capitulum diameter (cm)	Fruit mass (mg)	Pollen viability (%)
Leaf perimeter	Ligule length	Fruit length	
Leaf length	Ligule width	Fruit width	
Leaf width	Involucral bract width	Seed set (%)	
	Involucral bract length		
	Style branch length		

particles in the image. More specifically, an image of a leaf was taken with a ruler in the photo. The measurement feature was calibrated in ImageJ using the ruler, which allowed us to later make simple measurements of length and width, and also more complex measurements such as area and perimeter. The colour photo was converted to an 8-bit (greyscale) image and the threshold of the photo is increased so that vegetative particles are fully saturated/black against a white background (as in Fig. 1B). Since ImageJ was calibrated using the ruler, the program is able to analyse the pixels that represent the leaf and measures the leaf area. Similarly ImageJ can assign an outline to the cluster of particles/pixels and measure leaf perimeter. Instructions for these procedures are available online.

Following measurements of the diameter of 3 to 5 intact capitula per individual, the capitula were dissected and characters (ligule length and width, style branch length; bract length and width) were measured for 5 florets per capitulum. Structures were imaged using a digital Nikon dissecting microscope (×10) and measured using InfinityAnalyze (Lumenera, Ottawa, ON, Canada), which allows for simple length and width measurements. The software InfinityAnalyze produces a live image of the specimen under the lens of a digital microscope. After calibrating the measurement feature of the software using a ruler under the lens, it is possible to make length and width measurements on screen. These measurements were taken using software because the small and sometimes non-linear nature of the floral parts (e.g. curled style branches) precludes unmagnified measurement with a ruler. All seed data except per cent self-seed set and seed weight were collected using these techniques as well.

Means and standard errors were determined for each floral, vegetative and fruit character. Tukey–Kramer Honestly Significant Difference (HSD) *post hoc* tests and ANOVA were performed on each character singly to examine variance among the F_4 inbred lines. Varying criteria have been employed for scoring hybrid traits as transgressive (Rieseberg *et al.* 1999; Stelkens and Seehausen 2009); in the present study, traits were considered transgressive if the mean values were outside the ranges of the two parents (Stelkens and Seehausen 2009).

Floral and vegetative data were pooled for analyses in multivariate space. Principal component analysis (PCA) was performed on the floral and vegetative characters and an individual factor map was created. Missing data were estimated using a non-parametric multiple imputation approach via the missMDA package (Dray and Josse 2015; Josse and Husson 2016) for R and the final data set was plotted using the associated FactomineR

package. Ninety-five per cent barycentric confidence ellipses were assigned around group centroids.

Results

All hybrids were perennials, with none of the plants flowering once and senescing, as is typical of the SC annual parent *T. coronopifolia*. Means and standard errors for floral, vegetative and fruit characters are shown in Figs 3–5, together with the means and ranges of the traits of their parents. For hybrids, each ANOVA test yielded *P*-values of <0.05, suggesting that each character examined varies significantly among inbred lines. Results of the Tukey–Kramer HSD *post hoc* tests are shown by the horizontal bars above the plots of means and standard errors shown in Figs 3–5. Involucral bract length is the only character that failed to delimit inbred lines into two or more groups (Fig. 4).

Line 15 was notable in that all leaf traits were transgressive to the parents, including being the only line that was transgressive for leaf perimeter (Fig. 3). By contrast, lines 11 and 24 were the only lines that were intermediate for all leaf traits. Two traits, basal leaf area and length, were transgressive in the majority of lines, whereas leaf perimeter was, with the exception of line 15, intermediate between the parents (Fig. 3). With regard to floral traits, neither ligule length nor capitulum diameter was transgressive in any of the lines (Fig. 4). Eight of the families had style branch lengths outside the means of the parents. The more cryptic floral traits, such as ligule width and involucral bract length and width, varied from being transgressive in all lines to being intermediate in about half of the lines (Fig. 4). Only one of four fruit traits was transgressive in some lines and self-seed set in hybrids was intermediate between the parental species in every inbred line (Fig. 5).

Results of the PCA of floral and vegetative characters are shown in Fig. 6. The 95 % barycentric confidence ellipses around group centroids show a large cluster of phenotypic overlap, but there is evidence of the formation of distinct phenotypes, e.g. lines 3, 15, 22 and 23 (Fig. 6). The first principal component has large eigenvectors for vegetative characters, which are relatively much smaller in magnitude on PC2 **[see Supporting Information—Table S1]**. On the other hand, PC1 has mostly negative eigenvectors for floral characters, which are positive on PC2. Only one floral trait, style branch length, loaded with vegetative characters, and no vegetative characters loaded with floral characters. Because principal components are uncorrelated by nature, it is clear that there exists little linkage between floral and vegetative characters in hybrid lines.
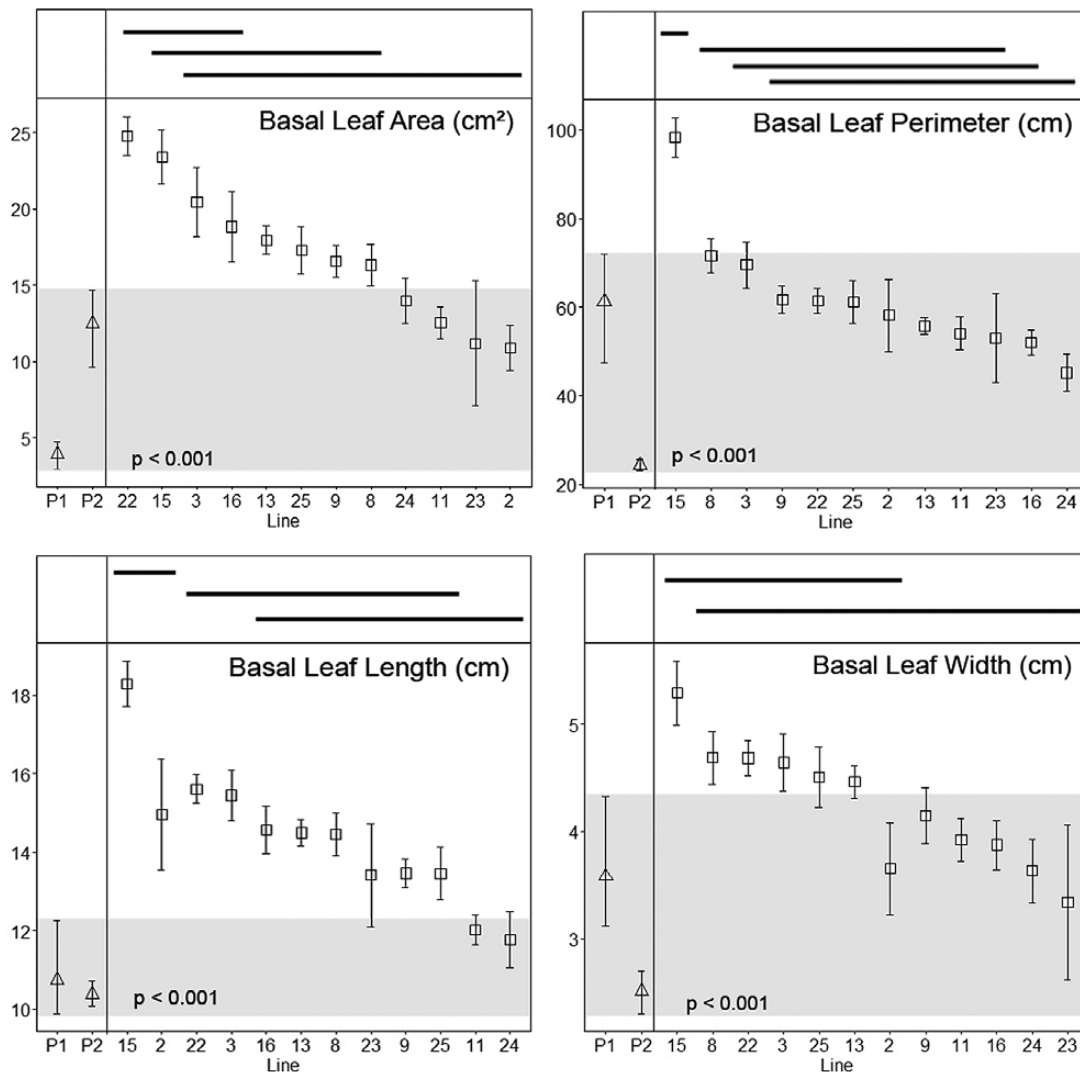
Figure 3. Plots of mean values (squares) and standard errors of inbred hybrid lines for vegetative characters. An ANOVA yields *P*-values < 0.05 for all characters, suggesting differences among lines. Bars above the graphs indicate which families are not significantly different from one another (*P* > 0.05, Tukey–Kramer HSD *post hoc* tests). Mean values (triangles) and ranges for the original progenitors, *Tolpis coronopifolia* (P1) and *T. santosii* (P2) are shown in the left-hand side of each plot. The greyed regions depict the ranges for the two parents. Hybrid line means falling outside this range represent transgressive traits. Lines are sorted mostly by decreasing mean values except lines 2 and 23 which exhibit large variability for some characters.

In all F_4 generation lines, mean values for pollen viability were above 40 % (Fig. 7). However, there was variation in mean values among lines (42–79 %) and extensive variation among individuals within some lines (Fig. 7). Four of the lines were fixed for pappus presence, three lines had only plants lacking a pappus, and both conditions existed among individuals in the other lines.

A character that emerged in the hybrids that has not been detected in any other *Tolpis* endemic to the Canary Islands is white corollas in the outer florets of the capitula (Fig. 8A). This character was not fixed in any of the F_4 inbred lines; rather, it was seen in four of the lines and only in line 13 was it present in more than one plant. The

F_3 maternal plant of line 13 represented the first appearance of this floral trait in any of the hybrid lines, and five of the 34 progeny (*ca.* 13 %) from this plant displayed white florets.

Discussion

Our results demonstrate experimentally the generation of considerable variation in several characters within and across the 12 F_4 inbred lines derived from a single cross between two species of *Tolpis*. For example, self-seed set varied within the F_4 lines; this was more pronounced in some than in others (Fig. 5). An earlier study by Soto-Trejo
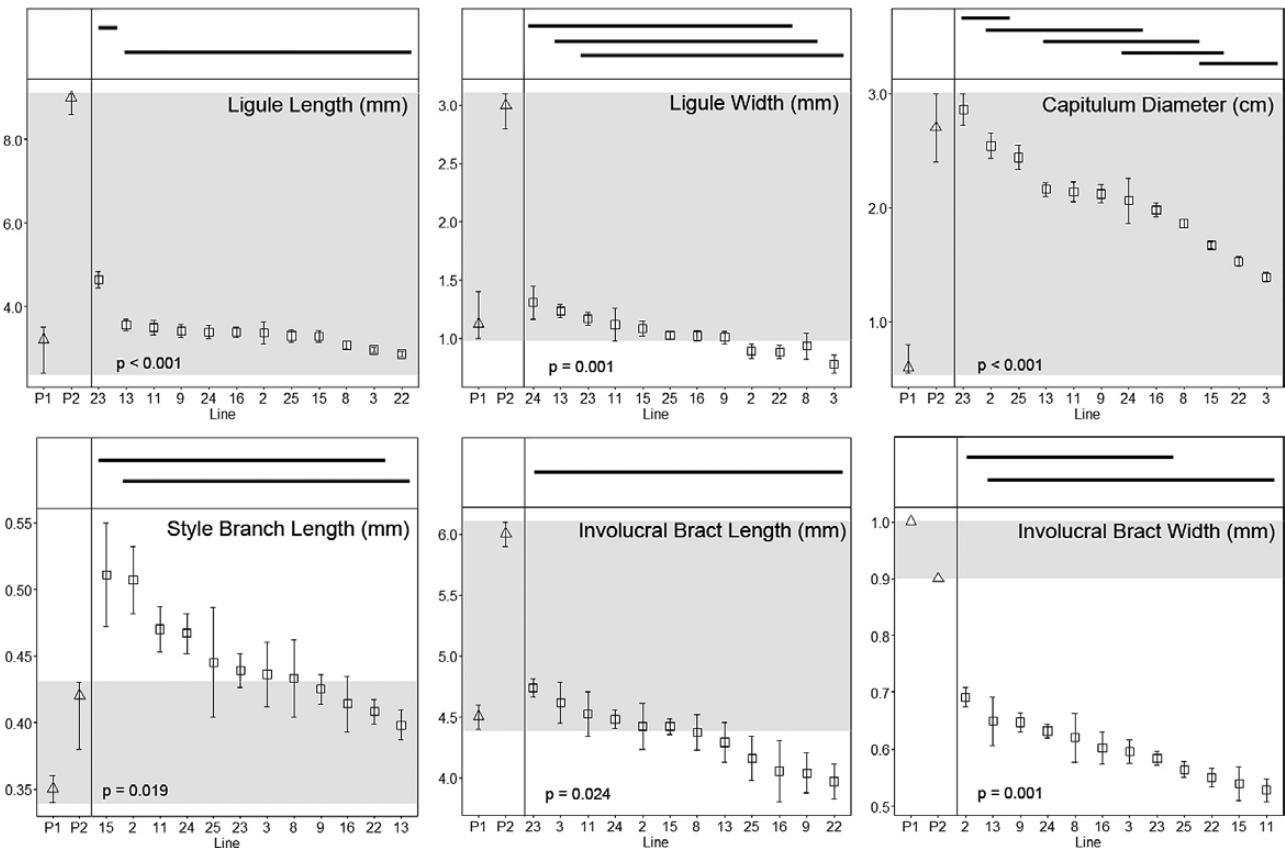
Figure 4. Plots of mean values (squares) and standard errors of inbred hybrid lines for floral characters. An ANOVA yields *P*-values < 0.05 for all characters, suggesting differences among lines. Bars above the graphs indicate which families are not significantly different from one another (*P* > 0.05, Tukey–Kramer HSD *post hoc* tests). Mean values (triangles) and ranges for the original progenitors, *Tolpis coronopifolia* (P1) and *T. santosii* (P2) are shown in the left-hand side of each plot. The greyed regions depict the ranges for the two parents. Hybrid line means falling outside this range represent transgressive traits. Involucral bract length was the only character that did not delimit lines into more than one group. Lines are sorted by decreasing mean values.

et al. (2013) indicated that self-seed set is controlled by a major locus because there was a bimodal distribution of seed set in the F_2 generation, although with a range of values in each of the groups. Even when Soto-Trejo *et al.* (2013) used a cut-off of 30 % or less seed set for SI and 50 % or more for SC, there still was a ratio of 2.94:1 for SI:SC (expected 3:1). Since seeds for succeeding generations originated from selfing, and there is evidence that self-seed set is controlled largely by a recessive allele at a single locus, one would expect the fixation for high self-seed set in each of the lines. The lower seed set may be a reflection of one or a combination of several factors discussed by Soto-Trejo *et al.* (2013), including the influence of modifier loci and pollen viability of parents.

In natural populations of Canary Island *Tolpis*, fruits without a pappus are rarely seen (Fig. 2B; Jarvis 1980; D. J. Crawford, University of Kansas, unpubl. data); however, plants lacking a pappus are unusually common in the population of the SI/PSC parent used in the initial cross in this study (10 %; D. J. Crawford, University of

Kansas, unpubl. data). Three F_4 lines appear to be fixed for pappus absence, and if verified with additional progeny, this would distinguish those lines from all other *Tolpis* endemic to the Macaronesian archipelagos. In addition to being an easily recognizable phenotypic trait (Fig. 2), the lack of a pappus in *Tolpis* diminishes the capacity for wind dispersal (K. J. Niklas *et al.*, Cornell University, unpubl. data).

There are few transgressive traits in *Tolpis* hybrid lines, especially for floral and seed characters. Stelkens and Seehausen (2009) concluded that genetic distance between parental species is positively correlated with transgressive traits in their hybrids, and that strong directional selection on traits is not conducive to the appearance of transgressive traits in the hybrids It is not known whether these two factors are responsible for the low frequency of transgressive traits in *Tolpis* hybrids. However, the lack of ITS sequence variation between the parental species (Mort *et al.* 2007) and likely strong selection for the selfing syndrome (Ornduff 1969; Foxe
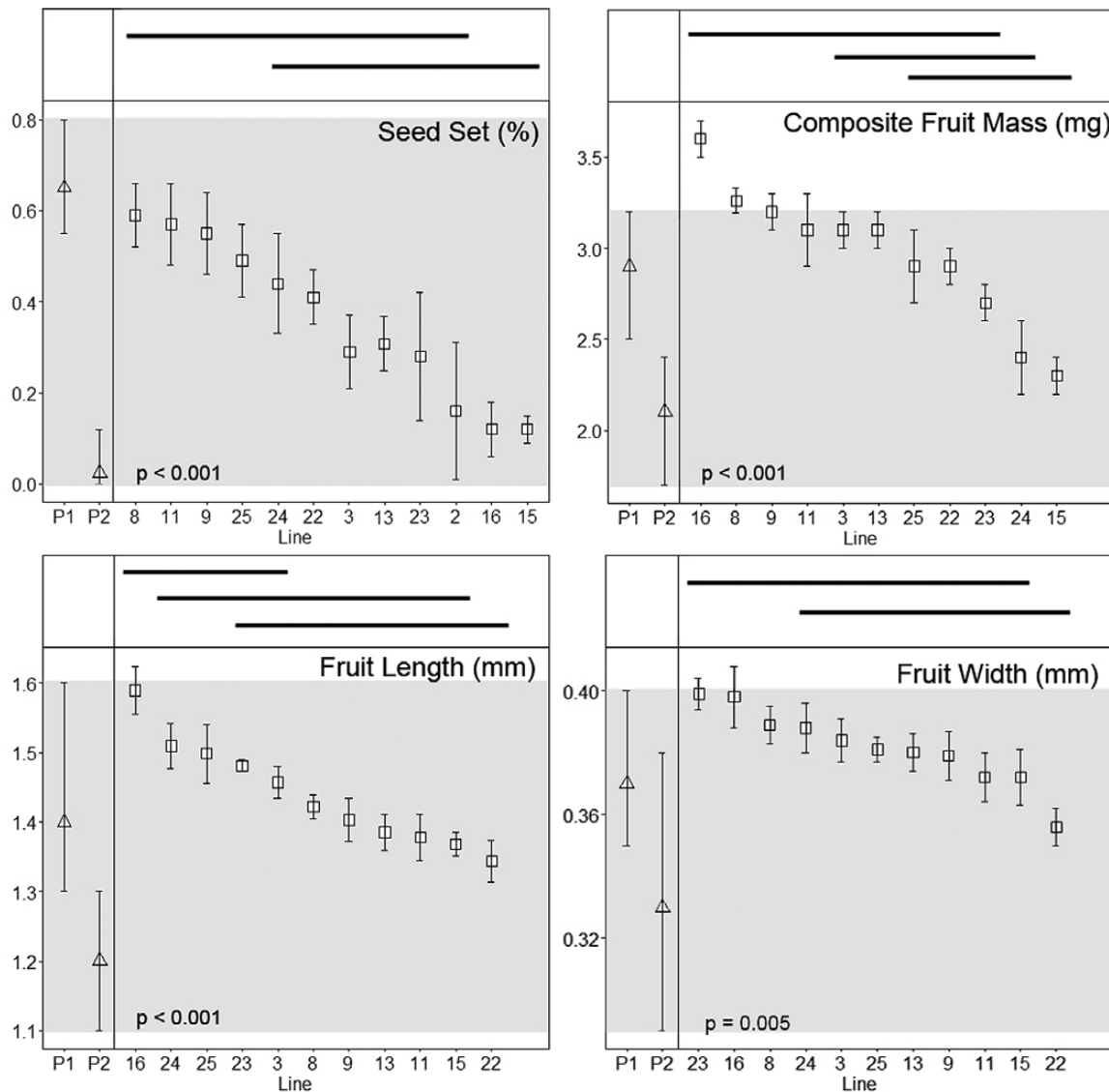
Figure 5. Plots of mean values (squares) and standard errors of inbred hybrid lines for fruit characters. An ANOVA yields *P*-values of < 0.05 for all characters, suggesting differences among lines. Bars above the graphs indicate which families are not significantly different from one another (*P* > 0.05, Tukey–Kramer HSD *post hoc* tests). Mean values (triangles) and ranges for the original progenitors, *Tolpis coronopifolia* (P1) and *T. santosii* (P2) are shown in the left-hand side of each plot. The greyed regions depict the ranges for the two parents. Hybrid line means falling outside this range represent transgressive traits. Inbred lines are sorted by decreasing mean values. Line 2 had an insufficient population size for fruit characters and was not included in the size analyses. Composite fruit mass is a measurement of 20 seeds from each inbred line.

et al. 2009; Guo *et al.* 2009; Slotte *et al.* 2012; Soto-Trejo *et al.* 2013) suggest that the two factors are viable hypotheses for the relative lack of transgressive traits.

The most visibly striking transgressive character in the hybrids is the presence of white corollas on the outer florets of capitula (Fig. 8A) as opposed to the common condition of only yellow corollas (Fig. 8B). As far as we are aware, this colour variant has not been detected in any *Tolpis* endemic to the Canary Islands. However, a similar pattern of pigmentation is sometimes seen in

Tolpis barbata (Fig. 8C), a species that occurs in, but is not endemic to the Macaronesian islands (Jarvis 1980). It is widely distributed in southern Europe and northern Africa (Jarvis 1980). Whether the similar colour patterns have the same genetic basis is not known.

Like floral traits, leaf characters in the hybrids are a mixture of intermediate and transgressive traits (Fig. 3), but all seed traits are intermediate between the parents (Fig. 5). In addition to transgressive traits, intermediate phenotypes can be viewed as novel in the sense that
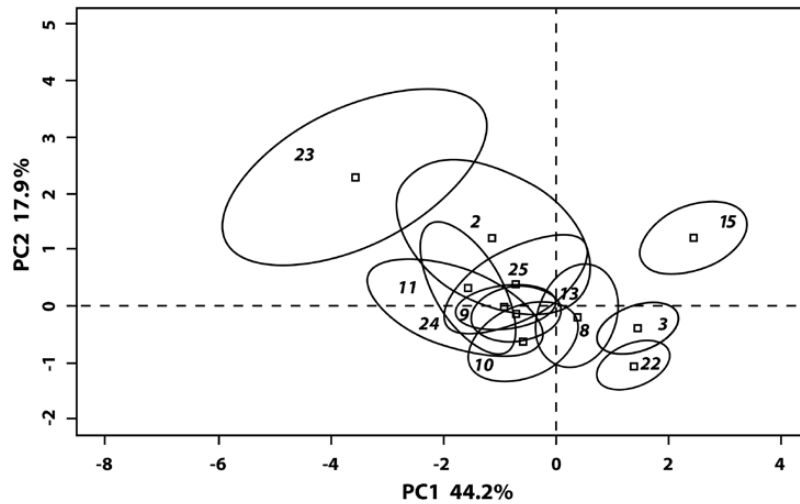
Figure 6. PCA of floral and vegetative characters with 95 % confidence ellipses around the barycentre of each inbred line. The first principal component (PC1) explains 44.2 % of the variance in the data set, and the second principal component (PC2) explains 17.9 %. A complex cluster of hybrid intermediacy is seen, but lines 3, 15, 22 and 23 are clearly distinct from others.
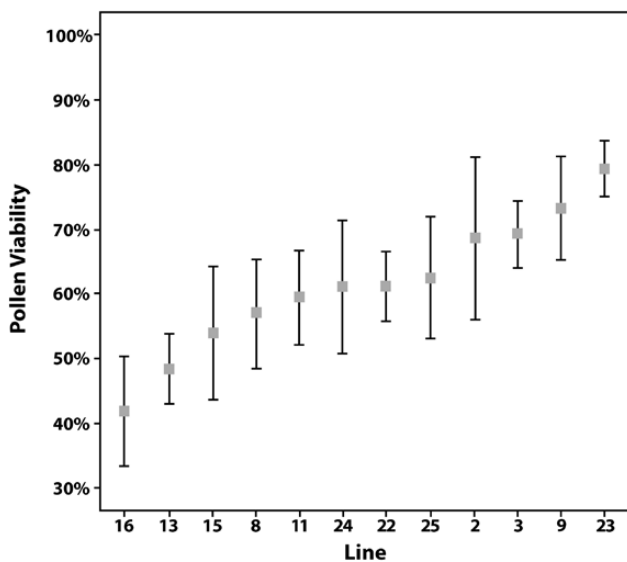
Figure 7. Plot of pollen viability in the F_4 generation lines. Squares denote means and lines standard errors in each of the lines.

they do not occur in either of the parents. For example, García-Verdugo *et al.* (2013) pointed out that interspecific hybrids in Hawaiian *Dubautia* with leaf areas intermediate between the parental species occupy habitats distinct from each of the parents. They suggested that the variation in area and other leaf traits could facilitate the establishment and persistence of the hybrids in microenvironments where the parents were not seen.

In *Tolpis*, leaf perimeter (which reflects leaf dissection) was intermediate between the extremes of the parental species in nearly all hybrid lines (Figs 1B and 3). An array of leaf forms similar to those seen in the hybrids is known

within *Tolpis* in the Canarian archipelago (Jarvis 1980; Crawford *et al.* 2009). *Tolpis* occurs in different vegetation zones (Bramwell and Bramwell 2001), but there are no studies of the correlation between leaf morphology and habitat.

Several of the inbred lines form morphologically distinct cohesive lineages (Fig. 6), and may be identified using a combination of characters. Whether or not any of these phenotypically distinct lines would be recognized as distinct species if they were found in nature is an open question, and ultimately a matter of judgment. Although there is no direct evidence that any of the synthetic hybrid lines would be reproductively isolated from each other, or from their parents in nature, there is reason to believe that gene flow could be reduced. All of the hybrid lines have relatively high levels of self-seed set and can self-pollinate, two attributes that would reduce gene flow among the lines (Levin 2002). Empirical and simulation studies indicate that selfing reduces gene flow via pollen, which would be effective in isolating hybrids from an outcrossing progenitor (Wright *et al.* 2013; Brys *et al.* 2014; Hu 2015). Although different inbred lines would initially be isolated primarily by mating system, other isolating barriers could subsequently evolve (Wright *et al.* 2013). The ability to self could drive the rapid evolution and fixation of characters (Foxe *et al.* 2009; Guo *et al.* 2009). Selfing could facilitate the initial establishment and persistence of small sexually reproducing founder hybrid populations because selfing could provide 'reproductive assurance' when compatible mates and pollinators are limited (Wright *et al.* 2013; Barrett *et al.* 2014; Barrett and Crowson 2016). In the heterogeneous landscape of the Canaries (Carracedo and Day 2002; Carracedo 2011),

Figure 8. Capitula colour variation among several *Tolpis* lineages. (A) The rare, white floral form seen in some hybrid lines. Photo J. Ressler. (B) The typical yellow ligules found in most hybrid plants and all endemic Canarian species of *Tolpis*. Photo J. Ressler. (C) The capitulum of the non-endemic *Tolpis barbata*, grown from seed of *Crawford, Santos & Mort 1840A* collected near Santiago del Tiede, Tenerife. Photo J. K. Archibald.

this may facilitate the colonization of open or disturbed areas not occupied by their parents (e.g. Brochmann *et al.* 2000; Francisco-Ortega *et al.* 2000; van Hengstum *et al.* 2012). The level of inbreeding depression in the selfing hybrids is not known and this could be a factor reducing fitness in the hybrids. For example, Layman *et al.* (2017) demonstrated that high seed discounting and inbreeding depression likely accounted for the maintenance of outcrossing despite the constant input of SC mutations.

Although one of the parents (*T. coronopifolia*) is an annual, all of the hybrids match the other parent (*T. santosii*) in being perennial. The combination of the perennial habit with the capacity for self-seed set would be an advantage in the establishment and persistence of new hybrid populations because individuals could persist even if there were low seed set or suboptimal conditions for seed germination in any given year, particularly in the early stages of population establishment.

Conclusions

Twelve synthetic hybrid lines were generated from two species endemic to the Canary Islands. The parental species are closely related and genetically similar, but divergent in a number of phenotypic traits and in reproductive biology. The hybrid lines are pollen fertile, SC, perennials. Progeny from these lines exhibit combinations of characters not seen in either parent, have characters intermediate between their parents and display some transgressive traits relative to their parents. Morphometric analyses of floral and vegetative traits resolved several of the 12 lines as phenotypically distinct. The phenotypic novelty seen in the synthetic hybrids suggests evolutionary potential, including the possible origin of new homoploid hybrid species in heterogeneous landscapes such as those on the Canary Islands.

Accession Numbers

Vouchers of the parental species used to generate F_4 progeny are deposited in KANU under accession numbers: *Tolpis santosii* 397175 and *T. coronopifolia* 397158. High-quality digital images of the leaves of F_4 progeny are available upon request.

Supporting Information

The following additional information is available in the online version of this article—

Table S1. Loading of PCA analysis.

Sources of Funding

Contributions by the Authors

B.K., J.R. and M.J.S.B. collected and analysed the data; J.K.K. advised on data analyses; M.E.M. assisted in the collection of materials in the field, in the propagation of the experimental hybrids and facilitated funding for the research; A.S.-G. directed field work and the collection of materials; J.C.-C. facilitated field work and provided interpretations of the results; D.J.C. conceived of the project, made the crosses in the greenhouse, and was the primary writer, with extensive input from all other authors.

Conflicts of Interest

None declared.

Acknowledgements

Literature Cited

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJE, Bierne N, Boughman J, Brelsford A, Buerkle CA, Buggs R, Butlin RK, Dieckmann U, Eroukhmanoff F, Grill A, Cahan SH, Hermansen JS, Hewitt G, Hudson AG, Jiggins C, Jones J, Keller B, Marczewski T, Mallet J, Martinez-Rodriguez P, Möst M, Mullen S, Nichols R, Nolte AW, Parisod C, Pfennig K, Rice AM, Ritchie MG, Seifert B, Smadja CM, Stelkens R, Szymura JM, Väinölä R, Wolf JB, Zinner D. 2013. Hybridization and speciation. *Journal of Evolutionary Biology* **26**:229–246.

Abbott RJ, Hegarty MJ, Hiscock SJ, Brennan AC. 2010. Homoploid hybrid speciation in action. *Taxon* **59**:1375–1386.

Anderson E, Stebbins GL. 1954. Hybridization as an evolutionary stimulus. *Evolution* **8**:378–388.

Arnold ML. 2016. Anderson's and Stebbins' prophecy comes true: genetic exchange in fluctuating environments. *Systematic Botany* **41**:4–16.

Arnold ML, Hamlin JAP, Brothers AN, Ballerini ES. 2012. Natural hybridization as a catalyst of rapid evolutionary change. In: Singh RS, Xu J, Kulathinal RJ, eds. *Rapidly evolving genes and genetic systems*. Oxford, UK: Oxford University Press, 256–265.

Barrett SCH, Arunkumar R, Wright SI. 2014. The demography and population genomics of evolutionary transitions to self-fertilization in plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **369**:20130344.

Barrett SCH, Crowson D. 2016. Mating systems in flowering plants. *Encyclopedia of Evolutionary Biology* **2**:473–479.

Bramwell D, Bramwell Z. 2001. *Wild flowers of the Canary Islands*, 2nd edn. Madrid, Spain: Editorial Rueda SL.

Brennan AC, Woodward G, Seehausen A, Muñoz-Fuentes V, Moritz C, Guelmami A, Abbott RJ, Edelaar P. 2014. Hybridization due to changing species distributions: adding problems or solutions to conservation of biodiversity during global change? *Evolutionary Ecology Research* **16**:475–491.

Brochmann C, Borgen L, Stabbetorp OE. 2000. Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Plant Systematics and Evolution* **220**:77–92.

Brys R, Vanden Broeck A, Mergeay J, Jacquemyn H. 2014. The contribution of mating system variation to reproductive isolation in two closely related *Centaurium* species (Gentianaceae) with a generalized flower morphology. *Evolution* **68**:1281–1293.

Carr GD. 2003. Hybridization in Madiinae. In: Carlquist S, Baldwin BG, Carr G, eds. *Tarweeds and silverswords*. St. Louis, MO: Missouri Botanical Garden Press, 79–104.

Carracedo JC. 2011. *Geología de Canarias. Origen, evolución, edad y volcanismo*. Madrid, Spain: Editorial Rueda, E.L.

Carracedo JC, Day S. 2002. *Classic geology in Europe 4: Canary Islands*. Hertfordshire, UK: Terra Publishing.

Crawford DJ, Archibald JK, Mort ME, Santos-Guerra A. 2009. Pollen viability of intra and interspecific F$_1$ hybrids in Canary Island *Tolpis* (Asteraceae). *Plant Systematics and Evolution* **279**:103–113.

Crawford DJ, Anderson GJ, Borges Silva L, Menezes de Sequeira M, Moura M, Santos-Guerra A, Kelly JK, Mort ME. 2015. Breeding systems in *Tolpis* (Asteraceae) in the Macaronesian islands: the Azores, Madeira and the Canaries. *Plant Systematics and Evolution* **301**:1981–1993.

Crawford DJ, Archibald JK. 2016. Island floras as model systems for studies of plant speciation: Prospects and challenges. *Journal of Systematics and Evolution* doi:10.1111/jse.12234.

Crawford DJ, Archibald JK, Stoermer D, Mort ME, Kelly JK, Santos-Guerra A. 2008. A test of Baker's law: the radiation of *Tolpis* (Asteraceae) in the Canary Islands. *International Journal of Plant Sciences* **169**:782–791.

Crawford DJ, Mort ME, Archibald JK. 2013. *Tolpis santosii* (Asteraceae: Cichorieae), a new species from La Palma, The Canary Islands. *Vieraea* **41**:163–169.

Crawford DJ, Stuessy TF. 1997. Plant speciation on oceanic islands. In: Iwatsuki K, Raven PH, eds. *Evolution and diversification of land plants*. Tokyo: Springer-Verlag, 249–267.

Dobzhansky T. 1970. *Genetics of the evolutionary process*. New York: Columbia University Press.

Dray S, Josse J. 2015. Principal component analysis with missing values: a comparative survey of methods. *Plant Ecology* **216**:657–667.

Foxe J, Sotte T, Stahl E, Neuffer B, Hurka H, Wright S. 2009. Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences of the United States of America* **106**:5241–5245.

Francisco-Ortega J, Crawford DJ, Santos-Guerra A, Jansen RK. 1997. Origin and evolution of *Argyranthemum* (Asteraceae). In: Givnish TJ, Sytsma KJ, eds. *Molecular evolution and adaptive radiation*. Cambridge: Cambridge University Press, 407–431.

Francisco-Ortega J, Santos-Guerra A, Kim SC, Crawford DJ. 2000. Plant genetic diversity in the canary islands: a conservation perspective. *American Journal of Botany* **87**:909–919.

García-Verdugo C, Friar E, Santiago LS. 2013. Ecological role of hybridization in adaptive radiations: a case study in the *Dubautia arborea–Dubautia ciliolata* (Asteraceae) complex. *International Journal of Plant Sciences* **174**:749–759.

Gillett GW, Lim EKS. 1970. An experimental study of the genus *Bidens* in the Hawaiian Islands. *University of California Studies in Botany* **56**:1–63.

Gruenstaeudl M, Santos-Guerra A, Jansen RK. 2013. Phylogenetic analyses of *Tolpis* Adans. (Asteraceae) reveal patterns of adaptive radiation, multiple colonization and interspecific hybridization. *Cladistics* **29**:416–434.

Guo Y, Bechsgaard J, Slotte T, Neuffer B, Lascoux M, Weigel D, Schierup M. 2009. Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences of the United States of America* **106**:5246–5251.

Henderson A. 2006. Traditional morphometrics in plant systematics and its role in palm systematics. *Botanical Journal of the Linnean Society* **151**:103–111.

Hu X-S. 2015. Mating system as a barrier to gene flow. *Evolution* **69**:1158–1177.

Jarvis C.E. 1980. *Systematic studies in the genus Tolpis Adanson*. PhD Thesis, University of Reading, UK.

Josse J, Husson F. 2016. missMDA: a package for handling missing values in multivariate data analyses. *Journal of Statistical Software*. doi:10.18637/jss.v070.i01.

Kearns CA, Inouye DW. 1993. *Techniques for pollination biologists*. Niwot, CO: University Press of Colorado.

Kincaid DT, Schneider RB. 1983. Quantification of leaf shape with a microcomputer and Fourier-transform. *Canadian Journal of Botany* **61**:2333–2342.

Layman NC, Fernando MT, Herlihy CR, Busch JW. 2017. Costs of selfing prevent the spread of a self-compatibility mutation that causes reproductive assurance. *Evolution* **71**:884–897.

Levin DA. 2002. *The origin, expansion, and demise of plant species*. New York: Oxford University Press.

Lowrey TK. 1986. A biosystematic revision of Hawaiian *Tetramolopium* (Compositae: Astereae). *Allertonia* **4**:203–265.

Mallet J, Besansky N, Hahn MW. 2016. How reticulated are species? *Bioessays* **38**:140–149.

Mayer SS. 1991. Artificial hybridization in Hawaiian *Wikstroemia* (Thymelaeaceae). *American Journal of Botany* **78**:122–130.

Mayr E. 1942. *Systematics and the origin of species*. New York: Columbia University Press.

Mort ME, Archibald JK, Gibson MJS, Bontrager H, Hauber DP, Silva LB, Sequeira MM, Moura M, Santos-Guerra A, Kelly JK, Gruenstaeudl M, Caujapé-Castells J, Crawford DJ. 2016. Analyses of multiplexed-shotgun-genotyping (MSG) data reveal cryptic biodiversity in Macaronesian *Tolpis*. In: Gabriel R, Elias RB, Amorim IR, Borges PAV, eds. Conference program and abstracts of the 2nd International Conference on Island Evolution, Ecology and Conservation: Island Biology 2016, 18–22 July 2016, Angra do Heroísmo, Azores, Portugal. Arquipelago. Life and Marine Sciences. Supplement 9, 235–236.

Mort ME, Archibald JK, Randle CP, Levsen ND, O'Leary TR, Topalov K, Wiegand CM, Crawford DJ. 2007. Inferring phylogeny at low taxonomic levels: utility of rapidly evolving cpdna and nuclear its loci. *American Journal of Botany* **94**:173–183.

Mort ME, Crawford DJ, Kelly JK, Santos-Guerra A, Menezes de Sequeira M, Moura M, Caujapé-Castells J. 2015. Multiplexed-shotgun-genotyping data resolve phylogeny within a very recently derived insular lineage. *American Journal of Botany* **102**:634–641.

Ornduff R. 1969. Reproductive biology in relation to systematics. *Taxon* **18**:121–133.

Raven PH. 1976. Systematics and plant population biology. *Systematic Botany* **1**:284–316.

Rieseberg LH, Archer MA, Wayne RK. 1999. Transgressive segregation, adaptation and speciation. *Heredity* **83**:363–372.

Rieseberg LH, Ellstrand NC. 1993. What can molecular and morphological markers tell us about plant hybridization? *Critical Reviews in Plant Sciences* **12**:213–241.

Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C. 2003a. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301**:1211–1216.

Rieseberg LH, Widmer A, Arntz AM, Burke JM. 2003b. The genetic architecture necessary for transgressive segregation is common in both natural and domesticated populations. *Philosophical Transactions of the Royal Society of London. Series b: Biological Sciences* **358**:1141–1147.

Schneider CA, Rasband WS, Eliceiri KW. 2012. nih image to ImageJ: 25 years of image analysis. *Nature Methods* **9**:671–675.

Schumer M, Rosenthal GG, Andolfatto P. 2014. How common is homoploid hybrid speciation? *Evolution* **68**:1553–1560.

Slotte TK, Hazzouri M, Stern D, Andolfatto P, Wright SI. 2012. Genetic architecture and adaptive significance of the selfing syndrome in *Capsella*. *Evolution* **66**:1360–1374.

Soltis DE, Visger CJ, Marchant DB, Soltis PS. 2016. Polyploidy: pitfalls and paths to a paradigm. *American Journal of Botany* **103**:1146–1166.

Soto-Trejo F, Kelly JK, Archibald JK, Mort ME, Santos-Guerra A, Crawford DJ. 2013. The genetics of self-compatibility and associated floral characters in *Tolpis* (Asteraceae) in the Canary Islands. *International Journal of Plant Sciences* **174**:171–178.

Stelkens R, Seehausen O. 2009. Genetic distance between species predicts novel trait expression in their hybrids. *Evolution* **63**:884–897.

Stebbins GL. 1959. The role of hybridization in evolution. *Proceedings of the American Philosophical Society* **103**:231–251.

Vallejo-Marín M, Hiscock SJ. 2016. Hybridization and hybrid speciation under global change. *New Phytologist* **211**:1170–1187.

van Hengstum T, Lachmuth S, Oostermeijer JGB, den Nijs HJCM, Meirmans PG, van Tienderen PH. 2012. Human-induced hybridization among congeneric endemic plants on Tenerife, Canary Islands. *Plant Systematics and Evolution* **298**:1119–1131.

Wagner WH Jr. 1970. Biosystematics and evolutionary noise. *Taxon* **19**:146–151.

Whitney KD, Ahern JR, Campbell LG, Albert LP, King MS. 2010. Patterns of hybridization in plants. *Perspectives in Plant Ecology, Evolution, and Systematics* **12**:175–182.

Wright SI, Kaliszand S, Slotte T. 2013. Evolutionary consequences of self fertilization in plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **280**:20130133.

Yakimowski SB, Rieseberg LH. 2014. The role of homoploid hybridization in evolution: a century of studies synthesizing genetics and ecology. *American Journal of Botany* **101**:1247–1258.