

Breeding systems in *Tolpis* (Asteraceae) in the Macaronesian islands: the Azores, Madeira and the Canaries

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Abstract Plants on oceanic islands often originate from self-compatible (SC) colonizers capable of seed set by self-fertilization. This fact is supported by empirical studies, and is rooted in the hypothesis that one (or few) individuals could find a sexual population, whereas two or more would be required if the colonizers were self-incompatible (SI). However, a SC colonizer would have lower heterozygosity than SI colonizers, which could limit radiation and diversification of lineages following establishment. Limited evidence suggests that several species-rich island lineages in the family Asteraceae originated from SI colonizers with some “leakiness” (pseudo-self-compatibility, PSC) such that some self-seed could be produced. This study of *Tolpis* (Asteraceae) in Macaronesia provides first reports of the breeding system in species from the Azores and Madeira,

and additional insights into variation in Canary Islands. *Tolpis* from the Azores and Madeira are predominately SI but with PSC. This study suggests that the breeding systems of the ancestors were either PSC, possibly from a single colonizer, or from SI colonizers by multiple disseminules either from a single or multiple dispersals. Long-distance colonists capable of PSC combine the advantages of reproductive assurance (via selfing) in the establishment of sexual populations from even a single colonizer with the higher heterozygosity resulting from its origin from an outcrossed source population. Evolution of *Tolpis* on the Canaries and Madeira has generated diversity in breeding systems, including the origin of SC. Macaronesian *Tolpis* is an excellent system for studying breeding system evolution in a small, diverse lineage.

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Introduction

Globally, endemic insular floras make up about 25 % of described species of vascular plants, yet oceanic islands constitute only about 5 % of the land surface of the Earth (Kreft et al. 2008). Some 5–10 % of oceanic endemic species are highly threatened (Caujapé-Castells et al. 2010), making them of special conservation concern. As for all organisms, successful reproduction is critical for the survival and evolution of island plants, and any conservation plan that does not include knowledge of reproductive biology is destined to fail (Anderson et al. 2001; Bernardello et al. 2001; Crawford et al. 2011). A widely held generalization about the reproductive biology of oceanic island flowering plants that has emerged from empirical evidence gathered over the past several decades is that they frequently originate from self-compatible (SC) ancestors capable of self-pollination (Baker 1955; Ehrendorfer 1979; McMullen 1987, 1990; Barrett 1996; Anderson et al. 2001; Bernardello et al. 2001; Crawford et al. 2011; Chamorro et al. 2012). The advantages of these attributes in a colonizer are obvious: long-distance dispersal is likely a rare event and one disseminule could establish a sexually reproducing population without the need for specific pollinators. These advantages of SC colonizers were put forward by Baker (1955), and have become known as Baker's Law (Stebbins 1957), although Baker (1967) himself later suggested that the "law" is better considered as a "rule", and should be viewed more in terms of the higher probability of SC colonizers becoming established following long-distance dispersal. Carlquist pointed out (1974, p. 513) that the weakness of this strategy is that a SC colonizer originating from a highly selfing source population would have low genome-wide heterozygosity and limited allelic variation at many loci, which could limit radiation and diversification following establishment. Conversely, although a strictly self-incompatible (SI) colonizer would presumably have higher heterozygosity than a SC colonizer, establishment of a sexually reproducing population would require either a minimum of two disseminules in a single colonization event (and likely more, depending on the alleles at the self-incompatibility or S-locus) or multiple dispersals. This would mean that the founders would have more alleles contributing to later genetic diversity. Furthermore, the existence of SC within a species does not mean that all the individuals are selfing all of the time, or that the colonizers would only self-cross—only that they could do so. Although compatibility in plant breeding systems has often been categorically scored as SI or SC, it has long been realized that the situation is often more complex and variable than two contrasting character states, and that

basically SI plants can occasionally self-fertilize, that is exhibit pseudo-self-compatibility, hereafter designated as PSC (e.g., de Nettancourt 1977; Levin 1996; Cheptou et al. 2002; Barrett 2003; Busch and Schoen 2008; Good-Avila et al. 2008; Brennan et al. 2011). In addition to the initial dispersal to islands, the dynamic landscapes of oceanic archipelagoes (Whittaker and Fernández-Palacios 2007, chapter 2; Fernández-Palacios 2010), with dispersal to other islands in an archipelago or colonization of open areas following disturbances on an island (Roderick et al. 2012; López de Heredia et al. 2014), could select for a combination of establishment and diversification strategies (Wright et al. 2013). These considerations suggest that breeding system variation beyond the extremes of obligate selfing and outcrossing could be significant in adaptation to the changing landscape in oceanic archipelagoes.

There is evidence from Asteraceae, the angiosperm family with the highest number of endemic oceanic angiosperm species (Wagner et al. 1990; McMullen 1999; Bramwell and Bramwell 2001; Danton et al. 2006), that some of the larger lineages in several oceanic archipelagoes are ancestrally SI, e.g., silversword alliance in Hawaii (Carr et al. 1986) and *Scalesia* in the Galápagos Islands (Nielsen et al. 2000, 2003), both presumably with the sporophytic system typical of Asteraceae (Hiscock and Tabah 2003; Brennan et al. 2011). Baker (1967, p. 853) wisely and perceptively stated, "The results of tests on further taxa will be awaited with interest, bearing in mind that more is required in the test than noting that an isolated plant growing away from its native habitat does not set seed, for which reason the acquisition of sufficient data to estimate the frequency of self-incompatibility will be a long task." It can be argued that despite Baker's plea for more data, there has been, with notable exceptions (e.g., Sakai et al. 1997, 2006; Weller et al. 2005; Sun and Ganders 1988), relatively little progress the past half century on understanding the breeding–mating systems in Asteraceae and other insular endemic plant lineages. For clarity, in this paper, breeding system will be used to refer to the aspects of individuals, such as SC; whereas mating system will denote *who mates with whom in nature* (e.g., levels of selfing vs. outcrossing; Neal and Anderson 2005).

The specific purpose of the present study is to provide additional insight into the breeding system of Asteraceae, a family with considerable diversity in many oceanic archipelagoes. The general goal, is that by adding data on a leaky breeding system (PSC) from a diverse clade of insular species, we will advance a more fundamental understanding of how colonization, and subsequent radiation work most effectively after long-distance dispersal (Pannell 2015). Specifically, we focus on self-seed set as a measure of physiological compatibility, which is one of the

key elements of the breeding system (see discussion in Neal and Anderson 2005).

The genus *Tolpis* Adans. (Asteraceae) is monophyletic (Gruenstaeudl et al. 2013) and is primarily distributed in Macaronesia on the Azores, Canary Islands, Cape Verde Islands, and Madeira, with continental occurrences in the Mediterranean regions and North Africa (Jarvis 1980). Of the 12 *Tolpis* species recognized by Jarvis (1980), 10 are insular and two are continental. Six of the strictly insular species recognized by Jarvis (1980) are endemic to the Canaries, with the Azores, Cape Verde, and Madeira each having one endemic species, and another species shared by the Azores and Madeira. A new species from the Canaries has been described recently (Crawford et al. 2013), and there are likely additional cryptic species in the Canaries (Crawford et al. 2009; Gruenstaeudl et al. 2013; A. Santos-Guerra unpubl.), the Azores (Silva et al. 2012), Madeira (M. Menezes de Sequeira unpubl.; A. Santos-Guerra unpubl.) and the Cape Verde Islands (M. Romeiras pers. comm.).

A prior survey (Crawford et al. 2008) of the Canarian *Tolpis* lineage showed that, with one notable exception, all species are obligately SI, but with low self-seed set (PSC) found in some plants. “Self-seed set” is used in the present study in the same sense as it was by Soto-Trejo et al. (2013): the percent seed set following hand pollination of florets from the same plant. There are no absolute values of self-seed set that distinguish PSC from SC, although SC plants typically have much higher seed set than PSC plants. Levin (1996) indicated several criteria that distinguish the two, including that PSC plants typically have higher seed production with outcross than self pollen, and a more continuous distribution of seed set among progeny. One endemic species, *T. coronopifolia* (Defs.) Biv., was shown to be highly SC (uniformly very high self-seed set) and displays several floral features characteristic of the selfing syndrome, including fewer florets per capitulum, smaller florets (i.e., shorter anthers, style branches, and ligules) and lower pollen to ovule ratios (Ornduff 1969; Barrett 2002; Crawford et al. 2008; Sicard and Lenhard 2011; Slotte et al. 2012). The native, non-endemic *T. barbata* (L.) Gaertn. was also shown to be SC; it is widely distributed in the Azores, Canaries and Madeira (Jarvis 1980), and does not occur within the insular clades in molecular phylogenetic analyses (Gruenstaeudl et al. 2013). The only known insular *Tolpis* polyploid, the very rare *T. glabrescens* Kämmer, is endemic to Tenerife in the Canary Islands and showed some breakdown of the SI system, although the number of plants examined was limited by the rarity of the species (Crawford et al. 2008). The first survey (Crawford et al. 2008) provided a general overview of breeding systems in Canary Island *Tolpis*, but included a limited sample

of populations. While all of the Canarian *Tolpis* species known at the time were studied, sampling within populations was limited; seed set from hand pollinations was examined for 83 plants from 33 populations (mean of 2.5 plants per population), and the sample size was effectively reduced by the fact that 33 of the plants from 11 populations focused on the two SC species and the tetraploid species. As a consequence, the evaluation of the levels and prevalence of PSC was based on only 50 plants from 22 populations. However, even with limited sampling, there were intriguing results, with variation in self-seed set evident in several populations. These potentially interesting results prompted the present investigation with the goal of using more species, and more plants within populations to provide a clearer picture of whether and how the SI, SC and PSC system operates in this diverse and ecologically important Canarian group. We also expanded the scope to include all the known *Tolpis* in three Macaronesian archipelagos, with emphasis on providing the first assessments of the breeding systems of *Tolpis* in the Azores and Madeira. *Tolpis* is very rare in the Cape Verde Islands, and has not yet been examined for breeding system. Gruenstaeudl et al. (2013) proposed two plausible biogeographic hypotheses for *Tolpis* in the Macaronesian archipelagoes. The first inferred a single dispersal from the mainland to Madeira, which then served as a stepping stone to the Azores, whereas the second involved two independent dispersals from the continent to Madeira and the Azores. Regardless of which of the scenarios is correct, it is clear that there was an independent dispersal from a continental source area to the Canaries. Knowledge of the breeding system of *Tolpis* in Madeira and the Azores allows inferences about their colonizing ancestors and whether they, like the ancestors of the Canary Islands, were SI or PSC. Another question is whether true SC (loss of functional alleles at S locus) has evolved from a PSC system in the Azores or Madeira, as it has in the Canaries (Jarvis 1980; Crawford et al. 2008). In the attempt to understand the links between breeding systems, and long-distance dispersal and successful colonization, first approximations of breeding systems call for analysis of many species and few individuals (e.g., Anderson et al. 2001; Bernardello et al. 2001). But for a more complete understanding of breeding systems, and the variation and lability of breeding systems, a number of individuals from multiple populations have to be studied carefully and in detail. Collectively, these data help project the features of the first insular colonists in Macaronesian archipelagoes as well as provide insights into the evolution of breeding systems within the island setting, and hence to add the type of information that Baker (1967) called for to gain more refined insights into island plant colonization, establishment and diversification.

Table 1 Population of *Tolpis* used in breeding system studies

Species	Archipelago-Island	Collection	Locale
<i>T. azorica</i>	Azores-São Miguel	Moura TA-SMLC-S1 to S8.	Lagoa do Canário, trackside, UTM (609186, 4188689), 716 m a.s.l.
<i>T. azorica</i>	Azores-São Miguel	Borges SilvaTA-SMME-S1 to S10	Monte Escuro, UTM (637454, 4182984), 798 m a.s.l.
<i>T. succulenta</i>	Azores-São Miguel	Borges SilvaTS-SMPA-S1 to S13	Porto da Ajuda, Bretanha, on a steep rock wall next to the road leading to the harbor, UTM (609857, 4195351), 41 m a.s.l.
<i>T. succulenta</i>	Azores-Santa Maria	H. Schäfer s. n.	Vila do Porto Harbour, exposed steep cliffs, 20 m a.s.l.
<i>T. crassiuscula</i>	Canary Islands-Tenerife	Crawford et al. 1993	Tunnel west of El Fraile
<i>T. laciniata</i>	Canary Islands-La Palma	Crawford et al. 2012	Along highway to Roque de los Muchachos
<i>T. laciniata</i>	Canary Islands-La Palma	Crawford et al. 2015	Along Highway LP-1 between Hoya Grande and Roque de los Muchachos
<i>T. laciniata</i>	Canary Islands-La Palma	Crawford et al. 2016	Road between Las Manchas and Roque de Molina
<i>T. laciniata</i>	Canary Islands-La Palma	Crawford et al. 2017	Along Highway LP-1, just north of Jeday
<i>T. laciniata</i>	Canary Islands-El Hierro	Crawford et al. 1869	Along road above La Frontera
<i>T. laciniata</i>	Canary Islands-El Hierro	Crawford et al. 2002	Scattered in lava along old road above Valverde
<i>T. laciniata</i>	Canary Islands-El Hierro	Crawford et al. 2006	In lava near Mirador de Jinama
<i>T. proustii</i>	Canary Islands-El Hierro	Crawford et al. 2008	Riscos de Bascos
<i>T. santosii</i>	Canary Islands-La Palma	Crawford et al. 2010	Along road just above beach at Playa de Nogales
<i>T. sp. nov. 3</i>	Canary Islands-Tenerife	Crawford et al. 2003	Barranco del Infierno
<i>T. macrorrhiza</i>	Madeira-Madeira	Menezes de Sequeira 7100	Water-channel to Caldeirão-Verde, 500 m from Caldeirão-Verde, on the vertical north exposed walls, as a chasmophyte
<i>T. macrorrhiza</i>	Madeira-Madeira	Menezes de Sequeira 7106	Pico Cidrão. North, exposed rocky walls as a chasmophyte, 32° 44' 26.1''N 16° 56' 20.1'', 1750 m a.s.l.
<i>T. macrorrhiza</i>	Madeira-Madeira	Menezes de Sequeira 7120	Encumeada, at the beginning of the Folhadal water-channel, on the vertical embankments of the road
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7025	Machico, "Pico do Facho", chasmophyte plant community, south exposed, 32°43' 26.35''N16°45' 30.75''W, 272 m a.s.l.
<i>T. succulenta</i>	Madeira- Madeira	Menezes de Sequeira 7058	Formosa beach, on the promenade that leads to Câmara de Lobos, vertical disturbed embankments. 32° 38.581'N 16° 57.698W, 15 m a.s.l.
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7059	Porto Novo, on the west side of the river, on NE exposed rocky walls, 32° 45.622'N 16° 48.607'W, 85 m a.s.l.
<i>T. succulenta</i>	Madeira- Madeira	Menezes de Sequeira 7061	Marçoços, near the entrance to the tunnel to Porto da Cruz, on the road to Portela, 32° 44.127'N 16° 48.312' W, 83 m a.s.l.
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7062	Porto da Cruz, close to the beach, on rocky walls, 32° 46.473'N 16° 49.685'W, 50 m a.s.l.
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7083	Road from Ponta do Sol to Canhas, rocky walls
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7094	On the road from Ribeira do Inferno to Seixal, Lajido; chasmophyte on vertical embankments
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7095	Porto Moniz, near the ocean
<i>T. succulenta</i>	Madeira-Madeira	Menezes de Sequeira 7104	Road to Pico Areeiro, circa Poço da Neve. 1667 m a.s.l. N32 43,560W 16 55,483

Materials and methods

Seeds collected from natural populations served as sources for plants grown in the University of Kansas greenhouses. Seeds from the Canary Islands were collected by D. J. Crawford et al. and A. Santos-Guerra, those from the Azores by M. Moura, L. Borges Silva and H. Schäfer, and those from Madeira by M. Menezes de Sequeira (Table 1).

Progeny were most often generated from individual plants, and less frequently from bulked seed from natural populations (Table 1). Only healthy and vigorous plants were selected for study in the greenhouse to minimize resource limitation as a factor in low seed set. Three species included in Crawford et al. (2008), *Tolpis barbata*, *T. coronopifolia*, and *T. glabrescens*, were not considered in the present study because the former two species were shown to be uniformly

SC, whereas additional material of the very rare *T. glabrescens* was not available. All other species recognized by Jarvis (1980) for the Azores, Canaries, and Madeira were included, as was the recently described *T. santosii* Crawford, Mort and Archibald (Crawford et al. 2013). Voucher specimens are deposited in the McGregor Herbarium (KANU) at the University of Kansas.

The methods of Crawford et al. (2008) were employed to use the capacity for self-seed set as the measure of SC. Pollen fertility was determined for each plant using the lactophenol aniline blue method described by Kearns and Inouye (1993), with at least 200 pollen grains scored per plant. The darkly stained, plump viable pollen grains were easily distinguished from the shriveled, lightly stained nonviable grains. Documentation of pollen fertility assured that seed set was not a reflection of the quality/viability of the pollen (Gottlieb 1973; Soto-Trejo et al. 2013). Plants with lower than 30 % pollen fertility, which occurs rarely in populations (Online resource 1), were excluded. In some instances, instead of bagging individual capitula, plants were isolated spatially. Results for 10 SI plants showed that non-bagged capitula had no self-seed set, a verification of the lack of biotic and abiotic (e.g., wind) pollen vectors in the glasshouses. To test for SC, the flowers of a capitulum were gently brushed across the open flowers of a second capitulum from the same plant on several successive days as the anthers of florets sequentially dehisced and the style branches became receptive. This was done to assure pollination, even though it has been shown that SC *Tolpis* plants have high self-seed set without manipulation (Soto-Trejo et al. 2013). Because of the large number of plants selfed, it was not possible to test all individuals for out-crossed-seed set to calculate the compatibility index (i.e., percent self-seed set/percent seed set after cross pollination; Lloyd and Schoen 1992), which corrects for factors such as pollination techniques and variation in plant vigor (see “Results”). Crosses were made between pairs of plants from the same populations, or sometimes between two plants from different populations of the same species from the same archipelago. In some cases, percent seed set was determined for reciprocal crosses.

Subsequently, percent self-seed set was used as the comparative measure of compatibility. In all instances, fruits were examined from 2 to 5 capitula per plant, with shriveled fruits taken to indicate a lack of embryos (Fig. 1; Crawford et al. 2008; Soto-Trejo et al. 2013). That is, seed set, and more to the point, the percent seed set, was determined by actual counts and calculations (% seed set = number viable seeds/number viable + number of shriveled seeds). In some instances, the same selfing crosses were made on successively maturing capitula (beyond the first 5) on individual plants to determine whether seed set changed with the sequence of maturity on the



Fig. 1 Mature capitula of *Tolpis macrorrhiza*. Capitulum on left with non-viable, shriveled fruits and one on right with viable fruits

plant. In all instances, unless otherwise indicated, “self-seed set” means the percent seed set from these experimental populations. Using standard techniques (Martin 1959), observations were made of pollen tube growth in the styles of four randomly selected SC (individuals of *Tolpis coronopifolia*) and SI plants following hand self-pollination. There were many pollen tubes in the styles of SC plants; whereas there was no pollen tube growth into the styles of SI plants, a pattern characteristic of the sporophytic SI system of Asteraceae (Allen et al. 2011, and references therein). These observations indicate that lack of self-seed set is the result of SI and not early acting inbreeding depression. For statistical analysis of pollen viability and self-seed set (e.g., testing for differences among populations), we applied the angular transformation to proportions. However, we report differences below on the original scale of measurement.

Results

The populations from which data were obtained are presented in Table 1. A summary of the salient aspects of the results is presented in Table 2, with the data on which the summaries are based given in the Online resource 1. Details not amenable to inclusion in tabular form are presented in this section. Prior to testing for differences between particular populations, we tested for an effect of maternal plant on both pollen viability and self-seed set using Nested ANOVAs (maternal plant within population). We find a highly significant effect of maternal plant on pollen viability ($F_{59, 230} = 2.18, p < 0.001$), but not on self-seed set ($F_{54, 244} = 0.63, p > 0.1$). The correlation between pollen viability and self-seed set was not significant. Differences among populations were significant for both pollen viability ($F_{26, 59} = 2.21, p < 0.01$) and self-seed set ($F_{28, 54} = 2.71, p < 0.01$).

Table 2 Overview of self-seed set in Macaronesian *Tolpis* (see Table 1 for population localities, and Online resource 1 for the data on which the summaries are based); “bulked seed” denotes seed from unknown number of plants combined

Species (collection)	No. source plants/no. populations	No. progeny fully SI	No. progeny setting seed	% Seed set
<i>T. azorica</i> ^a	5/2	25	1	0.5 %
<i>T. succulenta</i> ^a (Azores)	3/2	13	1	8 %
<i>T. succulenta</i> ^a (Madeira)	29/8	95	18	$\bar{\chi}$ = 8 % (first capitula) r = 0.4–30 % (first capitula) $\bar{\chi}$ = 8 % (later capitula) r = 0.6–45 % (later capitula) See text
<i>T. macrorrhiza</i>	11/3	See text	See text	See text
<i>T. crassiuscula</i> (1993)	4/1	16	25	$\bar{\chi}$ = 5 % r = 0.3–14 % See text
<i>T. sp. nov. 3</i> (2030)	Bulked seed/1	5	4	$\bar{\chi}$ = 4 % r = 2–40 %
<i>T. laciniata</i> (1869)	Bulked seed/1	6	3	$\bar{\chi}$ = 2.3 % r = 0.4–6 %
<i>T. laciniata</i> (2002)	13/1	6	7	$\bar{\chi}$ = 37 % r = 4–82 %
<i>T. laciniata</i> (2006)	6/1	24	0	$\bar{\chi}$ = 0 %
<i>T. laciniata</i> (2007)	7/1	37	12	$\bar{\chi}$ = 2 % r = 0.4–13 %
<i>T. laciniata</i> (2012)	1/1	4	2	$\bar{\chi}$ = 0.75 % r = 0.6–9 %
<i>T. laciniata</i> (2015)	1/1	9	1	9 %
<i>T. laciniata</i> (2016)	4/1	0	4	$\bar{\chi}$ = 3.8 % r = 2–6 %
<i>T. laciniata</i> (2017)	1/1	5	2	$\bar{\chi}$ = 1.9 % r = 0.7–3 %
<i>T. proustii</i> (2008)	2/1	7	4	$\bar{\chi}$ = 1.2 % r = 0.1–3.4 %
<i>T. santosii</i> (2010)	3/1	See text	See text	See text

Progeny are plants grown from seed of individuals or from bulk seed from plants in natural populations

$\bar{\chi}$ mean and r denotes range when two or more progeny have seed set, See text additional details in “Results” section of text

^a Data combined for a species, where one species occurs on more than one archipelago, the locations given in parentheses

Outcross-seed set

Outcross-seed set, used to insure that the crossing methods employed for assessing SC were effective, was above 80 % for crosses between pairs of plants in the same population or between populations of the same species (Online resource 1). Given the very high outcross-seed set, the values for self-seed set are given as the percent seed set, rather than calculating a self-compatibility index (Lloyd and Schoen 1992) because the results are similar with the two methods. In several crosses, particularly between plants from the same population, or between progeny of the same maternal plant, there were reciprocal differences between seed set of the parental plants, with one having high seed

set and the other none. This pattern is indicative of the sporophytic SI system found in Asteraceae, where there may be dominance relationships among S-alleles, including differing patterns of dominance for the pollen and stigma (Hiscock and Tabah 2003; Lafuma and Maurice 2007).

Self-seed set

Azores

T. azorica (Nutt.) P.Silva. Self-seed set was very low in this species, with one plant having viable seed (Table 2; Online resource 1). *T. succulenta* Lowe. As with *T. azorica*, 1 tested plant from the Azores had self-seed set (Table 2; Online

resource 1). There were no significant differences in pollen viability or seed set between the two species.

Madeira

T. succulenta Lowe. Sixteen percent of the progeny had self-seed set, with 5 of 8 Madeiran populations having 1 or more maternal plants with viable seed (Table 2; Online resource 1). Although there were no significant differences among populations, nearly 67 % (12 of 18) of the progeny with seed set were from the 2 populations referred to as 7058 and 7059 (Table 1; Online resource 1). Six progeny had increased seed set in later-maturing capitula, the largest being the increase from 2 to 45 % for 1 progeny from 7094 (Online resource 1).

T. macrorhiza (Lowe ex Hooker) DC. This is the only species of Macaronesian *Tolpis* in which a small percentage of plants flower in the greenhouse during any one cycle. The flowering “season” or “cycle” denotes an episode of flowering in which the perennial plants flower for several weeks, followed by the senescence of the flowering branches, and after several months, another cycle of flowering commences. Less than 10 % of the individuals from 3 populations flowered in the greenhouse in the first year, and overall, less than 20 % have flowered over 2 years. In this species, the first capitulescence on a plant is typically a central, upright unit; whereas later-maturing capitulescences, if they develop, occur laterally (Fig. 2). In the following results, progeny from the seed of individual maternal plants collected from natural populations are designated by letters in bold and numbers in parentheses refer to plants grown from seeds from each of the progeny. Some progeny developed only a central capitulescence. **Population 7100: Plant A:** (1) 94 % self-seed set for 10 capitula on the central capitulescence. **Plant B:** (1) no self-seed set on central flowering branch; (2) central capitulescence with 9 % seed set; (3) 0.4 % self-seed set for 6 capitula on central capitulescence, first lateral capitulescence with 2 % for 5 capitula, and a second lateral branch with 44 % seed set for 6 capitula; (4) no seed set on central flowering branch. **Plant D:** (1) 15 % seed set for 6 capitula on first-formed capitulescence and 50 % on lateral flowering branch. **Plant E:** (1) no seed set on 6 capitula of central flowering branch; (2) 46 % self-seed for 8 capitula on central capitulescence. **Plant F:** (1) no seed set on central capitulescence; (2) central capitulescence with 33 % seed set. **Plant G:** (1) 16 % self-seed set on central flowering branch. **Population 7106: Plant A:** (1, 2) no seed set on their central or lateral flowering branches; (3) 1 % seed set on central capitulescence; (4) 4 % seed set on central capitulescence. **Plant F:** (1) 24 % seed set on central flowering branch, 90 % seed set on lateral capitulescence. **Population 7120: Plant A:** (1) 15 % seed set on



Fig. 2 Plant of *T. macrorhiza* showing a central capitulescence and several lateral flowering branches

central capitulescence. **Plant D:** (1) no seed set on central flowering branch; (2) 8 % seed set on a central capitulescence. **Plant E:** (1) 5 % seed set on central flowering branch.

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Tenerife. *T. crassiuscula* Svent. As summarized in Table 2, self-seed set is generally low, if present at all. All 4 maternal plants had 1 or more progeny with seed. This is the only known population of this rare species consisting of more than a few individuals. There was no increase in seed set with later-maturing capitula. ***T. sp. nov. 3.*** Seed set was generally low for population 2030, but there was a wide range among the plants (Table 2). **El Hierro. *T. laciniata*** (Schl Bip. ex Webb & Berthel.) Webb: The 4 populations from this island show extensive variation in self-seed set within and among populations (Tables 1; 2). ***T. proustii*** Pit. Seed set was generally low (Table 2); 2 of the 3 maternal plants had progeny with self-seed set. **La Palma. *T. laciniata.*** All populations had some progeny with low seed set (<10 %, Tables 1, 2). ***T. santosii*** (reported as *T. sp. nov. 1* in Crawford et al. 2008). **Plant B:** 1 progeny had 4 % seed set for the first 5 capitula, 30 %, on the next 6 capitula, and 32 % on the next 5 capitula; a second progeny had 17 % seed set for the first 5 capitula, the next 6 with 41 %, and the next 5 capitula had 60 %. **Plant C:** 1 progeny had 20 % seed set for first 3 capitula and 36 % on

next 6 capitula. **Plant G:** 1 progeny had 0.3 % self-seed set on first 15 capitula.

Discussion

This study provides further evidence of the subtle variation in the breeding systems in insular Asteraceae, even in a relatively small lineage such as Macaronesian *Tolpis*. In a recent review, Pannell (2015), argues for making the distinction between the ability to set low levels of self-seed on occasion, and selection on overall selfing rate. The first data presented here for the Azores, while based on limited sampling, indicate that both *T. azorica* and *T. succulenta* are highly SI, with rare plants exhibiting low levels of PSC (first of Pannell's two categories). It appears that the colonizing ancestors of the Azores, as in the Canaries (Crawford et al. 2008), were SI or weakly PSC. The very low PSC in contemporary Azorean *Tolpis* raises the possibility of a single colonizer (fruit) establishing a sexual population via self-seed. Obviously, other scenarios are possible, in particular a situation with multiple disseminules in a single event, or multiple dispersal events could yield colonizers bearing S-alleles that permit compatible crosses.

Molecular phylogenetic studies (Mort et al. unpubl.) provide strong support for the monophyly of Azorean *Tolpis*, indicating that they have evolved from a common ancestor. There apparently has been little, if any, divergence from the ancestral breeding system during speciation and radiation in the Azores. For example, there is no evidence of the evolution of SC and the floral selfing syndrome (Ornduff 1969; Barrett 2002; Slotte et al. 2012) in the Azores, as was documented for *T. coronopifolia* in the Canary Islands (Crawford et al. 2008). In concordance with the low self-seed set, very preliminary data on capitulum diameters and other floral measurements for *T. azorica* and *T. succulenta* (Jarvis 1980; Crawford et al. unpubl.) fall into the range for outcrossing species on the Canaries (Crawford et al. 2008; Soto-Trejo et al. 2013), and pollen-ovule ratios range from 2400:1 to 2800:1 for the Azorean plants (Crawford et al. unpubl.), which is indicative of outcrossers (Cruden 1977).

In Madeira, the morphologically variable *T. succulenta* is SI, with some self-fertilization found in about 15 % of the progeny, but with a mean of less than 5 % seed set per plant for the plants capable of selfing. Plants capable of selfing are distributed among the populations on Madeira, but some (e.g., populations 7058 and 7059; Online resource 1) do have a higher percentage of plants with viable seed. With rare exceptions (Online resource 1), there is no increased seed set on later-maturing capitula. Overall, *T. succulenta* in Madeira has, in the words of Pannell (2015,



Fig. 3 On left, two-year-old individual of *T. macrorrhiza* that has not flowered in greenhouse; on right, individual that is flowering in first year, with the central and one lateral capitulescence

p. 3), “a simple capacity for occasional self-fertilization”, which is the prevalent breeding system in populations of Macaronesian *Tolpis*.

The pattern of flowering and self-seed set in *Tolpis macrorrhiza* differs in several respects from all other *Tolpis* species in the Macaronesian archipelagoes. As indicated earlier, flowering is infrequent in cultivation, with a low percentage of the plants flowering over more than 2 years (=two flowering cycles). The greenhouse-grown plants are uniformly large and vigorous, and there is no evidence that lack of flowering is caused by limited resources. In fact, several small plants flowered during the first season whereas others from the same population have grown much larger and have not flowered after 2 years (Fig. 3). Field observations (M. Menezes de Sequeira) parallel the greenhouse results in that they indicate that fewer than 10 % of the adult plants of *T. macrorrhiza* in natural populations are in flower in any given year.

Tolpis macrorrhiza is unusual, and perhaps distinctive, in another respect: we are not aware of reports, either in Asteraceae or other plants, of one of the phenomena seen in this species, namely, selfing success varying between flowering branches on a given plant. Of course, most studies have not had the luxury of focusing on an array of genotypes from each species, where variation such as we describe is revealed. However, similar temporal patterns of self-seed set have been shown where the strength of SI may decrease with the age of a given flower (Vogler et al. 1998; Goodwillie et al. 2004; Good-Avila et al. 2008). In addition, Travers et al. (2004), working with *Solanum carolinense* (Solanaceae), showed increased levels of successful self crosses in later flowers where no fruits had developed from earlier-developing flowers on the same plant. It would seem that the lack of developing fruits lowers the barrier to selfing in normally SI plants (gametophytic SI in this case) plants. The pattern in *T. macrorrhiza* is similar to *Solanum carolinense* except that in *Tolpis* later-maturing capitula set higher self-seed even with earlier capitula developing fruit. The variation of self-seed set among individuals within a

population such as herein reported for *T. macrorhiza* has been found in other plants where seed set varies among individuals within the same and different populations (Brauner and Gottlieb 1987; Maddux et al. 1996; Goodwillie et al. 2004; López-García and Maillet 2005; Ortiz et al. 2006; Good-Avila et al. 2008). This is perhaps analogous to the pattern of communication among disparate branches on a plant that Diggle (1991) found with andromonoecious solanums, where the development (or lack thereof) of fruits in inflorescences determined the gender of subsequently developing flowers (e.g., higher fruit set in earlier developing flowers dictated a higher proportion of staminate-only flowers in later developing inflorescences).

The capitula of *T. macrorhiza* are the smallest of the SI species of *Tolpis* in Macaronesia (Jarvis 1980; Crawford et al. unpubl.) and the pollen/ovule ratios (1800:1–2200:1, Crawford unpubl.) are lower than reports for other *Tolpis* SI species (most values are over 2500:1; Crawford et al. 2008). While this species does not exhibit a highly evolved selfing syndrome, certain floral characters (i.e., smaller capitula and fewer flowers) tend toward those seen in the SC *T. coronopifolia* (Soto-Trejo et al. 2013). Studies of *Hypochaeris salzmanniana* (Ortiz et al. 2006) and *Crepis tectorum* (Andersson 2012), which like *Tolpis* belong to tribe Cichorieae of Asteraceae, showed that the SC plants also had smaller capitula with fewer florets than plants from SI populations. Whether the smaller capitula of *T. macrorhiza* could be associated with the transition to selfing and represent early stages in the evolution of the selfing syndrome, as seen in *T. coronopifolia* in the Canaries, remains an open question and awaits further study.

As with Azorean *Tolpis*, molecular phylogenetic studies (Mort et al. unpubl.) also provide strong support for the monophyly of Madeiran *Tolpis*, which means that *T. succulenta* is not monophyletic, something that has been inferred from other studies (Silva et al. 2012; Gruenstaedl et al. 2013). However, in contrast to the Azores, there has been more divergence in the reproductive biology and breeding system associated with speciation in Madeira. The breeding system of the colonizing ancestor(s) of *T. succulenta* in Madeira were likely similar to those in the Azores, either SI or PSC.

The present results generally confirm the earlier conclusions of low self-seed set for *Tolpis* in the Canary Islands (Crawford et al. 2008), but more intensive sampling within populations has revealed variation not detected with the limited sampling in the prior study. Most notably, variation in seed set, as discussed above for *T. macrorhiza*, was also found among capitula of individual plants of *T. santosii*, with seed set values generally higher in later-maturing capitula. Clearly, in some instances, as has been reported in other plants (Vogler et al. 1998; Goodwillie et al. 2004; Good-Avila et al. 2008), there is considerable

plasticity in the SI response in individual plants. However, the underlying causes of the variation and the physiological basis of how such a system operates are not poorly known.

There can be little doubt that the ability to set some seed from self-fertilization could be critical to the colonization and establishment of a sexual population, especially with the chance dispersal of a single disseminule to an oceanic island (Baker 1955, 1967; Stebbins 1957; Barrett 1996; Bernardello et al. 2006; Pannell 2015). However, as emphasized by Pannell (2015) and mentioned earlier, the ability to self does not mean that there is selection for an overall higher selfing rate. Data presented in this paper and in earlier studies (Crawford et al. 2008, 2010; Soto-Trejo et al. 2013) suggest that this important point is relevant to Macaronesian *Tolpis*, and that *Tolpis* may be a suitable system for studying the factors that could influence selfing rate, including the origin of true SC. Initially, the ability to set some seed by self-fertilization could have facilitated the establishment of *Tolpis* in the Macaronesian archipelagoes, and this remains the predominant breeding system in most populations. However, this is not the situation for all *Tolpis*. As described earlier and discussed further below, variation from this basic pattern of low, occasional self-seed set is seen in both Madeira and the Canary Islands, culminating in the transition to true SC (loss of functional S-locus) and the evolution of typical morphological features characteristic of the selfing syndrome in *T. coronopifolia* in the Canaries (Crawford et al. 2008; Soto-Trejo et al. 2013; J. K. Kelly unpubl.).

The transition to selfing is one of the most common transitions in flowering plants and available evidence from many studies are consistent in indicating that the loss of a functional S-locus (SC) is irreversible and has occurred multiple times in some lineages (Igic et al. 2008; Pettengill and Moeller 2011; Wright et al. 2013; Barrett et al. 2014). The two most commonly invoked factors for the transition to selfing are reproductive assurance and the “automatic selection hypothesis”, also referred to as the “automatic transmission” hypothesis of Fisher (Barrett et al. 2014; Pannell 2015). The first posits that when compatible mates or pollinators limit outcross seed set, higher selfing rates would be favored. The second hypothesis is based on an idea that a selfing gene has a 3:2 advantage because it could be transmitted through both the pollen and ovule of an individual as well as contributes pollen to outcrossing. By contrast, pollen transmission of an SI gene can occur only through outcrossing. Inbreeding depression is generally accepted as the primary factor maintaining outcrossing. It has long been recognized that distinguishing between the two alternative hypotheses for increased selfing is not a trivial task, and indeed elucidating the factors affecting selfing rates are complex and difficult to disentangle (Busch and Delph 2012; Barrett et al. 2014; Pannell

2015). While the results of the present study and a prior investigation of Macaronesian *Tolpis* (Crawford et al. 2008) do not address directly these complex, basic questions about the transition to selfing, the documented variation in breeding system do raise some relevant questions that could serve as a general framework or context for discussion and future studies. In addition, the advantages of the insular setting for studying breeding system variation are considerable.

The transition to SC (loss of functional S-locus) and the evolution of the selfing syndrome is generally considered to be irreversible (Igic et al. 2008; Pettengill and Moeller 2011; Wright et al. 2013; Barrett et al. 2014). However, this is not invariably the case for PSC, where variation in seed set from self-fertilization in a PSC system may be influenced by modifier loci that may or may not be associated with the S-locus, and selection may favor higher or lower seed set (Levin 1996; Good-Avila et al. 2008; Brennan et al. 2011; Pannell 2015). As indicated earlier, the transition to SC has occurred at least once in *Tolpis* in the Canaries, with *T. coronopifolia* occurring primarily in open habitats, i.e., areas of more recent volcanic activity and landslides, on the island of Tenerife (Jarvis 1980). Genetic data show that the SI–SC response in *Tolpis* is associated with a single locus or several tightly linked loci (Soto-Trejo et al. 2013; J. K. Kelly et al. unpubl.). Whether or not the paucity of compatible mates during the initial colonization and establishment on open habitats on Tenerife was a factor in the evolution of SC in *T. coronopifolia* is an open question. However, the occurrence of the species on several areas of recent landslides and volcanic activity (Jarvis 1980), suggests that SC may be a factor in the present distribution of the species.

There are reports of species with populations consisting of plants that are either basically SC or highly to obligately SI (e.g., Foxe et al. 2010; Busch et al. 2011) and other reports of polymorphism for presence–absence of functional S-alleles within populations (e.g., Brauner and Gottlieb, 1987). In this regard, it is of interest to note that in addition to SC in *T. coronopifolia*, observations of variation in self-seed set in other Macaronesian *Tolpis* suggest the possibility of the rare occurrence of nonfunctional S-alleles within two of the populations examined in the present study, although more data are needed. The variation we report in self-seed set among progeny (0–94 %) in one population (*Menezes de Sequeira 7100*, see “Results”) is suggestive of polymorphism for functional S-alleles. Another possible example of variation occurs in population 2012 (Table 1) from El Hierro in the Canaries where half the progeny had no self-seed; while two other progeny had over 60 and 80 % seed set. Determining whether the high seed set is the result of nonfunctional S-alleles or variation at modifier loci could be assessed by crossing to SI plants

and looking at segregation in the F₂ generation, as was done by Soto-Trejo et al. (2013) with *T. coronopifolia*. If seed set is controlled by the S-locus, then there should be segregation for high and low selfing (Soto-Trejo et al. 2013); whereas if seed set is the result of modifier loci, then, the progeny should exhibit continuous seed set (Levin 1996; Brennan et al. 2011).

Variation in the occurrence and level of self-seed set among plants from the same population, as seen in some populations of *Tolpis*, has been reported in many other angiosperm species (Good-Avila and Stephenson 2002; Mable et al. 2005; Ortiz et al. 2006; Stone et al. 2006; Good-Avila et al. 2008). Consider next plants that are basically SI but with PSC. In most populations of *Tolpis*, seed set from self-fertilization is generally low (<10 %) but there are populations on Madeira and the Canary Islands where seed set is considerably higher in some progeny (Table 2, Online resource 1). This variation in seed set by self-fertilization raises the issue, emphasized by Pannell (2015), of whether the variation is the result of selection for selfing rate or just reflects the ability to set some seed. Oceanic archipelagoes provide excellent settings for examining this question because natural disturbances such as landslides and volcanic activity on single islands create shifting, dynamic landscapes (Whittaker and Fernández-Palacios 2007, chapter 2; Fernández-Palacios 2010) and there are opportunities for inter-island dispersal within archipelagoes (Roderick et al. 2012; López de Heredia et al. 2014). The colonization of newly open habitats created by disturbances on single islands and interisland dispersal within an archipelago could result initially in a paucity of compatible mates and select for higher selfing rates, whereas values could be lower when more compatible mates and/or pollinators are present in larger populations. Landslides and volcanic activities in the Canary Islands have occurred repeatedly, from over a million years before present to historical times (Carracedo and Day 2002; Hoernle and Carracedo 2009), with recurrent colonization and establishment of populations. *Tolpis* occurs on the substrate of different ages, and it could be informative to examine breeding systems in populations of various sizes on substrates of different ages to see if there are correlations between age of substrate, population size and breeding system. Correlations between high self-seed and recent disturbance would be concordant with, but not prove, that reproductive assurance is selecting for higher selfing rates. In this regard, it is of interest to note that population Crawford et al. 2002 (Table 1) in which there are individuals with high seed set was also included in an earlier study (Crawford et al. 2010) demonstrating a mixed mating system in what was considered to be a highly outcrossing population. This population is from a disturbed roadside on the island of El Hierro (Table 1).

Although the present study of breeding systems in *Tolpis* in three Macaronesian archipelagoes is primarily descriptive, the intensive sampling done at the populational level has revealed complex and subtle diversity. The results of this study suggest that the variation seen in *Tolpis*, when combined with the dynamic island setting in which it occurs, provide an excellent system for examining some of the basic questions in the evolution of plant mating systems.

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