

Molecular and quantitative trait variation across the native range of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-adaptation?

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Abstract

To understand the success of invasive species, it is important to know whether colonization events are facilitated by adaptive evolution or are limited to sites where a species is pre-adapted to thrive. Studies of the ancient colonization patterns of an invader in its native range provide an opportunity to examine its natural history of adaptation and colonization. This study uses molecular (internal transcribed spacer sequence and amplified fragment length polymorphism) and common garden approaches to assess the ancient patterns of establishment and quantitative trait evolution in the invasive shrub *Hypericum canariense*. This species has an unusually small and discrete native range in the Canary Islands. Our data reveal two genetic varieties with divergent life histories and different colonization patterns across the islands. Although molecular divergence within each variety is large (pairwise F_{ST} from 0.18 to 0.32 between islands) and nearly as great as divergence between them, life-history traits show striking uniformity within varieties. The discrepancy between molecular and life-history trait divergence points to the action of stabilizing selection within varieties and the influence of pre-adaptation on patterns of colonization. The colonization history of *H. canariense* reflects how the relationship between selective environments in founding and source populations can dictate establishment by particular lineages and their subsequent evolutionary stasis or change.

Keywords: founding event, island endemic, Macaronesia, plant invasion, radiation, selective filter

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Introduction

Successful colonization events pose fundamental questions about the factors that shape species distributions. Species could be limited to colonization of environments to which they are pre-adapted, and this reasoning is the basis of algorithms that use native habitat parameters to define expected range limits of recently introduced species (e.g. Stockwell & Peters 1999; Elith *et al.* 2006). In contrast, colonization events are also associated with many striking cases of adaptive radiation and niche exploitation (e.g. Givnish 1998; Reznick & Ghalambor 2001; Gavrillets & Vose 2005; Losos *et al.* 2006). Recently introduced populations have demonstrated rapid evolutionary changes (Thompson 1998; Reznick & Ghalambor 2001; Cox 2004), and these

may be contributing directly to further establishment and spread (Blossey & Notzold 1995; Ellstrand & Schierenbeck 2000; Sakai *et al.* 2001; Lee 2002; Allendorf & Lundquist 2003; Müller-Schärer *et al.* 2004). Therefore, it is not clear how often colonization patterns are limited by a lack of adaptive evolution (i.e. governed by pre-adaptation), and this issue is garnering increasing theoretical and empirical attention (e.g. Kirkpatrick & Barton 1997; Kawecki 2000; Sax 2001; García-Ramos & Rodríguez 2002; Angert & Schemske 2005; Holt *et al.* 2005).

The high volume of human-mediated dispersal across continents has sparked an urgent need to understand where and how non-native species might invade native ecosystems (e.g. Parker *et al.* 2003; Kolbe *et al.* 2004; Kliber & Eckert 2005). Holt *et al.* (2005) recently explored theoretical predictions regarding the ecological and evolutionary conditions under which founding populations are expected to establish and invade. In brief, they suggest that colonization is most likely to be successful under conditions

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requiring little adaptive change (pre-adaptation), and that as recipient habitats deviate more strongly from the historical range, larger numbers of founders and more substantial adaptive evolution are required for successful expansion. From this perspective, natural and human-mediated colonization events share the same requirements for success, and both could be useful for testing these predictions. Comparative approaches between ancient and modern invasions should be particularly useful for elucidating both general features of colonization events and any distinctive features of human-mediated cases (Hierro *et al.* 2005). The ancient colonization history of modern invaders is largely unexplored, however, limiting such comparative approaches (but see May *et al.* 2006).

Here, we take advantage of the unusually small and discrete native range of the invasive plant *Hypericum canariense* L. (Hypericaceae or Clusiaceae), Canary Island St. Johns wort, to examine the role of adaptive evolution in its ancient colonization history. *Hypericum canariense* is a large (up to 3 m tall) perennial shrub that is currently invading areas of Australia (Hansford & Iaconis 2004), New Zealand (Allen Herbarium accession CHR542688), and the USA (Talbot 1993; in California; Wagner *et al.* 1999; in Hawaii), but it is a native endemic of the Canary Islands (Robson 1996). The Canary Islands have been the focus of many phylogeographical studies owing to their exceptional endemism and diversity, which are thought to be the result of both high intra-island habitat heterogeneity and repeated (though rare) inter-island colonization events (Francisco-Ortega *et al.* 2000; Juan *et al.* 2000). The Canary archipelago includes seven small volcanic islands (from 278 km² to 2034 km²), the western five of which are mountainous and support several distinct habitat types within each island (Bramwell 1976). The islands range in age from 1 to 20 million years old (Juan *et al.* 2000 and references therein), generating repeated opportunities for colonization of new terrain.

In contrast to the narrow distribution of many Canary Island endemic plants (Francisco-Ortega *et al.* 2000), *H. canariense* occupies all five of the western islands, where it occurs in xerophytic scrub and mesic forest habitat types, and where it can become locally dominant, particularly in mesic sites (Bramwell & Bramwell 1984; personal observation). Each plant produces hundreds of showy insect-pollinated flowers, and the fruits contain thousands of small (< 0.1 mg) seeds. Its seeds are not adorned for wind or animal dispersal, however, so long-distance seed movement should be rare (Willson 1993). Pollen dispersal at the scale of islands is also unlikely (Ellstrand 1992); the only demonstrated pollen movement among islands was from a wind-pollinated species (Gómez *et al.* 2003). Therefore, we expect low dispersal and strong genetic differentiation among islands. Morphological differences among islands, particularly in sepal shape,

caused early taxonomists to divide *H. canariense* into several species, but inconsistencies in these geographical patterns led to the conclusion that *H. canariense* is a single taxonomic unit (Robson 1996 and references therein).

We use DNA sequencing and amplified fragment length polymorphisms (AFLP) to reconstruct the natural expansion history of this species across the Canary Islands. Using greenhouse common gardens to assess morphological and life-history traits, we then contrast patterns of molecular and quantitative trait differentiation across the islands. Mismatches among measures of genetic differentiation imply that directional selection has driven change, or that stabilizing selection has driven stasis, of particular traits in opposition to patterns of random genetic drift (Merilä & Crnokrak 2001; McKay & Latta 2002). We use this information to assess whether patterns of quantitative trait evolution suggest that colonization has occurred via pre-adaptation or that adaptive radiation might have facilitated establishment of *H. canariense* across its native range.

Materials and methods

Collections

During August of 2002 and 2003, seeds and leaf material were collected from plants at 33 sites that span the distribution of *Hypericum canariense* in the Canary Islands (Fig. 1, Appendix). Where multiple collections were made at a site, plants were chosen at least 5 m apart along rough transects. This species is capable of limited rhizomatous expansion, and sampling was designed to reduce the chance of collecting clonal ramets. Seeds were removed from mature fruits immediately, and seeds and leaves were stored on silica desiccant. Genomic DNA was isolated from approximately 20 mg of each leaf collection using DNeasy plant extractions kits (Qiagen).

Sequencing

The internal transcribed spacer (ITS) region of nuclear ribosomal DNA (708 positions including ITS1 and ITS2 internal transcribed spacers and the intervening 5.8S coding sequence) was amplified from one individual at each collection site, as well as from *H. canariense*'s putative mainland progenitor *Hypericum revolutum* (Robson 1996) (field collected by B & T World Seeds, Pagnuignan, France; voucher available at University of California Santa Cruz Herbarium). The ITS4 and ITS5 universal primers (White *et al.* 1990) were used in a 25- μ L polymerase chain reaction (PCR) cocktail, including approximately 10 ng genomic DNA with 200 pmol of each primer, 200 μ M dNTPs, 2 mM MgCl₂, 1 U *Taq* polymerase (Promega) and 1 \times Promega PCR buffer. PCR was executed by a GeneAmp 9700

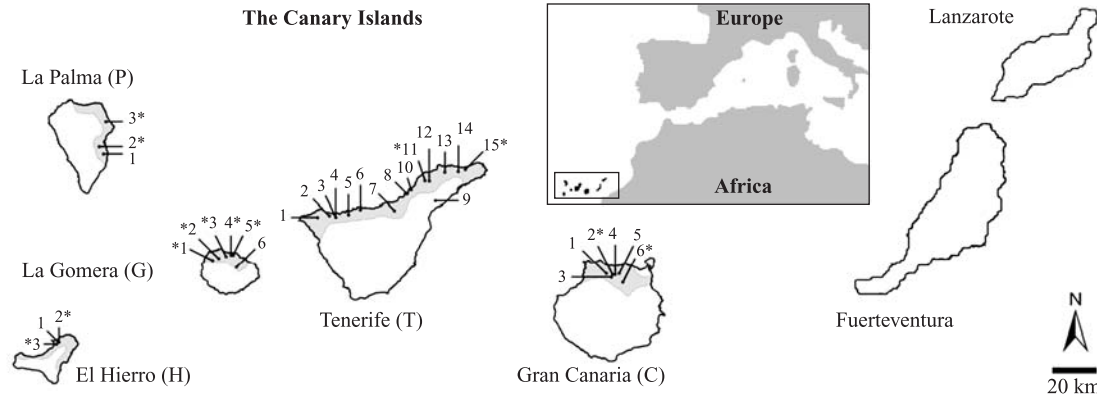


Fig. 1 Map of the Canary Islands showing the primary distribution (grey) of *Hypericum canariense* (Barquin-Díez & Voggenreiter 1988; personal observation) and sampling locations (numbered) used in this study. Locations marked with an (*) provided seed used in the common garden experiments.

thermocycler (Applied Biosystems) using 5 min at 95 °C, 34 cycles of 94 °C, 50 °C, and 72 °C at 1 min each, followed by 7 min of 72 °C. PCR product was cleaned from an agarose gel using the Zymoclean Gel DNA Recovery kit (Zymo Research). Sequencing reactions were carried out using ABI PRISM BigDye 2.0 chemistry and conditions (Applied Biosystems), but with 1/2 volume reactions (10 µL). Sequences were visualized on an Applied Biosystems 3100 capillary array with POP6 polymer medium. Base calls were edited using CHROMASLITE 2.01 (Technelysium) and alignments were performed using CLUSTALW 1.83 (European Bioinformatics Institute). Edited sequences were deposited in GenBank (accession nos EF015303–4, EF034032–69, EF638821). Relationships among sequences were constructed using maximum parsimony in PAUP* 4.0 beta 10 (Swofford 1998). Insertion/deletion (indel) mutations were coded as binary characters, nonparametric bootstraps were conducted using 1000 replicates, and the species *Clusia rosea* Jacquin (Hypericaceae) was used as an outgroup (GenBank no. AJ509230, Gehrig *et al.* 2003).

AFLP

AFLPs were screened from the genomic DNA of 5–10 individuals (with some exceptions, see Appendix) from each site. AFLPs were generated using the AFLP Plant Mapping Kit from Applied Biosystems. Fragments were selectively amplified with the primer pairs *EcoRI*-TG/*MseI*-CAT, *EcoRI*-ACT/*MseI*-CTG, *EcoRI*-ACC/*MseI*-CTC, *EcoRI*-ACC/*MseI*-CAG, *EcoRI*-AG/*MseI*-CAG. Products of each primer pair were individually injected onto an Applied Biosystems 3100 capillary array with POP4 polymer. Sizing and peak identification were performed using GENEMAPPER 4.0 software (Applied Biosystems). Highly repeatable and reliably detectable loci were identified using duplicate samples and manual inspec-

tion before being scored automatically for presence in each individual. A scoring repeatability rate for these loci was calculated by averaging the proportion of matching scores between duplicate amplifications of several individuals within each primer pair (86 duplicates total).

Genetic structure was assessed using a variety of approaches. Large-scale groupings of individuals were identified using a maximum-likelihood approach implemented in the program STRUCTURE 2 (Pritchard *et al.* 2000), which assigns individuals to groups in an effort to maintain constant allele frequencies and Hardy-Weinberg equilibrium (HWE) within groups. AFLP data were input as unlinked loci with one unknown allele at each locus, and no admixture among groups (appropriate for dominant markers). The program was unconstrained by any geographical information. Convergence of models on stable likelihood estimates was verified through multiple runs at each group number. Diversity (*H*), and F_{ST} values within and among these groups were generated using the program AFLP-SURV (Vekemans *et al.* 2002), which assumes HWE and that the frequency of the null (band absent) allele reveals the frequency of homozygous recessive individuals. Significance of F_{ST} values was determined using 500 bootstrapped data sets. Private alleles that occurred in at least two individuals (no singletons) within each group were identified using allele frequencies generated by AFLP-SURV. Distance structure was visualized using a neighbour-joining phenogram of Nei's genetic distances (generated in AFLP-SURV) constructed using PAUP. Bootstrap values were based upon 1000 resampled data sets and were calculated using the 'Neighbour' and 'Consense' features in PHYLIP 3.65 (see Felsenstein 1989). The variance in AFLP banding patterns was partitioned within and among groups by analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) using the RFLP function in ARLEQUIN 2.0 (Schneider

et al. 2000). Only the 28 sites with five or more individuals each were included in the AMOVA (Appendix). Significance of variance components was assessed with permutation tests (using the default 1023 permutations).

Growth and flowering

Seeds collected from each of the five islands with *H. canariense* were reared together in a common garden at the University of California campus in Santa Cruz, CA. Three offspring from each of 10 maternal individuals per island were used ($3 \times 10 \times 5$ islands = 150 plants total). Maternal individuals were located at two or more geographically separated sites per island (Fig. 1). Seeds were germinated during November 2002 by placing 20–30 seeds per maternal plant on the surface of moistened soil (Pro-mix HP, Premier Horticulture) in 5-by-5-cm pots. Flats of pots were covered with a clear, ventilated lid and kept in a greenhouse at 21 °C 16-h days/13 °C 8-h nights for 8 weeks. After 4 weeks, seedlings were thinned as needed to prevent shading among individuals. During week 8, seedlings were transplanted individually to Ray Leach pine cell 'conetainers' (Stuewe & Sons) and fully randomized. Conetainers were watered daily and fertilized weekly with 130 p.p.m. equal parts NPK fertilizer (JR Peters). On February 15 (week 13), plants were moved outside to the greenhouse roof for the spring and summer, where they experienced ambient conditions similar to those found in their native range (Mediterranean climate with coastal fog input), with additional watering to deliver weekly fertilizer. In June (week 30) plants were potted into 7.6-L pots, re-randomized, and fertilized with applications of slow-release fertilizer (Osmocote, Scotts Miracle-Gro).

Changes in biomass were monitored using a non-destructive size index developed from a study of naturally occurring *H. canariense* in California, where it is introduced. The above-ground wet weight of *H. canariense* plants is strongly predicted by the product of basal stem area and maximum height (linear regression: $N = 20$, $r_{\text{adj}}^2 = 0.96$, $P < 0.0001$), yielding the relationship: wet biomass (g) = $218.5 \times$ basal area (cm²) \times maximum height (cm). Exponential growth rates were fit to estimated biomass values from July 1, October 1, and November 22, 2003. Growth exponents were compared among islands and among families within islands using restricted maximum likelihood (REML) estimation of variance components (Lynch & Walsh 1998). Planned contrasts were performed between and within groups of islands that represented two major clades identified by the molecular analyses above. All statistical analyses of common garden data were conducted using JMP version 6 (SAS Institute), and assumptions of normality and homoscedasticity were verified before analysis.

Beginning in July, plants were checked three times per week for the appearance of the first fully open flower. Flowering date was compared among islands and families using REML variance component estimation. Total flower production was assessed on October 1 for one individual from each family. Flower number was compared among islands with REML variance component estimation. Planned contrasts within and between clades were performed for both flowering date and number. Pairwise correlations were calculated between flower number (square root transformed to conform to assumptions of bivariate normality), flowering date (family means), and growth rate (family means).

For growth rate and flowering date, our estimation of among-family variance permitted comparisons between the structure of quantitative trait variation (Q_{ST}) and the structure of molecular variation (F_{ST}). Q_{ST} was computed as the ratio $V_{\text{GP}}/(V_{\text{GP}} + 2V_{\text{a}})$, where V_{GP} is the among-island variance and V_{a} is the genetic variance within islands (Spitze 1993; Merilä & Crnokrak 2001). V_{a} was estimated as $4V_{\text{d}}$, where V_{d} is the variance among maternal families. Before these calculations, the significance of variance among maternal families was tested using likelihood-ratio tests comparing full models to models without families as a factor. Variance components were estimated by REML, and 95% confidence intervals were generated for Q_{ST} using 1000 bootstrap replicates of families programmed with the LME4 package (Bates & Sarkar 2007) functions 'lmer' and 'VarCorr' in R 2.5.0 (R Development Core Team 2007). F_{ST} values for comparison were computed among populations within each variety and between the two varieties in AFLPSURV using the AFLP data and 500 permutations to generate 95% confidence intervals.

Because seeds for common garden plants were field-collected, maternal contributions could drive observed differences between islands and inflate estimates of among-family variance. Seed mass was used to quantify variation in maternal resource contribution, since it is often driven by maternal environment (Roach & Wulff 1987). Average seed mass was quantified by weighing a subset of 40 seeds per fruit. Seed mass was assessed as a covariate in all analyses, but did not explain significant variation in any trait, or change the conclusions of any tests (seed mass effects: $P_{\text{growth}} = 0.50$, $P_{\text{flowerdate}} = 0.21$, $P_{\text{flower\#}} = 0.38$). Seed mass was therefore not used as a covariate in the final analyses. Seed provisioning was assumed to contribute little to differences among locations or families.

Floral morphology

An additional offspring from each of the families above was reared from January to November of 2005 for floral

measurements. The protocol for germination, thinning and fertilization was as above, although seeds were germinated directly into conetainers. Plants were moved outside in May and re-potted in June. Between four and eight individuals from each island produced flowers for morphological comparisons. Three fully open but unwilted flowers were collected from each plant and preserved in 70% ethanol. Flowers were dissected and petals and sepals were scanned as digital images. The software NIH IMAGE 1.62 (National Institutes of Health) was used to measure the angle of the apex of the sepals (the 'sepal acuity', see Fig. 7a), and the maximum length of each petal from its abscission zone at the receptacle to its outer tip (see Fig. 7b). The maximum angle observed from measurements of 1–2 sepals per flower across all three flowers per individual was compared between clades using a Wilcoxon nonparametric rank test. Averages of petal lengths per individual were also compared between clades using a Wilcoxon test. Spearman rank correlations were calculated between petal size and both sepal acuity and flower number (in the 2002/2003 garden).

Results

Sequencing

DNA sequences revealed only two genetic variants within the Canary Islands (Fig. 2). With one exception, samples from Tenerife, La Gomera, and El Hierro were entirely of one variant while those from Gran Canaria and La Palma were entirely of the other (Fig. 3). This distribution coincides with a disputed taxonomic subdivision of *H. canariense* into the varieties *Hypericum canariense* var. *floribundum* (hereafter *H. c. var. floribundum*) on Gran Canaria and La Palma and

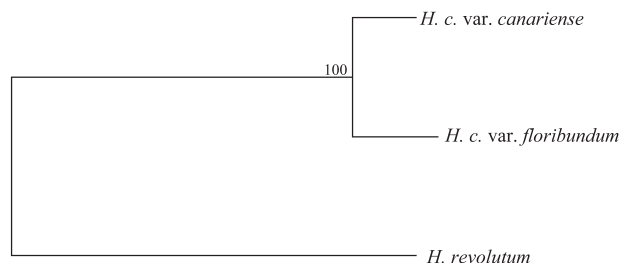


Fig. 2 Cladogram of the two *Hypericum canariense* varieties and their putative mainland progenitor *Hypericum revolutum*, based upon ITS sequences. *Clusia rosea* was used as an outgroup but is not shown. The bootstrap value is based upon 1000 replicates. Branch lengths reflect the number of mutations (substitutions and indels).

Hypericum canariense var. *canariense* (hereafter *H. c. var. canariense*) on Tenerife, La Gomera, and El Hierro (Robson 1996), and these conventions will be used here. The exception to this pattern is a single occurrence of the *H. c. var. floribundum* ITS genotype at site H1 on the island of El Hierro (Fig. 3), which was confirmed by re-extracting and sequencing this individual. While material from only one individual was collected at this site, further sequencing of the ITS region in six more individuals from each of the other two sites (H2, H3) on El Hierro failed to reveal additional *H. c. var. floribundum* samples. One individual from site H3 (GenBank no. EF034047) was heterozygous for one of the polymorphisms in ITS1, suggesting possible recombination.

The two varieties were much more similar to one another than to their putative mainland progenitor *Hypericum revolutum*. Based upon our phylogenetic reconstruction, 19–20 mutations (substitutions or indels) separate each of the three taxa from a common ancestor (Fig. 2). According to an assessment of the average rate of evolution in the ITS region for woody angiosperms (2.15×10^{-9} substitutions/site/year, Kay *et al.* 2006), 19–20 fixed differences would suggest a divergence time on the order of 19 million years BP. In contrast, the separation of the two varieties from one another (3–4 substitutions), would suggest that they diverged on the order of 3.5 million years ago.

AFLP

AFLP analysis generated 244 polymorphic loci, distinguishing each of 285 individuals as unique (indicating that there were no clones in our data set). Scoring repeatability was 95.6%. STRUCTURE modelling showed large gains in likelihood for up to six genetic groups: one corresponding to each of the five islands, and two within Tenerife, one of which included some individuals from nearby La Gomera (Fig. 3). Individuals in mixed sites on Tenerife and La Gomera showed nonzero assignment probabilities to both groups represented at a site, indicating recombination. The single *H. c. var. floribundum* individual from El Hierro was grouped with individuals from its own island with 100% probability, suggesting that its ITS genotype has introgressed into an otherwise El Hierro genetic background. Further partitioning of individuals continued to increase the likelihood of the model, but these new groupings were not along geographical lines and many different combinations produced models of similar likelihood (data not shown).

The division between the two ITS varieties was reiterated in the AFLP data. Pairwise F_{ST} values were greatest between islands of different variety (Fig. 3), and the two major clades were clearly delineated in the distance

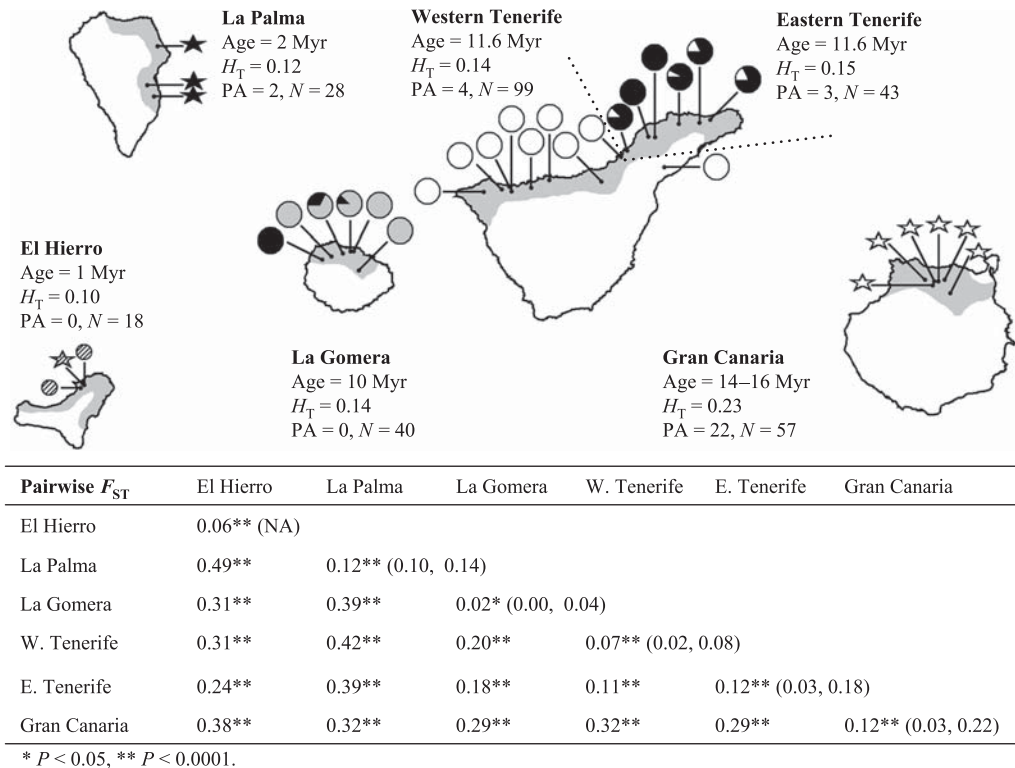


Fig. 3 Genetic structure of *Hypericum canariense* on the Canary Islands. Two varieties were differentiated by ITS genotypes and are indicated by stars (*H. c. var. floribundum*) and circles (*H. c. var. canariense*). Six major genetic groups were identified by STRUCTURE analysis of AFLP data, and these are shown as follows: dark stars (La Palma), open stars (Gran Canaria), stripes (El Hierro), grey circles (La Gomera), open circles (western Tenerife), and dark circles (eastern Tenerife). Mixed circles on La Gomera and Tenerife show the proportion of individuals in the populations that were assigned to each group. The geological age of the island (Juan *et al.* 2000; references therein), total allelic diversity (H_T), private alleles (PA), and sample size (N) are given next to each genetic group. Pairwise F_{ST} values are given for each pair of groups (off-diagonal). F_{ST} values within each group are shown on the diagonal, with the range of values for individual collection sites shown in parentheses.

	d.f.	Sum of squares	Variance	Percentage of total variance
All individuals				
Between ITS varieties	1	976.9	3.57	13.7
Among islands within variety	3	1018.0	7.89	30.2
Within islands	280	4095.3	14.63	56.1
<i>H. c. var. floribundum</i>				
Among islands	1	512.9	11.99	38.3
Among sites within islands	7	362.6	3.81	12.2
Within sites	77	1190.5	15.46	49.5
<i>H. c. var. canariense</i>				
Among islands	2	505.0	5.16	27.9
Among sites within islands	16	541.3	2.21	12.0
Within sites	180	2001.0	11.12	60.1

Table 1 Partitioning of AFLP variation among *Hypericum canariense* individuals in the Canary Islands by analysis of molecular variance (AMOVA). Analyses include the 28 sites with samples of five or more individuals (see Appendix). An analysis including all of these individuals shows the variation within and between the two ITS varieties. Two additional analyses show the variance structure within each variety separately. All variance components are significant at $P < 0.0001$.

phenogram of collection sites (Fig. 4), although differentiation within each variety was nearly as great in magnitude. AMOVA identified a significant proportion of variation (13.7%) associated with differences between the

H. c. var. floribundum and *H. c. var. canariense* islands, although the analysis attributed much greater variation to differences among islands within variety (Table 1). Nevertheless, no other multi-island clusters explained

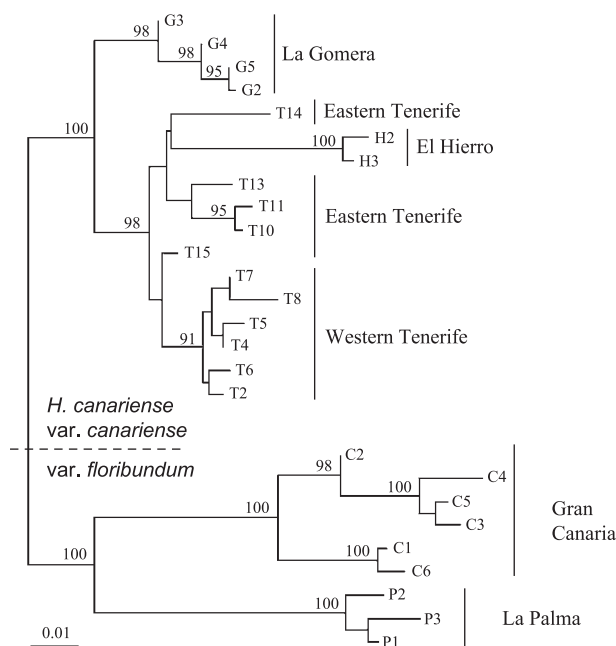


Fig. 4 Neighbour-joining phenogram of Nei's genetic distances between AFLP allele frequencies at different collection sites. Site names correspond to those in Fig. 1. Bootstrap values based upon 1000 resampled data sets are shown for each node where they exceeded 75%. Two major clades match the two varieties identified by ITS genotype and are indicated by the dashed line.

significant variation (data not shown), and eight private alleles also distinguished *H. c. var. floribundum* islands from *H. c. var. canariense* islands.

Variance structure was similar between the two varieties and highly scale-dependent. A large proportion of variation was associated with differences among islands (Table 1). Apart from some gene exchange between Tenerife and La Gomera (as identified by STRUCTURE analysis), it appears that postcolonization gene flow is rare at this scale. This interpretation is reinforced by the high pairwise F_{ST} values among islands (Fig. 3) and the strong support for nodes associated with different islands in the distance tree (Fig. 4). In contrast, while site differences explained significant variation (Table 1) and generated significant F_{ST} values (Fig. 3), this differentiation was both lower than that among islands and far exceeded by variation among individuals within sites. The high ratio of within- to among-site variation is consistent with an outcrossing mating system for *H. canariense* in its native range.

Patterns of genetic distance and diversity (Figs 3 and 4) reveal the colonization history of the *H. canariense* varieties in the Canary Islands. The younger, smaller islands more distant from the mainland have lower total AFLP diversity, fewer private alleles, and generally lower differentiation among sites (F_{ST}), suggesting more recent colonization via Gran Canaria (for La Palma) and Tenerife (for La Gomera

Table 2 Variance partitioning of *Hypericum canariense* life-history traits in a common garden using REML analysis. Growth rates (exponent) and flowering dates (Julian day) were measured for 2–3 offspring per maternal family, whereas flower number was assessed using a single individual per family. F -statistics (ndf, ddf) are given for fixed effects and G statistics (from likelihood-ratio tests) are given for random effects.

Effect	N	Test statistic or V_e	P value
Growth Rate			
Island	5	$F_{(4,45.5)} = 11.25$	< 0.0001
Family w/in island	50	$G = 5.05$	0.02
Whole model	150	$V_e = 4.17 \cdot 10^{-5}$	< 0.0001
Flowering Date			
Island	5	$F_{(4,44.9)} = 3.10$	0.02
Family w/in island	50	$G = 52.91$	< 0.0001
Whole model	139	$V_e = 161.2$	< 0.0001
Flower Number			
Island	5	$F_{(4,40)} = 11.31$	< 0.0001
Whole model	45	$V_e = 33317$	< 0.0001

and El Hierro) to the East. Large changes in allele frequencies between sites on the eastern islands and those on the western islands of La Palma and El Hierro (Fig. 4) are also consistent with founder effects (e.g. Thorpe *et al.* 1994). El Hierro sites are nested within sites on eastern Tenerife, strongly indicating a source region there, as opposed to one on nearby La Gomera. The partitioning of Tenerife into eastern and western clades is likely to reflect volcanic activity rather than colonization history, as Tenerife has experienced a geologically recent connection of older volcanic terrains on the east and west of the island (summarized in Juan *et al.* 2000).

Growth and flowering

Growth and flowering data revealed striking differences among islands, particularly between the *H. c. var. floribundum* and *H. c. var. canariense* varieties (Table 2). Individuals from Gran Canaria and La Palma collections grew at a ~50% greater rate, started flowering more than 2 weeks later, and produced far fewer flowers than individuals from other islands, although La Gomera individuals also produced a low number of flowers during the course of this experiment (Fig. 5). Growth rate and flowering date did not differ among islands within a variety, although significant family variation was detected for both traits (Table 2), suggesting a potential for further evolution. Families that grew faster flowered later in general ($N = 50, r = 0.61, P < 0.0001$), consistent with the nearly identical patterns of differentiation of these traits among the islands (Fig. 5). Looking within varieties, where neither trait differed among islands, growth

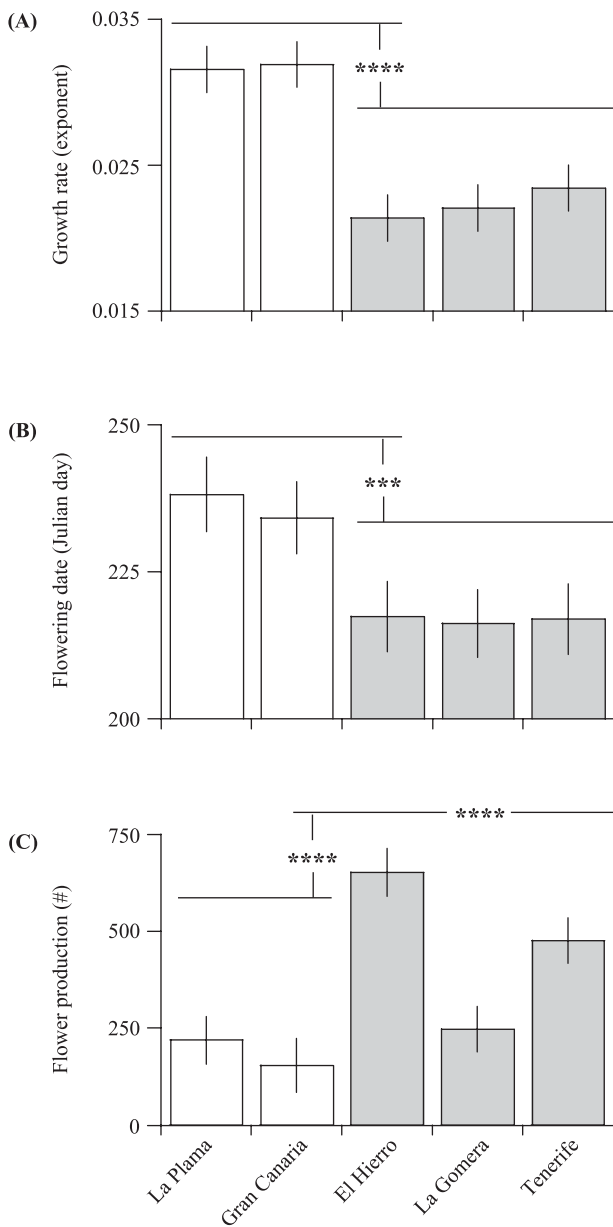


Fig. 5 Least squares means of *Hypericum canariense* (a) growth rate (b) date of first flower, and (c) flower production per plant for each island of origin. Error bars show one standard error, based upon REML analyses of variance (with families nested within islands for a and b). Plants were reared in a rooftop common garden in Santa Cruz, California. The ITS variety found on each island is indicated by an open (*H. c. var. floribundum*) or shaded (*H. c. var. canariense*) bar. Planned contrasts were conducted within and between varieties (** $P = 0.001$, **** $P < 0.0001$).

and flowering date were again (at least weakly) correlated, supporting the possibility of a genetic covariance between them (within *H. c. var. floribundum*: $N = 19$, $r = 0.62$, $P = 0.005$; within *H. c. var. canariense*: $N = 31$, $r = 0.34$, $P = 0.06$).

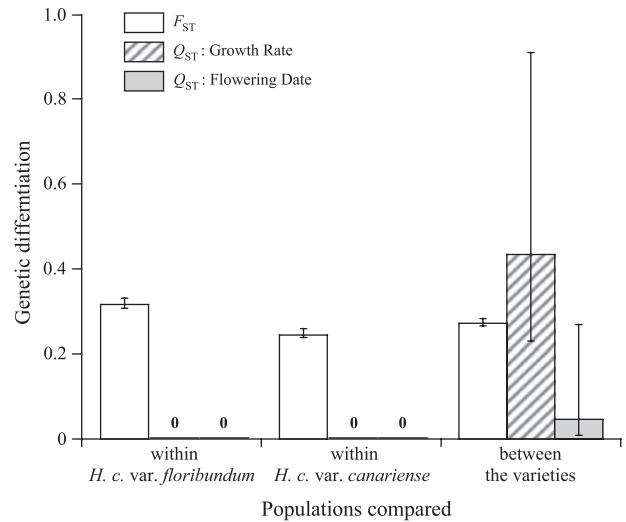


Fig. 6 Q_{ST} estimates for growth rate and flowering date contrasted with F_{ST} values. Error bars show 95% confidence intervals (calculated using 1000 nonparametric bootstraps across families for Q_{ST} and 500 across loci for F_{ST}).

Flowering in *H. canariense* is indeterminate, and many individuals from all islands had not ceased flowering by the date on which flower number was assessed, so differences in flower number may be influenced by other phenological differences among the islands. Flower production by October 1 was not correlated with flowering date ($N = 45$, $P = 0.31$), however, indicating that differences in flower production were not driven by differences in the duration of the flowering period. Flower production was lower in families with faster growth rates overall ($N = 45$, $r = -0.48$, $P = 0.0008$), but not within either variety (*H. c. var. floribundum*: $N = 16$, $P = 0.42$; *H. c. var. canariense*: $N = 29$, $P = 0.16$), suggesting that flower number and growth rate vary independently. Sample sizes within each variety were low, making correlations difficult to detect, so growth rate was also examined as a covariate in the ANOVA of flower number. There was no effect of growth rate on flower number ($F_{1,39} = 0.24$, $P = 0.63$) in this model, supporting the interpretation of these traits as independent.

Comparisons of Q_{ST} and F_{ST} suggest that selection may be driving patterns of stasis within the varieties and the level of differentiation between them (Fig. 6). Because of the low differentiation among islands within varieties for both growth rate and flowering date, REML estimates of among-island variance were zero (in fact negative in all cases when variance estimation was unbounded), generating zero values for Q_{ST} within varieties. These contrasted with substantial and highly significant values of F_{ST} , suggesting that selection has maintained low quantitative trait differentiation despite significant family variation within islands and limited gene flow among them.

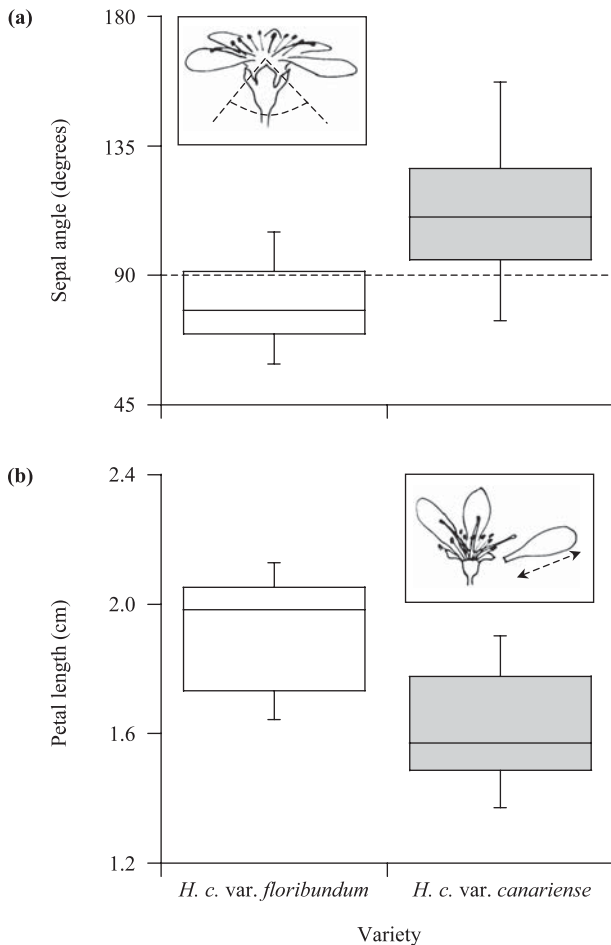


Fig. 7 Box plots showing *Hypericum canariense* sepal shape (a) and petal length (b) for each ITS variety. Insets show measurement locations. Plants were reared in a rooftop common garden in Santa Cruz, California. Boxes enclose 50% of the data and bars indicate the full extent of the distribution.

Between varieties, growth rate showed much higher Q_{ST} than F_{ST} while flowering time demonstrated the reverse pattern, although confidence intervals overlapped somewhat in both comparisons. The striking difference between the two traits in this regard stems from a much larger among-family variance in flowering date, such that the evolutionary change that occurred appears to be much less than predicted on the basis of drift alone. In contrast, growth rates may have diverged much more than predicted by drift.

Floral morphology

The variation in maximum sepal acuity was substantial, ranging from 36 to 157 degrees (Fig. 7a), and often spanning much of this range within a single flower (data not shown). Maximum angles did differ between the varieties ($\chi^2_1 = 12.8$, $P = 0.0003$): sepals from La Palma and Gran

Canaria (*H. c. var. floribundum*) were on average acute, while those from El Hierro, La Gomera and Tenerife (*H. c. var. canariense*) were typically obtuse (Fig. 7a). Individuals of both varieties deviated strongly from this pattern, however, making this trait unreliable as a taxonomic indicator.

Average petal length was also highly variable, ranging from 1.3 to 2.2 cm. Petals were larger in *H. c. var. floribundum* plants ($\chi^2_1 = 11.0$, $P = 0.0009$), although again there was substantial overlap between the varieties (Fig. 7b). There was a marginal negative correlation between mean petal length and maximum sepal angle for each individual ($N = 29$, $r = -0.35$, $P = 0.06$), and petal lengths were negatively correlated with the number of flowers made by their siblings in the common garden ($N = 25$, $r = -0.54$, $P = 0.005$).

Discussion

This study takes advantage of a rare opportunity to examine the role of evolution in the ancient colonization history of a modern invasive species. Molecular surveys of *Hypericum canariense* in the Canary Islands reveal two genetic varieties with different establishment patterns. While molecular divergence within each variety rivals that between them, the life-history traits examined here show uniformity within varieties. This discrepancy between molecular and quantitative traits suggests the action of stabilizing selection and the influence of pre-adaptation on patterns of colonization. We discuss these findings and their implications below.

Two varieties and their colonization histories

ITS sequence and AFLP variation describe two major lineages of *H. canariense* in the Canary Islands. The geographical distribution of these lineages corresponds to the distribution of two previously proposed morphological varieties: *H. canariense* var. *floribundum* on Gran Canaria and La Palma, and *H. canariense* var. *canariense* on Tenerife, La Gomera, and El Hierro (Robson 1996). Taxonomists based these distinctions on differences in the acuity of the sepal at its apex, and floral morphology of common garden plants supports this pattern, in that sepals are typically acute in *H. c. var. floribundum* and can be obtuse in *H. c. var. canariense*. This trait is highly variable, however, such that many individuals do not fit the acute/obtuse dichotomy, making it unreliable as an identifying characteristic.

Several scenarios for the origin and distribution of these two varieties are possible. They may be the result of divergence on the mainland, divergence on the Canary Islands, or differentiation of one island lineage followed by a second colonization from the mainland. AFLP analyses indicate that the molecular divergence between varieties

is low relative to variation within and among islands. This suggests that colonization of the westernmost islands occurred not long after the separation of the varieties. Such a pattern would seem unlikely if the varieties were already established as unique lineages on the mainland. Based on detailed studies of morphological characters, *Hypericum revolutum* has been postulated to be the mainland progenitor of the *H. canariense* lineage (Robson 1996). Our ITS results suggest that *H. canariense* and *H. revolutum* diverged much longer ago than did the two varieties. Using an average rate of substitution in the ITS regions of woody angiosperms (Kay *et al.* 2006), we estimate that they diverged on the order of 19 million years ago. Colonization of the Canary archipelago at approximately this time is plausible, particularly if the eastern islands of Lanzarote and Fuerteventura were previously more mesic than they are today (summarized in Juan *et al.* 2000). Unfortunately, relationships within the genus have not yet been evaluated by a comprehensive molecular study, and neither *H. canariense* nor *H. revolutum* have been included in molecular surveys to date (Crockett *et al.* 2004; Park & Kim 2004). Future studies of the genus could identify a different extant sister species for *H. canariense*, with a more recent divergence.

Regardless of relationships with mainland taxa, it is clear that the two varieties separated recently in comparison with the ages of the older islands in their distribution (10–16 million years, Juan *et al.* 2000 and references therein). Average rates of ITS evolution suggest that this occurred on the order of 3.5 million years ago. Other studies of diversification in the Canary Islands have found evidence for radiations of taxa 2–5 million years ago (Francisco-Ortega *et al.* 1995; Kim *et al.* 1996), and Kim *et al.* (1996) suggested that glaciation and desertification events 2–3 million years ago may have contributed to these patterns. Our life history data also suggest that the divergence of the *H. canariense* varieties was associated with adaptation to contrasting habitats (see below), perhaps representing differences between Tenerife and Gran Canaria, which as the largest, oldest, and closest mesic islands, would have been the primary targets for colonization from the mainland up to that time.

The westernmost islands of La Palma, El Hierro, and La Gomera appear to have been colonized more recently via Gran Canaria and Tenerife to the East. This general pattern makes sense given that the western islands are both more distant from the African mainland and geologically younger. Radiations from Tenerife and Gran Canaria are also supported for other Canary Islands taxa (e.g. Thorpe *et al.* 1994; Bohle *et al.* 1996; Kim *et al.* 1996; Ribera *et al.* 2003). More specifically, evidence suggests that most Canary Island taxa have followed an east–west ‘progression rule’ model of island colonization (Juan *et al.* 2000; Francisco-Ortega *et al.* 2001; Francisco-Ortega

et al. 2002; Allan *et al.* 2004; Kvist *et al.* 2005), wherein western islands are colonized via the nearest island to the east (Cowie & Holland 2006). Our data support this model for *H. canariense* var. *canariense*, in which El Hierro and La Gomera have been colonized via Tenerife. This colonization route is also consistent with trade-wind direction and water currents that may have sent rafting vegetation from Tenerife southwest to the other islands.

In contrast to the ‘progression rule’ model, La Palma has been colonized via the most distant island in the current range, Gran Canaria, counter to wind and water currents. This pathway has never been noted in the Canary Islands before; however, it seems probable for some other taxa (Percy 2003; Lee *et al.* 2005). Given that the island of Madeira (450 km to the north) has been colonized repeatedly via the Canary archipelago (Francisco-Ortega *et al.* 2002; Mort *et al.* 2002; Carine *et al.* 2004; Lee *et al.* 2005; Trusty *et al.* 2005), dispersal from Gran Canaria to La Palma is not implausible. The large molecular divergence between La Palma and Gran Canaria also suggests a rare dispersal event, with a strong founder effect during colonization (*sensu* Desalle & Templeton 1988; Thorpe *et al.* 1994). Individuals from Gran Canaria could have colonized La Palma via Tenerife, although there is no molecular evidence of migrants from Gran Canaria in the Tenerife samples.

Perhaps more surprising than propagules reaching La Palma from Gran Canaria, is that there is no signature of migration from the nearby *H. c.* var. *canariense* islands, as is common in other taxa (Emerson *et al.* 2000a, b; Hess *et al.* 2000; Kvist *et al.* 2005). It is possible that hybridization with *H. c.* var. *canariense* genotypes was simply missed in the sampling for this study. This is particularly likely if *H. c.* var. *floribundum* individuals populated the island initially, and subsequent gene flow from other islands is only a small part of the gene pool (Herben *et al.* 2005). The substantial difference in flowering date in a common environment may also act to isolate these two varieties to some degree. Nevertheless, the presence of an *H. c.* var. *floribundum* ITS genotype in an otherwise *H. c.* var. *canariense* AFLP background in one El Hierro individual indicates that the varieties are capable of natural hybridization. Life-history data (discussed below) suggest instead that selection for different strategies may keep *H. c.* var. *canariense* colonists and genes at a fitness disadvantage on La Palma, maintaining the genetic division between the lineages.

Trait divergence and the role of pre-adaptation in colonization

Common garden experiments reveal large morphological and life-history differences between the two varieties. In the greenhouse, individuals from *H. c.* var. *floribundum*

islands grow much more quickly and flower more than 2 weeks later than individuals from *H. c. var. canariense* islands. Variety *floribundum* individuals produce fewer and larger flowers than most *H. c. var. canariense* individuals (except those from La Gomera), although it is unknown how many flowers they might make given a longer season or multiple years. It is striking that these traits have not also diverged *within* the varieties along with the rest of the genome. It is clear from variance partitioning, STRUCTURE analysis, and the abundance of private alleles, that gene flow between islands is very limited and molecular divergence among them is strong. Gran Canaria and La Palma lineages are nearly as distant in AFLP allele frequencies as are the two varieties, yet individuals from those islands do not differ in growth rate, flowering date, or flower production. Indeed, Q_{ST} estimates for both varieties are zero, despite significant genetic variation within islands and large F_{ST} among them. This lack of concordance between patterns of molecular and quantitative trait differentiation strongly implies the action of selection (e.g. Spitze 1993; Bonnini *et al.* 1996; Lynch *et al.* 1999; Pfrender *et al.* 2000; Merilä & Crnokrak 2001; Reed & Frankham 2001; Lee & Frost 2002; Edmands & Harrison 2003; Morgan *et al.* 2005; Porcher *et al.* 2006), and has been noted in other colonists (Koskinen *et al.* 2002; Lavergne & Molofsky 2007). Because AFLP data on average are expected to reflect noncoding (selectively neutral) variation (Sunnucks 2000; Bensch & Akesson 2005), our data indicate that it is the life-history traits that are experiencing stabilizing selection within each variety, and that this selection is maintaining two different life-history strategies (or similar strategies under different ecological conditions).

Our comparisons of molecular and quantitative trait variation across varieties are also consistent with divergence shaped by selection. Our Q_{ST} estimate for flowering date was a small fraction of F_{ST} , while the value for growth rate was twice as high as F_{ST} . These patterns suggest that particularly large changes in growth rate between varieties may have been favoured by selection, while adjustments in flowering time were much more restrained than they might have been. These differences between varieties, coupled with significant quantitative variation found within islands, indicate that a lack of genetic variation or the presence of genetic constraints cannot explain the complete stasis of traits within varieties. Moreover, our data from invading populations that originated on Tenerife reveal that these lineages do have the ability to evolve striking changes in quantitative traits over short time periods (K.M. Dlugosch and I.M. Parker, unpublished manuscript).

If selection is maintaining divergent patterns in life history between the varieties, then (i) the selective environments on the islands must be similar within variety and different between them, and (ii) successful

colonization must have followed these paths of ecological similarity (i.e. pre-adaptation). No other studies to date have proposed ecological constraints on island-wide colonization patterns in the western Canary Islands, although few have examined both molecular and life-history traits (but see Francisco-Ortega *et al.* 1996; Sahuquillo & Lumaret 1999; Gubitz *et al.* 2005; Trusty *et al.* 2005). Groups that have diverged in habitat use within islands do seem to colonize those same habitats as they reach new islands (Francisco-Ortega *et al.* 1996; Sahuquillo & Lumaret 1999; Francisco-Ortega *et al.* 2001; Francisco-Ortega *et al.* 2002). Reciprocal transplants and selection analyses are needed to confirm this hypothesis, but there is some support that the varieties have dispersed in accordance with habitat differences. Guerrero *et al.* (2005) compared the abiotic environments among the islands using an ordination of 15 different temperature, moisture and light variables, and found that La Palma and Gran Canaria are indeed most similar to one another. Detailed analyses of environmental variables within *H. canariense* populations would be particularly useful as a basis for climate-matching approaches that address the hypothesis of colonization by pre-adaptation and for making predictions about the spread of introduced populations.

Conclusions

Our data indicate that two ecologically divergent lineages of *Hypericum canariense* have differentially colonized the Canary Islands in response to distinct selective environments. This pattern implies that pre-adaptation has guided patterns of establishment, and is consistent with the idea of a selective filter favouring particular pre-adapted lineages during founding events (Nueffer & Hurka 1999; Mack 2000; Simons 2003; Brown & Eckert 2005). Does this mean that there is little role for post-establishment adaptive evolution in the modern colonization success of this species? In light of the initial life-history divergence between the varieties and substantial genetic variation among individuals, the maintenance of two life-history strategies appears to be a response to stabilizing selection rather than evidence of constraint. If the environment of a founding population closely resembles that of its source, as we might expect in this small group of islands, evolutionary change will not be advantageous. In distant and more divergent environments, such as those where *H. canariense* has been recently introduced, adaptive change is likely to be more critical (Holt *et al.* 2005). Consistent with this idea, we do see major life-history changes in populations introduced to California and Hawaii (K.M. Dlugosch and I.M. Parker, unpublished manuscript). We suggest that the colonization history of *H. canariense* supports a general principle that the relationship between

adaptive optima in founding and source populations will dictate patterns of evolutionary stasis or change, and that similarity among environments is likely to promote colonization by pre-adapted lineages.

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Appendix

Sampling locations and AFLP sample sizes for *Hypericum canariense* plants in the Canary Islands. Locations marked with an (†) were estimated to the nearest $1/4$ min of distance and 10 m of elevation from maps, while all other locations were recorded using a handheld GPS unit (Geo Explorer CE, Trimble). Locations with an (*) provided seed for the common garden study.

Site	Location	Elevation	Nos sampled
La Palma			
P1	28°37'11.2"N 17°46'10.2"W	227 m	10
P2*	28°39'7.0"N 17°47'19.1"W	381 m	9
P3*	28°45'25.5"N 17°45'41.3"W	353 m	10
El Hierro			
H1	27°48'17.0"N 17°58'31.0"W	694 m	1
H2*	27°48'59.6"N 17°58'11.8"W	459 m	10
H3*	27°48'46.2"N 17°57'56.7"W	560 m	10
La Gomera			
G1*	28°9'51.5"N 17°17'55.7"W	628 m	1
G2*	28°10'24.7"N 17°16'20.5"W	301 m	10
G3*	28°10'54.8"N 17°14'23.9"W	464 m	10
G4*	28°11'13.7"N 17°13'5.5"W	369 m	10
G5*	28°11'16.0"N 17°12'35.2"W	403 m	7
G6	28°8'3.6"N 17°11'46.0"W	549 m	2
Tenerife			
T1	28°21'8.4"N 16°50'42.0"W	384 m	3
T2	28°21'34.8"N 16°47'40.3"W	611 m	25
T3	28°22'2.6"N 16°46'18.0"W	316 m	5
T4	28°22'2.5"N 16°46'22.8"W	299 m	5
T5	28°21'52.2"N 16°42'43.7"W	331 m	5
T6	28°23'5.9"N 16°39'41.4"W	233 m	25
T7	28°22'54.4"N 16°30'54.7"W	555 m	10
T8	28°27'1.0"N 16°27'37.7"W	337 m	25
T9	28°25'19.6"N 16°20'20.0"W	441 m	1
T10	28°30'24.0"N 16°23'2.4"W	398 m	8
T11*	28°30'18.2"N 16°21'55.0"W	401 m	9
T12	28°32'36.2"N 16°17'54.0"W	668 m	1
T13	28°32'47.9"N 16°14'25.3"W	435 m	10
T14	28°33'15.4"N 16°12'38.0"W	335 m	5
T15*	28°28'5.1"N 16°26'35.9"W	465 m	10
Gran Canaria			
C1†	28°6'30"N 15°36'30"W	590 m	10
C2*†	28°5'30"N 15°35'15"W	680 m	8
C3†	28°6'15"N 15°35'00"W	490 m	10
C4†	28°6'15"N 15°34'15"W	385 m	10
C5†	28°6'30"N 15°33'15"W	390 m	10
C6*†	28°4'11.3"N 15°32'15.6"W	535 m	9