Trait evolution and adaptive radiation in *Crambe* L. (Brassicaceae)

Minor research project, August 2023

Noor van den Berg (6122574)

Daily supervisor and first examiner: Ryan Brewer, Frederic Lens, Luís Valente

Second examiner: Hans ter Steege

Universiteit Utrecht

Naturalis Biodiversity Center

Acknowledgement

This final product would not have been possible without the help and support of the people around me. I'm extremely grateful to my daily supervisor Ryan Brewer for his great insights, recommendations, feedback and overall support in the last 6 months. Furthermore, I would like to thank Frederic Lens and Luís Valente for lending their expertise. A special thanks to Etienne Rampal for his recommendations for the modelling. And lastly, I very much appreciate Hans ter Steege for making it possible for me to do the internship.

Abstract

Species richness, form, and function vary greatly throughout the tree of life, leading many to link characteristics with diversification. Which traits affect diversification and whether this pattern is different between continents and islands remain largely unknown. Using the angiosperm genus *Crambe*, the differences in traits (both abiotic and biotic) between the island and mainland species, and their possible effects on diversification were explored. We generate new phylogenies, based on 353 markers, which reveal the current taxonomic classifications, based on morphology, at both the species and subspecies levels are possibly inaccurate. Subspecies within this genus were found not to be closely related in the phylogeny, and the previously debated synonyms *Crambe gigantea* and *Crambe santosii* do not seem to be sister species. Furthermore, our time-calibrated phylogeny and diversification modelling suggest the evolution of *Crambe* on the Canary island is possibly an adaptive radiation and it is likely there was a key innovation that influenced this clade to radiate. Ancestral state reconstructions were performed for multiple abiotic and biotic traits, showing differences between the island and the mainland species. The key innovation could be the availability of new habitat (the island) itself or the abiotic factors of this habitat in particular continentality (difference between the warmest and coldest month), and UV. However, there are also indications that it could be the plant characteristics, upper segment length, or maximum plant height. Lastly, the island species occur in more arid conditions hinting towards a key innovation that made it possible for the species to survive there.

Layman's summary

By studying how plants evolve over time it is possible to build a tree of how different plants are related. This study has generated the most complete tree for the plant group *Crambe*. It was discovered that the relationships found in this tree are different than previously thought. Furthermore, due to the distribution of the species, with almost half of the species in this group only being present in the Canary Islands, it has been previously thought that a characteristic or combination of characteristics of the species influenced the distribution. This study found further arguments for this theory. It was discovered that likely a specific trait of

the species on the islands, such as the maximum height of the plant or that they can survive in drier conditions, is the reason behind the many species present on the islands today. This means due to the traits of the island species being different to the mainland species, more species are present on the islands.

Content

Introduction

Since the publication of Darwin's theory of evolution (Darwin & Kebler, 1859), numerous studies have tried to identify whether species have differential rates of evolution and whether a single key trait can explain potential shifts in species diversification rates (Koutroumpa et al., 2021; Sauquet & Magallon, 2018). Here, diversification rate is defined as the difference between speciation and extinction rates (Donoghue & Sanderson, 2015). If a species trait significantly increases the diversification rate we refer to this as a key innovation (Donoghue & Sanderson, 2015). Current studies classify traits responsible for key innovations as either extrinsic (abiotic) or intrinsic (biotic) (Helmstetter et al., 2023). An important, unanswered question in the literature, is whether there is a difference between the effects of extrinsic and intrinsic traits on the rate of diversification (Patiño et al., 2018). As key innovations can change the niche of species in novel ways, and subsequently how a species interacts with its environment (Miller, Stroud & Losos, 2022), they are thought to be able to promote diversification and drive radiations.

The term radiation applies when an ancestor proliferates into multiple species rapidly (Losos & Ricklefs, 2009; Matsubayashi & Yamaguchi, 2022). Non-adaptive radiations occur without ecological differentiation between emergent species, whereas adaptive radiation involves species adaptations in response to ecological opportunities (Stroud & Losos, 2016; Losos & Ricklefs, 2009; Matsubayashi & Yamaguchi, 2022). New ecological opportunities typically become available following geographic colonisation of new areas, extinction of surrounding species, availability of new resources or through the evolution of key innovations that can aid in exploitation of the environment (Donoghue & Sanderson, 2015). This study follows the definition of adaptive radiation given by Stroud and Losos (2016, p.507): "the proliferation of species from a single ancestor and diversification into many ecologically different forms". Furthermore, questions remain around functional traits, defined here as "morpho-physio-phenological traits which impact fitness indirectly via their effects on growth, reproduction and survival" (Violle et al., 2007) within the context of adaptive radiation. These questions include whether functional traits are associated with the elevated diversification rates that characterise many radiations, whether they differ between continental and island systems, and how these traits are linked to each other, if at all (Patiño et al., 2018).

Evolutionary processes and the traits associated are often researched on islands. This is not only due to the isolation and clear boundaries of insular systems, but also due to their high level of biodiversity and their simplified biotas compared to the mainland (Helmus, Mahler & Losos, 2014; Losos & Ricklefs, 2009). With only 3.5% of the global land surface area, islands manage to hold 20% of the world's terrestrial biodiversity (Whittaker et al., 2017). Often a high percentage of the species present on an island or island archipelago are endemic (Fernández-Palacios et al., 2021). For example, the Mediterranean islands hold between 7 to 18% endemic species, a figure that goes up to 40% at higher elevations (Médail, 2017). It is important to realise that while islands are well-suited to research the evolutionary processes due to their biodiversity, they are likely not representative of the processes on the mainland (Patiño et al., 2018). Indeed, an important question in the field of biogeography is whether and how the evolution of traits differs fundamentally between islands and the mainland (Patiño et al., 2018). For example, island disharmony, the theory that islands hold atypical subsets of species and that traits and species can be over- or underrepresented on an island in comparison to the mainland, could partly be a result of differences in trait evolution between the two systems (Taylor et al., 2019; König et al., 2021). Therefore, it is important to not only distinguish what traits influence diversification on islands, but also to compare them to the mainland.

A genera that has species present on both islands and the mainland is *Crambe* L. (hereafter "*Crambe*"). Furthermore, it is one of the most species-rich genera in the Brassicaceae family (Rudloff & Wang, 2011; Warwick, Francis & Gugel, 2009). There are currently 39 species in the genus, mainly distributed across the northern hemisphere. *Crambe* species are native to Europe, the Middle East, regions of Africa and East Asia (Figure 1). Throughout the years, species have been introduced in Australia and the United States of America (Plants of the World Online, 2023). The genus is found both on the Canary Islands, with 14 endemic species, and continentally, with 25 species on the mainland (Plants of the World Online, 2023), making it a good candidate for comparison of traits between mainland and islands. In addition, previous research has highlighted that hybridization has not played a significant role in the history of *Crambe* (Francisco-Ortega et al., 2002), making it a more accessible genus to study evolution and biogeography, as hybridization can have contradicting and difficult to measure effects on diversification rates and phylogeny (Abbott et al., 2013).

It has been estimated that *Crambe* arrived on the Canary Islands between 1.3 and 8.2 million years ago (Hooft van Huysduynen et al., 2020; Kim et al., 2008). Once on the Canary Islands, *Crambe* has undergone a large radiation, which is thought to be adaptive (Kim et al., 2008). A remarkable feature of this radiation is that all the species present on the Canary Islands are woody (Hooft van Huysduynen et al., 2021), whereas the rest of the genus on the continent is herbaceous. Since the rate of diversification of herbaceous species is (generally) higher than that of woody species (Givnish, 2010; Hamrick & Godt, 1996), this pattern raises an interesting question arises: why did the island species radiate widely in a relatively small area and what functional traits are associated with this island radiation?

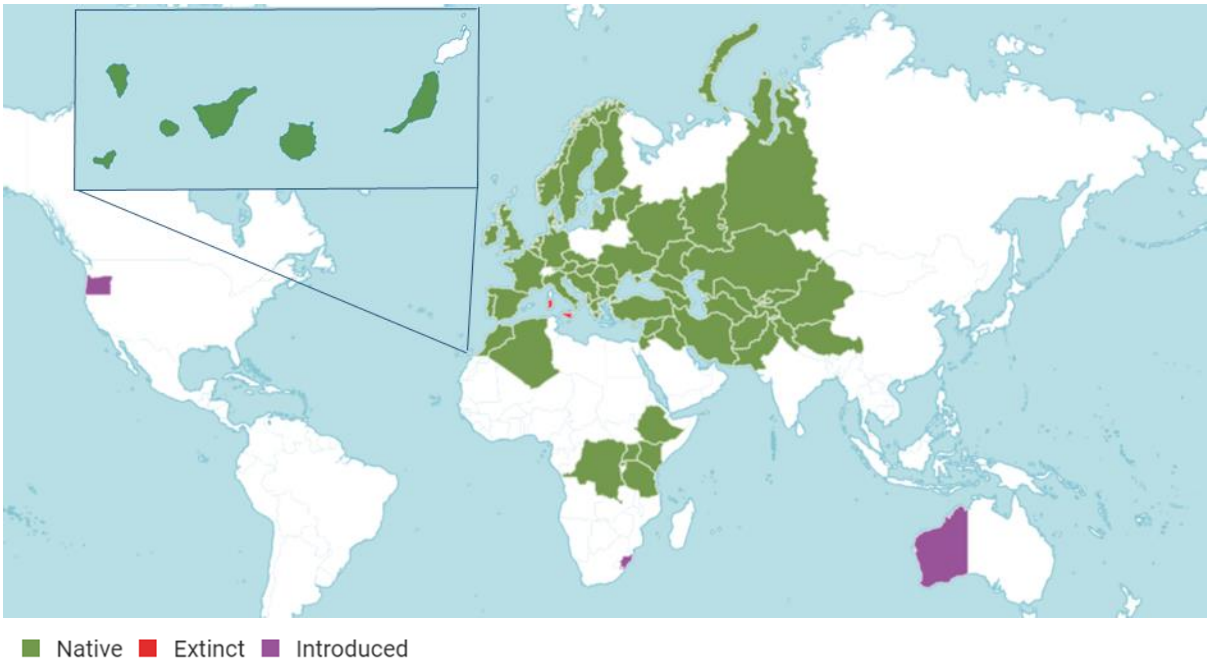


Figure 1: Distribution of the Crambe *genus. Highlighted are the Canary Islands. Taken and edited from: Plants of the World Online (2023).*

Canary Islands

The Canary Islands themselves have long held the attention of many botanists, palaeontologists and phylogeneticists. The archipelago has been of importance in the understanding of island colonisation (García-Verdugo, Caujapé-Castells & Sanmartín, 2019); they have an exceptional fossil record for most organisms except for plants, which is preserved in the context of active oceanic volcanic islands (Jiménez-Gomis et al., 2019); and island models and theories have been tested on the islands, such as the effect of dispersal on diversification (Sanmartín, Van Der Mark & Ronquist 2008). The Canary Islands hold over 2,000 native plant species (Beierkuhnlein et al., 2021), of which it has been estimated that over a third are endemic to the region (Hanz et al., 2022). The Mediterranean realm, of which the Canary Islands are part of, is the third richest plant biodiversity hotspot in the world (Koutroumpa et al., 2021). Due to its incredibly rich diversity, botanists have paid much attention to its flora, and detailed vegetation maps have been made (Del Arco & Rodrigue, 2018), though some gaps remain. Furthermore, the variations in habitat types and vegetation zones on the Canaries have been speculated to be (partially) responsible for the presence of both species-rich and species-poor genera (Juan et al., 2000). Overall multiple radiations have been documented (see Jackson et al., 2023; Jorgensen & Olesen, 2001).

The island closest to the continent (Lanzarote) is situated approximately 96 kilometres away from the west coast of Morocco, Africa (Fernández-Palacios et al., 2011). The Canarian archipelago currently comprises seven main large volcanic islands: La Gomera, La Palma, Tenerife, Gran Canaria, El Hierro, Fuerteventura, Lanzarote. The maximum elevation is 3,718 m (Teide volcano on Tenerife; Kunkel, 2012). Fuerteventura is the oldest of the seven islands, being approximately 20 million years old, while the youngest (El Hierro) is only one million years old (Juan et al., 2000) (Figure 2). In the early Miocene, the presence of up to 26 sea mounts between the Canary Islands and the Iberian Peninsula made it possible for a stepping-stone model of migration between the mainland and the archipelago (Fernández-Palacios et al., 2011).

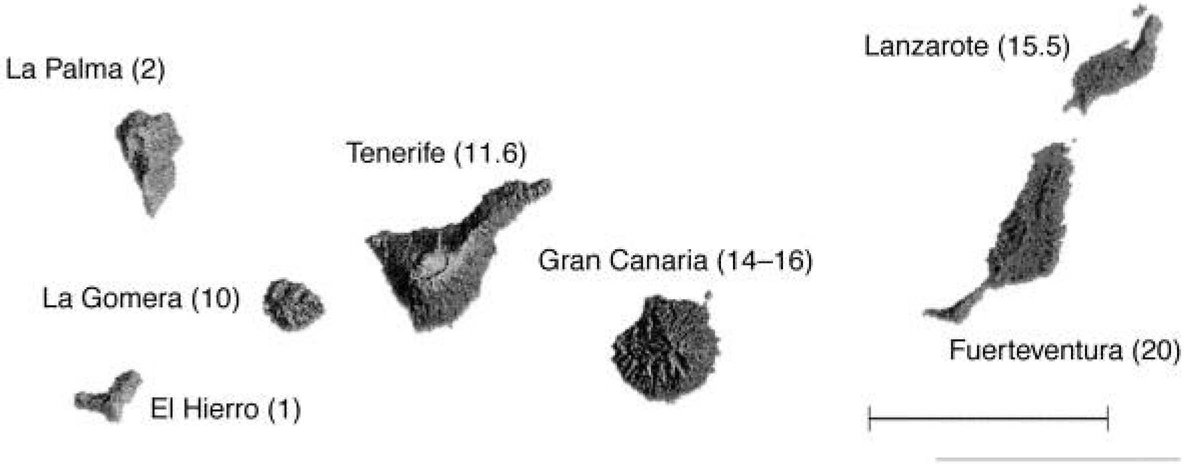


Figure 2: Map of the seven Canary Islands with their given age in mya, scale is in 100 km. Taken from Juan et al., (2000)

A phylogenetic tree of the genus *Crambe* has previously been reconstructed by Francisco-Ortega et al., (2002) for the species on the Canary Islands. However, this data is based on very few genetic markers and phylogenetic data from species occurring outside of the islands is limited. Furthermore, traits for this genus are yet to be consolidated into a single source. This project aims to reconstruct the most complete phylogenetic tree of the *Crambe* genus to date and to create an overview of the morphological and abiotic traits for the species within this genus. Then, taking advantage of *Crambe's* interesting distribution pattern (many species on both continent and Canary Islands), using these new datasets, what extrinsic and intrinsic traits might be linked to the island radiation of *Crambe* on the Canary Islands will be investigated. This project will also address whether, and how, island species of *Crambe* differ from those on the mainland.

Methods

Species Distinctions

The taxonomic scheme of *Crambe* species has seen frequent changes in the last decades (cite differing examples), with numbers of species, nomenclature and classification between sources differing among treatments. For this research, we used the taxonomic scheme by the Plants of the World Online (2023) which considers 35 species. Furthermore, the sub-species of *Crambe hispanica, C. gomeraea, C. orientalis, C. tataria and C. cordifolia* were also researched (*C. hispanica* subsp. *abyssinica, C. hispanica* subsp. *glabrata, C. gomeraea* subsp. *hirsuta, C. orientalis* subsp. *sulphurea* and *Crambe tataria* subsp. *aspera*). Due to the lack of distinction in traits between *C. cordifolia* and *C. kotschyana* (a proposed sub-species, *C. cordifolia* subsp. *kotschyana*), the latter was treated as a sub-species and not as a different species. If no data for the subspecies was found, it was assumed that they did not differ from the main species. The one hybrid species *Crambe x tchalenkoae* was not considered part of the *Crambe* as it is not considered a distinct species. In total, 42 *Crambe* taxa were investigated.

Herbarium Sampling, Library Preparation, and Phylogenetics

This research builds on the ongoing work of Hendriks et al., (2023, unpublished data), who has sequenced 20 *Crambe* species using more than 1000 molecualr markers as part of their attempt to reconstruct a species-dense Brassicaceae Tree of Life. The remaining unsequenced species housed in the Naturalis Biodiversity Center, Leiden, The Netherlands, herbaria (Amsterdam, Leiden, Utrecht, and Wageningen collections; AMD, L, U, WAG, respectively) had their DNA sequenced from leaf material collected from herbarium specimens. This increased the sequenced species from 20 to 32, representing 76% coverage of the genus. The subspecies were included in this phylogeny due to the controversies in the taxonomic classification of *Crambe.* Furthermore, *Hemicrambe fruticulose, Coincya monensis* and *Diplotaxis harra* were used as the outgroup, as Hendriks et al., (2023, unpublished data) found species from these genera to be closest relatives to the *Crambe* genus.

Phylogenetic trees were built following the methodology of Hendriks et al., (2022). Briefly, genomic DNA was extracted with the DNeasy Powerplant Pro Kit (Qiagen, Germany) from dried leaf material. Target capture sequencing was done with a mixed bait approach (Hendriks et al. 2021), using the Angiosperm353 and Brassicaceae-764 baitsets (Johnson et al., 2019; Nikolov et al., 2019). An Illumina HiSeq 2500 sequencer at a 100x technical coverage was used for sequencing. The sequenced data was quality controlled and trimmed with Trimmomatic v0.39 and mapped to a reference file using HybPiper v2.0 with the setting from Baker et al. (2022) (Bolger et al. 2014; Johnson et al., 2016). Unpaired reads were combined for use in HybPiper. The A353 'mega353' reference file of McLay et al. (2021) was used as the target file, as well as B764. Gene alignments were constructed using MACSE (Ranwez, Chantret & Delsuc, 2021), due to its ability to handle large numbers of sequences and for its ability to generate codon aware alignments. Sequences were aligned with the OMM_MACSE pipeline. Different phylogenomic approaches were used to analyse the gene alignments. Astral Pro 2 was used to reconstruct a coalescent-based species tree based on all gene trees (Zhang & Mirarab, 2022), to give insight into the most supported topology. IQtree was used to generate a time-calibrated maximum likelihood phylogenetic tree with the settings from Hendriks et al. (2023, unpublished data), which was then used for ancestral state reconstructions and diversification analyses (Minh et al., 2020). In order to compare the topologies produced by these two methods, a tanglegram was produced using the R package *dendextend* (R core team, 2022).

Traits

Trait selection for this study was guided by the literature, and includes those that have been hypothesised to promote diversification. Traits were classified into two types: extrinsic and intrinsic traits. The third trait classification based on Helmstetter et al., (2023) 'interaction', was not taken into account due to the limited available data. First, the papers by Prina (2000; 2009) and Prina and Laborde (2008) were used as the basis for this research. Furthermore, the databases TRY and GIFT (Global Inventory of Floras and Traits) were examined (Kattge et al., 2020; Weigelt, Konig, and Kreft, 2020). If information was not found in these papers, Google Scholar was used for specific species and traits. Lastly, the herbaria of the Naturalis Biodiversity Center were examined. The four Naturalis herbaria currently hold 18 of the *Crambe* species. The descriptions on the labels were used to score trait information. The information from the literature and additional measurements was compiled and scored for as many *Crambe* species as possible. In total 17 traits were scored.

Extrinsic traits

Extrinsic traits are not inherent to the plants but to the environments in which they can be found. However, they can influence how a plant species evolves and how far a species can migrate (Givnish, 2010). Questions remain concerning how area, elevation and - in the case of islands - isolation, influence dynamics (Patiño et al., 2018).

Islands vs mainland

One of the main research questions this study aims to address is whether the traits which may affect diversification and radiation are different between islands and the mainland. For this, a simple discrete scale was used with 0 indicating species that are present only on the Canary Islands while 1 means they are present on all other localities, further referred to as mainland.

No *Crambe* species were documented to occur on other islands (Plants of The World Online, 2023).

Habitat heterogeneity

Higher heterogeneity is associated with higher levels of species diversity (Nurk et al., 2020). Therefore, this paper looked at multiple aspects of landscapes that can be diverse for a species.

Habitats occupied

The presence of species was compared with the Global Habitat Type Map (Jung et al., 2020), the ESA CCI Land Cover series 1992-2015 (ESA, 2017), and the Copernicus Global Land Service map (cite). For this, distribution maps were built using coordinates found on GBIF (Global Biodiversity Information Facility 2023), accessed via the *rgbif* package in R. If not available, the coordinates were proxied using georeferenced data per individual for each species used in Prina (2000; 2009) and Prina and Laborde (2008). Habitat type occupancy data were then compared to the Global Habitat Type Map and extracted from the Copernicus Global Land Service map and ESA CCI Land Cover series 2015 land cover map using the R package *raster*. Habitats were scored based on the criteria/legend of these maps: forest, savanna, shrubland, grassland, wetland, rocky and desert following the land service map classification scheme(s). Multiple habitats for single species had multiple numbers. The proportion of habitat states was calculated by dividing the number of habitats a species is present in by the total amount of habitats *Crambe* can be present in.

Vegetation zones

Vegetation zones were classified as the range in elevation level at which the species exists. Based on the lack of distinct categories seen within the data (Annex I) it was chosen to make the categories as follows:

Table 1: Categories for the elevation.

Category	Elevation
1: low	<1000 m
2: mid	1000-2000 m
3: high	>2000 m
4:low/mid	0-2000 m
5:mid/high	>1000 m
6: full range	>0

This still makes a distinction between island species possible, as *Crambe* is found on elevations up to 2400 m.

Annual potential evapotranspiration

The definition used is based on the ENVIREM definition where they define annual potential evapotranspiration as: “the ability of the atmosphere to remove water through evapotranspiration processes” in mm/year (Title & Bemmels, 2018). Coordinates were plotted against the Annual Potential Evapotranspiration map after which the mean was calculated.

Continentality

Continentality is defined as the temperature of the warmest month - the temperature of the coldest month (Title & Bemmels, 2018). The coordinate data gathered from GBIF and Prina was plotted in R against the ENVIREM map (Title & Bemmels, 2018). The mean per species was calculated.

Mean UV

Taking a look at the present distribution might show some insights into the past *Crambe*. For this the coordinates were mapped in R against the UV annual mean map (Beckmann et al., 2014). The mean per species was calculated.

Aridity

The aridity index of the ENVIREM was used to determine the mean aridity of *Crambe* species based on coordinates. The aridity index refers to the degree of water deficit below water.

A PCA was performed to see if aridity, UV, continentality and evapotranspiration could be explained together by one component. The components of the PCA did not explain enough of the variation for a single component to be used (Annex V). Further analyses for these traits were, therefore, carried out individually.

Intrinsic traits

Herbaceous vs woody

This trait was scored on a scale of 0 (herbaceous), or 1 (insular woody).

Life cycle time

Depending on the difference between species, the life cycle time was also taken into account. This will be based on 1 (annual life cycle), 2 (biennial life cycle), and 3 (perennial life cycle).

Maximum plant height

Maximum plant height was measured in centimetres (cm). The categories were decided based on the results of the data points (Annex I). 3 categories were chosen due to the limited number of species in the phylogeny (Table 2).

Table 2: Categories for the max height.

Category	Height
1: low	<70 cm
2: mid	70-120 cm
3: high	>120 cm

Dispersal & reproductive traits

Reproductive plant traits have been measured frequently in the literature, and have been found in half of the cases to have had an effect on speciation and diversification (Helmstetter et al., 2023). However, when looking at dispersal in particular, even though this has barely been researched, the percentage lays substantially higher (Helmstetter et al., 2023). For this research, both reproductive and dispersal traits were taken into account. However, more focus was put onto the dispersal and characteristics of the seeds.

Fruit upper segment length

For this project the length was measured in millimetres. Crambe has segmented fruit. Due to the upper segment containing the fruit, being more influential on the distribution, and this data being more available the length of the upper segment was used (Prina 2000 & 2009).

Table 3: Categories for the upper segment length.

Category	Upper segment length
1: small	<2 mm
2: mid	2-3.5 mm
3: large	>3.5 mm

Seed mass/seed size

Islands tend to have plants with larger seeds, independent of dispersal and plant size, than the mainland (Burns, 2016; Biddick, Hendriks & Burns, 2019; Kavanagh & Burns, 2014). If an island is more isolated, the seeds tend to be smaller (Negoita et al., 2016). Smaller seed size has been linked to significantly elevated rates of diversification (Igea et al., 2017), as smaller seeds facilitate long-distance dispersal thereby increasing the possibility of colonising novel areas. However, opposite patterns have also been discovered (Thomson et al., 2011). Seed size, measured in seed mass, could therefore have an influence on diversification. Unfortunately, due to limited available data, measurements for only 14 species were found. Therefore, this trait was not used in the diversification modelling.

Fruit and flower colours

The colour of fruits can influence distribution of plant species as well as their rates of dispersal and diversification (Lu et al., 2019). Evolutionary changes in flower colour have also been linked to cause diversification (Delph & Kelly, 2014). This trait was not included in further analysis due to lack of shifts.

Ancestral State Reconstruction

To reconstruct the evolution of the traits, identify the shifts between trait states and to identify the ancestral state for the whole genus and for the island clade an ancestral state reconstruction was performed. This was done using the make.simmap and contMap functions of *phytools* package in R(Revell, 212). The outgroups were removed from the phylogeny, as this could give a skewed view of the ancestral trait state. If the trait state was unknown for a species they were set to be able to have any of the states. A stochastic character mapping was performed for the discrete characters, while the maximum probability was used for the continuous characters to perform a maximum likelihood analysis. Specifically, 100 stochastic models were simulated of which the likelihood for each state was summarised, while for the continuous characters a gradient for the trait was made. This was done for each traits individually.

Key-innovation (KI) Model

Diversity-dependent diversification (DDD) models were used to find the best fit to explain the temporal diversification pattern in the phylogeny (Etienne et al., 2012). Dd_ML, a maximisation of the log likelihood under a diversity-dependent diversification model was first performed to the whole tree to estimate parameters of this model. The missing number of species was put on 15, the branching times were calculated from the IQtree with ape::branching.times, for initparsopt the speciation rate, extinction rate and carrying capacity were set to 0.5, 0.1 and 40 respectively. Afterwards a key-innovation model was fitted (dd_KI_ML). Here, the island clade was taken as the subclade that had a putative key innovation that influences the diversification rate. The branching times for both the subclade and main clade were calculated using ape::branching.times. The time of the subclade splitting from the main clade was set 4.69 million years ago based on the phylogeny results. The carrying capacity, speciation rate of the main clade, extinction rate of the main clade were set to 60, 0.885 and 2e-14 respectively. While for the subclade carrying capacity and speciation were set to 26 and 0.9 while extinction rate was set to there being no shift. Maximum likelihood optimization was performed three times using a different set of parameters. Once without change in parameters in the island clade (dd_KI_ML), once with a decoupling of the carrying capacity between the island clade and the rest of the genus (dd_KI_ML K), and once with decoupling of the speciation rate between the island clade and the rest of the genus (dd_KI_ML S). The AIC was used to account for the varying amount of parameters between models

Results

Molecular Data

In total, 272,152,145 reads were recovered for the 32 sequenced species (range: 552,742 - 11,586,839, for *C. juncea* and *C. filimformis*, respectively. On average, around 329 A353 (178:347) and 736 B764 (408:759) genes were recovered. Only *Crambe laeviata* had no genes recovered and subsequently was not included in the phylogenetic reconstructions. A complete overview is given in supplementary material (Annex II).

Phylogenetic Reconstructions

The species-level phylogeny of genus *Crambe*is overall consistent with previous findings (Figures 3 & 4). Two major clades are recovered: one containing the species found currently in Northern Africa, Middle East, Eastern Europe and Asia, branching around 5 million years ago (henceforth 'Afro-Eurasian clade'); and the other containing Mediterranean North-African/European species and the Canary Islands radiation. The species found in the Canary Islands are recovered as monophyletic (*C. santosii* to *C. sventenii),* with the ancestor of the island clade colonising the archipelago 2.58 million years ago. However, the deep relationships within the radiation are unresolved in a polytomy. The closest related species to the island clade seems to be the species found in Mediterranean North-Africa/Europe (henceforth 'European clade').

Interestingly, the three *C. hispanica* individuals included in the analyses do not cluster together in the phylogeny. One of the individuals, *C. hispanica* subsp. *abyssinica* (S0395) was retrieved within the Canary Island clade while the individuals of *C. hispanica* subsp. *glabrata* and the other sampled *C. hispanica* subsp *abyssinica* were retrieved in the European clade and the Afro-Eurasian clade, respectively. These specimens, therefore, do not seem to be closely related. As *C. hispanica* is widespread throughout Europe and has previously been recovered as part of the European clade (Plants Of the World Online, 2023), the individual nested within the Canary Island clade was removed from the phylogeny in further analyses due to its apparent aberrant placement. For example, in Hendriks et al., (2023, unpublished) *C. hispanica* was recovered in a similar position to *C. hispanica* subsp. *glabrata* recovered this study, i.e. at the tip of the European clade. After rerunning the maximum likelihood tree without *C. hispanica,* no significant changes in topology were found (Annex III), justifying its removal from subsequent analyses. Overall, in other species with two or more subspecies, the subspecies sampled do not seem to cluster together. For instance, *C. cordifolia* and *C. cordifolia* subsp. *kotschyana* were retrieved in separate locations on the tree . Furthermore, *C. gigantea* and *C. santosii* who are considered synonyms by POWO are not sister species; *C. microcarpa* is closer related in both the coalescent-based and maximum likelihood trees.

The topology of the Astral tree is largely similar to the IQtree (Figure 5). Most of the differences are found within the island clade. These are also the least supported nodes in the IQtree (Figure 3). The main differences between the IQtree and the Astral tree are the relation of *C. grandiflora* to *C. pinnatifida* and *tataria,* and the position of *C. tamadabensis*. *C. grandiflora* is sister to *C. tataris* in the Astral tree but sister to *C. pinnatifida* in the IQtree. *C.* tamadabensis is basal in the Astral Canary Islands clade but nested deep within the radiation in the IQtree Canary Islands clade.

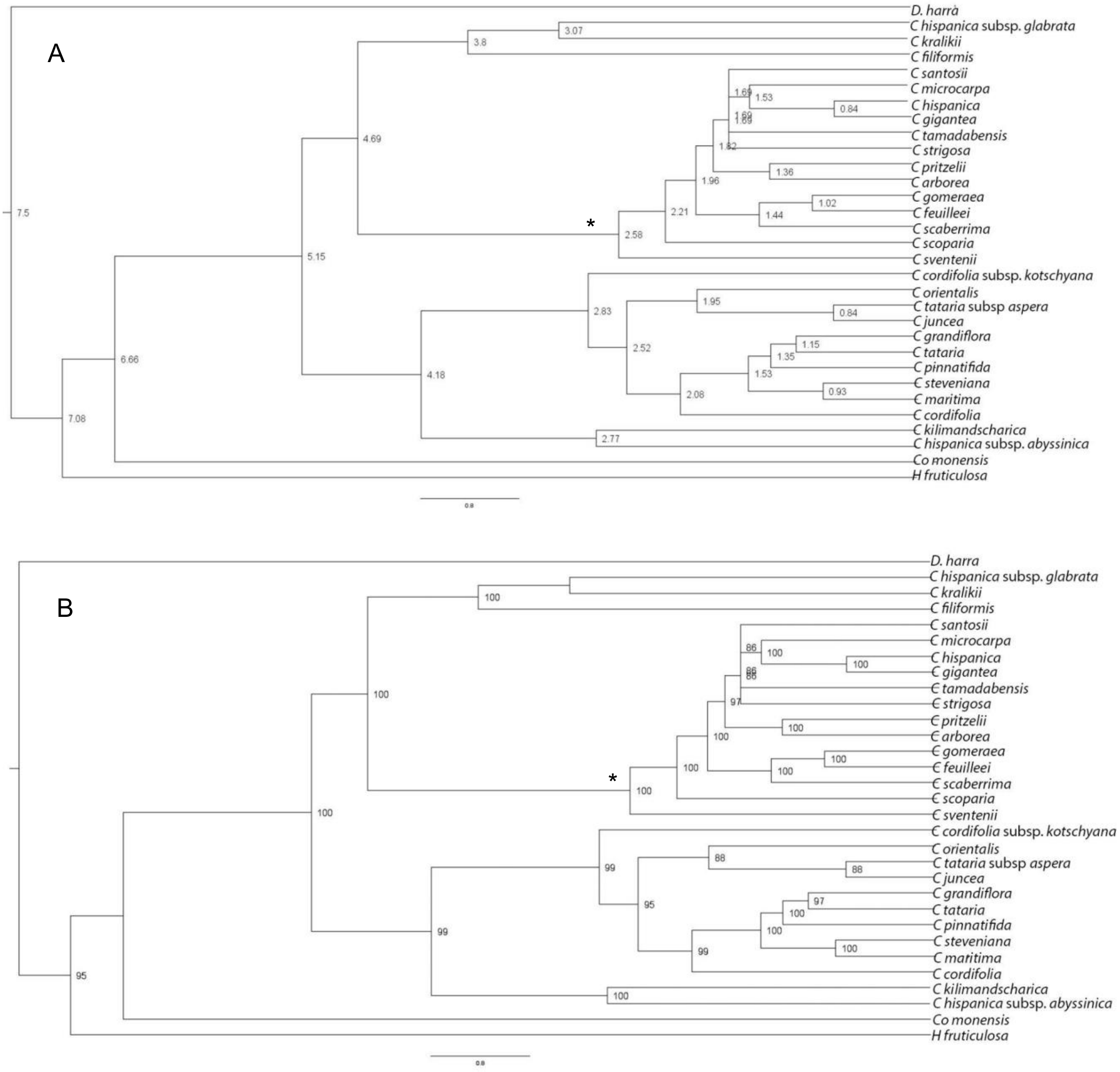


Figure 3: IQtree phylogeny of the Crambe genus with A: Age in millions of years at the nodes. B: support values at the nodes. The asterisk (*) indicates the crown node of the island clade.

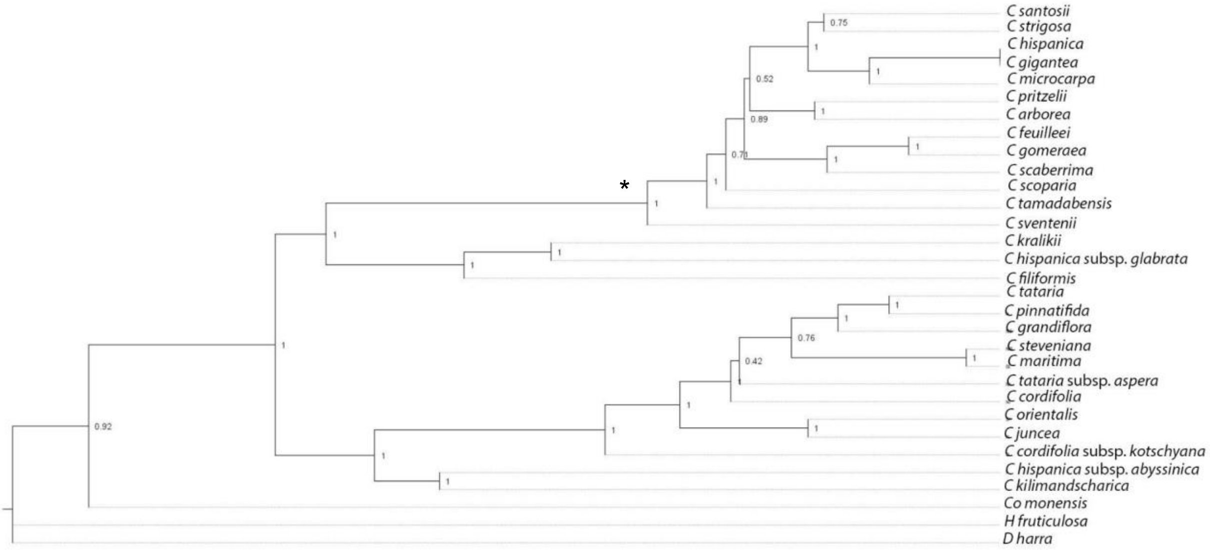


Figure 4: Astral phylogeny of the Crambe genus. Maximum likelihood at the nodes.The asterisk (*) indicates the crown node of the island clade.

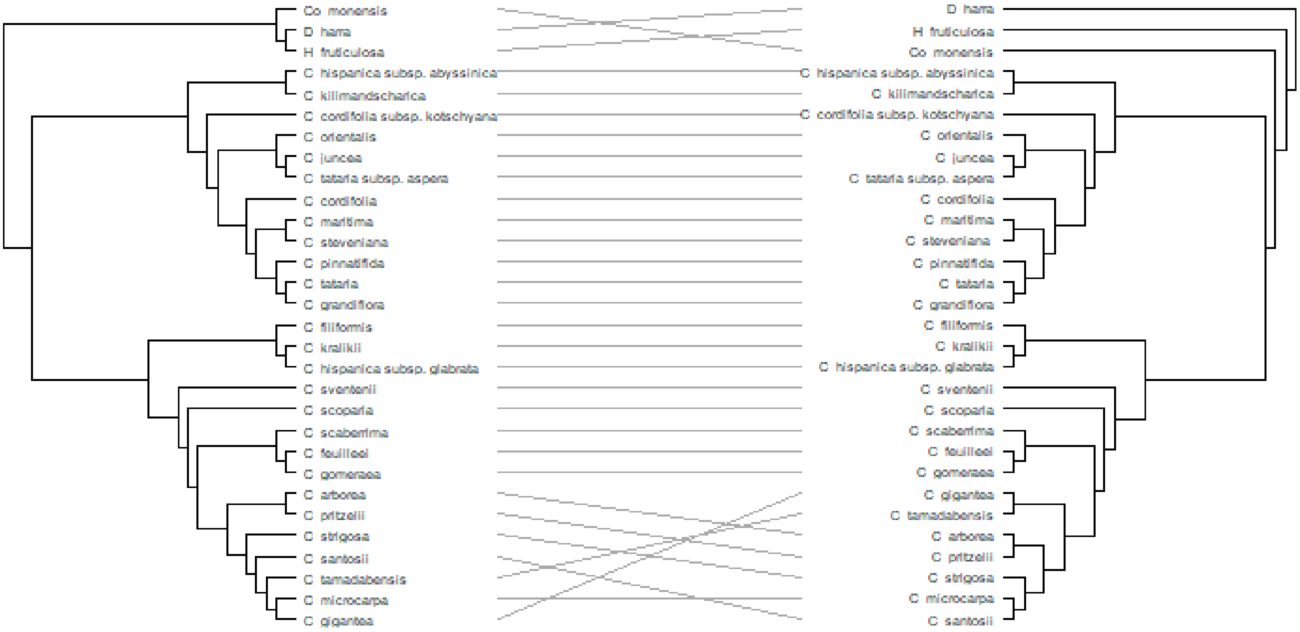


Figure 5: Dendogram of the IQtree (left) and the Astral tree (right)

Trait Scoring and Ancestral State Reconstructions

Overall there is disparity within the traits (Table 4). For most traits data was found for above 80% of the total species. The full data set can be found in the supplementary I.

Table 4: Overview of the traits measured

Trait	Range	Completeness (%)	Distribution
Island/mainland		100	Island=15 Mainland=27
Habitats occupied ESACCI		92.9	
Habitat coverage ESACCI	0.048-0.857	92.9	
Habitat Copernicus global land service		83.3	
Habitat coverage Copernicus	0.12-0.87	83.3	
Vegetation zones (m)	0-4200	92.9	
Herbaceous/woody		100	Herbaceous=27 Woody=15
Life cycle		100	Annual=5 Biannual=1 Perennial=36
Maximum height (cm)	60-400	92.9	
Upper segment length (mm)	1.3-7	97.6	
Seed mass (mg)	1.3-46.5	31	

Fruit colour	Brown-dark violet	10	
Flower colour	White-yellow	95.2	
evapotranspiration	654.6-1653.0	95.2	
Mean UV (mW/cm2)	1908.1-5862.4	95.2	
Continentality	4.1-30.4	95.2	
Aridity	40.75-88.2	95.2	

Ancestral state reconstructions were performed for all 11 traits except for flower colour, seed size, and island versus mainland inhabitance, due to the lack of differences between species, lack of data, and clear difference between the clades, respectively. For ancestral state reconstruction of vegetation zones, evapotranspiration and life cycle, see Annex IV.

Fruit upper segment length has a range of 1.2 - 7., found in *C. scoparia* and *C. steveniana,* respectively. The Canary Islands clade mostly has upper segment lengths of 2-3.5 mm or lower (Figure 6); only *C. sventenii* has an upper segment length of over 3.5 mm. Most species outside of this clade have larger segment lengths than 3.5 mm except for *C. hispanica* subsp. *abyssinica, C. maritima, C. pinnatifida* and *C. filiformis.* Furthermore the island clade are all insular woody while all other species are herbaceous (Figure 7).

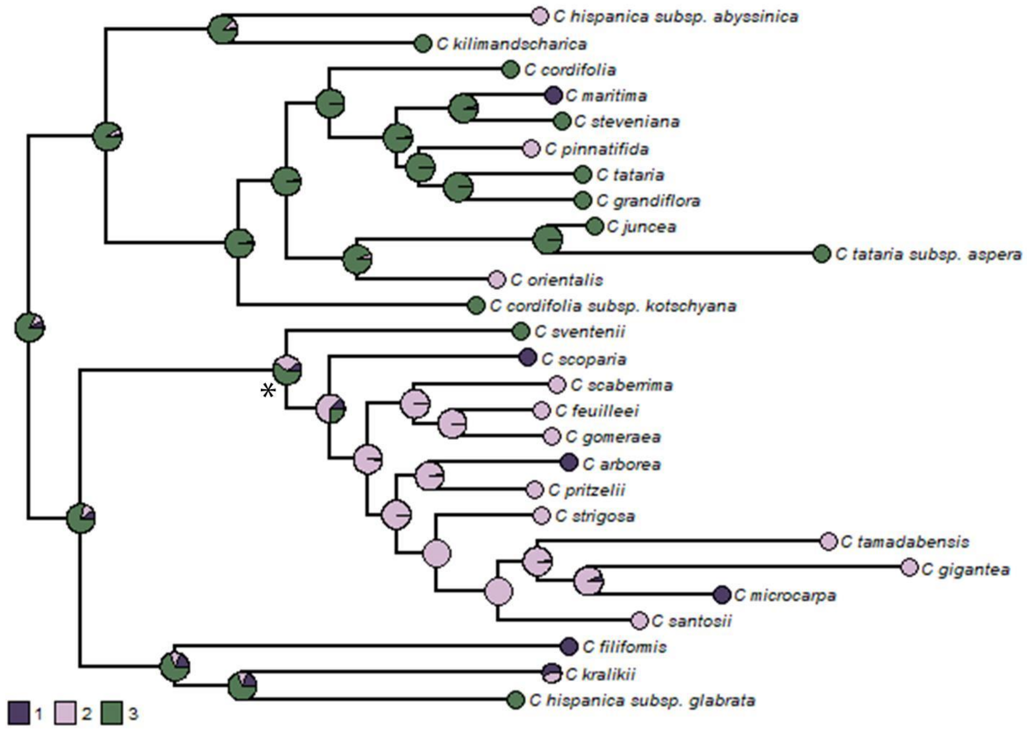


Figure 6: Ancestral state reconstruction of the upper segment length of the Crambe *genus. Circles at the tips indicate the trait state of the species at present, pie charts at the nodes indicate the likelihood of the state ; 1: <2.0 mm, 2: 2-3.5 mm, 3:>3.5 mm. For species for which the trait state was unknown, the possibility of it being any of the states was assumed. The asterisk (*) indicates the crown node of the island clade.*

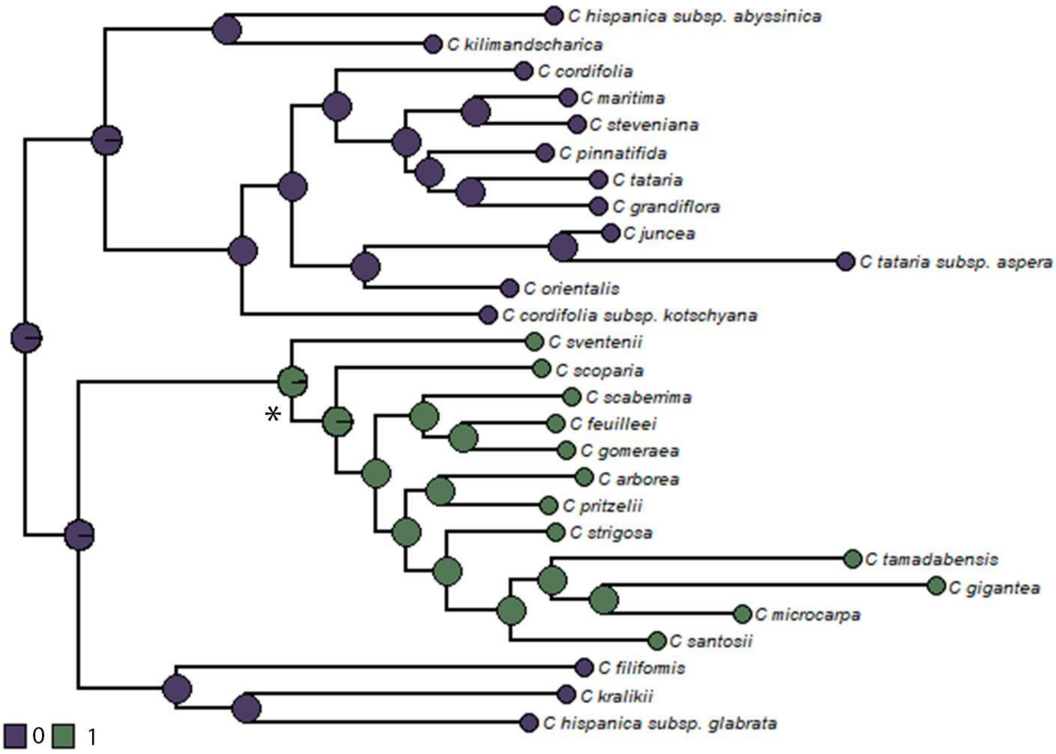


Figure 7: Ancestral state reconstruction for the Crambe *genus. 0: herbaceous, 1: insular woody. The asterisk (*) indicates the crown node of the island clade*

Most species on the Canary Islands were found to have a maximum height above 120 cm, except for: *C. sventenii, C. scaberrima* and *C. tamadabensis,* at 70 cm, 100 cm, and 100 cm, respectively (Figure 8). Most of the species in the Afro-Eurasian clade are below 120 cm except for: *C. cordifolia C. steveniana* and *C. tataria,* at 250 cm, 164 cm, and 160 cm, respectively. The ancestors of the island group and the Afro-Eurasian clade were likely above 120 cm.

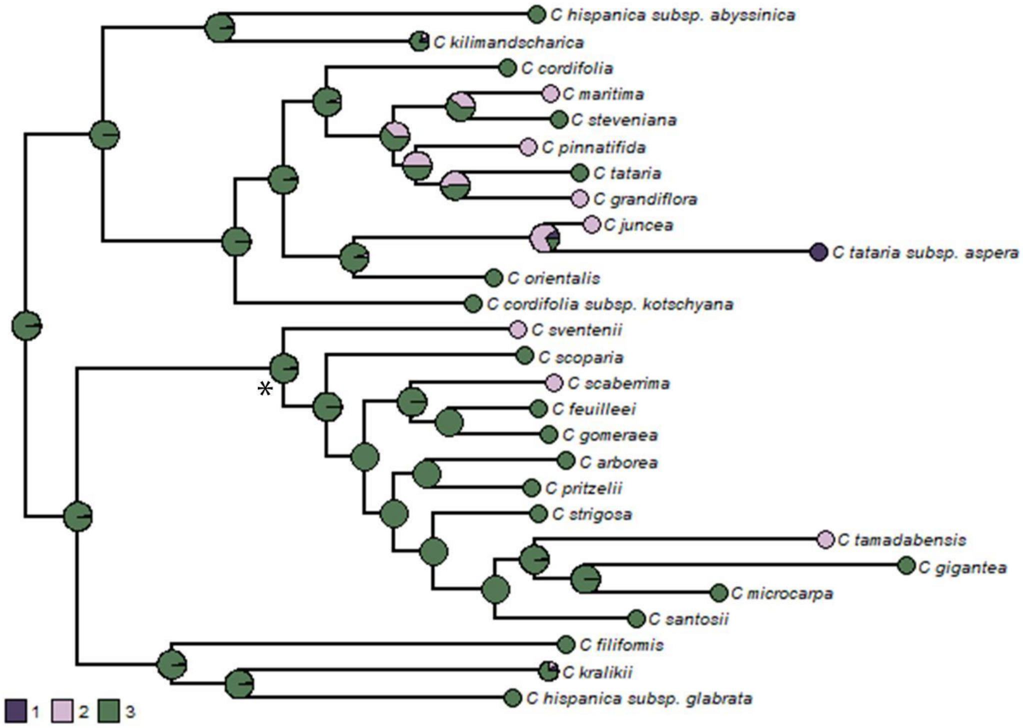


Figure 8: Ancestral state reconstruction for the maximum height of the Crambe *genus. 1:<70 cm, 2:70-120 cm, 3:>120cm. The asterisk (*) indicates the crown node of the island clade*

Aridity in *Crambe* ranges from 38 to 90.5 (based on the Aridity Index), which species have the lowest & highest. The island and European clade both score high on the aridity index (>76.4) while the Afro-Eurasian clade is low to mid low on the index (<62). The only exceptions to this are *C. cordifolia* subsp. *kotschyana* and *C. orientalis* (81.4, 66.2) (Figure 9). The opposite trend can be seen in Figure 10 where the island clade overall scores low (<9.2) on the continentality while the other clades score higher (>14.2). Overall continentality ranges between 3.3 and 27.5.

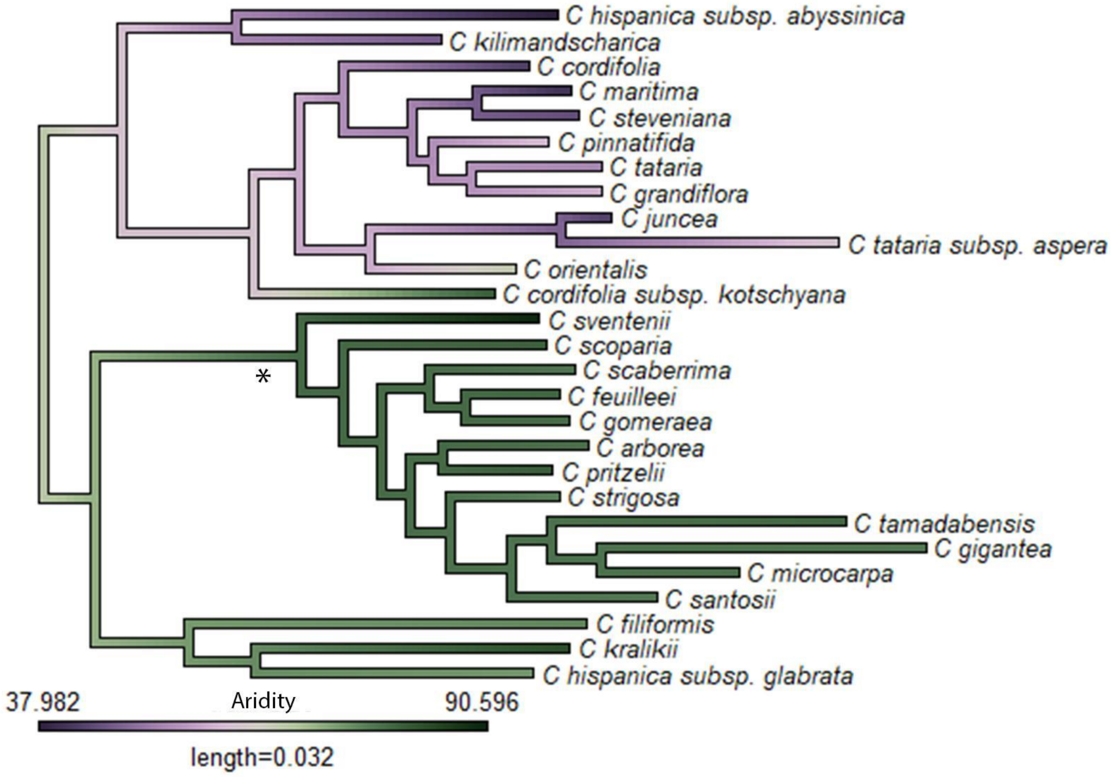


Figure 9: Ancestral state reconstruction of the aridity of Crambe. The asterisk (*) indicates the crown node of the island clade. Colour of branches indicate the reconstructed trait state.

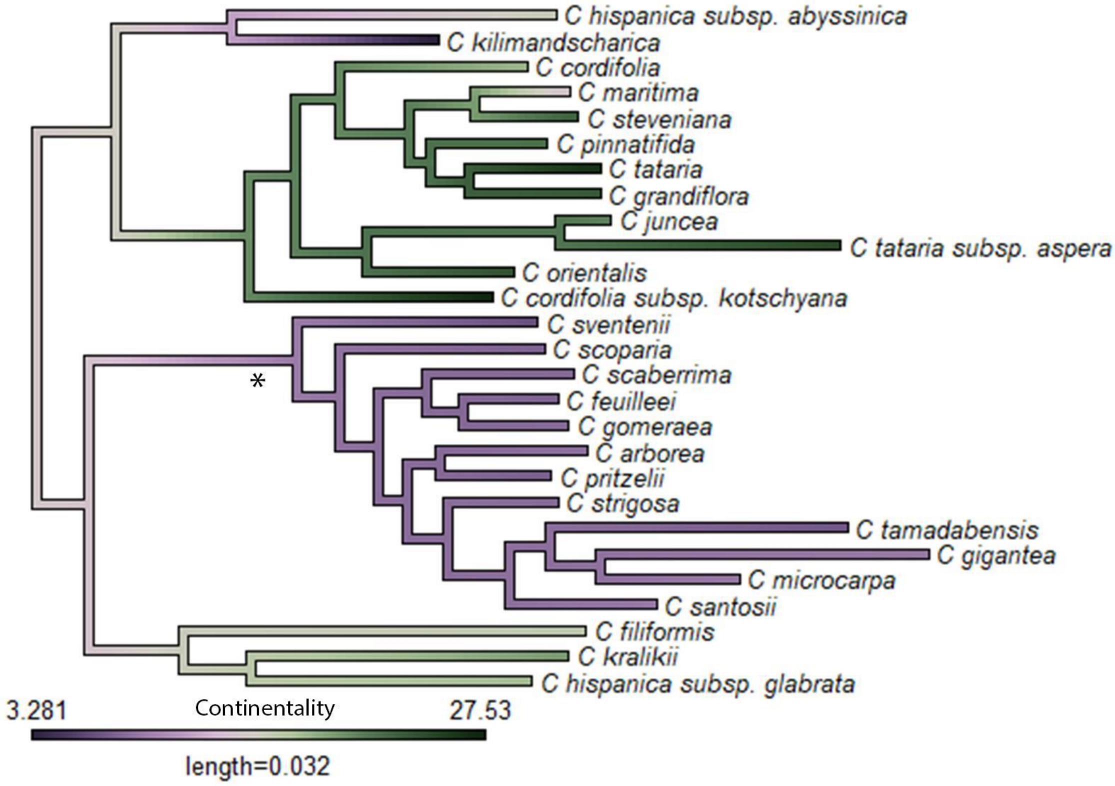


Figure 10: Ancestral state reconstruction for the continentality index of Crambe. The asterisk (*) indicates the crown node of the island clade. Colour of branches indicate the reconstructed trait state.

The island clade covers a low amount of the 21 total habitats of the *Crambe* genus, often only inhabiting one to two habitats (Figure 11). Outside of this clade there is disparity with *C. filiformis* inhabiting the most while *C. hispanica* subsp. *glabrata* inhabits the least. The highest and lowest species ranges from 0.048 to 0.857 which is equals to 4.8% and 85.7%, the island clade generally has lower values than the continent.

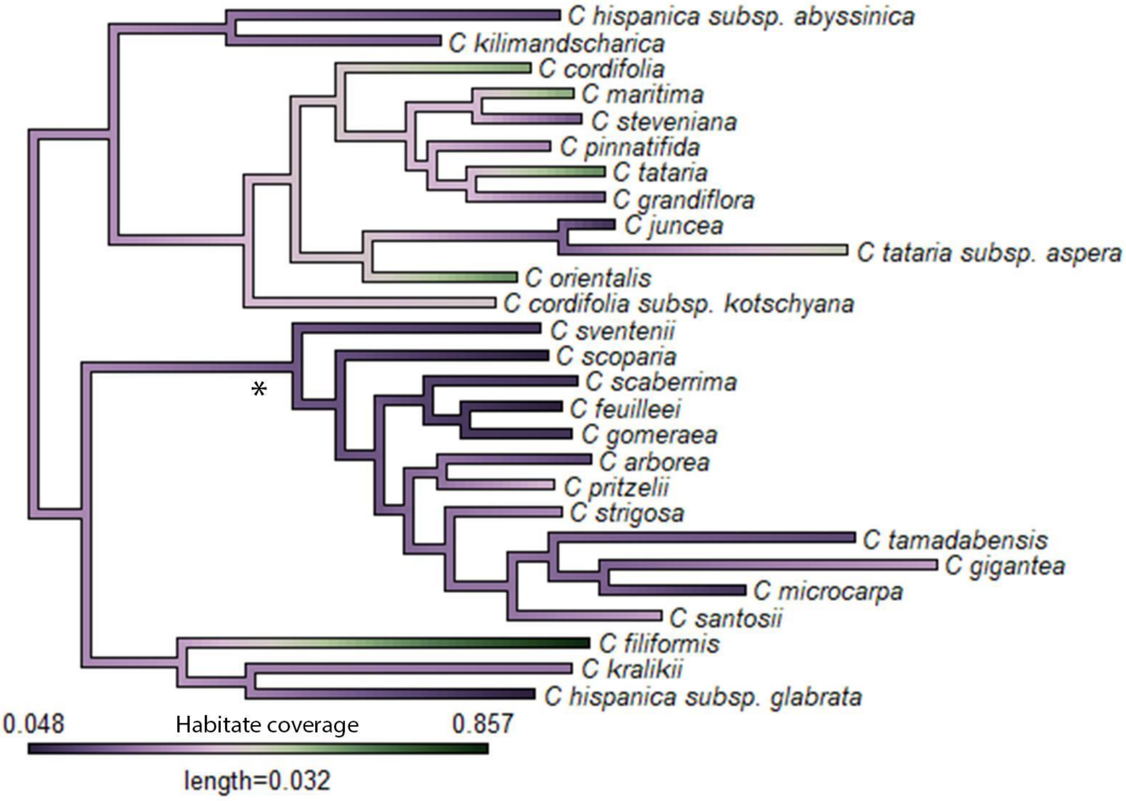


Figure 11: Ancestral state reconstruction for the habitat coverage of Crambe *where 1 is a species is present in all habitats, and 0 is present in no habitats. The total number of habitats is 21. The asterisk (*) indicates the crown node of the island clade. Colour of branches indicate the reconstructed trait state.*

The UV level on the island clade is very similar among species being in the mid high range(4029-4249) (Figure 12). The Afro-Eurasian clade's range in comparison is lower (<3594), with the only exception being *Crambe kilimandscharica* (5862). The European clade has a range from 3223 to 4288. Therefore, the European clade overlaps with both the Afro-Eurasian and the island clade. However, the island and Afro-Eurasian clade do not overlap with the island clade's lowest value 400 higher than the Afor-Eurasian.

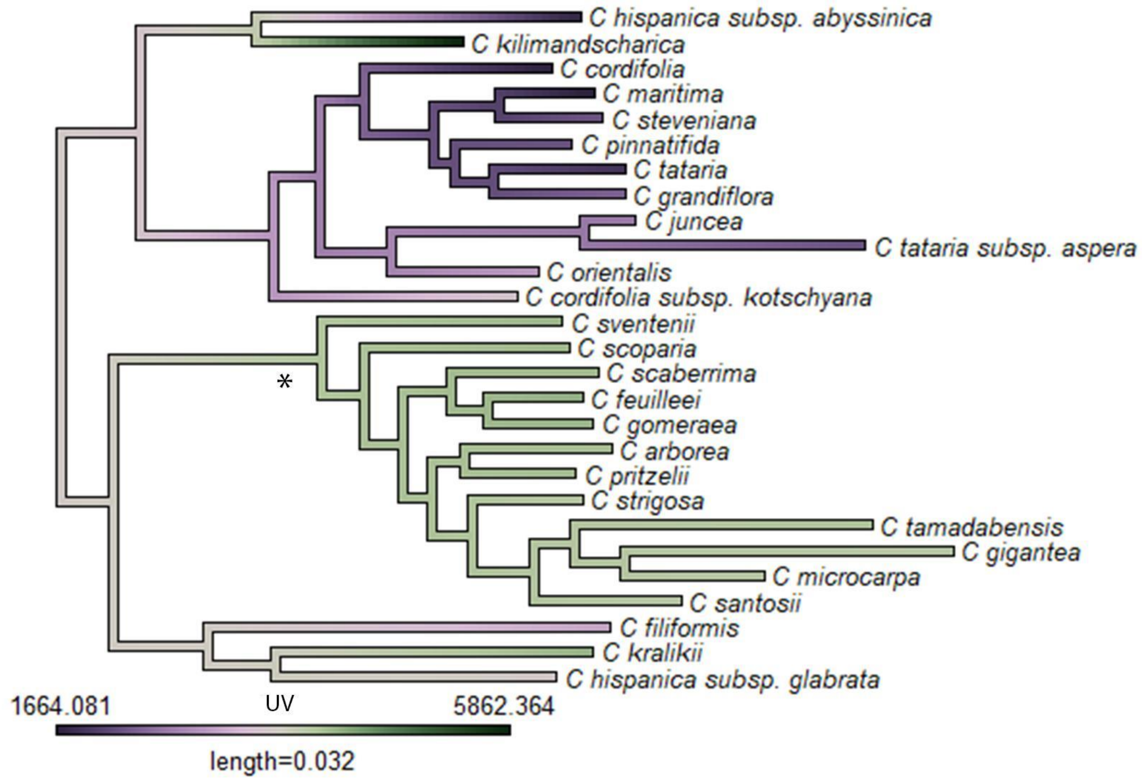


Figure 12: Ancestral state reconstruction for UV for the Crambe *genus (mW/cm2). The asterisk (*) indicates the crown node of the island clade. Colour of branches indicate the reconstructed trait state.*

Key Innovation Model

Based on the AIC scores, the key innovation model where the speciation rate is decoupled between the island and the mainland species is the preferred model (Table 5). According to the preferred model, the clade of Canary Island *Crambe* species has a higher rate for speciation (3.42, 1.15) and the ancestor species developed or encountered a key innovation.

Table 5: Results from the DDD models. dd_ml: diversity dependent diversification model, dd_ki_ml: diversity dependent diversification model with a decoupling of a subclade, dd_ki_ml K: decoupling of a subclade and the carrying capacity, dd_ki_ml: decoupling of a subclade and speciation rate, AIC: Akaike Information Criterion, lambda_S: speciation rate of the subclade, lambda_M: speciation of the main clade.

Model	Maximum likelihood	Parameters	AIC	lambda_S	lambda_M
dd_ml	-41.46	3	88.91		1.30
dd_ki_ml	-37.35	4	82.70	1.48	1.48
dd_ki_ml K	-37.17	5	84.33	1.54	1.54
dd_ki_ml SR	-34.22	5	78.44	3.42	1.15

Discussion

This study aimed to update the phylogenetic tree of the genus *Crambe* by reconstructing the most complete phylogeny of the genus to date, at the same time creating the most detailed trait database for any Brassicaceae genus and, due to *Crambe's* interesting island-mainland distribution pattern, investigate unanswered biogeographic questions by addressing how extrinsic and intrinsic traits differ between the Canary Islands radiation and the mainland representatives of the genus. We also test for the potential evolution of a key innovation within *Crambe*, using birth-death phylogenetic models of the DDD family, and discuss which traits may underlie this radiation.

Phylogenetic reconstructions

Overall, the phylogenetic trees built using the hyb-seq dataset were robust, well-resolved and generally agreed with previous studies. A noteworthy pattern we found was that for all cases where multiple subspecies of the same species were sampled, they did not seem to cluster together in the phylogeny, and were separated by (in some cases) several species, suggesting these intra-specific taxa may may be more distantly related than morphological data suggests. Therefore, it is possible that these actually are distinct species instead of subspecies. This can also be seen in the traits where, especially for the *C. hispanica* group, the traits scored differ highly between subspecies (Supplementary I). For example, the maximum height of *C. hispanica* subsp. *hispanica* is 100 cm while *C. hispanica* subps. *abyssinica* is 200 cm. A similair case can be made for *C. tataria* where *C. tataria* is 160 cm while *C. tataria* subsp. a*spera* only has a max height of 40cm. Some traits do not vary between conspecific subspecies but this is often due to a methodological assumption during scoring: if the trait is not mentioned as different for the subspecies and no further information is available, it was assumed that it was the same as the nominate subspecies or for the whole species. Further information on these subspecies is required to clarify this assumption. For *C. hispanica,* further indication of the three subspecies being distinct species can be found in their distribution. The distribution is highly disjunct between the subspecies, ranging from Spain, Portugal, and Morocco (*C. hispanica* subsp. *glabrata*), the east of Africa (*C. hispanica* subsp. *abyssinica*), to the Eastern Mediterranean and the Middle East (*C. hispanica* subsp. *hispanica)* (Plants of the World Online, 2023).

A similar picture can be seen for *C. santosii* and *C. gigantea.* Plants of the World Online (2023) treat these two species as synonymous, while BioCan (2023) and Beierkuhnlein et al. (2021) treat them as different species. From the phylogenies, it seems that these species could have diverged over a million years ago. However, due to the polytomy this is uncertain. Furthermore, *C. microcarpa* in both the IQtree and Astral tree is sister to *C. gigantea*. In the IQtree, *C. tamadabensis* is more closely related to *C. gigantea* than to *C santosii*. However, as one of the species that has a different placement between the two trees, this relationship is not present in this clade in the Astral tree. As the Astral tree reconstructs the phylogeny using all available gene trees, rather than concatenation (which can amplify issues typical in phylogenetics such as long-branch attraction and rapidly evolving DNA; Lui et al., 2015) it can be argued that the placement of *C. tamadabensis* outside of this clade is more likely. Nonetheless, these phylogenies indicate that *C. gigantea* and *C. santosii* are likely different species or that they, together with *C. microcarpa,* form a hitherto unrecognised species complex. *C.* m*icrocarpa* has a similar range of abiotic traits though their biotical traits are different (supplementary I). Unfortunately, due to *C. gigantea* and *C. santosii* previously being grouped together as one species, nothing can be said about their differences in traits.

There are some differences in the topology of the phylogeny produced here and the most recent Canary Islands *Crambe* phylogeny by Francisco-Ortego et al., 2002. This is partially due to the different species sampled in the previous study. For example, *C. wildpretti, laevigata,* and *fruticosa* are sampled in the previous phylogeny, but missing from this study. Interestingly, the relationships between species are quite similar for the species on the island, with the main differences being *C. arborea* now being a sister of *C. pritzelii*, fewer polytomies and better supported nodes, and *C. microcarpa* being retrieved in a different clade. While in the previous study this species was in a polytomy with *C. scaberrima, C. laevigata, C. gomerae* and *C. feuilleei,* now it is closest related to *C. gigantea*. These differences are however expected as the previous study used fewer genetic markers to reconstruct the phylogeny (2 vs the 353 used here). Furthermore, due to the 353 molecular markers, our phylogeny is less likely to be affected by incomplete lineage sorting. Therefore, our more deeply sampled phylogeny and the relationships between the outgroup species are likely to be different than the phylogeny of Francisco-Ortego and colleagues (2002).

Overall, the topologies of the molecular phylogenies of *Crambe* do not fully agree with the current taxonomic classification of *Crambe* of Plants of the World Online (2023). As there are already differences between sources on the classification further investigation is needed into the classification of this genus.

Trait evolution in *Crambe* and key innovation model

The diversification rate model with a key innovation was found to have a lower AIC than the diversity-dependent model with no key innovation. Therefore, the key innovation model with a decoupling of speciation rates between the Canary Island clade and the rest of the genus is the preferred scenario. Furthermore, the speciation rate of the island clade is higher than for the main clade. This finding suggests that there may have been a key innovation that increased the diversification rate on the Canary Islands. While the analyses conducted here are not sufficient to identify what the precise innovation may have been, a few possibilities can be hypothesised, and these are discussed below.

First, the key innovation leading to increased diversification rates in the Canary Islands could simply be that *Crambe* colonised the Canarian archipelago. This would have created a novel opportunity for the colonising population as it gained access to abiotic and biotic conditions unique to the islands and unavailable on the mainland. An argument can be made that a new area available itself can cause an increase in the diversification, however the area of the Canary Islands is small in comparison to the remainder of the range in Europe, Asia, Africa and the Middle East. Research has shown that the likelihood of speciation increases with area (Kisel & Barraclough, 2010). Therefore, it would be expected that on the mainland *Crambe* species should have a higher speciation rate based on area alone. However, the opposite is shown in the models. Possibly due to the mainland species having access to more suitable habitats, increasing dispersal and therefore decreasing their heterogeneity (Figure 11). This can lead to less speciation due to lack of isolation by distance. Furthermore, the island species mostly occur in small areas on the islands while mainland species are widespread (Plants of the World Online, 2023). Besides this, *Crambe* only colonised the islands 2.5 million years ago while there are now already 14 different species (not including the aforementioned subspecies that might be species), which, especially given the small geographical area of the Canarian archipelago, can be considered many species produced in a short period of time. Therefore, our study confirms the Canary Island forms a radiation as it is recent and monophyletic. It also suggests it is adaptive, given that a key innovation might be involved and that there are changes in traits potentially related to its environment. However, this would need to be further investigated.

Occurrence on the islands itself is not the only possible candidate for the key innovation. Only the *Crambe* species on the Canary Islands are (insular) woody. This shows a clear difference between the island and mainland clades and is therefore a good candidate trait to underlie the radiation of *Crambe* on the islands. A shift from herbaceousness to woodiness after the colonisation of an island (insular woodiness) has been proposed to be one of the key drivers for radiations on islands (Lens et al., 2013; Nürk et al., 2019). Zizka et al., (2022) argued that due to the Canary islands harbouring most insular woody genera there appears to be a link between woodiness and diversification. It could also be inferred that insular woodiness may confer evolutionary benefits within the Canarian climate, akin to how deciduousness provides benefits in temperate climates in woody angiosperms (Zanne et al., 2014; Wiens, 2023). However, research based on other species on the Canary islands (e.g. *Limonium*; Koutroumpa et al., 2021) has argued that the evolution of insular woodiness was not associated with an increase in the rate of diversification. Nevertheless, without further statistical tests (for co-evolution of trait syndromes and island occurrence, for example) we cannot currently conclude that woodiness influenced the radiation of *Crambe* on the Canary Islands.

Fruit size influences dispersal rates, and, as fruit and seed size can be highly inheritable, this could lead to effects on diversification (Galetti et al., 2013; Schupp et al., 2019). Indeed, fruit size has been hypothesised to influence diversification rates (Helmstetter et al., 2023). In

Crambe, seeds are only produced in the upper segment (Prina 2000 & 2009). As the ancestor of the island clade most likely had a larger upper segment (Figure 6), the species evolved a smaller upper segment length after being on the island. Interestingly, *Crambe* follows a classic island syndrome (Schrader et al., 2021), with reduction in fruit mass and subsequently seed investment and smaller seed size, reflected in the lower fruit upper segment length (Figure 6). As this is likely a response to promote fitness in island systems by reducing the number of propagules lost to the sea, it is possible this shift to shorter dispersal distances has facilitated within-archipelago dispersal, promoting allopatric cladogenesis. Therefore, this could potentially be a the key innovation.

It is interesting to note that while the fruit size in *Crambe* is lower on the islands, the size of the plant itself was found to be higher on the islands. Some indications in previous studies have been given for a difference in maximum plant height between islands and mainland. Maximum plant height tends to be lower on islands (island dwarfism), with the exception of some becoming substantially larger instead (island gigantism) (Burns, 2016; Biddick, Hendriks & Burns, 2019). The *Crambe* clade on the Canary Islands seems to favour the hypothesis of the existence of island gigantism. Little research has been done on the effect of maximum plant height on diversification rates, though some indications show that the smaller the plant size the faster the diversification rate (Boucher et al., 2017). If this is the case, it would mean for *Crambe* that the evolution of larger plant sizes on the islands and other potential diversification-boosting effects had enough of a positive effect to counteract the generally negative effect of plant height on diversification rate. Another hypothesis could be that plant height might have the opposite effect for diversification on islands. The possible common occurrence of gigantism on islands might indicate a benefit for the plants on the islands to become bigger and thus possibly influencing diversification rates positively. Further research on maximum plant height of species on the mainland and island and the effect of this trait on diversification rates may yield interesting results.

The continentality for the island clades is much lower than the mainland clades (Figure 10). The consistency of the temperature between the warmest and the coldest months (continentality) can influence diversification rates. Temperature has been found to influence plant morphology, metabolic processes and speciation (Allen et al., 2006; Larcher, 2003). It has been speculated that regions that experience less disturbances will have greater numbers of species due to decreased extinction rates (Schluter & Weir, 2007). However, the relationship between consistent temperatures and less disturbances is still unclear. Furthermore, there currently is still debate over if there are higher diversification rates at low latitudes and/or high latitudes (Willis, Bennett & Birks, 2009). Therefore, this may have been a reason as to why *Crambe* has diversified in 2.5 million years from one to 14 species.

The results of this study show that UV for the species on the island is higher than species on the mainland (Figure 12). UV tends to change with latitude and altitude, the closer a spot is to the sun the more UV it gets (Willis, Bennett & Birks, 2009). Another study found that higher environmental energy, which includes UV, has been linked to a strong effect on species richness and evolutionary rates (Daves et al., 2004). Therefore, UV could be the extrinsic trait that explains the increase in diversification. Furthermore, as temperature is also included in environmental energy it could be a combination of these two traits.

Lastly, aridity and aridity-induced vicariance on diversification have been shown in a multitude of species (GonÇalves et al., 2018). Furthermore, this is often the result of key innovations, such as in C4 photosynthesis in cacti (Arakaki et al., 2011). So as the *Crambe* species on the island are found on more arid environments than mainland taxa (Figure 9) this might be due to an adaptation that made it possible for these species to survive in more arid conditions. It is therefore possible that the key innovation was a trait not included in this study.

The species on the island have seen some changes in the traits in comparison to the other species (Figures 6-8). Their woodiness, segment length and size differs between island and mainland clades. Furthermore, the extrinsic traits of the island are overall different from those where any of the other species are present (Figures 9-12). Especially aridity, continentality and UV. Either of these traits, besides the fact that the species colonised the islands could be a key innovation that has caused this species to radiate on the Canary Islands.

Limitations

This research was based on the new trait database built for this study (Supplementary I). However, the amount of information available per species varied widely This could have an effect on the completeness of the data, especially the data that was based on coordinates. For example, the vegetation zone is a range that becomes more certain when more data points are available. Furthermore, it was assumed that if no publications mentioned a difference for the subspecies, that the trait would be the same as that in the main species description. All these factors could result in some biases in the trait data. Another limit is that the maps used were on a different resolution (5 and 30 arc seconds), but as no comparison between the map results was made, the impact should be limited.

While the phylogeny produced here has included more *Crambe* species than ever before, not all (sub)species were included. Though this is unfortunate, a 76% coverage was still reached, therefore, the results are still applicable. The phylogeny also is based on only the crown age. Lastly, an interesting observation was that *Crambe laeviata* failed to be sequenced four different times (including three not in this study), from three different specimens. Whilst this could be related to specific properties of the chosen herbarium specimens, there could possibly be a species-specific property preventing DNA from being effectively recovered, one that is not present in other *Crambe* species. As Francisco-Ortega et al., (2002) used only short fragments of two genes, it is possible this was not a limiting factor for their study. For future studies, it would be pertinent to compare DNA extraction methods with more *C. laevigata* specimens.

Conclusion

In short, a new phylogeny was generated which revealed that the current taxonomic classifications, based on morphology, at both the species and subspecies level are inaccurate. Particularly subspecies are not closely related and *Crambe gigantea* and *Crambe santosii*, previously debated synonyms, are two different species. Furthermore, the phylogeny was used to compare the traits between the island and mainland species within genus *Crambe*. It was found that a key innovation was involved in the radiation of *Crambe* on the islands. The species on the island has seen some changes in the traits in comparison to the other species. Their woodiness, segment length size has changed in response to the island. Furthermore, the extrinsic traits of the island are overall different from where any of the other species are present. Especially aridity, continentality and UV. Any of these traits besides the fact that the species came to colonise could be the key innovation that has caused this species to radiate on the Canary Islands. In addition to the key innovation, and changes in traits potentially related to its environment, the species colonised 2.5 million years ago, suggesting that the radiation was adaptive. However, further investigation is needed.

Bibliography

Abbott, R., Albach, D., Ansell, S., Arntzen, J. W., Baird, S. J., Bierne, N., ... & Zinner, D. (2013). Hybridization and speciation. *Journal of Evolutionary biology,* 26(2), 229-246.

Arakaki M, Christin P.A., Nyffeler R., Lendel A., Eggli U., Ogburn R.M., Spriggs E., Moore M.J., Edwards E.J. (2011). Contemporaneous and recent radiations of the world's major succulent plant lineages. *Proceedings of the National Academy of Sciences of the United States of America,* 108, 8379–8384.

Baker, W. J., Dodsworth, S., Forest, F., Graham, S. W., Johnson, M. G., McDonnell, A., & Wickett, N. J. (2021). Exploring Angiosperms353: An open, community toolkit for collaborative phylogenomic research on flowering plants. *Americal Journal of Botany*, *108*(7).

Beckmann M., Václavík T., Manceur A.M., Šprtová L., von Wehrden H., Welk E., Cord A.F. (2014) glUV: A global UV-B radiation dataset for macroecological studies, *Methods in Ecology and Evolution*, 5, 372–383. doi: 10.1111/2041-210X.12168

Beierkuhnlein, C., Walentowitz, A., & Welss, W. (2021). FloCan—A revised checklist for the flora of the Canary Islands. *Diversity*, *13*(10), 480.

Biddick, M., Hendriks, A., & Burns, K. C. (2019). Plants obey (and disobey) the island rule. *Proceedings of the National Academy of Sciences*, *116*(36), 17632-17634.

Bolger AM, Lohse M, Usadel B. (2014). Trimmomatic: A Flexible Trimmer For Illumina Sequence Data. *Bioinformatics,* 30, 2114–2120.

Boucher, F. C., Verboom, G. A., Musker, S., & Ellis, A. G. (2017). Plant size: a key determinant of diversification?. *New Phytologist*, *216*(1), 24-31.

Burns, K.C. (2016) Size changes in island plants: independent trait evolution in Alyxia ruscifolia (Apocynaceae) on Lord Howe Island. *Biological Journal of the Linnean Society. 119*, 847–855

Carracedo, J. C., S. J. Day, H. Guillou, and P. J. Gravestock. (1997). Geological map. Cumbre Vieja volcano (La Palma, Canary Islands). *Viceconsejería de Medio Ambiente del Gobierno de Canarias and Consejo Superior de Investigaciones Científicas, Santa Cruz de Tenerife, Canary Islands, Spain*.

Chao Zhang , Siavash Mirarab, ASTRAL-Pro 2: ultrafast species tree reconstruction from multi-copy gene family trees, *Bioinformatics*, Volume 38, Issue 21, 1 November 2022, Pages 4949–4950, https://doi.org/10.1093/bioinformatics/btac620

Consejería de Medio Ambiente y Ordenación Territorial. Gobierno de Canarias. (n.d.). *Biodiversity data bank of the Canary Islands*. Biota. https://www.biodiversidadcanarias.es/biota/

Darwin, C. & Kebler, L. (1859) On the origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life. London: J. Murray. [Pdf] Retrieved from the Library of Congress, https://www.loc.gov/item/06017473/.

del Arco Aguilar, M. J., & Rodríguez Delgado, O. (2018). Vegetation of the Canary Islands (pp. 83-319). *Springer International Publishing*.

Delph, L. F., & Kelly, J. K. (2014). On the importance of balancing selection in plants. *New phytologist*, *201*(1), 45-56.

Donoghue, M. J., & Sanderson, M. J. (2015). Confluence, synnovation, and depauperons in plant diversification. *New Phytologist*, *207*(2), 260-274.

Emerson, B. C. (2017). A roadmap for island biology: 50 fundamental questions after 50 years of The Theory of Island Biogeography. *Journal of Biogeography*, *44*(5), 963-983.

ESA. Land Cover CCI Product User Guide Version 2. Tech. Rep. (2017). Available at: maps.elie.ucl.ac.be/CCI/viewer/download/ESACCI-LC-Ph2-PUGv2_2.0.pdf

Etienne Rampal S., Haegeman Bart, Stadler Tanja, Aze Tracy, Pearson Paul N., Purvis Andy, and Philimore Albert B. (2012). Diversity-dependence brings molecular phylogenies closer to agreement with the fossil record. *Proceedings of the Royal Society B*, *279*, 1300–1309.

EuroVegMap - Home. (n.d.). https://www.synbiosys.alterra.nl/eurovegmap/

Fernández-Palacios, J. M., De Nascimento, L., Otto, R., Delgado, J. D., García-del-Rey, E., Arévalo, J. R., & Whittaker, R. J. (2011). A reconstruction of Palaeo-Macaronesia, with particular reference to the long-term biogeography of the Atlantic island laurel forests. *Journal of Biogeography*, *38*(2), 226-246.

Fernández-Palacios, J. M., Kreft, H., Irl, S. D., Norder, S., Ah-Peng, C., Borges, P. A., ... & Drake, D. R. (2021). Scientists' warning–The outstanding biodiversity of islands is in peril. *Global Ecology and Conservation*, *31*, e01847.

Francisco-Ortega, J., Fuertes-Aguilar, J., Kim, S. C., Santos-Guerra, A., Crawford, D. J., & Jansen, R. K. (2002). Phylogeny of the Macaronesian endemic Crambe section Dendrocrambe (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal DNA. *American Journal of Botany*, *89*(12), 1984-1990.

Galetti M, Guevara R, Côrtes MC, Fadini R, Von Matter S, Leite AB, Labecca F, Ribeiro T, Carvalho CS, Collevatti RG, Pires MM, Guimarães PR Jr, Brancalion PH, Ribeiro MC, Jordano P. (2013). Functional extinction of birds drives rapid evolutionary changes in seed size. *Science* 340:1086–1090.

García-Talavera, F.. (1999). La Macaronesia. Consideraciones geológicas, biogeográficas y paleoecológicas. In J. M. Fernández-Palacios, J. J. Bacallado, and J. A. Belmonte [eds.], *Ecología y cultura en Canarias,* 39– 63. Múseo de la Ciencia, Cabildo Insular de Tenerife, Santa Cruz de Tenerife, Canary Islands, Spain.

García-Verdugo, C., Caujapé-Castells, J., & Sanmartín, I. (2019). Colonization time on island settings: lessons from the Hawaiian and Canary Island floras. *Botanical Journal of the Linnean Society*, *191*(2), 155-163.

GonÇalves, D. V., Pereira, P., Velo-AntÓn, G., Harris, D. J., Carranza, S., & Brito, J. C. (2018). Assessing the role of aridity-induced vicariance and ecological divergence in species diversification in North-West Africa using Agama lizards. *Biological Journal of the Linnean Society*, 124(3), 363-380.

Givnish, T. J. (2010). Ecology of plant speciation. *Taxon*, *59*(5), 1326-1366.

Hamrick, J. L., & Godt, M. W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *351*(1345), 1291-1298.

Hanz, D. M., Cutts, V., Barajas-Barbosa, M. P., Algar, A. C., Beierkuhnlein, C., Fernández-Palacios, J. M., & Irl, S. D. (2022). Climatic and biogeographical drivers of functional diversity in the flora of the Canary Islands. *Global Ecology and Biogeography*, *31*(7), 1313-1331.

Helmstetter, A. J., Zenil-Ferguson, R., Sauquet, H., Otto, S. P., Méndez, M., Vallejo-Marin, M., ... & Käfer, J. (2023). Trait-dependent diversification in angiosperms: Patterns, models and data. *Ecology Letters*, *26*(4), 640-657.

Helmus, M. R., Mahler, D. L., & Losos, J. B. (2014). Island biogeography of the Anthropocene. *Nature,* 513(7519), 543–546. https://doi.org/10.1038/nature13739

Hendriks, K. P., Kiefer, C., Al-Shehbaz, I. A., Bailey, C. D., Hooft van Huysduynen, A., Nikolov, L. A., ... & Lens, F. (2022). Global Phylogeny of the Brassicaceae Provides Important Insights into Gene Discordance. *bioRxiv*, 2022-09.

Hooft van Huysduynen, A. H., Janssens, S., Merckx, V., Vos, R., Valente, L., Zizka, A., ... & Lens, F. (2020). Multiple origins of insular woodiness on the Canary Islands are consistent with palaeoclimatic aridification. *bioRxiv*, 2020-05.

Hooft van Huysduynen, A., Janssens, S., Merckx, V., Vos, R., Valente, L., Zizka, A., ... & Lens, F. (2021). Temporal and palaeoclimatic context of the evolution of insular woodiness in the Canary Islands. *Ecology and Evolution*, *11*(17), 12220-12231.

Igea, J., Miller, E.F., Papadopulos, A.S. and Tanentzap, A.J. (2017). Seed size and its rate of evolution correlate with species diversification across angiosperms. *PLoS biology*, *15*(7), p.e2002792.

Jackson, A. C., White, O. W., Carine, M., & Chapman, M. A. (2023). The role of geography, ecology, and hybridization in the evolutionary history of Canary Island Descurainia. *American Journal of Botany*, *110*(5), e16162.

Jorgensen, T. H., & Olesen, J. M. (2001). Adaptive radiation of island plants: evidence from Aeonium (Crassulaceae) of the Canary Islands. *Perspectives in Plant Ecology, Evolution and Systematics*, 4(1), 29-42.

Jiménez-Gomis, C., García Frank, A., Sarmiento Chiesa, G., & Castillo Ruiz, C. (2019). Conservation status of the listed marine fossil sites in the Macizo de Anaga Biosphere Reserve (Tenerife, Canary Islands, Spain). *Geoheritage*, *11*, 1757-1769.

Juan, C., Emerson, B. C., Oromí, P., & Hewitt, G. M. (2000). Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology & Evolution*, *15*(3), 104-109.

Jung, M., Dahal, P. R., Butchart, S. H., Donald, P. F., De Lamo, X., Lesiv, M., ... & Visconti, P. (2020). A global map of terrestrial habitat types. *Scientific data*, *7*(1), 256.

Kattge, J., S. Díaz, S. Lavorel, I. C. Prentice, P. Leadley, G. Bönisch, E. Garnier, M. Westoby, P. B. Reich, I. J. Wright, J. H. C. Cornelissen, C. Violle, S. P. Harrison, P. M. v. Bodegom, M. Reichstein, B. J. Enquist, N. A. Soudzilovskaia, D. D. Ackerly, M. Anand, O. Atkin, M. Bahn, T. R. Baker, D. Baldocchi, R. Bekker, C. Blanco, B. Blonder, W. J. Bond, R. Bradstock, D. E. Bunker, F. Casanoves, J. Cavender-Bares, J. Q. Chambers, F. S. Chapin, J. Chave, D. Coomes, W. K. Cornwell, J. M. Craine, B. H. Dobrin, L. Duarte, W. Durka, J. Elser, G. Esser, M. Estiarte, W. F. Fagan, J. Fang, F. Fernández-Méndez, A. Fidelis, B. Finegan, O. Flores, H. Ford, D. Frank, G. T. Freschet, N. M. Fyllas, R. V. Gallagher, W. A. Green, A. G. Gutierrez, T. Hickler, S. Higgins, J. G. Hodgson, A. Jalili, S. Jansen, C. Joly, A. J. Kerkhoff, D. Kirkup, K. Kitajima, M. Kleyer, S. Klotz, J. M. H. Knops, K. Kramer, I. Kühn, H. Kurokawa, D. Laughlin, T. D. Lee, M. Leishman, F. Lens, T. Lenz, S. L. Lewis, J. Lloyd, J. Llusià, F. Louault, S. Ma, M. D. Mahecha, P. Manning, T. Massad, B. Medlyn, J. Messier, A. T. Moles, S. C. Müller, K. Nadrowski, S. Naeem, Ü. Niinemets, S. Nöllert, A. Nüske, R. Ogaya, J. Oleksyn, V. G. Onipchenko, Y. Onoda, J. Ordoñez, G. Overbeck, W. A. Ozinga, S. Patiño, S. Paula, J. G. Pausas, J. Peñuelas, O. L. Phillips, V. Pillar, H. Poorter, L. Poorter, P. Poschlod, A. Prinzing, R. Proulx, A. Rammig, S. Reinsch, B. Reu, L. Sack, B. Salgado-Negret, J. Sardans, S. Shiodera, B. Shipley, A. Siefert, E. Sosinski, J.-F. Soussana, E. Swaine, N. Swenson, K. Thompson, P. Thornton, M. Waldram, E. Weiher, M. White, S. White, S. J. Wright, B. Yguel, S. Zaehle, A. E. Zanne, C. Wirth. 2011. TRY – a global database of plant traits. *Global Change Biology*, 17:2905–2935.

Kavanagh, P. H., & Burns, K. C. (2014). The repeated evolution of large seeds on islands. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1786), 20140675.

Kim, S. C., McGowen, M. R., Lubinsky, P., Barber, J. C., Mort, M. E., & Santos-Guerra, A. (2008). Timing and tempo of early and successive adaptive radiations in Macaronesia. *PLoS One*, *3*(5), e2139.

Kisel, Y. & Barraclough, T.G. (2010). Speciation has a spatial scale that depends on levels of gene flow. *The American Naturalist*, *175*, 316–334.

König, C., Weigelt, P., Taylor, A., Stein, A., Dawson, W., Essl, F., ... & Kreft, H. (2021). Source pools and disharmony of the world's island floras. *Ecography*, *44*(1), 44-55.

Koutroumpa, K., Warren, B. H., Theodoridis, S., Coiro, M., Romeiras, M. M., Jiménez, A., & Conti, E. (2021). Geo-climatic changes and apomixis as major drivers of diversification in the Mediterranean sea lavenders (Limonium Mill.). *Frontiers in Plant Science*, *11*, 612258.

Kunkel, G. (Ed.). (2012). *Biogeography and ecology in the Canary Islands* (Vol. 30). Springer Science & Business Media.

Larcher, W. (2003) *Physiological plant ecology,* 4th edn. Springer-Verlag, Berlin.

Lens, F., Davin, N., Smets, E., & del Arco, M. (2013). Insular woodiness on the Canary Islands: a remarkable case of convergent evolution. *International Journal of Plant Sciences*, *174*(7), 992-1013.

Losos, J. B., & Ricklefs, R. E. (2009). Adaptation and diversification on islands. *Nature*, *457*(7231), 830-836.

Lu, L., Fritsch, P. W., Matzke, N. J., Wang, H., Kron, K. A., Li, D. Z., & Wiens, J. J. (2019). Why is fruit colour so variable? Phylogenetic analyses reveal relationships between fruit-colour evolution, biogeography and diversification. *Global Ecology and Biogeography*, *28*(7), 891-903.

Médail, F. (2017). The specific vulnerability of plant biodiversity and vegetation on Mediterranean islands in the face of global change. *Regional Environmental Change, 17*(6), 1775-1790.

Matsubayashi, K. W., & Yamaguchi, R. (2022). The speciation view: Disentangling multiple causes of adaptive and non-adaptive radiation in terms of speciation. *Population Ecology*, *64*(2), 95-107.

Miller, A. H., Stroud, J. T., & Losos, J. B. (2022). The ecology and evolution of key innovations. *Trends in Ecology & Evolution*, *38*(2), p122-131.

Minh BQ, Hahn MW, Lanfear R. (2020). New Methods to Calculate Concordance Factors for Phylogenomic Datasets. *Molecular biology and evolution,* 37(9), 2727-2733.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, *37*(5), 1530-1534.

Negoita, L., Fridley, J. D., Lomolino, M. V., Mittelhauser, G., Craine, J. M., & Weiher, E. (2016). Isolation-driven functional assembly of plant communities on islands. *Ecography*, *39*(11), 1066-1077.

Nürk, N. M., Atchison, G. W., and Hughes, C. E. (2019). Island woodiness underpins accelerated disparification in plant radiations. *New Phytologist*, *224*, 518–531. doi: 10.1111/nph.15797

Patiño, J., Whittaker, R. J., Borges, P. A., Fernández-Palacios, J. M., Ah-Peng, C., Araújo, M. B., ... & Sauquet, H., & Magallón, S. (2018). Key questions and challenges in angiosperm macroevolution. *New Phytologist*, *219*(4), 1170-1187.

Plants of the World Online | Kew Science. (n.d.).*Crambe L.* Plants of the World Online. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:12477-1

Prina, A. (2000). A taxonomic revision of Crambe, sect. Leptocrambe (Brassicaceae). *Botanical Journal of the Linnean Society*, *133*(4), 509-524.

Prina, A. O. (2009). Taxonomic review of the genus" Crambe" sect." Crambe"(Brassicaceae, Brassiceae). *In Anales del Jardín Botánico de Madrid* (Vol. 66, No. 1, pp. 7-24). Real Jardín Botánico.

Prina, A. O., & Martinez-Laborde, J. B. (2008). A taxonomic revision of Crambe section Dendrocrambe (Brassicaceae). *Botanical Journal of the Linnean Society*, *156*(2), 291-304.

Ranwez, V., Chantret, N., & Delsuc, F. (2021). Aligning Protein-Coding nucleotide sequences with MACSE. *Multiple Sequence Alignment: Methods and Protocols*, 51-70.

R Core Team (2022). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Revell L (2012). “phytools: An R package for phylogenetic comparative biology (and other things).” *Methods in Ecology and Evolution*, 3, 217-223. doi:10.1111/j.2041-210X.2011.00169.x.

Rudloff, E., & Wang, Y. (2011). Crambe. *Wild crop relatives: genomic and breeding resources: oilseeds*, 97-116.

Sanmartín, I., Van Der Mark, P., & Ronquist, F. (2008). Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. *Journal of Biogeography*, *35*(3), 428-449.

Sauquet, H., & Magallón, S. (2018). Key questions and challenges in angiosperm macroevolution. *New Phytologist*, *219*(4), 1170-1187.

Schupp, E. W., Zwolak, R., Jones, L. R., Snell, R. S., Beckman, N. G., Aslan, C., ... & Shea, K. (2019). Intrinsic and extrinsic drivers of intraspecific variation in seed dispersal are diverse and pervasive. *AoB Plants*, *11*(6), plz067.

Schluter, D. & Weir, J.T. (2007) Explaining latitudinal diversity gradients: response. *Science*, 317, 452–453.

Stroud, J. T., & Losos, J. B. (2016). Ecological opportunity and adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics*, *47*, 507-532.

Taylor, A., Weigelt, P., König, C., Zotz, G., & Kreft, H. (2019). Island disharmony revisited using orchids as a model group. *New Phytologist*, *223*(2), 597-606.

Thomson FJ, Moles AT, Auld TD, Kingsford RT. 2011. Seed dispersal distance is more strongly correlated with plant height than with seed mass. *Journal of Ecology,* 99, 1299-1307

Title P.O., Bemmels J.B. 2018. ENVIREM: an expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography, 41*, 291–307.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional!. *Oikos*, *116*(5), 882-892.

Warwick, S. I., Francis, A., & Gugel, R. K. (2009). Guide to wild germplasm of Brassica and allied crops (tribe Brassiceae, Brassicaceae). *Canada: Agriculture and Agri-Food Canada*, *1*(6).

Weigelt, P. and Kreft, H. (2013) Quantifying island isolation insights from global patterns of insular plant species richness. *Ecography* 36, 417–429

Whittaker, R. J., Fernández-Palacios, J. M., Matthews, T. J., Borregaard, M. K., & Triantis, K. A. (2017). Island biogeography: Taking the long view of nature's laboratories. *Science*, 357(6354), eaam8326. https://doi.org/10.1126/science.aam8326

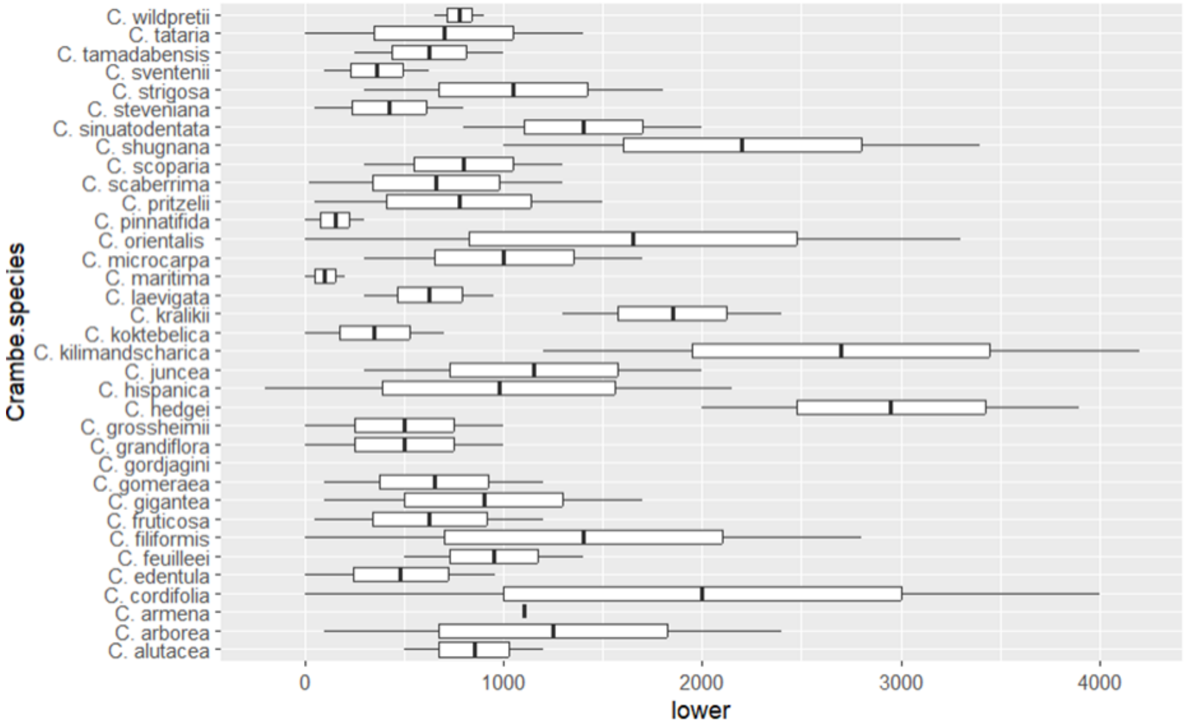
Wiens, J. J. (2023). Trait-based species richness: ecology and macroevolution. *Biological Reviews*.

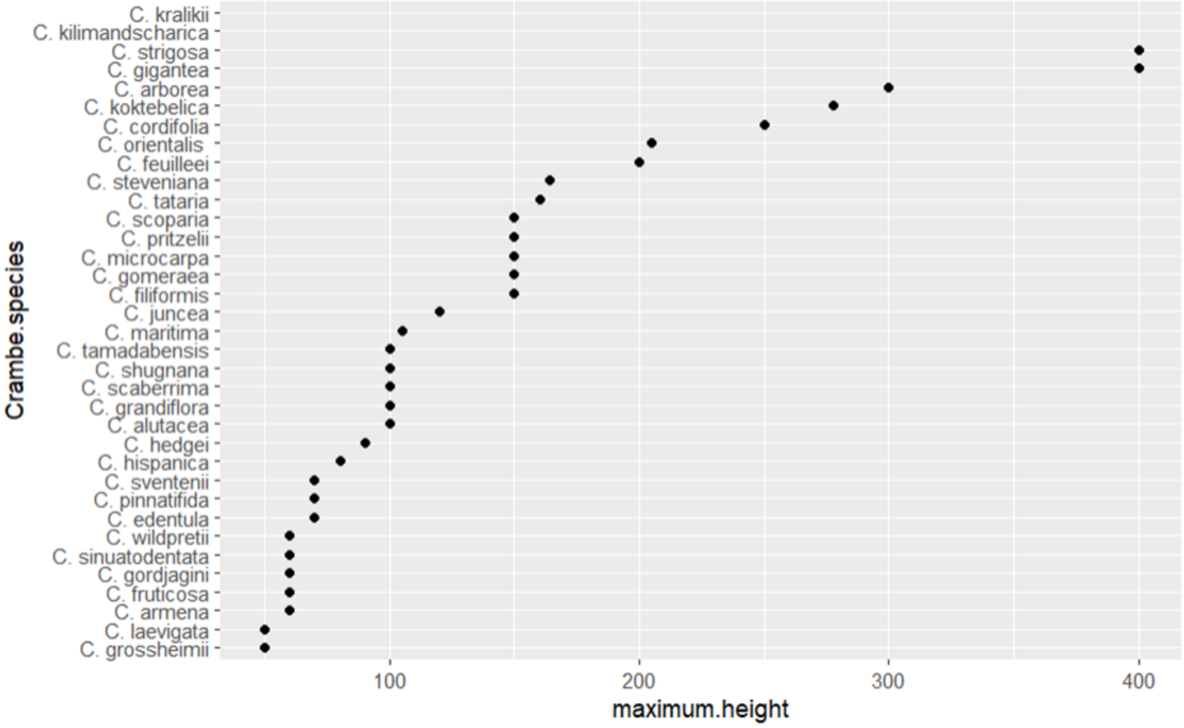
Willis, K. J., Bennett, K. D., & Birks, H. J. B. (2009). Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation. *Journal of Biogeography*, *36*(9), 1630-1644.

Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FitzJohn, R. G., ... & Beaulieu, J. M. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature*, *506*(7486), 89-92.

Zizka, A., Onstein, R. E., Rozzi, R., Weigelt, P., Kreft, H., Steinbauer, M. J., ... & Lens, F. (2022). The evolution of insular woodiness. *Proceedings of the National Academy of Sciences*, *119*(37), e2208629119.

Annex I Categories



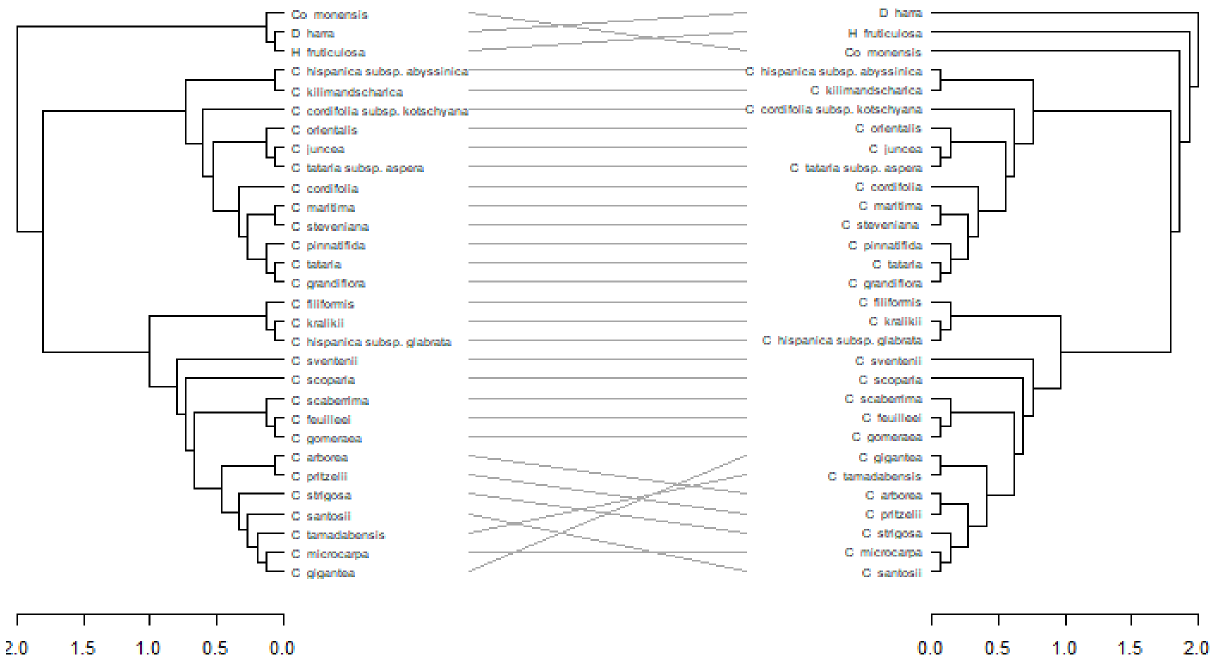


Annex II sequencing

Sample	**Species**	**DNA Conc. Extract1**	**DNA Conc. Final FA**	**A353 Genes Recovered**	**Genes w/Seqs A353**	**% onTarget A353**	**Genes at 75% A353**	**B764 Genes Recovered**	**Genes w/Seqs B764**	**% onTarget B764**	**Genes at 75% B764**
NB1	*Crambe grandiflora*	4.039	10.3	345	345	38.4	315	759	759	45.2	743
NB2	*Crambe filiformis*	6.4524	17.4	347	347	28.5	338	759	759	51.8	750
NB3	*Crambe steveniana*	1.6144	5.92	346	346	35.7	325	759	759	46.1	745
NB4	*Crambe pinnatifida*	28.3601	7.37	346	346	25.3	332	755	755	44.1	749
NB5	*Crambe abyssinica*	12.1573	13.6	347	347	31.7	331	757	757	40.9	749
NB6	*Crambe laeviata*	0.492	1.4	1	1	78.8	0	1	1	13.6	0
NB7	*Crambe cordifolia*	51.4812	5.03	347	19	28.8	69	759	759	46	745
NB8	*Crambe aspera*	1.1646	0.536	155	155	72.8	6	408	408	34	19
NB9	*Crambe tataria*	2.1175	8.36	343	343	52	303	752	752	46.1	726
NB10	*Crambe juncea*	2.9137	1.36	198	198	43.1	28	509	509	37.2	45
NB11	*Crambe hispanica* subsp. *glabrata*	17.0354	6.41	345	345	27.7	327	753	753	42.4	740
S0395	*Crambe hispanica*	NA	NA	320	320	41.7	132	681	681	27.4	328
S0460	*Crambe strigosa*	NA	NA	343	343	27.4	316	747	747	40.5	731
S0570	*Crambe pritzelii*	NA	NA	343	343	30.9	299	748	748	47.7	723
S0597	*Crambe gigantea*	NA	NA	231	231	47.7	19	504	504	38.8	36
S0617	*Hemicrambe fruticulosa*	NA	NA	178	178	23.1	39	750	750	47.6	740
S0661	*Crambe kotschyana*	NA	NA	344	344	20.1	330	741	741	29.8	725
S0698	*Crambe tamadabensis*	NA	NA	256	256	22.1	50	631	631	22.4	159
S0699	*Crambe sventenii*	NA	NA	324	324	35.1	279	749	749	37.4	695
S0702	*Crambe microcarpa*	NA	NA	271	271	39.3	133	706	706	12.7	395

S0714	*Crambe scoparia*	NA	NA	340	340	12.8	295	697	697	6.6	554
S0715	*Crambe scaberrima*	NA	NA	340	340	41	300	737	737	28.1	677
S0731	*Crambe maritima*	NA	NA	345	345	15.6	327	758	758	19.4	748
S0851	*Diplotaxis harra*	NA	NA	347	347	31	325	753	753	22.7	726
S0924	*Crambe kralikii*	NA	NA	344	344	31.9	264	744	744	28.4	674
S1149	*Crambe orientalis*	NA	NA	340	340	16.9	321	748	748	30	726
S1307	*Crambe gomeraea*	NA	NA	344	344	12	327	736	736	6.9	688
S1310	*Crambe kilimandscharica*	NA	NA	343	343	18.1	315	746	746	28.4	733
S1401	*Coincya monensis*	NA	NA	348	348	27.4	334	755	0	35.5	0
S1554	*Crambe arborea*	NA	NA	345	345	20.9	332	744	744	23.9	721
S1555	*Crambe feuilleei*	NA	NA	345	345	20.5	319	753	0	30.7	38
ODN163	*Crambe santosii*	NA	NA	344	344	28.2	312	694	694	6	534

Annex III Hispanica tanglegram



Annex IV ASR

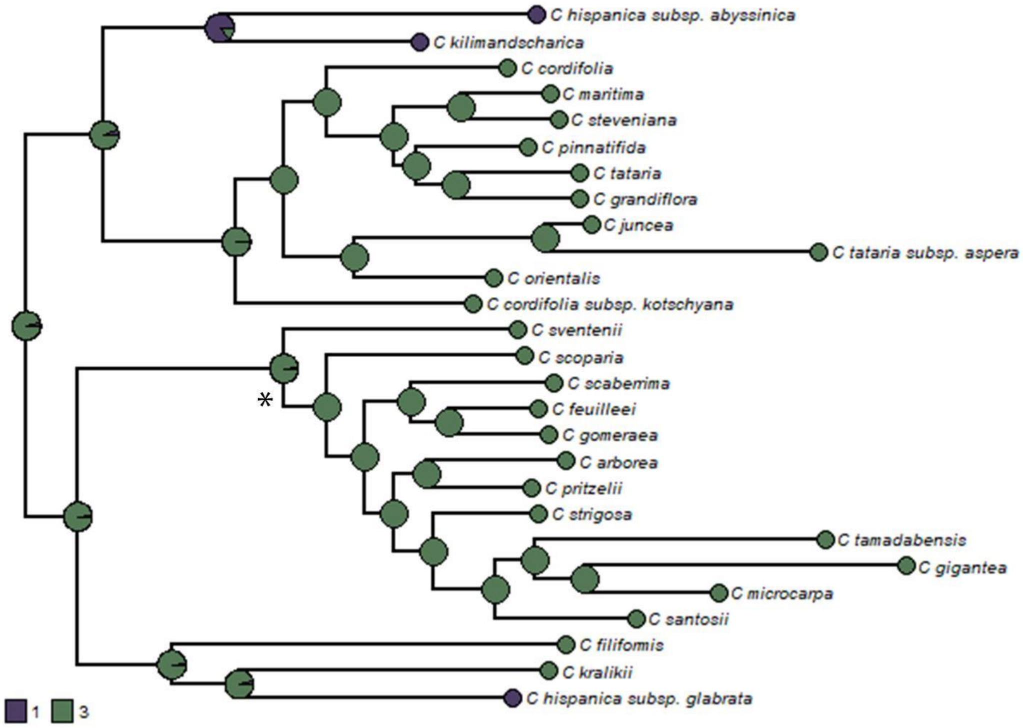


Figure: ancestral state reconstruction for the life cycle for the Crambe genus. 1: annual, 3: perennial. Uncertainty for the outgroups was added. The asterisk (*) indicates the crown node of the island clade

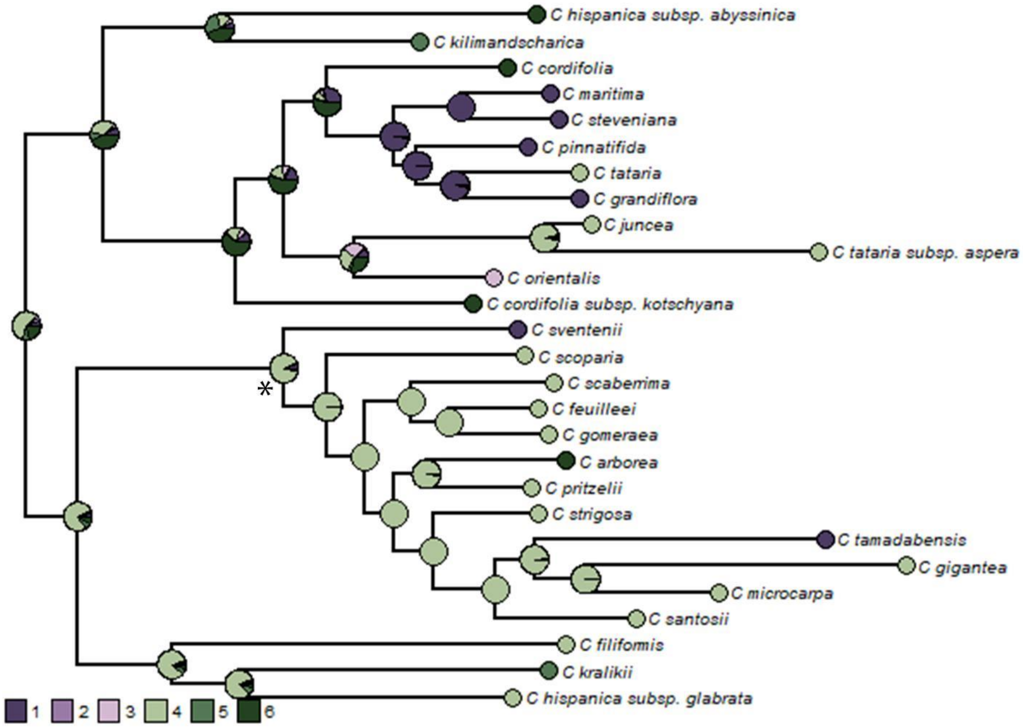


Figure: ancestral state reconstruction for vegetation zones of the Crambe genus. 1: low, 2:mid, 3:high, 4:low/mid, 5:mid/high, 6:full range. The asterisk (*) indicates the crown node of the island clade

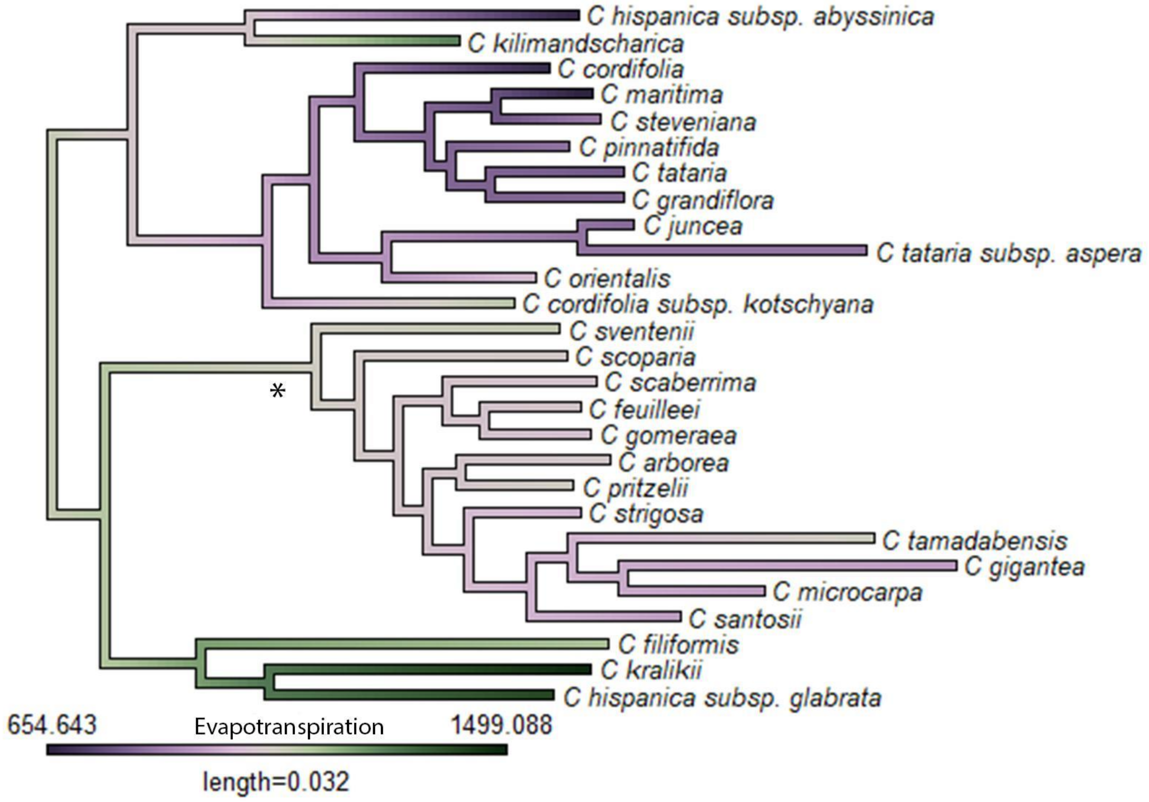
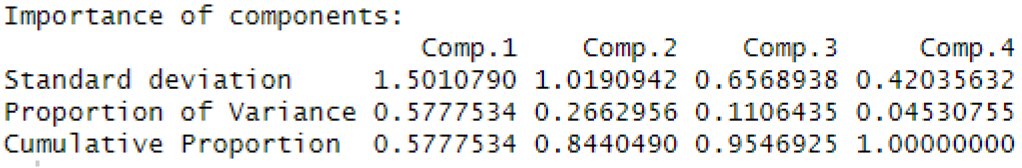


Figure: ancestral state reconstruction for evapotranspiration in mm/year for the Crambe genus. The asterisk (*) indicates the crown node of the island clade.

Annex V PCA



```
Importance of components:
                          Comp.1    Comp.2    Comp.3     Comp.4
Standard deviation     1.5010790 1.0190942 0.6568938 0.42035632
Proportion of Variance 0.5777534 0.2662956 0.1106435 0.04530755
Cumulative Proportion  0.5777534 0.8440490 0.9546925 1.00000000
```