# Genomic stability in the Galápagos *Scalesia* adaptive radiation: Consistent transposable element accumulation despite hybridization and ecological niche shifts

José Cerca[1,2], Patricia Jaramillo Díaz[3,4,5], Clément Goubert[6], Heidi Yang[7], Vanessa C. Bieker[1], Mario Fernández-Mazuecos[8,9], Pablo Vargas[10], Rowan Schley[11], Siyu Li[12], Juan Ernesto Guevara-Andino[13], Bent Petersen[14,15], Gitte Petersen[16], Neelima R. Sinha[12], Lene R. Nielsen[17], James H. Leebens-Mack[18], Gonzalo Rivas-Torres[19], Loren H. Rieseberg[20], Michael D. Martin[1]

1 - Department of Natural History, University Museum, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

2 - Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Oslo, Norway

3 . Estación Científica Charles Darwin, Fundación Charles Darwin, Santa Cruz, Galápagos, Ecuador.

4. Department of Botany and Plant Physiology, University of Málaga, Málaga, Spain.

5. IUCN SSC Galapagos Plant Specialist Group, Puerto Ayora, Galapagos 200102, Ecuador.

6. McGill Genome Centre, McGill University, Montreal H3A 0G1, QC, Canada

7. Department of Ecology & Evolutionary Biology, University of California, Los Angeles, Los Angeles, California, United States of America

8. Departamento de Biología (Botánica), Facultad de Ciencias, Universidad Autónoma de Madrid, Calle Darwin 2, 28049 Madrid, Spain

9. Centro de Investigación en Biodiversidad y Cambio Global, Universidad Autónoma de Madrid (CIBC-UAM), Calle Darwin 2, 28049 Madrid, Spain

10. Biodiversity and Conservation, Real Jardín Botánico, 28014 Madrid, Spain

11. Department of Geography, University of Exeter, Laver Building, North Park Road, Exeter, Devon, UK

12. Department of Plant Biology, University of California, Davis, Davis, CA, 95616, USA

13. Grupo de Investigación en Ecología y Evolución en los Trópicos-EETrop- Universidad de las Américas, Quito-Ecuador

14. Center for Evolutionary Hologenomics, Globe Institute, University of Copenhagen, DK-1353, Copenhagen, Denmark

15. Centre of Excellence for Omics-Driven Computational Biodiscovery (COMBio), Faculty of Applied Sciences, AIMST University, Kedah, Malaysia

16. Department of Ecology, Environment and Plant Sciences, Stockholm University, 106 91 Stockholm, Sweden
17. Department of Geosciences and Natural Resource Management, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark
18. Department of Plant Biology, University of Georgia, Athens, GA, 30602, USA
19. Colegio de Ciencias Biológicas y Ambientales, Galapagos Science Center, Universidad San Francisco de Quito USFQ, Quito 170901, Ecuador
20. Department of Botany and Biodiversity Research Centre, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada

**Abstract**

Transposable elements (TEs) have been hypothesized to play a pivotal role in driving diversification by facilitating the emergence of novel phenotypes and the accumulation of divergence between species. The rapid speciation and ecological diversification characteristic of adaptive radiations offer a unique opportunity to examine the link between TE accumulation and speciation, diversification and adaptation. Here, focusing on all 15 species of the genus *Scalesia* (Asteraceae), a radiation endemic to the Galápagos Islands, we test whether hybridization or shifts in ecological niche are associated with changes in TE accumulation in genomes. Our analyses reveal little to no variation in TE accumulation among *Scalesia* species nor its hybrid populations. Shifts in ecological niches, linked to climatic variation, did not result in discernible changes in TE accumulation, a surprising finding given the anticipated selective pressure imposed by aridity, a factor often linked to genome size reduction. We found no distinct patterns in the temporal accumulation of TEs, with the exception of a taxon (var. *atractyloides*) of *Scalesia atractyloides*. Our findings challenge the assertion that TEs have directly driven diversification, speciation and local adaptation. Instead, TEs may simply be 'along for the ride,' rather than actively contributing to plant diversification.

# Introduction

Transposable elements are DNA sequences capable of relocating within the genome (McClintock 1984). Through their mobility and ability to replicate, TEs are able to generate a diverse range of mutations encompassing changes in promoter gene sequences, frameshifts, intronic modifications and gene duplications within their hosts (Choi and Lee 2020; Kidwell and Lisch 1997; Bennetzen and Wang 2014; Bourque et al. 2018; Pimpinelli and Piacentini 2020; Oliver, McComb, and Greene 2013; Almeida et al. 2022). Some of these mutations have been linked to the evolution of novel phenotypes. For instance, the evolution of the melanic phenotype of the peppered moth (*Biston betularia*) showcases the potential functional consequences of TE insertions as the insertion of the 'carb-TE' in an intronic region of the *cortex* gene alters its expression, likely contributing to the melanic phenotype (Van't Hof et al. 2016).

Advances in genomic sequencing technology and processing algorithms have greatly facilitated the detection and characterization of TEs, particularly in non-model organisms with limited genomic resources (Clement Goubert et al. 2022). The evident adaptive potential of TEs in shaping phenotypes (Kratochwil et al. 2022; Conart et al. 2022; Bennetzen and Wang 2014), along with the evidence of substantial variation in TE diversity and accumulation across different reference genomes (e.g. (Cerca et al. 2021; Schley et al. 2022), has intensified interest in a potential relationship between TEs and diversification. One particularly intriguing hypothesis is that lineages with high speciation rates also exhibit elevated rates of TE accumulation on their genomes (Ricci et al. 2018; Pimpinelli and Piacentini 2020; Oliver and Greene 2009, 2012; Belyayev 2014; Ray et al. 2008; Falcon, Tanaka, and Rodriguez-Terrones 2023; Pace and Feschotte 2007; de Boer et al. 2007). However, a key limitation of these studies has been their reliance on a small number of genomes, often with limited taxonomic coverage while encompassing large clades, and primarily based on correlations. Consequently, there may be an overestimation of the role of TEs as major drivers of speciation and ecological diversification at macroevolutionary scales.

Adaptive radiations represent evolutionary experiments where extensive ecological variation aligns with rapid speciation, and they have been a cornerstone of our understanding of evolutionary change (Cerca et al. 2023; Gillespie et al. 2020; Schluter 2000). Adaptive radiations can also yield valuable insights into the interplay between TEs and evolutionary success; however, the evidence available is conflicting and largely originated from cichlid radiations. For example, a study examining four species within an African cichlid adaptive radiation found higher TE counts in the genomes and transcriptomes of the radiating species compared to non-radiating lineages (Brawand et al. 2014). More recent evidence, however, suggested a lack of correlation between species richness at the tribe level in the Lake Tanganyika adaptive radiation and TE content across 245 genomes (Ronco et al. 2021). Similarly, cichlid lineages in the Americas had no

discernible differences in TE content when contrasting a radiating lineage (*Amphilophus citrinellus*) with a non-radiating lineage (*Archocentrus centrarchus*; (Xiong et al. 2021). Notably, a golden phenotype in the cichlid genus *Amphilophus* is associated with a transposon-derived inverted repeat in the *goldentouch* gene (Kratochwil et al. 2022). This conflicting body of evidence underscores the necessity for further exploration of TEs as facilitators of diversifications, particularly extending beyond the scope of cichlid radiations.

The *Scalesia* radiation (Asteraceae, Asterales, Magnoliidae), endemic to the Galápagos archipelago, presents an excellent opportunity to investigate the potential relationship between TEs and diversification. This genus comprises ~15 species that evolved from a single common ancestor which diversified about 1 million years ago (Fernández-Mazuecos et al. 2020). The recent origin of this radiation may facilitate the reconstruction and tracking of TE accumulation over time. *Scalesia* exhibits remarkable ecological diversity, particularly in its occupation of various climate zones (*hereafter* referred to as climate niches) (Blaschke and Sanders 2009). The genus inhabits a wide range of ecological niches and soil types (Figure 1), from coastal or littoral zones characterized by saltwater habitats, aridity, low precipitation, elevated temperatures, and intense solar radiation, to more humid zones typically found at higher elevations, which experience increased rainfall throughout the year, lower temperatures, and frequent cloud cover. This ecological variation provides an opportunity to explore whether climatic niches exert selective pressures that influence TE dynamics, as observed in plants under arid conditions (Schley et al. 2022). Specifically, the accumulation of TEs can result in an increase in genome size, which is associated with larger stomatal cells with impaired gas exchange efficiency and poor water economy (Franks and Beerling 2009). Thus, natural selection may play a role in regulating genome size in species adapted to arid conditions, such as many *Scalesia* species. Furthermore, hybrid populations exist in the radiation (Fernández-Mazuecos et al. 2020), allowing testing whether hybridization triggers genome deregulation by inducing TE activity (L. Wang et al. 2023; Petrov et al. 1995; Serrato-Capuchina et al. 2020; Rubin, Kidwell, and Bingham 1982; Jiang et al. 2003; Parisod et al. 2010). Finally, the reference genome assembly for the group, *Scalesia atractyloides*, is composed of over 80% of repetitive content and TEs – making this lineage ideal to understand TE accumulation dynamics (Cerca et al. 2022).

Building on the premise that TEs play a role in diversification dynamics, here we test three hypotheses: *(H1)* Lineages undergoing rapid speciation and broadening of ecological variation exhibit an increased rate of lineage- or taxon-specific TE accumulation/expansion on their genomes; *(H2)* Shifts in climatic niches result in a change of selective regimes, with lineages in arid areas experiencing stronger selection for TE removal as a result of selection for smaller cells; and *(H3)*. Hybrids have an increased accumulation of TEs as a result of genome deregulation. To test these hypotheses, we employed whole-

genome resequencing of all 15 *Scalesia* species, four *Scalesia* hybrid populations and outgroup species, and contrasted genomic results with ecological data (Blaschke and Sanders 2009; Perez et al. 2023).

# Materials and Methods

## Study species and data collection

*Scalesia* (Asteraceae) is an endemic genus to the Galápagos Islands consisting of approximately 15 species derived from a single common ancestor. The divergence of *Scalesia* from members of a continental sister lineage, *Pappobolus*, occurred around 3 million years ago, while most diversification of the group occurred within the last million years (Fernández-Mazuecos et al. 2020). *Scalesia* occupies a wide array of ecological niches and soil types (Figure 1) (Blaschke and Sanders 2009), displaying clear environment-phenotype associations (Perez et al. 2023; Fernández-Mazuecos et al. 2020), as expected in an adaptive radiation (Schluter 2000). In this study, we sequenced samples representing the entire *Scalesia* genus, including: *S. affinis, S. aspera, S. atractyloides* (var. *atractyloides* and *darwinii*), *S. baurii* (subsp. *baurii* and *hopkinsii*), *S. cordata, S. crockeri, S. divisa, S. gordilloi, S. helleri, S. incisa, S. microcephala, S. pedunculata, S. retroflexa, S. stewartii,* and *S. villosa* (Supplementary Table 01). Additionally, we carried out sequencing of samples from populations that show evidence of extensive hybridization (Bieker, *in prep*). The hybridizing pairs include only pairs of sister species: *S. retroflexa* × *helleri*, *S. crockerii* × *aspera*, *S. incisa* × *divisa*, and *S. stewartii* × *atractyloides* (Supplementary Table 01). We incorporated individuals from the genus *Pappobolus* from the South American continent as the outgroup (sister group) in some analyses (Blaschke and Sanders 2009; Fernández-Mazuecos et al. 2020), including *P. ecuadoriensis*, *P. hypargyreus*, *P. juncosae*, *P. lehmanii*, and *P. nigrescens*. Detailed information and accession numbers regarding the sampling of outgroup species can be found in Fernández-Mazuecos et al. (2020).

### Data generation (library preparation and sequencing)

We extracted genomic DNA using the DNeasy 96 Plant Kit (Qiagen) following the manufacturer's protocol with minor modifications. These modifications consisted of grinding dried leaf tissues in a Qiagen collection microtube with a 3-mm tungsten carbide bead and repeating the membrane washing step with an additional 800 µl of buffer AW2 to remove residual greenish color from the samples. We eluted the DNA from the membranes using 50 µl of buffer AE to obtain a high-concentration DNA yield. The DNA extracts were then sent to the commercial provider Novogene UK for shearing to a mean insert size of 350

153 bp, double-stranded DNA genomic library preparation, indexing and amplification via PCR, and 150-bp
154 paired-end sequencing on the Illumina NovaSeq 6000 platform. The average sequencing depth was ~6x
155 (low-coverage). Three samples used in this study were obtained from herbarium specimens and were
156 prepared for genomic sequencing independently. Leaf tissue was disrupted using a Tissue Lyser II
157 (Quiagen), followed by DNA extraction using the DNeasy Plant Mini Kit following the manufacturer's
158 protocol except for an additional overnight incubation step with 20 µL of proteinase K after cell lysis as
159 described in (Martin et al. 2014). DNA was eluted using 62 µL buffer AE. Extracts were then sent to the
160 commercial provider Novogene for double-stranded DNA genomic library preparation, indexing and
161 amplification via PCR, and 150-bp paired-end sequencing on the Illumina NovaSeq 6000 platform. The
162 raw sequencing data generated for this study can be found at the European Nucleotide Archive under
163 accession code PRJEB70770.

## 164 Low-coverage phylogenomics

165 We started by conducting a distance-based phylogenetic reconstruction, accounting for low-
166 coverage sequencing, to investigate the evolutionary history of the *Scalesia* genus and to establish a
167 backbone for subsequent analyses and interpretations. We performed an additional phylogenetic
168 reconstruction, including hybrid populations to confirm the phylogenetic placement of these hybrids. We
169 started by quality-filtering the raw Illumina data by identifying and removing adapters and low-quality
170 basepairs using *AdapterRemoval v2.3.2* (Schubert, Lindgreen, and Orlando 2016). Subsequently, we used
171 *Skmer v3.2.1*, a pipeline designed to estimate genetic distances between genomes through a *k*-mer-based
172 approach. *Skmer* offers a robust approach for phylogenetic reconstruction without requiring an assembly
173 and accounts for low-coverage of the samples (Marçais and Kingsford 2011; Ondov et al. 2016; Sarmashghi
174 et al. 2019; Rachtman et al. 2022). Running *Skmer* involved running the module 'skmer reference' to
175 process the adapter-trimmed sequencing data, followed by 'skmer subsample' to create 100 subsamples of
176 the libraries, and 'skmer correct' to correct the distance matrices based on these subsample replicates. To
177 construct a distance-based phylogenetic tree we employed *fastme v2.1.5* (Lefort, Desper, and Gascuel 2015).

## 178 TE library construction

179 We downloaded the reference genome of the sunflower (*Helianthus annuus*) from its official
180 website (https://www.sunflowergenome.org/; version Ha412HOv2.0-20181130), and used it to generate a
181 sunflower-based TE and repeat library using *Repeat Modeler v2.0.2* (Flynn et al. 2020). Sunflower was
182 selected as the reference species because it has a high-quality reference genome, and it is equally evolutionary

183 divergent from *Scalesia* and *Pappobolus*. Building a TE and repeat library based on *Scalesia* alone could
184 introduce biases when conducting comparisons with the outgroup taxa (but see below). We concatenated
185 the sunflower-based library with Repbase (2017), and used the concatenated library as the base library for
186 subsequent TE detections using *dnaPipeTE*.

187 We used *dnaPipeTE* (Clément Goubert et al. 2015) to extract and characterize TEs and repeat
188 sequences. *dnaPipeTE* leverages the RNA-seq assembly pipeline Trinity to extract, quantify and assemble
189 sequences, including TEs and other repeats. While the primary objective of *dnaPipeTE* is to construct
190 complete TE and repeat sequences, it also detects and quantifies partially assembled TE and repeats (which
191 suffice for estimating the relative abundances on which we rely in this study). Upon the completion of the
192 assembly and collection process, *dnaPipeTE* categorises TEs and repeats into different classes based on their
193 homology with the TE and repeat library. In the established 'concatenated library', TE classes included
194 DNA, Helitron, Long interspersed nuclear element (LINE), Long terminal reads (LTR), rRNA, and Short
195 interspersed nuclear elements (SINE). Leveraging *RepeatMasker*, *dnaPipeTE* identifies low-complexity
196 sequences, simple repeats, and satellites, guided by a specific coverage threshold. *dnaPipeTE* has been
197 successfully applied to a diverse array of species, including arthropods, mollusks, and vertebrates (reviewed
198 in (Clément Goubert 2023)), even revealing substantial variation in TE and repeat content at the
199 population-level (e.g. (Lerat et al. 2019)). We ran *dnaPipeTE* on single-end forward reads for each
200 individual separately, downscaling the data to 0.1 coverage, specifying a genome size of 3.9 Gbp (Cerca et al.
201 2022), two Trinity assembly iterations, and a minimal query percentage of 0.2 (Clément Goubert et al.
202 2015).

## 203 Exploration of patterns

204 We extracted three outputs from *dnaPipeTE*: 1) counts of TEs and repeats, which allowed a direct
205 comparison of the number of TEs and repeats accumulated across species; 2) repeat landscape plots from all
206 resequenced samples, which offered insights into the accumulation of TEs through time (to complement
207 this data, we also ran a repeat accumulation analysis in the chromosome-resolved *Scalesia atractyloides*
208 genome assembly (Cerca et al. 2022) using RepeatMasker; and 3) fasta files containing TE and repeat
209 sequences. Using the sequences in a fasta format, we used *OrthoFinder* to group TEs and repeats based on
210 sequence similarity (Emms and Kelly 2015, 2019), and we examined the number of TE/repeat orthogroups
211 shared between the outgroup species and *Scalesia* using *UpSet plots* and *Venn diagrams*. To test for
212 differences between groups, we performed a Mann–Whitney U test comparing hybrids *vs* non-hybrids, and

humid (*S. cordata, S. microcephala, S. pedunculata*) *vs* arid (*S. villosa, S. stewartii, S. atractyloides, S. crockeri, S. aspera, S. retroflexa, S. helleri, S. gordilloi, S. incisa, S. divisa*).

We considered the possibility that the limited variation in TE and repeat content observed in some of our results was due to the use of a sunflower-based library, which might be too divergent from *Scalesia* and *Pappobolus*. To address this concern, we repeated all the aforementioned analyses using a *Scalesia*-specific library instead of the sunflower library. To obtain the *Scalesia*-specific library, we ran *RepeatModeler* on the *Scalesia atractyloides* genome (Cerca et al. 2022) and repeated all steps above (i.e. concatenating the output from *RepeatModeler* with Repbase, running *DNApipeTE* analyses using the same parameters, and running analyses involving the exploration of TE and repeats). Analyses of the *Scalesia*-specific library found similar patterns to the main results (Supplementary Figure 01).

# Results

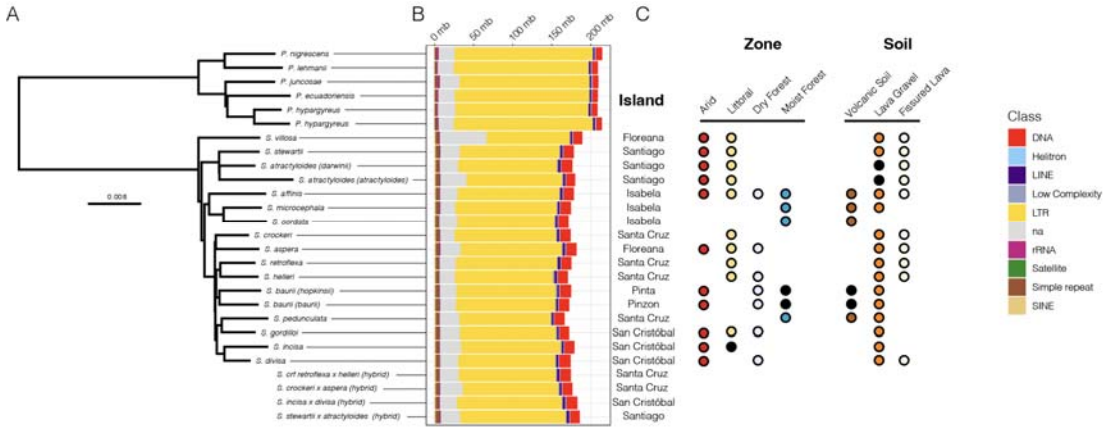


**Figure 01** - Phylogenetic relationships and transposable element (TE) variation in the *Scalesia* radiation and the outgroup (genus *Pappobolus*). a) Neighbor-joining phylogeny of *Scalesia* and the outgroup. Hybrid samples are shown below and were not included in the phylogenetic reconstruction. b) Stacked bar plot illustrating TE and repeat groups (for the analysis displayed here we used the sunflower library; the analysis using a *Scalesia*-based library is found in Supplementary Figure 01). Color-coding of the different repeat and TE groups is found in the rightmost part of the figure, where 'na' denotes unidentified TEs. c) Labelling of the different islands, environments, and soils occupied by different *Scalesia* species (for hybrids we include only island labelling). Environment and soil labelling follow (Blaschke and Sanders 2009), and black dots indicate uncertainty in environmental characterization.

The phylogenetic tree is characterized by mostly short internal branches in *Scalesia*, as expected in a rapid radiation. *Scalesia villosa* is positioned as the sister species to all the remaining *Scalesia* species (Figure 01A; Supplementary Figure 02). *S. stewartii* and *S. atractyloides* form a clade which is sister to the remaining 12 species. *S. affinis*, *S. microcephala*, and *S. cordata* are grouped together as a clade being sister to a clade including nine *Scalesia* species, namely *S. crockeri*, *S. aspera*, *S. retroflexa*, *S. helleri*, *S. baurii*, *S. pedunculata*, *S. gordilloi*, *S. incisa,* and *S. divisa* (Figure 01A; Supplementary Figure 02). The phylogenetic analysis including hybrid populations placed all hybrids as sister to one of the parental species (Supplementary Figure 03).

We observed little variation in TE counts among different species, both in relative and absolute terms (Figure 01B; Supplementary Table 02). The outgroup lineages displayed a higher count of TEs and repeats, with an average of 323,234,351 bp (max 328,160,920 bp, min 318,480,284 bp, standard deviation 3,752,276 bp). *Scalesia* species (excluding hybrids) exhibited a lower TE and repeat content, with an average of 266,935,585 bp (max 271,943,828 bp, min 259,698,199 bp, standard deviation 3,610,348 bp). Hybrid *Scalesia* populations displayed a TE and repeat content averaging 269,790,871 bp (max 274,454,284 bp, min 265,429,355 bp, standard deviation 4,796,678 bp; Figure 1B). Both Mann-Whitney U tests were non-significant (humid *vs* arid $P = 0.1264$; hybrid *vs* non-hybrid $P = 0.8972$)

Long terminal repeat (LTR) retrotransposons were the largest class of TEs found in the data. *Scalesia* species had an average of 129,559,033 bp of their genomes composed of LTRs (max 130,687,970 bp, min 106,894,927 bp, standard deviation 5,858,406 bp). The outgroup species had a mean of 172,863,512 bp of their genomes composed of LTRs (max 178,110,682 bp, min 166,411,171 bp, standard deviation 4,342,796 bp), while hybrid *Scalesia* populations had 129,559,033 bp of LTRs (max 135,389,443 bp, min 122,925,507 bp, standard deviation 6,440,151 bp). In the second largest class of TEs, DNA elements, *Scalesia* had an average of 13,256,247 bp composed of DNA elements (max 14,561,033 bp, min 11,743,596 bp, standard deviation 783,002 bp), while hybrid populations had 13,385,339 bp (max 14,377,105 bp, min 12,542,486 bp, standard deviation 853,847 bp). In contrast, the outgroup exhibited fewer DNA elements, with an average of 7,703,936 bp (max 8,258,881 bp, min 7,308,486 bp, standard deviation 348,650 bp).

**Repeat landscape plots**

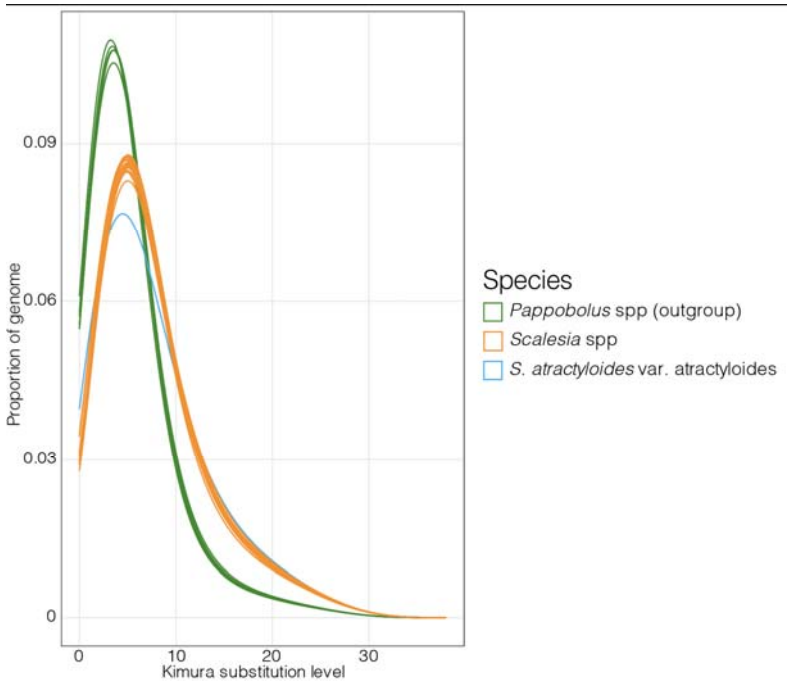


**Figure 02** - Repeat/transposable element landscape plot. The *x-axis* shows the sequence divergence (Kimura substitution level), an indirect measure of TE divergence/age, the *y-axis* shows the portion of the genome covered by TEs and repeats. Assuming a constant mutation rate, higher Kimura substitution levels indicate older TE ages. The outgroup (genus *Pappobolus*) is plotted in green, and all *Scalesia* species in orange with the exception of *S. atractyloides* var. *atractyloides* in blue since it has a distinct repeat accumulation pattern.

The repeat landscape plot shows that all *Scalesia* species exhibit a similar pattern of TE and repeat accumulation, with the exception of *S. atractyloides* var. *atractyloides* (Figure 02; Supplementary Figure 04). All outgroup species have a similar accumulation of TEs, which is distinct from that of *Scalesia*. Specifically, there is a younger accumulation of TEs in the outgroup relative to *Scalesia* (Figure 02; Supplementary Figure 04). In all the landscape plots, the TE/repeat accumulation peak of *Scalesia* is at a Kimura substitution level of approximately 7, while the genome assembly of *S. atractyloides* is around 12 (Supplementary Figure 05). The repeat landscape plots decomposed in different TE and repeat classes show that the accumulation of TEs and repeats does not differ when comparing the outgroup (Supplementary Figure 06), *Scalesia* (Supplementary Figure 07-09), and *Scalesia* hybrids (Supplementary Figure 10).

**Organization of genome-specific elements**

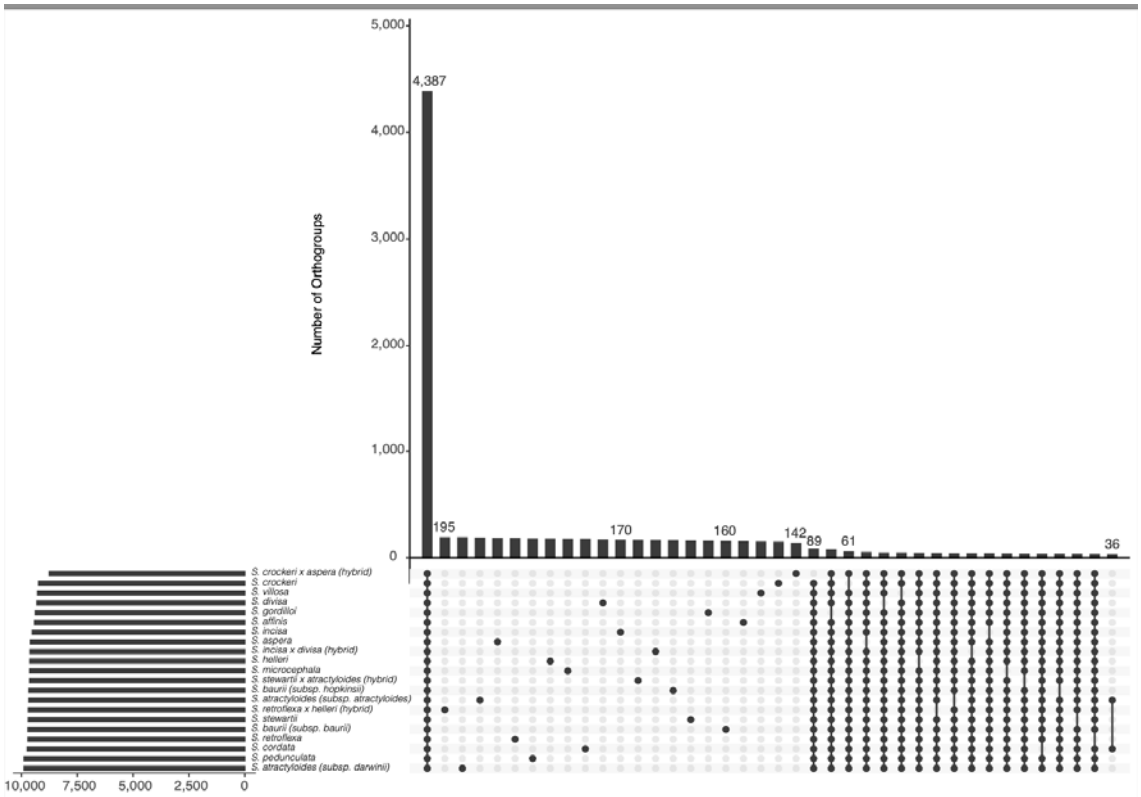


**Figure 03** - UpSet plot of long terminal repeat elements (LTR) identified within the *Scalesia* radiation (other TE and repeat groups are found in Supplementary Figures 11-15). The *UpSet* plot is an alternative visualisation to a Venn diagram where the y-axis on the top graphic shows a frequency of LTR orthogroups (groups of closely related LTRs) and the x-axis shows different combinations of the data (the first column includes orthogroups present in all *Scalesia*, the second column are orthogroups exclusive to S. *retroflexa x helleri* hybrids, the third column are orthogroups exclusive to *S. atractyloides* var. *atractyloides*). Dark grey circles highlight species combinations.

Using *OrthoFinder*, we grouped TEs and repeats into orthogroups, sets of orthologous sequences derived from the last common ancestor of all species under consideration (Figure 02; Supplementary Figures 11-15). In almost all the comparisons, we found that the majority of TE orthogroups are present in all *Scalesia*. The sole exception was in the satellite elements, for which we detected very few orthologs. In the largest class, LTR, we found 4,367 orthogroups present in all *Scalesia* genomes, while only 142-195 orthogroups were found to be unique to a single genome (Figure 03).

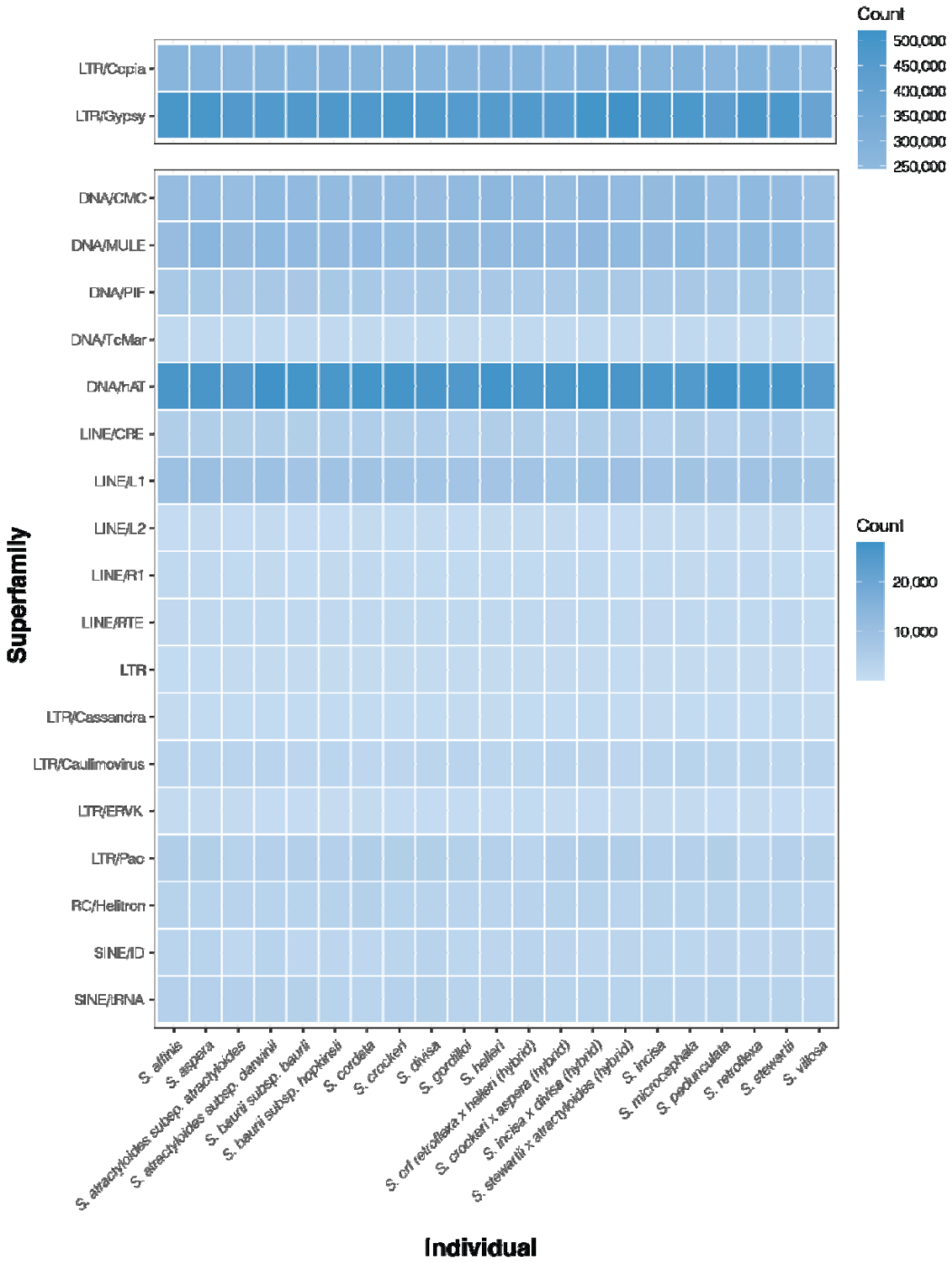


**Figure 04** - TE superfamily counts for different *Scalesia* species. The count scale is different for different TE super families, namely *LTR/Copia* and *LTR/Gypsy*, due to the large numbers of these elements.

Superfamily-level analyses show that TE figures were remarkably even in *Scalesia* (Figure 04) and the outgroup (Supplementary Table 16). With the exception of LTR/Gypsy and LTR/Copia, all TE

counts were below 30,000 elements and were remarkably even across *Scalesia* species. LTR/Gypsy and LTR/Copia included 250,000-500,000 elements (Figure 04).

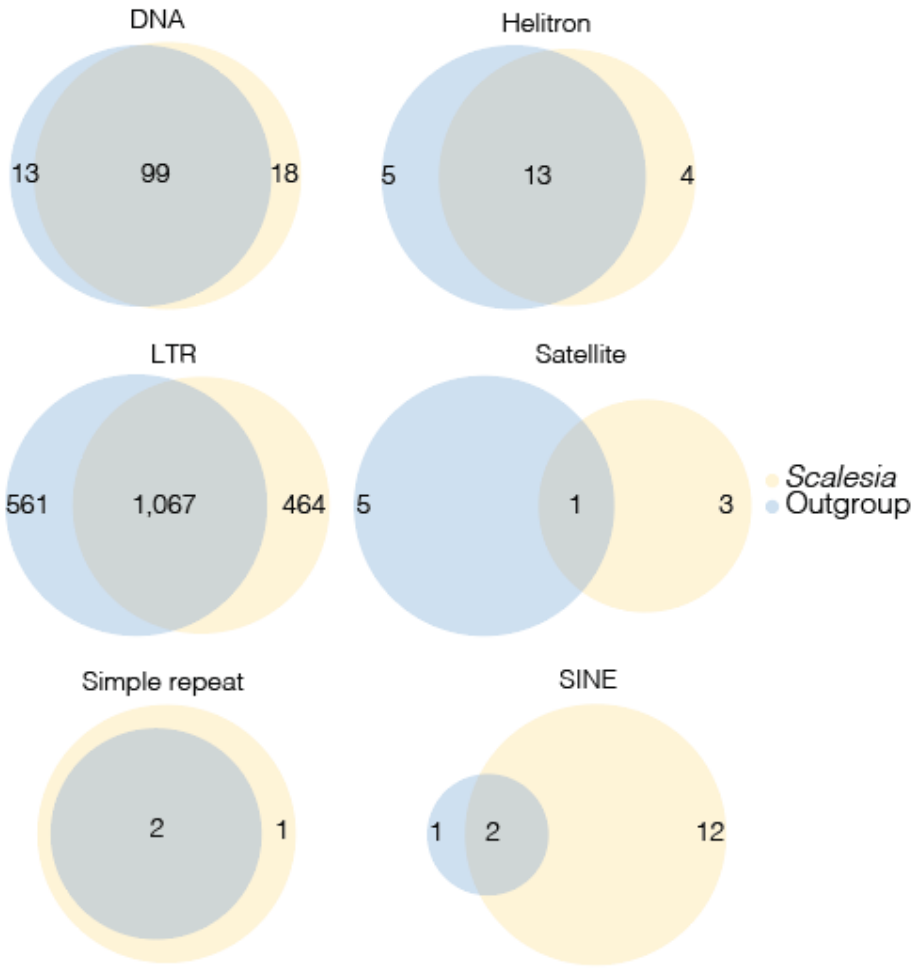


**Figure 05** - Representation of orthogroups present in *Scalesia* (yellow) or the outgroup (blue). Shared orthogroups are given as overlap for different TEs and repeats.

We used *OrthoFinder* to identify orthogroups of TEs and repeats in five randomly chosen *Scalesia* species and five outgroup *Pappobolus* species, and parsed the results to compare counts of *Scalesia*-specific orthogroups, outgroup-specific orthogroups, and orthogroups shared between the two genera (Figure 05). We chose to downscale the data to five random species because we observed imbalances in estimations that arose from analysing the 15 *Scalesia* species in comparison to only five outgroup species (two specimens of *P. hypargyreus* were included;Figure 05). Since we downscaled the data to only five *Scalesia* species, we repeated the analyses three times, finding that the results were consistent across the three iterations

(Supplementary Table 03). The largest three classes of TEs (LTR, DNA and Helitron) had a majority of its orthogroups common to *Scalesia* and the outgroup. *Scalesia* had more private SINEs than the outgroup (Figure 05).

# Discussion

Transposable elements (TEs) play a crucial role in shaping genomes and phenotype diversity. Here, we tested whether rapid diversification, shifts in climatic niches or hybridization are associated with an increase of TE accumulation in the genomes of the genus *Scalesia*, a radiation endemic to the Galápagos (Figure 01). Our results show that TE or repeat accumulation is consistent across the *Scalesia* phylogeny, with no major changes across species (Figure 01) or over time (with one exception; Figure 02). We also found no evidence of lineage-specific TE or repeat expansions, as most TE groups were found across all species (Figure 03), in similar numbers across the explored genomes (Figure 04), and shared with the outgroup (Figure 05). Overall, our findings indicate that neither rapid diversification, hybridization, nor ecological niche shifts are significantly associated with TE and repeat content variation within the *Scalesia* adaptive radiation.

**Limited variation in transposable element content in *Scalesia* despite rapid diversification**

We found no major differences in TE or repeat content in different *Scalesia* species in any of the analyses performed and focusing on phylogenetic comparisons, accumulation over evolutionary time, and class-specific expansions (Figures 01-5). The lack of variation may be attributable to the methods used, but this seems unlikely since *dnaPipeTE* retrieved different results for *Pappobolus* and *Scalesia*, suggesting that the two lineages have accumulated different TEs and repeats after their split, about 3 million years ago (Fernández-Mazuecos et al. 2020). *dnaPipeTE* has been shown to detect population-level differences (Kapun et al. 2020; Lerat et al. 2019; Blommaert et al. 2019; Vargas-Chavez et al. 2022), and between closely related species across the Tree of Life (Talla et al. 2017; Jaron et al. 2021; Wong et al. 2019; Hill and Betancourt 2018; Parisot et al. 2021; Lata et al. 2022; Castro et al. 2020). One tempting explanation for the lack of variation is that *dnaPipeTE* may be biased to find conserved old elements, thereby explaining the observed patterns. However, this is likely not the case as *dnaPipeTE* is very sensitive in detecting recent TE families, being less sensitive to older TE families due to increased divergence and short reads that limits alignments. For instance, when applied to the human genome, *dnaPipeTE* is biased towards more recent TEs (see supplementary data included in (Clément Goubert et al. 2015). As a result, we exclude method-

350 orient bias, and we suggest that TEs are under phylogenetic inertia, as shown for various *Drosophila* species
351 (Sessegolo, Burlet, and Haudry 2016). Our findings suggest that the fundamental transposable element
352 composition remains conserved across *Scalesia* lineages.

353 The lack of variation TE content in *Scalesia* is noteworthy. In our dataset, we observe a variation of
354 7.23 % in LTRs, and 0.28 % of variation in DNA elements. This variation is very little when compared to
355 other works focusing on congeneric species. For instance, in the plant genus *Passifora*, Tekay LTR-elements
356 varied between 8.52 - 35.71 % and Angela LTR-elements between 1.96 - 29.05% (absolute estimates
357 considering the whole genome; (Sader et al. 2021). In oaks (*Quercus*) LTRs varied between 34.07 - 55.05 %
358 (relative TE content; (Mascagni et al. 2019). In *Rhodnius* bugs, with different ecologies, Tc1/Mariner
359 elements varied between 14.3 - 25 % (Castro et al. 2020), while in *Diabrotica* beetles MITE elements varied
360 between 150 - 610 MegaBasepairs (Lata et al. 2022). In this regard, the variation in *Scalesia* is diminished
361 and noteworthy.

362 One potential explanation for the observed lack of species-specific TEs is the high repeat content of
363 the *Scalesia* genome. Our previous analyses revealed that approximately 80% of the *Scalesia atractyloides*
364 genome is composed of repetitive sequences (Cerca et al. 2022). This high repeat content could be due to
365 the presence of numerous repeat families that actively replicate with a strong phylogenetic signal, rather
366 than a dynamic turnover of species-specific repeat families with a low copy number. In this regard, Novák
367 and colleagues (2020) found that TE content reaches a plateau at around 80% across angiosperms with
368 genome sizes between 5 and 10 Gbp (Novák et al. 2020). It is possible that a plateau of TE content could be
369 maintained, with few families represented at high numbers, and that a dynamic turnover occurs within
370 families, rather than between TE families.

371 Our findings challenge the notion that lineages with high speciation rates also display elevated rates
372 of TE content accumulation. One caveat to this interpretation is that the sister lineage to *Scalesia*, including
373 *Pappobolus*, may have undergone similar diversification on the mainland (Fernández-Mazuecos et al. 2020).
374 Accordingly, we hypothesize that the entire *Pappobolus-Scalesia* clade possesses ancestrally high TE content
375 . However, recent TE activity is observed as in both lineages most TEs are relatively young (Figure 02). In
376 any case, the role of TEs in adaptive radiation remains debated, with some studies demonstrating their
377 importance as drivers of radiation (Feiner 2016; Carleton et al. 2020), while others proposing a less
378 significant role (Xiong et al. 2021; Ronco et al. 2021). This discrepancy could stem from the scale and data
379 employed. Specifically, the correlation between TE content and diversification based on limited data may
380 have led to an overestimation of TEs' role. For instance, an initial analysis of four transcriptomes and
381 genomes of East African cichlids, compared to tilapia and other teleosts, suggested a higher accumulation of

TE counts during the radiation (Brawand et al. 2014). In contrast, recent evidence involving analyses of hundreds of genomes found no relationship between species diversity and TEs within different tribes of the Tanganyika radiation (Ronco et al. 2021). However, this does not negate the compelling experimental evidence supporting the role of TEs in driving the evolution of specific phenotypic change, such as the elegant findings of Kratochwil and colleagues demonstrating that a TE insertion triggered the emergence of a novel phenotype, the cichlid gold morph (Kratochwil et al. 2022). Two conclusions can be drawn from our results and the literature. First, it is necessary to conduct additional studies that functionally validate the evidence and the mechanisms underlying TE insertions and their impact on phenotypic evolution. Second, caution should be taken with potential overestimation of the role of TEs as major driving forces in speciation and ecological diversification at the macroevolutionary scale in all cases (e.g. (Ricci et al. 2018; Pace and Feschotte 2007; Ray et al. 2008; Pritham and Feschotte 2007; de Boer et al. 2007; Falcon, Tanaka, and Rodriguez-Terrones 2023; Carducci et al. 2019; Belyayev 2014; Oliver and Greene 2009, 2012; Alioto et al. 2020)). This is particularly evident given the limitations of existing studies, which often rely on limited genomic data, correlational analyses, and a narrow taxonomic scope. Some of these correlations encompassing macroevolutionary scales might have overstated their impact on adaptive evolution, speciation, and their direct connection to evolutionary success. Indeed, it can be argued that TEs are unlikely to enhance diversification unless genetic variation is limiting, such as in a bottleneck – as expected in lineages such as *Scalesia*. While TEs offer a convenient explanation for rapid evolution, it is likely that they are merely "along for the ride" and may only be purged when they reach problematic levels of replication.

**TEs and ecological variation**

The minimal variation in TE accumulation observed in *Scalesia* suggests that the climatic niche does not significantly influence TE content within the radiation. In adaptive radiations, closely related species typically occupy distinct ecological niches (Gillespie et al. 2020; Schluter 2000; Arnegard et al. 2014). In *Scalesia*, species exhibit distinct climatic niches (Figure 01) (Blaschke and Sanders 2009; Fernández-Mazuecos et al. 2020; Perez et al. 2023), with temperature and the presence of arid environments being key factors shaping the evolution of the radiation (Fernández-Mazuecos et al. 2020; Cerca et al. 2022; Perez et al. 2023). Various studies have demonstrated that aridity exerts a significant selective pressure on plant genome size (e.g. (Doyle and Coate 2019; Schley et al. 2022). In arid regions, many plant species face strong selective pressures to maintain smaller genome sizes (Doyle and Coate 2019), which correlate with smaller cell size (Beaulieu et al. 2008). Smaller cells improve water economy through smaller stomata that respond more

414 efficiently to water stress, enhance gas exchange regulation, and optimize photosynthetic performance
415 (Franks and Beerling 2009; Lawson and Blatt 2014; Roddy et al. 2020). Given that arid environments favor
416 smaller cells, aridity is hypothesized to be an important selective pressure regulating genome size (Schley et
417 al. 2022). Therefore, it would be expected that TEs are being purged in species occupying arid coastal areas
418 (e. g. *S. affinis*), where temperatures frequently reach 40°C throughout the year and water stress is pervasive,
419 compared to species found in the humid highlands (e.g., *S. pedunculata*), where conditions are consistently
420 moist and cooler. This leads to an alternative hypothesis: perhaps selection is unable to effectively remove
421 transposable elements. The removal of a single TE, only a few kilobases in size, might be 'invisible' in a
422 genome of 4 gigabases (X. Wang et al. 2021).
423 Interestingly, the inclusion of an infraspecific taxon in the study helped observe a distinct pattern at
424 the microevolutionary scale, as *S. atractyloides* var. *atractyloides* exhibits a lower transposable element
425 content (Figure 02). This observation aligns with an emerging evolutionary change occurring in the arid
426 volcanic regions of Santiago Island, where a shift in leaf pubescence has also been observed (Wiggins and
427 Porter 1971)
428
429 **TEs and hybridization**
430 The four hybrids examined in this study exhibited similar TE content to non-hybrid populations,
431 excluding the possibility of genome deregulation through TE-release as a result of hybridization.
432 Traditionally, hybridization has been perceived as a detrimental force to the integrity of species (Mayr
433 1940), and it has been proposed that hybridization may lead to genome destabilisation by promoting TE
434 activity (Zhang et al. 2018; McClintock 1984). The presence of more TEs in hybrid populations would be
435 expected in a scenario involving genome shock triggered by multi-generational hybridization. In such cases,
436 increased TE transcription should lead to more TEs insertions in the hybrid genomes, and a consequent
437 increase of genome size. However, the case in *Scalesia*, as evidenced by the absence of supporting patterns in
438 the repeat accumulation curves (Figure 02), the lack of superfamily expansion or super-family level (Figure
439 03), and absolute accumulation (Figure 01). These findings are in agreement with those of (Kawakami et al.
440 2011), who demonstrated no evidence of LTR retrotransposon proliferation when comparing multiple
441 hybrid populations of *Helianthus annuus* (sunflower) × *H. petiolaris.* This result suggests that post-
442 transcriptional repression mechanisms may prevent the proliferation of LTRs (Kawakami et al. 2011). A
443 similar scenario may be at play in *Scalesia*, but additional data would be required to confirm this hypothesis.

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