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ARTICLE in BOTANICAL JOURNAL OF THE LINNEAN SOCIETY · OCTOBER 2015
Impact Factor: 2.53 · DOI: 10.1111/boj.12334

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From the Neotropics to the Namib: evidence for rapid ecological divergence following extreme long-distance dispersal

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Received 26 January 2015; revised 29 July 2015; accepted for publication 5 August 2015

Extreme long-distance dispersal is an important process in plant biogeography. Such events can lead to rapid diversification due to founder effects, genetic drift and novel selection in recipient environments. Balloon vines (Cardiospermum spp.) are mainly Neotropical, but include two native southern African species, the endemic desert-adapted C. pechuelii and the moist subtropical C. corindum (which also occurs in the Neotropics). We used phylogenetic approaches (internal transcribed spacer (ITS), rpl32 and trnL-trnF DNA sequencing data) and population genetics (amplified fragment length polymorphism (AFLP) analyses) to confirm the long-distance dispersal of C. corindum to southern Africa and to reveal the subsequent divergence of the morphologically and ecologically extreme but genetically close C. pechuelii. We could not judge whether incongruences between ecological requirements and morphology and gene trees for the African species resulted from ongoing gene flow or incomplete lineage sorting, but our findings do support recent divergence of C. pechuelii from C. corindum in Africa following transoceanic dispersal of the lineage. © 2015 The Linnean Society of London, Botanical Journal of the Linnean Society, 2015, ••–••.

ADDITIONAL KEYWORDS: balloon vine – Cardiospermum – endemic – speciation.

INTRODUCTION

Extreme long-distance dispersal is more prevalent in plants than previously thought, with numerous recently described examples of distant transoceanic exchanges (e.g. Givnish et al., 2004; Renner, 2004; Tremetsberger et al., 2005; Nettel & Dodd, 2007; Christenhusz & Chase, 2013; Takayama et al., 2013; Le Roux et al., 2014). Such dispersal events likely result in reduced genetic variation due to strong founder effects (Austerlitz et al., 1997) and strong local selection in novel environments (Carson & Templeton, 1984; Templeton, 2008). Radiations following single long-distance colonization events have been well-documented on islands (e.g. Wagner & Funk, 1995; Baldwin & Sanderson, 1998; Carlquist, Baldwin & Carr, 2003; Price & Wagner, 2004; Knope et al., 2012; García-Verdugo & Fay, 2014, and references therein), often leading to the evolution of floras that are distinct from those found in the areas from which they were derived (Whittaker, Jones & Partomihardjo, 1997). Here we investigate the possibility of rapid divergence in Cardiospermum L. (Sapindaceae) following extreme long-distance dispersal between the South American and African continents.

The genus Cardiospermum (17 species), commonly known as balloon vines, are mostly restricted to the Neotropics with a few exceptions (Gildenhuys et al., 2013). Numerous species have also been introduced globally to areas where they are now considered invasive (Gildenhuys et al., 2013, 2014). For example, in
southern Africa *C. grandiflorum* Sw. and *C. hali-cacabum* L. are thought to be introduced from South America and invasive, whereas *C. corindum* L. is regarded as native to both southern Africa and South America (Gildenhuys et al., 2014). A phylogenetic treatment of the group suggests that the latter represents an extreme long-distance dispersal event from South America to southern Africa, which occurred between 5.9 and 15.1 Ma (Gildenhuys et al., 2014).

African and South American *C. corindum* are similar morphologically, but *C. corindum* in Namibia is found in savannah regions (Fig. 1C), different from the more forested areas typical of the species in South America. *Cardiospermum corindum* is also widespread in both subtropical and arid areas in Africa and has been recorded in countries like Angola (J. Le Roux, personal observation), Tanzania (S Carroll, personal observation, Uganda (A. Witt, personal communication), Zimbabwe, Botswana etc. (Global Biodiversity Information Facility, GBIF). Some New World *C. corindum* populations are also found in arid regions such as the Sonoran Desert (S Carroll, personal observation). African *C. corindum* from southern Africa appears phylogenetically more closely related to the Namib Desert endemic, *C. pechuelii* Kuntze, than to South American *C. corindum*, despite its morphological resemblance to the latter (Gildenhuys et al., 2014). This paraphyletic grouping of *C. corindum* is peculiar given that *C. pechuelii* is distinct from African *C. corindum* based on morphology and habitat requirements (Fig. 1). *Cardiospermum pechuelii* has smaller leaf surface areas, a more compact shrub-like growth form and reduced fruit capsules compared to *C. corindum*, potentially xerophytic adaptations that may conserve water in the extremely arid Namib Desert habitats where it occurs (Fig. 1B, C). Morphological differences between *C. pechuelii* and *C. corindum* from South America and southern Africa persist when
grown under common garden conditions, indicating that phenotypic plasticity is an unlikely cause of these differences (E. Gildenhuys, unpubl. data). The latter, in conjunction with the paraphyletic nature of *C. corin-
dum*, suggests that African *C. corindum* constitutes a separate taxon rather than being a conspecific variant of *C. pechuelii*. The strikingly different morphological adaptations in African *C. pechuelii* and *C. corindum* and their peculiar phylogenetic relationship provide an interesting case to investigate how adaptation and divergence can occur following extreme long-distance dispersal.

Here, expanding on a previous study on the bioge-
ography of selected *Cardiospermum* spp. (Gildenhuys et al., 2014), we aim to better understand the historic relationships between *C. pechuelii* and *C. corindum* using phylogenetic and population genetic approaches. This will allow us to elucidate the dynamics of diver-
sification following intercontinental long-distance dis-
persal. First, using DNA sequencing data from multiple gene regions we hope to shed light on the dispersal. Second, a number and order of colonization events and the multiple gene regions we hope to shed light on the dispersal. First, using DNA sequencing data from multiple gene regions we hope to shed light on the dispersal. Second, a number and order of colonization events and the population genetic approach will allow us to compare patterns of diversification of African taxa. Finally, this study is informative concerning the diversification of African taxa. Further details of the PCR and cloning methods). For plastid DNA regions, the *trnL* intron and the *trnL-trnF* intergenic spacer (hereafter *trnL-F*) were amplified using the primers c (5′-CGA AAT CGG TAG AGC CTA CG-3′) and f (5′-ATT TGA ACT GGT GAC ACG TTC CAA AAA AAC GTA CTT-3′) (Shaw et al., 2007) were used for amplification of the *rpl32* region. Full details of amplification conditions used here are described in Gildenhuys et al. (2014). Forty-four individuals were sequenced for both plastid DNA regions (*rpl32* and *trnL-F*), of which a subset of 37 individuals were sequenced for ITS.

PCR amplification of AFLP fragments was per-
fomed using the ‘universal’ protocol described by Blignaut, Ellis & Le Roux (2013). Briefly, following digestion and pre-selective PCR, selective PCR amplification with one primer pair (EcoR1-CAT and MseI-CTT) resulted in good quality and repeatable profiles. Forty-six samples were amplified successfully for AFLP loci, all samples were duplicated either by PCR or re-extraction to account for an additional 49 AFLP profiles included in the repeatability analyses.

**MATERIALS AND METHODS**

**Material collection**

Leaf material was collected for 25 and nine *C. corin-
dum* plants from South America and southern Africa, respectively, and 32 *C. pechuelii* plants from Namibia (Fig. 2) (Supporting Information Table S1). Voucher specimens for each species have been deposited in the Geo-Potts Herbarium, Bloemfontein, South Africa (voucher numbers BLFU1336-1340). Whole genomic DNA was extracted from silica dried leaf material using the cetyltrimethyl ammonium bromide method as described by Doyle & Doyle (1990), modified by adding 1% PVP-40T. DNA quality and quantity were checked using a NanoDrop spectrophotometer (NanoDrop ND-1000, Inqaba Biotec, South Africa) and samples diluted to a final concentration of c. 50 ng/μL.

**Gene and AFLP amplification**

One nuclear internal transcribed spacer (ITS) and two plastid (*rpl32* and *trnL-trnF*) loci were used in this study. Briefly, the nuclear (nDNA) ITS region was amplified using the universal primers ITS1 (5′-TCC GTA GGT ACG CTA CCT GGG G-3′) and ITS4 (5′-TCC TCG CTT ATT TGA TAT GC-3′) and in certain cases ITS5 (5′-GGA AGG AGA AGT CTT AGT AAC G-3′) (White et al., 1990). These primers amplify the riboso-
mal transcribed spacer regions 1 and 2, and the 5.8S region. Some PCR products for the ITS region were cloned due to sequence ambiguity caused by polymerase drop-off (see Gildenhuys et al. (2014) for further details of the PCR and cloning methods). For plastid DNA regions, the *trnL* intron and the *trnL-trnF* intergenic spacer (hereafter *trnL-F*) were amplified using the primers c (5′-CGA AAT CGG TAG AGC CTA CG-3′) and f (5′-ATT TGA ACT GGT GAC ACG TTC CAA AAA AAC GTA CTT-3′) (Shaw et al., 2007) were used for amplification of the *rpl32* region. Full details of amplification conditions used here are described in Gildenhuys et al. (2014). Forty-four individuals were sequenced for both plastid DNA regions (*rpl32* and *trnL-F*), of which a subset of 37 individuals were sequenced for ITS.

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**Phylogenetic analysis**

DNA sequence data were edited and aligned in BioEdit v 7.0.5.3 (Hall, 1999) and MAFFT (Katoh & Standley, 2013). Two data matrices were created, a combined plastid matrix (*rpl32* and *trnL-F*) for 44 samples and a combined matrix including all three loci (ITS, *rpl32* and *trnL-F*) for 37 samples. Sequence data for all three loci for accessions of *C. hali-
cacabum*, the sister taxon to *C. corindum* and *C. pechuelii* (Gildenhuys et al., 2014), were included as outgroup data to root our phylogenetic trees.

A plastid DNA haplotype network was constructed from the *rpl32* and *trnL-F* dataset using statistical with 95% connection limit in TCS v 1.21 (Clement, Posada & Crandall, 2000). We chose network analysis in addition to phylogenetic tree building approaches to visualize and explore the data as networks are better suited to explore relationships between gene regions sampled within a species which are often not hierarchical as assumed by traditional phylogenetic
tree reconstruction methods (Posada & Crandall, 2001). A Bayesian inference phylogenetic tree was reconstructed from separate plastid DNA and nDNA partitions using Mr Bayes v 3.2 (Ronquist & Huelsenbeck, 2003). Tree topologies for the two datasets were congruent, but with lower nodal support for the plastid DNA data (results not shown). We therefore re-ran the analysis for all three loci. jModelTest (Posada, 2008) and the Akaike information criterion (Akaike, 1973) was used to determine the best-fit model for our data. The Bayesian model was run for 4 million generations sampling every 1000th generation and a consensus tree was built, discarding the first 25% of trees as burn-in. Posterior probabilities (PP) were calculated using a majority rule consensus method to assess tree topology support.

**AFLP GENOTYPING**

Genemarker Version 2.2.0 (SoftGenetics, LLC, CA, USA) was used to visually investigate data quality and remove failed AFLP profiles. AFLP loci scoring followed the methods proposed by Ley & Hardy (2013). Briefly, cleaned data were imported into PEAKSCANNER© software v 1.0 (Applied Biosystems, Foster City CA, USA) where peaks were automatically scored using default settings. The fragment sizing table was exported from PEAKSCANNER© and imported into tinyFLP v1.22 (Arthofer, 2010) to optimize peak selection. Settings for tinyFLP followed those described by Ley & Hardy (2013) with the following adjustments: minimum peak height, 1000; minimum fragment size, 80 bp; maximum fragment size, 400 bp; and maximum frequency, 100%. The modified dataset was transformed into a binary matrix using tinyCAT (Arthofer, 2010).

**AFLP REPEATABILITY**

Duplicate samples (re-extracted and/or duplicated PCRs) were used to estimate the broad sense herit-
levels of unique alleles in African African balloon vines in two ways. First, comparable informative concerning the evolutionary history of distance (Nei, 1978). Genetic diversity indices can be

tion divergences were estimated using Nei’s genetic 

C. corindum

tions were run for one to seven genetic clusters (K)

among South American accessions did not form reciprocally mono-

haptotypes identified as the general time reversible model (GTR + G). Cardiospermum pechuelii rendered C. corindum paraphyletic, with African C. corindum being more closely related to C. pechuelii than to any South American accessions (Fig. 3). Moreover, C. corindum from southern Africa and C. pechuelii were more closely related to each other than to any C. corindum haplotypes identified from South America, with the latter isolated by several mutational steps (Fig. 3).

HAPLOTYPENETWORK

The haplotype network consisted of 14 haplotypes, illustrating a close relationship between samples from southern African C. corindum and C. pechuelii, with these two taxa even sharing identical haplotypes (Fig. 3). Moreover, C. corindum from southern Africa and C. pechuelii were more closely related to each other than to any C. corindum haplotypes identified from South America, with the latter isolated by several mutational steps (Fig. 3).

PHYLOGENETICTREE

The best-fit model for the combined matrix was identified as the general time reversible model (GTR + G). Cardiospermum pechuelii rendered C. corindum paraphyletic, with African C. corindum being more closely related to C. pechuelii than to any South American accessions (Fig. 3). African and South American accessions did not form reciprocally monophyletic groups, with one South American sample (JLR291) sister to the remainder on the tree. Southern African accessions formed a single monophyletic group nested in South American C. corindum. This well supported southern African clade (PP = 1) included C. pechuelii and C. corindum, and further structure in this clade was only moderately supported (Fig. 3).

GENETIC DIVERSITY AND STRUCTURE ANALYSIS

Forty-one AFLP loci were considered reproducible ($H^2$ scores > 0.7). Bayesian assignment analysis identified two genetic clusters within the data (Fig. 2). The first cluster corresponded to all C. pechuelii and C. corindum individuals from southern Africa (indicated in blue in Fig. 2) and the second cluster to South American C. corindum (indicated in red in Fig. 2). AMOVA results showed that 37% of the AFLP genetic variation resided within populations and 63% of the variation resided among populations (Table 1). Even though sample sizes were higher for C. pechuelii than for C. corindum, all genetic diversity indices
(number of effective alleles, Shannon’s information index, expected heterozygosity, number of PA and percentage polymorphic loci) indicated the highest level of diversity for *C. corindum* from South America and lowest for African samples (Table 2). Pooled African samples (*C. corindum* and *C. pechuelii*) still harboured substantially lower genetic diversity than South American samples (Table 3). Nei’s pairwise genetic distances (Nei, 1978) not only show that genetic divergence is higher between South American and southern African *C. corindum* than between southern African *C. corindum* and *C. pechuelii*, but also that differentiation is lower between *C. pechuelii* and South American *C. corindum* than between *C. corindum* from southern Africa and South America (Table 4).

**DISCUSSION**

The genetic relationships within the *pechuelii-corindum* group are consistent with a model of speciation in response to novel environmental conditions following a single long-distance transoceanic dispersal. *Cardiospermum pechuelii*, a desert endemic from Namibia, arose after *C. corindum* dispersed from South America to southern Africa between 5.9 and 15.1 Ma (Gildenhuys et al., 2014). The drier conditions of the Namib Desert probably led to strong directional selection and, in the presence of genetic
drift (as evidenced by reduced genetic diversity in African taxa Tables 2 and 3), probably facilitated diversification of a morphologically and ecologically unique taxon. Although our data cannot distinguish the action of selection from drift, the low genetic differentiation between African *C. corindum* and *C. pechuelii* observed here (Tables 2 and 3) favours selection over isolation and genetic drift as the causal mechanism for the strikingly different morphologies and habitat of these two taxa (Fig. 1). Moreover, African *C. corindum* is genetically more closely related to the morphologically divergent *C. pechuelii* than to morphologically similar South American *C. corindum* (Figs 2, 3).

The mixed clade including southern African balloon vines is well supported (PP = 1) with two further clades that are moderately supported (Fig. 3). Again, for this African clade, *C. pechuelii* renders African *C. corindum* paraphyletic, i.e. some *C. pechuelii* accessions are phylogenetically more closely related to African *C. corindum* than some African *C. corindum* accessions are to each other. These relationships, along with population genetic structure based on AFLP data, possibly indicate that reproductive isolation is incomplete between these taxa and that gene flow is still ongoing. However, given their recent divergence, coupled with geographical isolation and distinct morphologies, we cannot dismiss the possibility of incomplete lineage sorting (the failure of gene copies to coalesce within the duration of the species) as an equally parsimonious explanation for the genetic patterns observed here. The placement of the *C. pechuelii* accession, CSMN47, within the African *C. corindum* plastid DNA clade (Fig. 3) also suggests that past hybridization between African *C. corindum* and *C. pechuelii* may have led to introgression and possible plastid capture. Taken together these results indicate that the evolution of *C. pechuelii* in Namibia probably occurred very recently and it is therefore conceivable that the extreme long-distance dispersal event of *C. corindum* from South America to Africa occurred towards the lower end of the 5.9–15.1 Ma estimation of Gildenhuys *et al.* (2014). Our phylogenetic analysis also illustrates the need for the revision of alpha taxonomy of the *pechuelii-corindum* group. Specifically, *C. pechuelii* should be treated as an

### Table 2. Genetic diversity indices based on AFLP analyses for *Cardiospermum corindum* from southern Africa and South America and *C. pechuelii* from Namibia.

<table>
<thead>
<tr>
<th></th>
<th>Ne</th>
<th>I</th>
<th>He</th>
<th>Pₐ</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pechuelii</em></td>
<td>1.030</td>
<td>0.035</td>
<td>0.019</td>
<td>3</td>
<td>21.95%</td>
</tr>
<tr>
<td><em>C. corindum</em> southern Africa</td>
<td>1.074</td>
<td>0.061</td>
<td>0.041</td>
<td>1</td>
<td>12.20%</td>
</tr>
<tr>
<td><em>C. corindum</em> South America</td>
<td>1.290</td>
<td>0.285</td>
<td>0.182</td>
<td>21</td>
<td>65.85%</td>
</tr>
</tbody>
</table>

Effective number of alleles (Ne), Shannon’s information index (I), expected heterozygosity (He), number of private alleles (Pₐ) and % polymorphic loci (PP) are shown.

### Table 3. Genetic diversity indices based on AFLP analyses for *Cardiospermum corindum* and *C. pechuelii*, grouping all African samples as a single population and South American *Cardiospermum corindum* samples as another.

<table>
<thead>
<tr>
<th></th>
<th>Ne</th>
<th>I</th>
<th>He</th>
<th>Pₐ</th>
<th>PP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern African samples</td>
<td>1.056</td>
<td>0.069</td>
<td>0.038</td>
<td>6</td>
<td>34.15%</td>
</tr>
<tr>
<td><em>C. corindum</em> South America</td>
<td>1.290</td>
<td>0.285</td>
<td>0.182</td>
<td>21</td>
<td>65.85%</td>
</tr>
</tbody>
</table>

Effective number of alleles (Ne), Shannon’s information index (I), expected heterozygosity (He), number of private alleles (Pₐ) and % polymorphic loci (PP) are shown.

### Table 4. Pairwise population matrix of Nei’s genetic distance based on AFLP analyses for *Cardiospermum corindum* from southern Africa and South America and *C. pechuelii* from Namibia

<table>
<thead>
<tr>
<th></th>
<th><em>C. pechuelii</em></th>
<th><em>C. corindum</em> southern Africa</th>
<th><em>C. corindum</em> South America</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. pechuelii</em></td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. corindum</em> southern Africa</td>
<td>0.050</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td><em>C. corindum</em> South America</td>
<td>0.141</td>
<td>0.153</td>
<td>0.000</td>
</tr>
</tbody>
</table>

African desert ecotype/variant or lineage of *C. corindum* if species delimitations are based on phylogenetic monophyly.

The recent divergence of African *C. pechuelii* and *C. corindum* and their relationship to South American *C. corindum* have important implications for ongoing biological control against invasive balloon vine species in southern Africa (Gildenhuys et al., 2014). First, our study highlights the need for sound taxonomy prior to implementing biological control programmes. Host-specificity trials of biological control agents currently considered for release in South Africa used mostly South American provenances of *C. corindum* (Mc Kay et al., 2010; Simelane et al., 2014). Given its genetic distinctiveness, it is evident that African *C. corindum* should be included as a separate taxon in host-specificity trials and that trials on South American provenances may not accurately predict host preferences in Africa. The complex provenances of balloon vines identified here also pose a unique opportunity to shift focus from classical to indigenous avenues of biological control, i.e. seeking and testing fauna associated with indigenous balloon vines as potential control agents against introduced balloon vines. Promoting native natural enemies to control non-native taxa probably has much untapped potential, but has hitherto been largely unexplored. Indigenous biological control may open the door to better understanding of how eco-evolutionary management may influence the structure of local and regional communities in general. For example, Andres et al. (2013) recently identified a host shift of hybrid lineages of native Australian *Leptocoris* soapberry bugs onto invasive balloon vine (*C. hali- cacabum*) in Australia. Parental, non-hybrid lineages of these soapberry bugs usually prefer distantly related native plant hosts (Andres et al., 2013).

The peculiar distribution and evolutionary history of African balloon vines also presents a system for testing and exploring numerous evolutionary hypotheses such as species concepts. Species concepts have been debated for decades and are still disputed with c. 26 different species concept definitions (Wilkins, 2011). The lack of agreement between scientists on species concepts often poses problems for identifying species. For example, a quick glance at *C. corindum* from South America and southern Africa is unlikely to reveal any immediate morphological differences, but to tease this relationship apart properly in-depth morphological and genetic analyses will have to be incorporated. Similarly, despite marked morphological differences between *C. pechuelii* and *C. corindum* our genetic results indicate recent diversification and therefore outcrossing experiments could add valuable information for understanding how complete reproductive isolation is between these two taxa.

The evolutionary diversification of *C. pechuelii* from *C. corindum* is also a remarkable example of the extent to which diversification can occur, from humid to subtropical to desert environments. This may not be unexpected, as others have found species of *Cardiospermum* and *Paullinia* L. to have significantly higher evolutionary rates compared to other genera in Sapindaceae (Harrington, 2008). Our study also adds to a growing body of evidence highlighting the importance of extreme long-distance dispersal in shaping regional biodiversity and how evolution can be triggered given novel environmental conditions.

ACKNOWLEDGEMENTS

We thank David Simelane and Andries Fourie who kindly provided us with *Cardiospermum* DNA samples from South America and Kate Le Roux, Hannes Wieze, Mandelise van Logerenberg, Ralph Riley and Jenella Loye for field assistance. We are grateful for sampling permits from the New South Wales Department of Environment and Conservation and Queensland Government Department of Environment and Resource Management (Australia) and the Ministry of Environment and Tourism (Namibia). We thank the Australian Tropical Herbarium and Australian National Herbarium for herbarium specimens. Financial support was provided by the DST-NRF Centre of Excellence for Invasion Biology and the Working for Water Programme through their collaborative project on ‘Research for Integrated Management of Invasive Alien Species’. E. G. acknowledges the South African National Research Foundation’s (NRF) Scarce Skills scholarship programme. J. L. R. also acknowledges Stellenbosch University’s Subcommittee B and the NRF Thuthuka Programme for research funding. S. C. acknowledges support from the School of Life Sciences at the University of Queensland, St. Lucia.

REFERENCES


Simelane DO, Mawela KV, Mc Kay F, Olieiro M. 2014. Field and laboratory studies to determine the suitability of *Cissaanthomonos tuberculipennis* (Curculionidae) for...
release against Cardiospermum grandiflorum in South Africa. *Biocontrol Science and Technology* **24**: 734–750.


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher’s website:

**Table S1.** Species, sample ID, geographical origin, site, latitude, longitude and GenBank accession numbers of samples used in this study.