

RESEARCH ARTICLE

Comparative phylogeographic analysis of *Cuscuta campestris* and *Cuscuta reflexa* in Kenya: Implications for management of highly invasive vines

Joel Masanga¹  | Richard Oduor¹ | Amos Alakonya² | Mathew Ngugi¹ |
Patroba Ojola¹ | Emily S. Bellis^{3,4} | Steven Runo¹ 

¹Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya

²Seed Health Unit, International Maize and Wheat Improvement Center, Texcoco, Mexico

³Arkansas Biosciences Institute and Department of Computer Science, Arkansas State University, Jonesboro, Arkansas, USA

⁴Center for No-Boundary Thinking, Arkansas State University, Jonesboro, Arkansas, USA

Correspondence

Steven Runo, Kenyatta University, Department of Biochemistry, Microbiology and Biotechnology, Nairobi, Kenya.
Email: runo.steve@ku.ac.ke

Emily S. Bellis, Arkansas State University, Arkansas Biosciences Institute and Department of Computer Science, Jonesboro, AR, USA.
Email: ebellis@astate.edu

Funding information

National Research Fund of Kenya, Grant/Award Number: NRF/PhD/02/76; Kenyatta University, Grant/Award Number: KU/DVCR/VRG/VOL.11/216

Societal Impact Statement

Eastern Africa is currently infested with dodders—highly invasive vines that threaten agriculture and biodiversity. We describe historical and ecological factors that have potentially shaped dodder invasion in this region. Our results reveal high levels of genetic diversity within dodder populations, which could enhance their ability to colonize new niches. Further, we identify two main population clusters in eastern and western Kenya, a pattern that is mirrored in an established invader (*Cuscuta campestris*) and a recent introduction (*C. reflexa*). Immediate control strategies are needed to limit deliberate or inadvertent seed/vine dispersal, eradicate existing populations, and monitor influx of propagules.

Summary

- Invasion by dodder (*Cuscuta* spp.), holo-parasitic plants of the Convolvulaceae family, has recently surged in Eastern Africa. Particularly, colonization by two species, *Cuscuta campestris* and *Cuscuta reflexa* that have a broad host range and rapid invasiveness, has escalated. We examined the first assessment of genetic diversity of these invasive species in Kenya to inform subsequent management practices.
- A comparative phylogeographic analysis, based on chloroplast and nuclear ribosomal DNA, was performed to determine genetic diversities, population structure, and isolation of *C. campestris* and *C. reflexa* in Kenya.
- Results reveal high genetic variation in both species within populations across localities. *C. campestris* had more haplotypes and higher gene diversity ($P = .0001$) and heterozygosity ($P = .004$) than *C. reflexa*. Both species were geographically separated, evidenced by two distinct lineages that mostly corresponded to localities east and west of Kenya. Correlation between genetic and geographic distances revealed evidence of isolation by distance (IBD).
- We attribute these genetic variation patterns to establishment via similar invasion routes for the studied *Cuscuta* spp. and suggest that *Cuscuta* invasion in Kenya is in the early stages of establishment. Rapid eradication is urgently needed to prevent shifts to economically important hosts, which would have devastating

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Plants, People, Planet* published by John Wiley & Sons Ltd on behalf of New Phytologist Foundation.

impacts. We recommend (i) development of targeted efforts to curb new introductions to limit the potential for genetic variability and adaptation, (ii) prevention of seed and vine dispersal from current incursions, and (iii) complete eradication of existing populations where this is feasible.

KEYWORDS

Cuscuta (dodder), gene flow, highly invasive vines, invasive alien species (IAS), isolation by distance, parasitic plants, phylogeography

1 | INTRODUCTION

Phylogeography examines distribution of natural populations and elucidates ecological and evolutionary processes that have shaped patterns of population structure (Avice et al., 1987). The ongoing surge in colonization by novel invasive alien species (IAS) or expansion by naturalized ones across habitats has increased the demand for insights into biological mechanisms that have influenced these invasions (Meyerson & Mooney, 2007; Schulz et al., 2019). The ability of an IAS to survive in a locality can depend on various factors, key among them being their introduction and adaptability to new environments, evolutionary history, and anthropogenic activities that either enhance or restrict dispersal (Dlugosch & Parker, 2008; Ward et al., 2008).

Understanding genetic variability can provide useful insights into patterns of local adaptation to changing environmental conditions and explain divergence among populations (Flanagan et al., 2018). In plants, pollen and seeds are key avenues for gene flow (Hamilton & Miller, 2002; Levin, 1981). A key determinant for the success of this process is dispersal of these materials, either biologically by vectors or abiotically by environmental agents, such as water and wind. Previous studies have shown that most plants adopt a leptokurtic gene flow pattern, where pollen and seeds are dispersed near a genotype's locality, and these are likely to be the most adapted (Bradshaw, 1972). Conversely, plants that lack long-distance dispersal mechanisms are expected to have highly restricted gene flow; hence, they are expected to exhibit a high genetic structure (Loveless & Hamrick, 1984; Sork & Smouse, 2006). Successful expansion of most invasive weeds into new ranges depends on their life history traits, particularly high reproductive outputs and wide dispersal abilities that ensure colonization and establishment over heterogeneous environments (Sork et al., 2016). This reproductive plasticity also ensures continuous establishment of new populations amid the changing environmental conditions. Since plants are sessile, their patterns of distribution and/or divergence across different geographical settings are subject to influence by isolation by distance (IBD), isolation by environment (IBE), and isolation by adaptation (IBA) (Wang & Bradburd, 2014). For instance, highly heterogeneous environments between populations may affect flowering times or pollinators, whereas long distances may restrict movement of pollen or seeds. Overall, these modes of isolation influence gene flow and affect phylogeography of plants.

All *Cuscuta* species are considered obligate holoparasites because they lack an established root system and therefore entirely depend on host plants for support and nourishment (Kaiser et al., 2015). They attach on a suitable host via haustorium, creating a conduit for siphoning of water, nutrients, and photosynthates (Dörr, 1969; Kuijt, 1969). These parasites multiply through both sexual and asexual reproduction, and parasitize a wide range of plants (Lanini & Kogan, 2005; Masanga et al., 2021). Overall, over 200 species, with a worldwide distribution, have been identified (Kaiser et al., 2015; Yuncker, 1932). Additionally, taxonomic and molecular identification as well as character evolution and distribution of these species have been studied (García et al., 2014; Ren et al., 2020; Stefanović et al., 2007; Yuncker, 1932). To date, little is known regarding genetic variation and demographic patterns of these noxious weeds across habitats, with only a handful of reports describing population genetics (Elsiddig et al., 2018; Mutikainen & Koskela, 2002). Evaluating the genetic basis underlying successful invasion could offer valuable insights to inform development of control and management measures, considering that genetic variability is an important determinant of invasiveness (Kolbe et al., 2004; Tsutsui et al., 2000; Ueno et al., 2015). Specifically, IAS with a high genetic diversity are expected to have superior adaptability that enables them to cope with perturbing and stressful conditions in the new environments (Prentis et al., 2008).

The overarching goal of this study was to explore patterns of genetic diversity in two distinct *Cuscuta* species occurring in Kenya and elucidate historical as well as evolutionary characteristics that have shaped their distribution and expansion across the landscapes. Field dodder (*Cuscuta campestris* Yuncker.), a North American species that is also native to the Caribbean islands, is naturalized in Africa, having been first identified in South Africa in 1918 (Yuncker, 1932) while the giant dodder (*Cuscuta reflexa* Roxb.), a tropical Asian species, was first reported in Mauritius in the 1970s (Holm et al., 1979). Recently, we provided the first evidence of presence of this Indian species in continental Africa (Masanga et al., 2021). Both species share ecomorphological traits (propagating by vines and seed) that allow them to colonize new localities across different environments, and in Kenya, they are co-distributed to the east and west of the country. Moreover, they are generalist parasites that infect numerous plants across various angiosperm orders (Masanga et al., 2021). To date, the role of other characteristics, such as physiological similarities, degree of virulence, habitat preference, reproductive plasticity, and human-aided dispersal remains unknown. We hypothesized that

populations of the two *Cuscuta* species could be genetically diverse, with this diversity potentially determining their success in colonizing current and subsequent expansion into new localities. Our findings suggest the existence of similar invasion routes for both species and emphasize the need to manage invasions prior to establishment.

2 | MATERIALS AND METHODS

2.1 | Sampling

A total of 10 and nine populations of *C. campestris* and *C. reflexa*, respectively, were collected from localities within their known distributions across Kenya. Identification and characterization of these species were carried out as earlier described in our previous work (Masanga et al., 2021). We focused on these species because (i) they are highly invasive and are currently expanding to various ecosystems across East Africa, and (ii) they have a wide host range, with potential to expand this parasitism to economically important tree crops, thereby posing danger to the agricultural sector. From each locality, we collected at least 5 individuals, at least 30 m apart, to minimize chances of resampling. Vines (5–10 cm), together with any flowers present, were collected and placed in silica gel, then stored at 4°C until DNA extraction. Sampled localities, across *Cuscuta* spp. ranges in Kenya are shown in Table 1. *C. campestris* populations west of the country included Nandi (NANC), Kisii (KSIC), Nyamira (NYAC), Vihiga (VIAC), Kakamega (KAKC), and Busia (BUSC), whereas those of *C. reflexa* were Kisumu (KSMR), Busia (BUSR), Kakamega (KAKR), Siaya (SIAR), Bomet (BOMR), Narok (NRKR), and Nandi (NANR). Those to the east included Machakos (MCKC), Kiambu (KIAC), Nakuru (NKUC), Nairobi (NRBC) (for *C. campestris*), and Nairobi (NRBR) and Machakos (MCKR) (for *C. reflexa*).

2.2 | DNA extraction, PCR, and sequencing

DNA was extracted from the sampled materials, using a standard DNA isolation protocol described by Doyle and Doyle (1990), and then used for PCR targeting the chloroplast (ribulose biphosphate carboxylase large subunit [*rbcl*]) and nuclear (internal transcribed spacer [ITS]) regions. For *rbcl*, we amplified a partial fragment using primers *rbcl*-512F and *rbcl*-1392R (McNeal et al., 2007), whereas in ITS, we targeted the 5.8S of the nuclear nrDNA region, alongside those flanking 18S and LSU (26S) genes (Baldwin, 1992). Amplifications were performed in 25- μ l reactions using MyTaq™ DNA polymerase kit (Bioline, Meridian Biosciences), according to the manufacturer's instructions. Reaction mixes were subjected to an initial denaturation step of 95°C for 1 min, followed by 35 cycles of 95°C for 15 s, 30-s annealing (at appropriate temperatures for each primer) and a 1-min extension at 72°C. A final extension at 72°C for 10 min was also included. PCR products were purified using the Qiaquick™ PCR purification kit (Qiagen, USA), then sequenced, using the forward primer, on the ABI system at Macrogen (Macrogen Inc.). Sequences

were cleaned in SeqMan Pro17 (DNASTAR Inc., Madison, WI, USA), to remove low quality reads and adapters and then aligned using ClustalX version 2.0 (Larkin et al., 2007). The resulting sequences were submitted to GenBank for the following accession numbers; *C. campestris rbcl*—MW581109-MW581141; ITS—MT845881-MT845895, MT849993-MT850001, and MT947606-MT9476223; *C. reflexa rbcl*—MW581080-MW581108; ITS—MT850025-MT850044 and MW080817-MW080836.

2.3 | Data analysis

The number of haplotypes (h), haplotype diversity (hd), and nucleotide diversity (π) were calculated using DnaSP 5.0 (Librado & Rozas, 2009). For phylogenetic analysis of haplotypes, we performed Bayesian inferencing (BI) using full sequences of the haplotypes. First, we used the program jModelTest version 2.1.6 (Posada, 2008) to determine the best fit model for both datasets and selected the general time-reversible (GTR) model with gamma distribution and assuming invariable sites (GTR + G + I) (Yang, 1994) in both Akaike information criteria (AIC) and Bayesian information criteria (BIC). Bayesian inferencing was performed using Mr. Bayes version 3.2.7 (Ronquist & Huelsenbeck, 2003) with four Markov chain Monte Carlo (MCMC) chains, according to the GTR + G + I model. The run included 1,000,000 generations with sampling every 100 generations. Sequences for *Ipomoea purpurea* were included as outgroups.

To further assess the relationship among haplotypes, we constructed median-joining (MJ) networks using popART v1.7 (Leigh & Bryant, 2015), with each indel treated as a single mutation event. Next, we investigated demographic history of both *Cuscuta* species using neutrality tests and mismatch analyses, by calculating Tajima's *D* (Tajima, 1989) and Fu's *F* (Fu, 1997) statistics, as well as SSD and Raggedness index in Arlequin version 3.5.2.2 (Excoffier & Lischer, 2010). The degree of genetic differentiation among localities was measured using pairwise F_{ST} , for both species in Arlequin. To identify phylogeographically homogeneous populations, we performed a spatial analysis of molecular variance (SAMOVA) using SAMOVA v.2.0 (Dupanloup et al., 2002). Consistency of our results was assessed by performing nine independent runs for each *K* (representing each cluster) value, from *K* = 2 to *K* = 10. Two groups, east and west of Kenya, emerged. We then used analysis of molecular variance (AMOVA), implemented in Arlequin, to test genetic relationships within and among these groups. We evaluated the relationship between genetic and geographical distances using isolation by distance (IBD) analysis. Briefly, we used the program Origin to generate 2D kernel density plots for our sample points and then chose the most densely populated localities (Nandi and Kisumu for *C. campestris* and *C. reflexa*, respectively) as reference points. Next, we calculated Euclidean geographical distances among populations using the “graph 4lg” package implemented in R v.3.5.2 and then correlated these distances with pairwise F_{ST} values using GraphPad Prism v.7 at $P \leq .05$.

TABLE 1 Sampling information for *Cuscuta* localities and genetic variation parameters among cpDNA and ITS regions

	Pop	Coordinat (lat/long)	Gene	n	H	Hd	Nucleotide diversity	Expected heterozygosity
<i>C. campestris</i>	MCKC	1°31'46"S/ 37°15'36"E	<i>rbcl</i>	5	3	0.7 ± 0.218	0.008 ± 0.006	0.500 ± 0.107
			ITS		3	0.7 ± 0.218	0.009 ± 0.006	0.533 ± 0.100
	KIAC	1°08'02"S/ 36°58'20"E	<i>rbcl</i>	5	5	1.0 ± 0.127	0.012 ± 0.008	0.450 ± 0.102
			ITS		5	1.0 ± 0.127	0.022 ± 0.014	0.4 ± 0.00000
	NKUC	0°12'46"S/ 35°51'45"E	<i>rbcl</i>	5	4	0.9 ± 0.161	0.010 ± 0.007	0.473 ± 0.127
			ITS		5	1.0 ± 0.127	0.021 ± 0.013	0.424 ± 0.066
	NRBC	1°12'30"S/ 36°54'43"E	<i>rbcl</i>	5	5	1.0 ± 0.126	0.038 ± 0.024	0.434 ± 0.091
			ITS		2	0.4 ± 0.237	0.005 ± 0.004	0.4 ± 0.00000
	NANC	0°09'25"S/ 35°12'08"E	<i>rbcl</i>	5	5	1.0 ± 0.126	0.026 ± 0.018	0.437 ± 0.099
			ITS		5	1.0 ± 0.127	0.049 ± 0.002	0.4 ± 0.00000
	KSIC	0°37'04"S/ 34°45'22"E	<i>rbcl</i>	5	5	1.0 ± 0.127	0.016 ± 0.011	0.432 ± 0.095
			ITS		4	0.9 ± 0.161	0.009 ± 0.002	0.66 ± 0.1000
	NYAC	0°36'55"S/ 34°58'18"E	<i>rbcl</i>	5	5	1.0 ± 0.127	0.028 ± 0.011	0.450 ± 0.107
			ITS		5	1.0 ± 0.127	0.012 ± 0.011	0.500 ± 0.141
	VIAC	0°10'11"N/ 34°55'22"E	<i>rbcl</i>	4	4	1.0 ± 0.127	0.014 ± 0.002	0.473 ± 0.127
			ITS		3	0.9 ± 0.161	0.050 ± 0.002	0.554 ± 0.088
	KAKC	0°10'29"N/ 34°45'04"E	<i>rbcl</i>	3	3	1.0 ± 0.127	0.004 ± 0.002	0.4 ± 0.09100
			ITS		3	1.0 ± 0.127	0.011 ± 0.011	0.533 ± 0.060
BUSC	0°29'08"N/ 34°08'10"E	<i>rbcl</i>	3	3	1.0 ± 0.127	0.003 ± 0.002	0.412 ± 0.071	
		ITS		2	1.0 ± 0.127	0.002 ± 0.011	0.4 ± 0.00000	
<i>C. reflexa</i>	KSMR	0°10'21"S/ 34°55'23"E	<i>rbcl</i>	5	1	0.0 ± 0.000	0.0 ± 0.00000	0.0 ± 0.00000
			ITS		4	0.9 ± 0.161	0.004 ± 0.003	0.4 ± 0.00000
	BUSR	0°30'12"N/ 34°07'42"E	<i>rbcl</i>	5	2	0.4 ± 0.237	0.001 ± 0.001	0.4 ± 0.00000
			ITS		3	0.7 ± 0.218	0.008 ± 0.005	0.5 ± 0.10600
	KAKR	0°11'38"N/ 34°45'50"E	<i>rbcl</i>	5	2	0.4 ± 0.237	0.001 ± 0.001	0.4 ± 0.00000
			ITS		2	0.6 ± 0.175	0.008 ± 0.006	0.6 ± 0.00000
	SIAR	0°04'22"N/ 34°15'48"E	<i>rbcl</i>	5	1	0.0 ± 0.000	0.0 ± 0.00000	0.0 ± 0.00000
			ITS		3	0.7 ± 0.218	0.011 ± 0.007	0.4 ± 0.00000
	NRBR	1°16'33"S/ 36°49'08"E	<i>rbcl</i>	5	2	0.4 ± 0.237	0.001 ± 0.001	0.4 ± 0.00000
			ITS		1	0.0 ± 0.000	0.0 ± 0.00000	0.0 ± 0.00000
	NRKR	0°51'34"S/ 35°23'32"E	<i>rbcl</i>	5	2	0.4 ± 0.237	0.002 ± 0.001	0.4 ± 0.00000
			ITS		3	0.7 ± 0.218	0.002 ± 0.002	0.5 ± 0.14100
	BOMR	0°43'48"S/ 35°20'54"E	<i>rbcl</i>	5	2	0.4 ± 0.237	0.001 ± 0.001	0.4 ± 0.00000
			ITS		3	0.7 ± 0.218	0.014 ± 0.009	0.554 ± 0.088
	NANR	0°09'25"S/ 35°12'08"E	<i>rbcl</i>	3	1	0.0 ± 0.000	0.0 ± 0.00000	0.0 ± 0.00000
			ITS		3	1.0 ± 0.272	0.019 ± 0.015	0.69 ± 0.089
	MCKR	1°31'11"S/ 37°13'49"E	<i>rbcl</i>	2	1	0.0 ± 0.000	0.0 ± 0.00000	0.0 ± 0.00000
			ITS		1	0.0 ± 0.000	0.0 ± 0.00000	0.0 ± 0.00000

Note: Pop = population; n = number of samples; H = number of haplotypes; Hd = haplotype diversity.

3 | RESULTS

3.1 | Genetic variation among *Cuscuta* taxa

rbcl alignments resulted in 470 sites for *C. campestris*, of which 50 were parsimony informative with 71 mutations, whereas those

for *C. reflexa* contained 494 sites, of which 23 were parsimony informative with 26 mutations. Average expected heterozygosity for *C. campestris* was 0.4461 (ranging from 0.4 to 0.5) and 0.4804 (ranging from 0.4 to 0.66), based on *rbcl* and ITS, respectively, whereas those for *C. reflexa* were 0.22 (range 0–0.4) and 0.4119 (range 0–0.69), based on *rbcl* and ITS, respectively (Table 1). A total

of 33 ($Hd = 0.97410$) and seven ($Hd = 0.62949$) haplotypes were obtained in *C. campestris* ($n = 45$) and *C. reflexa* ($n = 40$) populations, respectively. In *C. campestris* haplotypes, H1 was shared among three localities, east of Kenya, namely, MCKC, NKUC, and KIAC, H10 occurred between NANC and NKUC and H20 between KSIC and KAKC; H22 was shared among KSIC, NYAC, and BUSC and H23 between KSIC and KAKC, whereas H25 was shared between NYAC and VIAC populations. In *C. reflexa*, H1 was shared among all western populations (KSMR, BUSR, KAKR, and SIAR), whereas H4 occurred across all eastern populations and was shared with several populations west of the Rift valley depression (Figure 2b).

ITS alignments resulted in 131 and 47 segregating (polymorphic) sites for *C. campestris* and *C. reflexa*, respectively. Among these sites, 57 and 38 in *C. campestris* and *C. reflexa*, respectively, were parsimony informative and further contained a respective 146 and 52 mutations. Overall, a total of 33 ($Hd = 0.97677$) and 18 ($Hd = 0.92436$) haplotypes were obtained in *C. campestris* and *C. reflexa*, respectively. Two *C. campestris* haplotypes, namely, H22 (between KSIC and NYAC) and H29 (between VIAC and BUSC) were shared. In *C. reflexa*, H1 was shared among KSMR, BUSR, and SIAR and H11 occurred between NRBR and NRKR, whereas H13 was shared between NRKR and BOMR. Notably, H18 was shared between NANR (a western population) and MCKR (a population farthest east). Expected heterozygosity ranged between 0.4 and 0.66 for *C. campestris* populations and 0–0.69 for *C. reflexa*. In addition, gene (haplotype) diversity ranged between 0.4–1 and 0–1 for *C. campestris* and *C. reflexa*, respectively (Table 1). A comparison among genetic variation indices between species indicated that *C. campestris* had higher average gene diversity and heterozygosity across all populations than *C. reflexa*. Notably, this difference was statistically significant ($P < .05$) based on *rbcl* but not with regards to ITS (Table S1).

3.2 | Population and phylogeographic structure

We analyzed genetic differentiation among *Cuscuta* populations by comparing G_{ST} and N_{ST} , measures of differentiation that consider allelic frequencies and similarities among haplotypes, respectively. Generally, a significantly higher N_{ST} than G_{ST} value is indicative of phylogenetic subdivision, whereas the opposite is true for a significantly higher G_{ST} than N_{ST} (Pons & Petit, 1996). Estimates, based on cpDNA, revealed significantly higher N_{ST} (0.70949) than G_{ST} (0.26819) for *C. campestris*. Similarly, a significantly higher N_{ST} (0.78908) than G_{ST} (0.61359) was observed in *C. reflexa*, indicative of a phylogeographic structure. Estimates based on ITS corroborated these findings in both *C. campestris* ($N_{ST} = 0.66104 > G_{ST} = 0.2541$) and *C. reflexa* ($N_{ST} = 0.77794 > G_{ST} = 0.48417$). BI trees revealed two distinct clades in both species, across both nrDNA and cpDNA, which corresponded to western and eastern localities (Figure 1). Similarly, median joining networks corroborated these results, as evidenced by two clusters that also corresponded to the two geographical regions. Interestingly, taxa from Nandi populations (NANC and NANR) clustered with those from

eastern localities despite being geographically in the west. SAMOVA-based genetic analyses, targeting *rbcl* and ITS haplotypes, partitioned our data into two genetically distinct groups for both *C. campestris* and *C. reflexa*.

To assess variation across populations, we estimated and compared variance as well as proportions of variation among groups using F_{CT} and F_{ST} statistics. Results revealed maximum F_{CT} values of 0.64167 and 0.4666 for *rbcl* and ITS, respectively, in *C. campestris*, whereas *C. reflexa* recorded F_{CT} values of 0.81081 and 0.7654 for *rbcl* and ITS, respectively (Table 2). All F_{CT} values were significant ($P < .05$) and were obtained when $K = 2$ across all analyses. AMOVA revealed significantly moderate and high variability in *C. campestris* and *C. reflexa*, respectively. F_{ST} values, obtained after combining taxa from all populations, across cpDNA and ITS ranged from 0.76 to 0.98 ($P < .01$). In both species, the highest variation was attributed to groups and ranged between 46% and 98%, whereas the lowest variation resulted from populations (ranging between -0.05% to 20%) (Table 2).

3.3 | Demographic analyses and geographical distribution of haplotypes

Since SAMOVA partitioned our taxa into two distinct groups, we analyzed potential population expansion based on these groups, using mismatch analysis and neutrality tests. Generally, negative Tajima's D and Fu's F values, as well as significantly low SSD and raggedness values, indicate demographic expansion. Relatively small, but statistically insignificant, mismatch parameters were obtained in both species at both cpDNA and ITS levels. Neutrality tests revealed negative Tajima's D for only two populations of *C. campestris* (MCKC and KIAC) and for only one marker (Table 3), neither of which also had a negative value of Fu's F (Table 3). Based on these data, we found limited evidence of population expansion in both species across sampled localities. Spatial distribution of the haplotypes in Kenya is shown in Figure 2. In summary, geographically proximal localities shared haplotypes from the same clade, with an overlap observed among shared ones. Notably, no haplotypes were shared between populations to the east and west of the country (Figure 2). Interestingly, Nandi, which is on the west of the Rift Valley depression and is geographically close to the western group, was genetically more like eastern populations.

3.4 | Isolation by distance

Kernel density estimates indicated a high concentration of points in Nandi and Kisumu for *C. campestris* and *C. reflexa*, respectively (Figure 3). Consequently, we used these as reference points for calculating Euclidean distances between populations (Tables S2 and S3). A correlation between geographical and genetic distances (Tables S4–S7) appeared to suggest isolation by distance, although only *C. campestris* (based on ITS) was statistically significant.

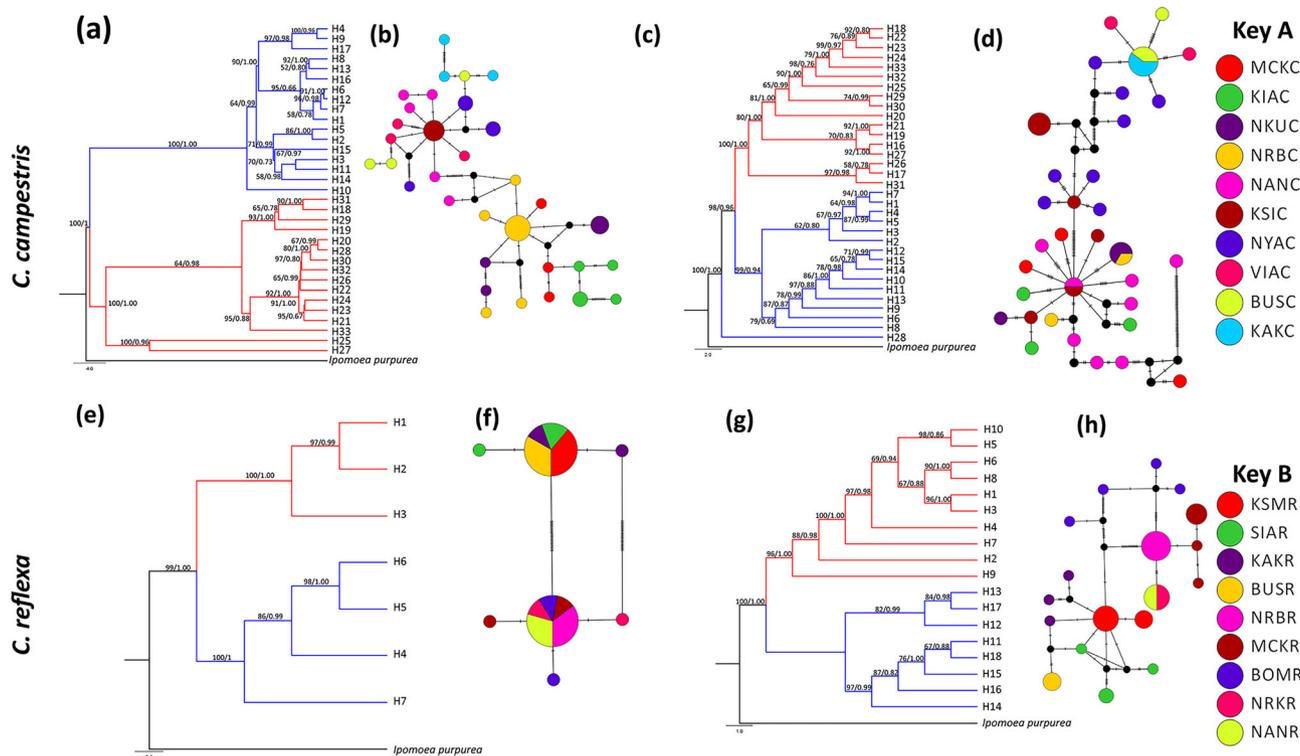


FIGURE 1 Relationships among dodder haplotypes. *Cuscuta campestris* Data are shown in the upper panel as follows; (a) and (c) are BI trees based on *rbcL* and ITS, respectively, whereas (b) and (d) are corresponding median joining networks based on *rbcL* and ITS, respectively. *Cuscuta reflexa* data are shown in the lower panel as follows; (e) and (g) are BI trees based on *rbcL* and ITS, respectively, whereas (f) and (h) are corresponding median joining networks based on *rbcL* and ITS, respectively. Red and blue clades (in the BI trees) represent taxa from western and eastern localities, respectively, whereas numbers denote bootstrap support/Bayesian posterior probabilities. Scale bars below each tree represent branch lengths. Circles in the median joining networks are color-coded according to sampling localities as represented by Keys A and B for *C. campestris* and *C. reflexa*, respectively. Circle sizes and pie segments are proportional to haplotype frequencies (calculated from 45 and 40 individuals for *C. campestris* and *C. reflexa*, respectively). Mutational steps between haplotypes are indicated by hatch marks. The small black circles represent median vectors

4 | DISCUSSION

We report genetic patterns that may have shaped successful invasion by *C. campestris* and *C. reflexa*, genetically distinct species, across Kenya. These two, alongside other *Cuscuta* species, have invaded various ecosystems across Eastern Africa (Masanga et al., 2021). We discuss these results with regards to how modes of reproduction and ability to disperse to new environments may have shaped the current invasion. These findings are expected to inform future management strategies for control of these noxious weeds.

The phylogeographic pattern of an invasive species is influenced by various factors, such as its life history characteristics, dispersal capabilities, environmental requirements, and association with ecology (Ditchfield, 2000). To understand the underlying invasion patterns, one requires to determine how much genetic divergence exists among individuals, haplotypes, and populations, as well as geographical distribution of the haplotypes (Ditchfield, 2000). Previous studies have recommended the use of multiple genes across genomes in a species, for detection of genetic structure among individuals, as these circumvent limitations associated with interpretation of historical

processes brought about by a single gene (Bermingham & Moritz, 1998; Schaal et al., 1998; Templeton, 2002). In the present study, we used two genetic markers to analyze genetic variation and population structure in *Cuscuta* spp. Specifically, we chose *rbcL* due to its low-level genetic diversity, which offers high differentiation among populations and a slow rate of evolution (Gaut et al., 1992), as well as nrDNA (ITS) because it offers more variability (relative to coding regions) and has a low level of intraspecific divergence (Zhang & Hewitt, 2003).

Gene trees revealed two distinct clades that corresponded to lineages in western and eastern Kenya. To confirm this pattern, we used tests of molecular variance (measures of genetic diversity and population structure), median joining networks as well as neutrality tests and mismatch distributions to depict relationships among haplotypes. SAMOVA and MJ networks corroborated results from BI analyses. Additionally, we observed high genetic variation, a strong population structure and expansion across both species. AMOVA indicated that this variation was due to differences among individuals within populations, with very little contribution among populations. Spatial analysis of haplotype distribution showed that most haplotypes

TABLE 2 Analysis of molecular variance (AMOVA) based on *rbcl* and ITS

Species	Gene	Source of variation	df	Sum of squares	Variance components	PV (%)	Fixation indices
<i>C. campestris</i>	<i>rbcl</i>	Among groups	1	192.532	8.48785	64.17	$F_{ST} = 0.62410^*$
		Among populations	8	31.457	-0.23247	-1.76	$F_{CT} = 0.64167^*$
		Within populations	35	174.033	4.97238	37.59	
		Total	44	398.022	13.22776		
	ITS	Among groups	1	191.309	7.66525	46.67	$F_{ST} = 0.67571^*$
		Among populations	8	165.547	3.43389	20.91	$F_{CT} = 0.46666^*$
		Within populations	35	186.433	5.32667	32.43	
		Total	44	543.289	16.42581		
<i>C. reflexa</i>	<i>rbcl</i>	Among groups	1	220.075	10.99848	98.88	$F_{ST} = 0.98840^*$
		Among populations	7	0.750	-0.00501	-0.05	$F_{CT} = 0.98885^*$
		Within populations	31	4.000	0.12903	1.16	
		Total	39	224.825	11.12250		
	ITS	Among groups		154.475	7.52582	76.54	$F_{ST} = 0.81081^*$
		Among populations	7	26.683	0.44646	4.54	$F_{CT} = 0.76540^*$
		Within populations	31	57.667	1.86022	18.92	
		Total	39	238.825	9.83250		

Note: Fixation indices represent average pairwise comparisons across all populations, $n = 10$ and $n = 9$ for *C. campestris* and *C. reflexa*, respectively. df, degrees of freedom; PV, percent variance; F_{ST} , proportion of variation among populations; F_{CT} , variance among groups.

* $P < .01$.

TABLE 3 Neutrality tests and mismatch distribution parameters in *Cuscuta* species

	Pop	Gene	Tajima's D (P)	Fu's F (P)	SSD (P)	Hrag (P)
<i>C. campestris</i>	MCKC	<i>rbcl</i>	-0.054 (.091)	1.872 (.043)*	0.232 (.090)	0.590 (.210)
		ITS	0.789 (.350)	2.225 (.160)	0.181 (.060)	0.470 (.180)
	KIAC	<i>rbcl</i>	-0.059 (.871)	-0.988 (.630)	0.060 (.500)	0.120 (.850)
		ITS	-0.076 (.043)*	1.325 (.053)	0.122 (.400)	0.470 (.670)
	NKUC	<i>rbcl</i>	-0.062 (.544)	0.552 (.350)	0.160 (.100)	0.43 (.150)
		ITS	-1.244 (.665)	-0.190 (.250)	0.083 (.350)	0.100 (.450)
	NRBC	<i>rbcl</i>	-0.093 (.323)	0.467 (.110)	0.109 (.080)	0.260 (.510)
		ITS	-0.019 (.010)*	-1.188 (.150)	0.098 (.230)	0.100 (.490)
	NANC	<i>rbcl</i>	-0.027 (.340)	0.010 (.023)*	0.132 (.160)	0.180 (.520)
		ITS	-0.868 (.100)	-0.261 (0.170)	0.106 (.080)	0.260 (.360)
	KSIC	<i>rbcl</i>	-0.028 (.665)	1.872 (.811)	0.185 (.250)	0.590 (.51)
		ITS	-0.024 (.061)	-1.361 (.630)	0.114 (.110)	0.260 (.63)
	NYAC	<i>rbcl</i>	-0.456 (.410)	-0.987 (.150)	0.060 (.480)	0.120 (.84)
		ITS	-1.134 (.090)	-1.475 (.430)	0.081 (.060)	0.290 (.55)
	VIAC	<i>rbcl</i>	-1.050 (.176)	0.646 (.537)	0.042 (.640)	0.075 (.75)
		ITS	-1.294 (.290)	-2.065 (.050)*	0.224 (.300)	0.260 (.860)
	KAKC	<i>rbcl</i>	-0.786 (.258)	-2.35 (.059)	0.095 (.040)*	0.277 (.040)*
		ITS	-2.743 (.060)	-1.103 (.320)	0.185 (.350)	0.660 (.783)
BUSC	<i>rbcl</i>	-0.382 (.444)	-0.510 (.205)	0.077 (.310)	0.160 (.440)	
	ITS	-1.215 (.410)	-1.188 (.130)	0.120 (.320)	0.230 (.980)	
<i>C. reflexa</i>	KSMR	<i>rbcl</i>	0.000 (.000)	0.000 (.000)	0.000 (.000)	0.000 (.000)
		ITS	-1.124 (.100)	-1.012 (.090)	0.057 (.990)	0.230 (.980)
	BUSR	<i>rbcl</i>	-0.817 (.287)	0.090 (.310)	0.007 (.990)	0.200 (1.000)

TABLE 3 (Continued)

Pop	Gene	Tajima's D (P)	Fu's F (P)	SSD (P)	Hrag (P)
	ITS	0.294 (.890)	1.872 (.900)	0.232 (.080)	0.590 (.160)
KAKR	<i>rbcL</i>	-0.817 (.287)	0.090 (.310)	0.007 (.990)	0.200 (1.000)
	ITS	1.743 (.890)	4.361 (.879)	0.394 (.000)	0.880 (.060)
SIAR	<i>rbcL</i>	0.000 (.000)	0.000 (.000)	0.000 (.000)	0.000 (.000)
	ITS	-1.215 (.020)*	2.535 (.100)	0.195 (.100)	0.470 (.140)
NRBR	<i>rbcL</i>	-0.028 (.299)	0.090 (.276)	0.008 (.040)*	0.200 (.890)
	ITS	0.000 (.000)	0.000 (.000)	0.000 (.000)	0.000 (.000)
NRKR	<i>rbcL</i>	-0.028 (.299)	0.090 (.276)	0.008 (.040)*	0.200 (.890)
	ITS	0.243 (.100)	-0.475 (.010)*	0.000 (.000)	0.110 (1.000)
BOMR	<i>rbcL</i>	-0.028 (.299)	0.090 (.276)	0.008 (.040)*	0.200 (.890)
	ITS	1.117 (.287)	3.065 (.100)	0.189 (.020)*	0.290 (.550)
NANR	<i>rbcL</i>	0.000 (.000)	0.000 (.000)	0.000 (.000)	0.000 (.000)
	ITS	0.000 (.000)	1.103 (.050)*	0.238 (.290)	0.444 (1.000)
MCKR	<i>rbcL</i>	0.000 (.000)	0.000 (.000)	0.000 (.000)	0.000 (.000)
	ITS	0.000 (.000)	0.000 (.000)	0.000 (.000)	0.000 (.000)

Note: *C. campestris* populations east of Kenya, include MCKC, KIAC, NKUC, and NRBC, whereas those to the west are NANC, KSIC, NYAC, VIAC, KAKC and BUSC. For *C. reflexa*, NRBR, NRKR, BOMR, and MCKR fall east, whereas NANR, KSMR, BUSR, KAKR and SIAR fall west of the country.

Pop = population; SSD = sum of square deviations; H_{RAG} = Raggedness index;

*Significant at $P \leq .05$.

(except H4 in *C. reflexa*) were shared within, but not between, localities in the east and west of the country. We also observed that populations farthest from each other were genetically distinct, albeit at no statistical significance (except *C. campestris* based on ITS), suggesting a small isolation-by-distance effect. The only exception to this was observed in taxa from Nandi, which despite being geographically closer to western localities, clustered and shared haplotypes with eastern populations. Taken together, these results suggest existence of more than one center of genetic diversity, on either side of the country. We attribute this allopatric pattern of distribution to multiple introductions of propagules, and a complex interaction between the invasive potential of *Cuscuta* and the environment. The discovery of multiple centers of genetic diversity is particularly concerning given evidence that parasitic plants are expected to have faster rates of molecular evolution across all three plant genomes, relative to their nonparasitic counterparts (Bromham et al., 2013).

Although dodder seeds lack specialized adaptations for dispersion, long-distance spread through bird endozoochory (Costea et al., 2016, 2019) and water (Ho & Costea, 2018) have been documented. In addition, we speculate that human-aided activities could be playing a key role in propagule exchange within populations. These activities could be direct, through movement of seeds and vines to new localities, as has previously been described (Knepper et al., 1990), or indirect, through habitat-modifying activities such as agriculture, hedge establishments and infrastructural developments, which have previously been shown to create alternative dispersal routes and contribute to the spread of other invasive weeds (Hodkinson & Thompson, 1997; Rouget & Richardson, 2003). Currently, naturalization of

invasive species, such as *Thevetia peruviana* and *Duranta erecta*, coupled with their extensive cultivation across Kenya (as hedges) have arguably modified habitats, and created new avenues for the spread of other (invasive parasitic) weeds, such as *Cuscuta* spp. that parasitize them.

Since *Cuscuta* spp. species propagate through both vines and seeds, we believe that this mode of propagation, coupled with their ability to parasitize multiple hosts, may be playing a key role in the parasites' successful invasion, and spread. The high genetic variation, observed herein, suggests frequent gene flow within regions, possibly through seed exchange or outcrossing. Previous evidence has indicated that successful invasion by alien species largely depends on their life history, population genetic parameters, particularly phenotypic plasticity (Barrett et al., 2008) and dispersal events (Arendt, 2015). These parameters shape the patterns of dispersal of propagules into a new environment, subsequent adaptation to the prevailing conditions and expansion. In many species, invaders that predominantly propagate vegetatively are expected to have a lower genetic diversity compared with their seed-propagated counterparts (Barrett et al., 2008). This asexual reproduction is advantageous when population sizes are small (Barrett et al., 2008; Lambrinos, 2001), although genetic bottlenecks may hinder successful expansion, especially in competitively more demanding environments. In addition, vegetative propagations limit a species' ability for genetic admixture, through reduced gene flow and low recombination (Eckert, 2002). As expected, all *Cuscuta* populations followed an isolation by distance pattern, with the only exception observed in Nandi. Despite being geographically closer to western localities, this population shared

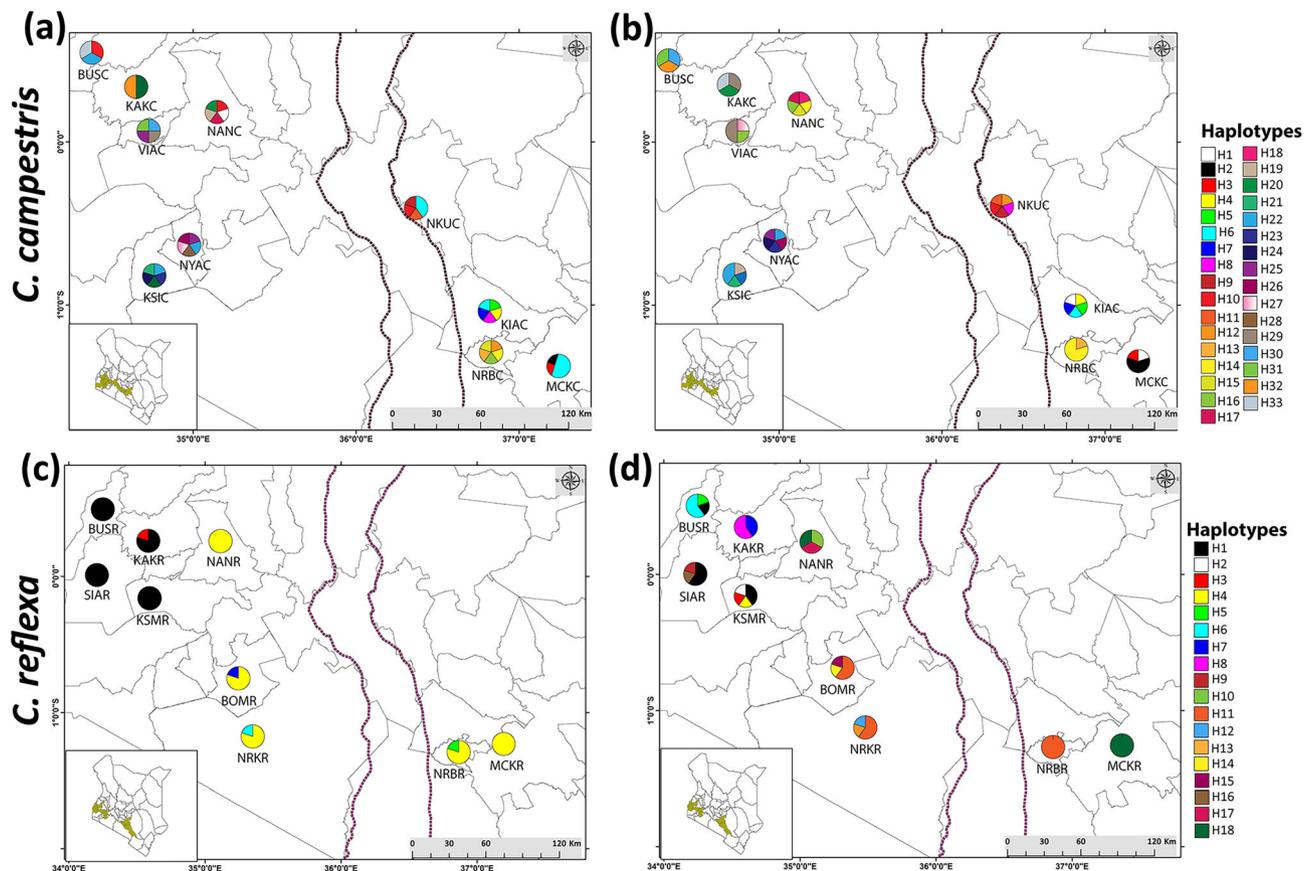


FIGURE 2 Geographical distribution of haplotypes across sampled localities. The upper panel represents *Cuscuta campestris* distribution, based on *rbcL* (a) and ITS (b), whereas the lower panel represents *Cuscuta reflexa* based on *rbcL* (c) and ITS (d). Pie charts are colored according to the haplotypes (as indicated in keys after each panel). The range of *Cuscuta* spp. occurrence is indicated on the full map of Kenya (inset), while the purple dotted lines indicate the Rift Valley depression. *C. campestris* populations east of Kenya include MCKC, KIAC, NKUC, and NRBC, whereas those to the west are KSIC, NYAC, VIAC, NANC, KAKC, and BUSC. For *C. reflexa*, NRBR and MCKR are east, whereas KSMR, BUSR, KAKR, SIAR, NRKR, BOMR, and NANR fall west of the country

haplotypes and clustered with those from east of the country, possibly because it could be an introduction from these localities. Overall, the lack of haplotype sharing and the IBD pattern suggests constrained long-distance dispersal across western and eastern groups.

Interestingly, we noted some striking similarities in genetic variation between the two *Cuscuta* species under this study. First, both species exhibited an allopatric pattern of distribution, as evidenced by two clades that corresponded to localities in the west and east of Kenya. Second, both species exhibited significantly higher genetic differentiation ($N_{ST} > G_{ST}$) across all populations, and third, the highest variation in both species was attributed to groups within, rather than among populations. Taken together, these findings suggest that both species may be employing near-similar historical characteristics during colonization and expansion into the observed localities. Conversely, *t* test results indicated that *C. campestris* populations had higher average gene diversities and heterozygosity than *C. reflexa*, across the sampled localities, possibly due to differences in demographic histories between the species. Particularly, *C. campestris* has been around longer than *C. reflexa*, thus has had more time for mixing and sharing of haplotypes among localities after introduction. We also speculate

that *C. campestris*, a naturalized invader, could be predominantly propagating through seed, hence enhanced mixing among populations, whereas *C. reflexa* may be largely spreading via vines although this remains to be confirmed. *C. reflexa* has larger seeds than *C. campestris*, although previous researchers found no evidence of any association between seed size and geographical distribution range among members of the genus *Cuscuta* (Olszewski et al., 2020). Future investigations are expected to elucidate the relationship between reproductive plasticity and the observed genetic variability in *Cuscuta* spp.

5 | IMPLICATIONS FOR MANAGEMENT

Our findings have far-reaching implications for strategic management of alien invasive species, such as *Cuscuta* spp. Management of alien species invasions falls into four stages, namely, prevention, eradication, containment, and asset-based protection, with substantially improved cost to benefit ratios for management during early stages of prevention and eradication, relative to later invasion stages (Harris et al., 2018). Evidence from our previous research revealed little

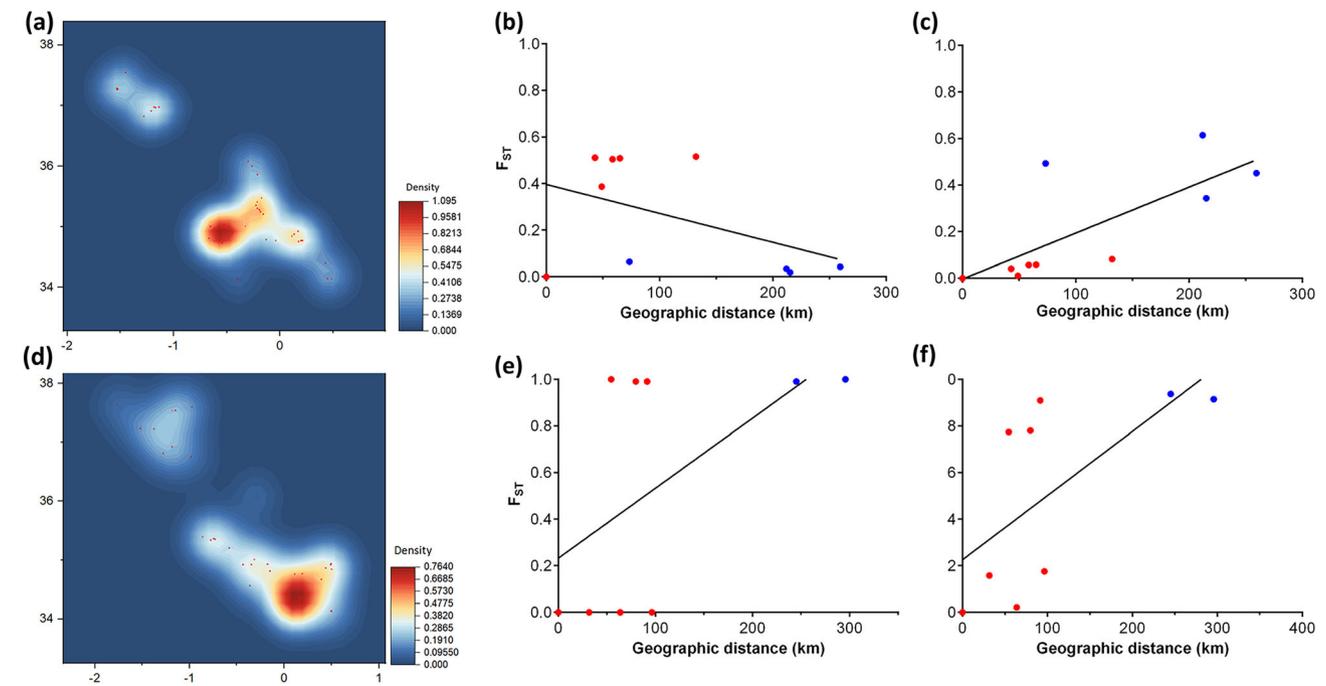


FIGURE 3 Isolation by distance showing the relationship between genetic and geographical distances among populations. The upper panel represents *Cuscuta campestris* datasets as follows; (a) density estimation of sampled points. (b) and (c) are correlations between genetic and geographical distances based on *rbcL* ($R^2 = .2065$, $P = .1871$) and ITS ($R^2 = .5564$, $P = .01130$), respectively. The lower panel represents *C. reflexa* datasets as follows; (d) density estimation of *C. reflexa* points. (e) and (f) are correlations based on *rbcL* ($R^2 = .3191$, $P = .1130$) and ITS ($R^2 = .4262$, $P = .0567$), respectively. Points marked in red (in graphs b, c, e, and f) indicate populations west of Kenya, whereas those in blue denote populations to the east. Density was estimated using a two-dimensional kernel density estimation in OriginPro v9.8. X and Y axes on the density plots represent latitude and longitude, respectively

parasitism on crops, where all hosts (except mango) were non-agricultural, suggesting that *Cuscuta* spp., have not yet shifted to parasitizing agricultural crops in Kenya, though they pose a significant risk to economically important crops such as tea and coffee (Masanga et al., 2021). In other places, such as neighboring Uganda, *Cuscuta* spp., have been found parasitizing coffee (*Coffea robusta*) (Kagezi et al., 2021). We suggest that current *Cuscuta* spp. invasions in Kenya are at the early stages of establishment; therefore, rapid eradication of existing populations is urgently needed before spread and adaptation to economically important hosts. Emphasis should be placed on limiting new introductions across populations, before genetic mixing, as this may enhance genetic variability and increase adaptability. Because seed plays an important role in gene flow and establishment of new populations, whereas vines ensure continuity of the existing ones, we propose that efforts should be put in place to prevent deliberate or inadvertent dispersal of these propagules, while eradicating existing populations.

ACKNOWLEDGEMENTS

We acknowledge efforts by Christine Namaemba and Anthony Kweyu during sample collection. This work was supported by funds from Kenyatta University, through the Vice Chancellors Research grant number KU/DVCR/VRG/VOL.11/216. JM's PhD is funded by the National Research Fund of Kenya grant number NRF/PhD/02/76.

AUTHOR CONTRIBUTION

SR conceptualized the study, provided funds, and edited the manuscript; JM performed experiments, analyzed data, and wrote the draft manuscript; ESB contributed to the study design, guided data analysis, and edited the manuscript; RO, AA, MN, and PO assisted with conceptual support and manuscript editing. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data used are provided in the manuscript text. Additional data are provided in the Supporting Information.

ORCID

Joel Masanga  <https://orcid.org/0000-0002-5933-2662>

Steven Runo  <https://orcid.org/0000-0002-9043-2338>

REFERENCES

- Arendt, J. D. (2015). Effects of dispersal plasticity on population divergence and speciation. *Heredity*, 115, 306–311. <https://doi.org/10.1038/hdy.2015.21>
- Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A., & Saunders, N. C. (1987). Intraspecific phylogeography:

- The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology, Evolution, and Systematics*, 18, 489–522. <https://doi.org/10.1146/annurev.es.18.110187.002421>
- Baldwin, B. G. (1992). Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the compositae. *Molecular Phylogenetics and Evolution*, 1(1), 3–16. [https://doi.org/10.1016/1055-7903\(92\)90030-K](https://doi.org/10.1016/1055-7903(92)90030-K)
- Barrett, S. C. H., Colautti, R. I., & Eckert, C. G. (2008). Plant reproductive systems and evolution during biological invasion. *Molecular Ecology*, 17, 373–383. <https://doi.org/10.1111/j.1365-294X.2007.03503.x>
- Bermingham, E., & Moritz, C. (1998). Comparative phylogeography: Concepts and applications. *Molecular Ecology*, 7, 367–369. <https://doi.org/10.1046/j.1365-294x.1998.00424.x>
- Bradshaw, A. D. (1972). Some of the evolutionary consequences of being a plant. *Evolutionary Biology*, 5, 25–47.
- Bromham, L., Cowman, P. F., & Lanfear, R. (2013). Parasitic plants have increased rates of molecular evolution across all three genomes. *BMC Evolutionary Biology*, 13, 126. <https://doi.org/10.1186/1471-2148-13-126>
- Costea, M., El Miari, H., Laczko, L., Fekete, R., Molnár, A. V., Lovas-Kiss, Á., & Green, A. J. (2019). The effect of gut passage by waterbirds on the seed coat and pericarp of diaspores lacking “external flesh”: Evidence for wide-spread adaptation to endozoochory in angiosperms. *PLoS ONE*, 14(12), e0226551. <https://doi.org/10.1371/journal.pone.0226551>
- Costea, M., Stefanović, S., García, M. A., De La Cruz, S., Casazza, M. L., & Green, A. J. (2016). Waterfowl endozoochory: An overlooked long-distance dispersal mode for *Cuscuta* (dodder). *American Journal of Botany*, 103, 957–962. <https://doi.org/10.3732/ajb.1500507>
- Ditchfield, A. D. (2000). The comparative phylogeography of Neotropical mammals: Patterns of intraspecific mitochondrial DNA variation among bats contrasted to nonvolant small mammals. *Molecular Ecology*, 9, 1307–1318. <https://doi.org/10.1046/j.1365-294x.2000.01013.x>
- Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, 17, 431–449. <https://doi.org/10.1111/j.1365-294X.2007.03538.x>
- Dörr, I. (1969). Fine structure of intracellularly growing *Cuscuta* hyphae. *Protoplasma*, 67, 123–137. <https://doi.org/10.1007/BF01248735>
- Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.
- Dupanloup, I., Schneider, S., & Excoffier, L. (2002). A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, 11, 2571–2581. <https://doi.org/10.1046/j.1365-294x.2002.01650.x>
- Eckert, C. G. (2002). The loss of sex in clonal plants. In J. F. Stuefer, B. Erschbamer, H. Huber, & J. I. Suzuki (Eds.), *Ecology and evolutionary biology of clonal plants*. Springer. https://doi.org/10.1007/978-94-017-1345-0_15
- Elsiddig, M. A., Mahadi, Y. M., Mohamed, E. H., Alamin, S. E., & Ismael, A. (2018). Genetic diversity of dodder (*Cuscuta* Spp.) collected from Khartoum and Gezira states. *International Journal of Science and Research*, 8, 634–640. <https://doi.org/10.29322/IJSRP.8.12.2018.p8482>
- Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567. <https://doi.org/10.1111/j.1755-0998.2010.02847.x>
- Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications*, 11, 1035–1052. <https://doi.org/10.1111/eva.12569>
- Fu, Y. X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, 147, 915–925.
- García, M. A., Costea, M., Kuzmina, M., & Stefanović, S. (2014). Phylogeny, character evolution, and biogeography of *Cuscuta* (Dodders; Convolvulaceae) inferred from coding plastid and nuclear sequences. *American Journal of Botany*, 101, 670–690. <https://doi.org/10.3732/ajb.1300449>
- Gaut, B. S., Muse, S. V., Clark, W. D., & Clegg, M. T. (1992). Relative rates of nucleotide substitution at the *rbcL* locus of monocotyledonous plants. *Journal of Molecular Evolution*, 35, 292–303. <https://doi.org/10.1007/BF00161167>
- Hamilton, M. B., & Miller, J. R. (2002). Comparing relative rates of pollen and seed gene flow in the island model using nuclear and organelle measures of population structure. *Genetics*, 162, 1897–1909.
- Harris, S., Elliott, C., Woolnough, A., & Barclay, C. A. (2018). Heuristic Framework for Invasive Species Research Planning and Measurement. Developing an Invasive Species Research Strategy in Tasmania. In Queen Victoria Museum and Art Gallery (Launceston, Tas.) Issue 117: ISSN 0085-5278.
- Ho, A., & Costea, M. (2018). Diversity, evolution and taxonomic significance of fruit in *Cuscuta* (dodder, Convolvulaceae); the evolutionary advantages of indehiscence. *Perspectives in Plant Ecology, Evolution and Systematics*, 32, 1–17. <https://doi.org/10.1016/j.ppees.2018.02.001>
- Hodkinson, D. J., & Thompson, K. (1997). Plant dispersal: The role of man. *Journal of Applied Ecology*, 34, 1484–1496. <https://doi.org/10.2307/2405264>
- Holm, L., Pancho, J. V., Herberger, J. P., & Plucknett, L. P. (1979). *A geographical atlas of world weeds*. Wiley. <https://www.cabi.org/isc/abstract/19802330214>
- Kagezi, G. H., Kyalo, G., Kobusinge, J., Nkuutu, E., Baluku, J., Arinaitwe, G., & Niyibigira, E. I. (2021). Rapid assessment of the invasive dodder weed, *Cuscuta* Spp. on Robusta coffee, *Coffea robusta* in Busoga coffee growing sub-region, eastern Uganda. *East African Scholars Journal of Agriculture and Life Sciences*, 4(3), 55–66. <https://doi.org/10.36349/easjals.2021.v04i03.003>
- Kaiser, B., Vogg, G., Fürst, U. B., Albert, M., & Westwood, J. H. (2015). Parasitic plants of the genus *Cuscuta* and their interaction with susceptible and resistant host plants. *Frontiers in Plant Science*, 6, 1–9. <https://doi.org/10.3389/fpls.2015.00045>
- Knepper, D. A., Creager, R. A., & Mussleman, L. J. (1990). Identifying dodder seed as contaminants in seed shipments. *Seed Science and Technology*, 18, 731–741.
- Kolbe, J. J., Glor, R. E., Schettino, R. L., Lara, A. C., Larson, A., & Losos, J. B. (2004). Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, 431, 177–181. <https://doi.org/10.1038/nature02807>
- Kuijt, J. (1969). *The biology of parasitic flowering plants* (Vol. 246). University of California Press.
- Lambrinos, J. G. (2001). The expansion history of a sexual and asexual species of *Cortaderia* in California, USA. *Journal of Ecology*, 89, 88–98. <https://doi.org/10.1046/j.1365-2745.2001.00524.x>
- Lanini, W. T., & Kogan, M. (2005). Biology and management of *Cuscuta* in crops. *Ciencia e Investigación Agraria*, 32, 165–179. <https://doi.org/10.7764/rcia.v32i3.317>
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Leigh, J. W., & Bryant, D. (2015). popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116. <https://doi.org/10.1111/2041-210X.12410>

- Levin, D. A. (1981). Dispersal versus gene flow in plants. *Annals of the Missouri Botanical Garden*, 68, 233–253. <https://doi.org/10.2307/2398797>
- Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>
- Loveless, M. D., & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual Reviews in Ecology and Systematics*, 15, 65–95. <https://doi.org/10.1146/annurev.es.15.110184.000433>
- Masanga, J., Mwangi, B. N., Kibet, W., Sagero, P., Wamalwa, M., Oduor, R., Ngugi, M., Alakonya, A., Ojola, P., Bellis, E. S., & Runo, S. (2021). Physiological and ecological warnings that dodder pose an exigent threat to farmlands in Eastern Africa. *Plant Physiology*, 185(4), 1457–1467. <https://doi.org/10.1093/plphys/kiab034>
- McNeal, J. R., Arumugunathan, K., Kuehl, J. V., Boore, J. L., & dePamphilis, C. W. (2007). Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus *Cuscuta* (Convolvulaceae). *BMC Biology*, 5, 1–19. <https://doi.org/10.1186/1741-7007-5-55>
- Meyerson, L. A., & Mooney, H. A. (2007). Invasive alien species in an era of globalization. *Frontiers in Ecology and the Environment*, 5, 199–208. [https://doi.org/10.1890/1540-9295\(2007\)5\[199:IASIAE\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2007)5[199:IASIAE]2.0.CO;2)
- Mutikainen, P., & Koskela, T. (2002). Population structure of a parasitic plant and its perennial host. *Heredity*, 89, 318–324. <https://doi.org/10.1038/sj.hdy.6800142>
- Olzewski, M., Dillio, M., García-Ruiz, I., Bendarvandi, B., & Costea, M. (2020). *Cuscuta* seeds: Diversity and evolution, value for systematics/identification and exploration of allometric relationships. *PLoS ONE*, 15, 1–23. <https://doi.org/10.1371/journal.pone.0234627>
- Pons, O., & Petit, R. J. (1996). Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, 144, 1237–1245.
- Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution*, 25, 1253–1256. <https://doi.org/10.1093/molbev/msn083>
- Prentis, P. J., Wilson, J. R. U., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Plant Science*, 13, 288–294. <https://doi.org/10.1016/j.tplants.2008.03.004>
- Ren, Z., Zagortchev, L., Ma, J., Yan, M., & Li, J. (2020). Predicting the potential distribution of the parasitic *Cuscuta chinensis* under global warming. *BMC Ecology*, 20, 28. <https://doi.org/10.1186/s12898-020-00295-6>
- Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Rouget, M., & Richardson, D. M. (2003). Inferring process from pattern in plant invasions: A semimechanistic model incorporating propagule pressure and environmental factors. *The American Naturalist*, 162, 713–724. <https://doi.org/10.1086/379204>
- Schaal, B. A., Hayworth, D. A., Olsen, K. M., Rauscher, J. T., & Smith, W. A. (1998). Phylogeographic studies in plants: Problems and prospects. *Molecular Ecology*, 7, 465–474. <https://doi.org/10.1046/j.1365-294x.1998.00318.x>
- Schulz, A. N., Lucardi, R. D., & Marsico, T. D. (2019). Successful invasions and failed biocontrol: The role of antagonistic species interactions. *Bioscience*, 69, 711–724. <https://doi.org/10.1093/biosci/biz075>
- Sork, V. L., Gugger, P. F., Chen, J.-M., & Werth, S. (2016). Evolutionary lessons from California plant phylogeography. *Proceedings of the National Academy of Sciences*, 113, 8064–8071. <https://doi.org/10.1073/pnas.1602675113>
- Sork, V. L., & Smouse, P. E. (2006). Genetic analysis of landscape connectivity in tree populations. *Landscape Ecology*, 21, 821–836. <https://doi.org/10.1007/s10980-005-5415-9>
- Stefanović, S., Kuzmina, M., & Costea, M. (2007). Delimitation of major lineages within *Cuscuta* subgenus *Grammica* (Convolvulaceae) using plastid and nuclear DNA sequences. *American Journal of Botany*, 94, 568–589. <https://doi.org/10.3732/ajb.94.4.568>
- Tajima, F. (1989). The effect of change in population size on DNA polymorphism. *Genetics*, 123(3), 597–601.
- Templeton, A. (2002). Out of Africa again and again. *Nature*, 416, 45–51. <https://doi.org/10.1038/416045a>
- Tsutsui, N. D., Suarez, A. V., Holway, D. A., & Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences*, 97, 5948–5953. <https://doi.org/10.1073/pnas.100110397>
- Ueno, S., Rodrigues, J. F., Alves-Pereira, A., Pansarin, E. R., & Veasey, E. A. (2015). Genetic variability within and among populations of an invasive, exotic orchid. *AoB Plants*, 7, plv077. <https://doi.org/10.1093/aobpla/plv077>
- Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, 23, 5649–5662. <https://doi.org/10.1111/mec.12938>
- Ward, S. M., Gaskin, J. F., & Wilson, L. M. (2008). Ecological genetics of plant invasion: What do we know? *Invasive Plant Science and Management*, 1, 98–109. <https://doi.org/10.1614/IPSM-07-022.1>
- Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: Approximate methods. *Journal of Molecular Evolution*, 39, 306–314. <https://doi.org/10.1007/BF00160154>
- Yuncker, T. G. (1932). The genus *Cuscuta*. *Torrey Botanical Society*, 18, 113–331.
- Zhang, D.-X., & Hewitt, G. M. (2003). Nuclear DNA analyses in genetic studies of populations: Practice, problems and prospects. *Molecular Ecology*, 12, 563–584. <https://doi.org/10.1046/j.1365-294x.2003.01773.x>

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Masanga, J., Oduor, R., Alakonya, A., Ngugi, M., Ojola, P., Bellis, E. S., & Runo, S. (2022). Comparative phylogeographic analysis of *Cuscuta campestris* and *Cuscuta reflexa* in Kenya: Implications for management of highly invasive vines. *Plants, People, Planet*, 4(2), 182–193. <https://doi.org/10.1002/ppp3.10236>