

Processes and drivers of *Prosopis* invasions in Eastern Africa

By

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DECLARATION

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third part rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature:

Maria Loreto Castillo

Date: December 2019

ABSTRACT

Increased movement of humans and goods around the world has facilitated the transportation of many species into new geographic ranges. A significant number of these have become invasive, resulting in substantial ecological, social, and economic impacts. In order to develop effective management strategies, it is necessary to elucidate the drivers underlying invasion and to understand what determines species invasiveness. Progress in the understanding and management of biological invasions depends on proper taxonomic identification of invasive species. However, the taxonomy of many alien taxa remains problematic due to unresolved species relationships, geographic distributions and/or inter-specific hybridization, among others.

Mequite trees from the genus *Prosopis* are problematic invasive species in many parts of the world. To resolve taxonomic uncertainty among *Prosopis* species globally, I used phylogenetic and population genetic approaches to examine evolutionary relationships and levels of genetic diversity and population genetic similarity among *Prosopis* species collected from four native regions (Argentina, Chile, Mexico and Peru) and six non-native regions (Australia, Hawaii, Kenya, Ethiopia, Tanzania and South Africa). The genetic analysis showed high phylogenetic similarity, low genetic differentiation between species from the native range and provided evidence for inter-specific hybridization between different *Prosopis* species in both native and non-native ranges. My findings suggest that hybridization between previously allopatric species may occur frequently when they are co-introduced into new ranges. In addition, polyploid individuals were detected in both native and non-native areas, with tetraploid *P. juliflora* being highly differentiated from other diploid *Prosopis* species. Polyploidy is therefore proposed as an additional mechanism that facilitates reproductive isolation between some *Prosopis* species. Lastly, levels of genetic diversity suggest that invasive populations in Eastern Africa (Kenya and Ethiopia) probably resulted from multiple introductions of two species, *P. juliflora* and *P. pallida*.

Prosopis invasion in Eastern Africa provided an excellent opportunity to examine how the ecological and genetic attributes of invasiveness, and drivers of invasion success, vary with context and taxon, because the founder trees of two species, tetraploid *P. juliflora* and

diploid *P. pallida*, are still present in the original plantations today. Here, I exploited these unique circumstances and examined the mechanisms – such as plasticity, rapid post-introduction evolution and hybridization – that contribute to the invasion success of these trees in Baringo County, Kenya and Afar Region, Ethiopia. I found that in Baringo County, despite the similar invasion history of *P. pallida* and *P. juliflora* and probable inter-species hybridization, only *P. juliflora* individuals became invasive in the region; indicating that the success of *Prosopis* invasion is not attributed to hybridization but potentially to the higher ploidy of *P. juliflora*. Similarly, in Ethiopia’s Afar Region, genotypes consisted exclusively of *P. juliflora*. In Kenya’s Baringo County, I performed common garden and reciprocal field transplant experiments that indicated that high levels of phenotypic plasticity and post-introduction evolution had contributed to the invasiveness of *P. juliflora*. Similar levels of plasticity were absent from introduced, but non-invasive, *P. pallida*. My results also showed that different demographic processes may be occurring in the Afar region (Ethiopia) and Baringo County (Kenya). In the latter, contemporary genetic change during the invasive spread, or founder effects during initial range expansion from plantations, may explain the genetic erosion I found along the range expansion of *Prosopis*. In Afar Region, successful spread may have been promoted by gene flow from “source” plantations to invasive genotypes, homogenizing standing genetic diversity across the invasion. Lastly, by using landscape resistance modelling in both areas, I showed that dispersal among *Prosopis* populations was not influenced by any of the attributes analysed: physical distance between populations, variables related to human and animal-mediated dispersal along roads and rivers, bioclimatic and altitudinal conditions. Therefore, the dispersal of *Prosopis* populations was not constrained by any landscape variable, and probably involved frequent human-assisted long-distance dispersal. Overall, this study showed that hybridization, polyploidy or both have contributed to the invasiveness of *Prosopis*.

Finally, this study formed part of a larger international collaborative project entitled “Woody invasive alien species (IAS) in Eastern Africa: assessing and mitigating their negative impacts on ecosystem services and rural livelihoods” (hereafter referred to as Woody Weeds), with the overall objective of mitigating the impacts of woody IAS on biodiversity, ecosystem services and rural livelihoods in Eastern Africa. For this, basic knowledge about the invasion process and the impacts of woody IAS are being evaluated and diverse control and sustainable

land management strategies are being proposed. In collaboration with PhD students involved in the Woody Weeds project, I propose key components of research projects addressing complex social-ecological topics that facilitate inter-disciplinary and, when interacting with stakeholders, trans-disciplinary research. Trans-disciplinary approaches should have a clear structure that transcends disciplines through a multidisciplinary team with common goals. To allow for integration and upscaling of findings, there should be a co-design of data collection using different methodologies in the same experimental units/scales. It is important to have the clear intention to identify management options with stakeholders, estimate their effects and test their implementation, as well as provide transdisciplinary training for all project participants.

To facilitate the integration of the drivers of alien plant invasion into the development of effective management options, I concluded with a discussion of two principal questions: (i) How does an improved understanding of the eco-evolutionary drivers of invasiveness help us to better manage the problem? (ii) What implications does the better understanding of genetic and ecological drivers have for the use of particular control methods, especially biological control?

OPSOMMING

Die toenemende beweging van mense en goedere oor die wêreld heen veroorsaak dat baie spesies na nuwe voorkomsgebiede vervoer word. 'n Beduidende aantal van dié spesies word indringerspesies, met wesenlike ekologiese, sosiale en ekonomiese impakte. Ten einde effektiewe bestuurstrategieë te ontwikkel is dit nodig om die drywers van indringing en die indringingsvermoë van spesies beter te verstaan. Vooruitgang in die verstaan en bestuur van biologiese indringings berus op behoorlike taksonomiese identifikasie van indringerspesies. Die taksonomie van baie uitheemse taksa bly egter problematies weens, onder andere, onopgeloste spesieverwantskappe en geografiese verspreidings en/of kruisteling tussen spesies.

Prosopis bome in die genus *Prosopis* is ernstige indringers in baie wêrelddele. Ten einde die taksonomiese onsekerheid van *Prosopis* spesies op te klaar, het ek filogenetika- en populasie genetika-benaderings gebruik om die evolusionêre verwantskappe, vlakke van genetiese diversiteit en populasie genetiese struktuur in vier inheemse streke (Argentinië, Meksiko, Chile en Peru) en ses nie-inheemse streke (Australië, Hawaii, Kenia, Etiopië, Tanzanië en Suid-Afrika) te ondersoek. Dié genetiese analise het hoë filogenetiese eendersheid aangedui, lae genetiese onderskeiding tussen spesies met soortgelyke inheemse verspreidings en dat kruisteling tussen verskillende *Prosopis* spesies in beide inheemse en nie-inheemse verspreidingsgebiede geskied. My bevindings dui daarop dat kruisteling dikwels kan plaasvind tussen voorheen-allopatriese spesies wanneer sulke spesies gesamentelik na nuwe areas ingevoer word. Poliploïed individue is gevind in beide inheemse en nie-inheemse areas, met tetraploïede *P. juliflora* wat hoogs gedifferensieer was van ander diploïede *Prosopis* spesies. Die voorkoms van poliploïed variasie word dus voorgestel as 'n addisionele meganisme wat voortplantingsisolasië tussen sekere *Prosopis* spesies fasiliteer. Laastens dui die vlakke van genetiese diversiteit daarop dat die indringerbevolkings in Oos-Afrika (Kenia en Etiopië) waarskynlik die gevolg is van veelvoudige vrylatings van twee spesies, *P. juliflora* en *P. pallida*.

Die indringing van *Prosopis* in Oos-Afrika bied 'n uitstekende geleentheid aan om die ekologiese en genetiese eienskappe van indringing en die drywers van suksesvolle indringing verskil tussen kontekste en taksa te ondersoek, aangesien die stigterbome van twee spesies, die tetraploïede *P. juliflora* en die diploïede *P. pallida*, steeds teenwoordig is in hulle

oorspronklike plantasies. In hierdie studie het ek dié unieke omstandighede benut om die meganismes – soos fenotipiese plastisiteit, kruisteling en evolusie – te ondersoek wat bygedra het tot die suksesvolle indringing van hierdie bome in Baringo-streek, Kenia en die Afar-streek in Etiopië. Ek het bevind dat slegs *P. juliflora* in die Baringo streek 'n indringer geword het, ten spyte van die soortgelyke indringingsgeskiedenis van *P. pallida* en *P. juliflora* en waarskynlike kruisteling tussen die spesies. Dit dui daarop dat die suksesvolle indringing van *Prosopis* nie toegeskryf moet word aan kruisteling tussen *P. pallida* en *P. juliflora* nie, maar moontlik aan die hoër poliploïd vlakke van *P. juliflora*. In die Afar-streek van Etiopië is al die genotipes die van *P. juliflora*. In Kenia se Baringo -streek het ek 'n tuinekperiment en 'n wedersydse oorplantingeksperiment uitgevoer, wat getoon het dat hoë vlakke van fenotipiese plastisiteit en evolusie bygedra het tot die indringing van *P. juliflora*. Soortgelyke vlakke van plastisiteit is nie gevind in *P. pallida* nie. My resultate wys ook dat daar moontlik verskillende demografiese prosesse plaasvind in die Baringo- en Afar-streke. In die Baringo -streek kan genetiese verandering gedurende die indringingsproses, of stigtereëffekte tydens die aanvanklike verspreiding vanuit plantasies, moontlik die verlaging van genetiese variasie wat ek waargeneem het in die verspreiding van *Prosopis* verklaar.

In die Afar-streek is die suksesvolle verspreiding moontlik bevorder deur deurlopende geenvloei tussen oorspronklike plantasies en indringerbevolkings, wat die genetiese diversiteit oor die indringerareas homogeniseer. Laastens het ek deur middel van landskapweerstandbiedendheids-modelle in beide areas getoon dat die beweging tussen *Prosopis* populasies nie beïnvloed word deur enige van die volgende eienskappe nie: fisiese afstand tussen bevolkings, veranderlikes verwant aan plant verspreiding deur mense en diere langs paaie en riviere, of bioklimatiese en topografiese faktore. Dus word die verspreiding van *Prosopis* bevolkings nie beperk weens landskapveranderlikes nie en word waarskynlik bevorder deur gereelde langafstand-verspreiding deur mense. Om op te som, bewys hierdie studie dat kruisteling, poliploïdvorming, of beide dié faktore bygedra het tot die suksesvolle indringing van *Prosopis* in Oos-Afrika.

Hierdie studie was deel van 'n groter internasionale projek, naamlik “Woody invasive alien species (IAS) in Eastern Africa: assessing and mitigating their negative impacts on ecosystem services and rural livelihoods” (hierna verwys as “Woody Weeds”), met die oorhoofse doel om die impakte van indringerplante op biodiversiteit, ekosisteedienste en landelike

gemeenskappe in Oos-Afrika te verminder. Dít benodig kennis van die indringingsproses en die impakte van indringerplante, sowel as die ontwikkeling van verskeie beheermetodes en volhoubare grondbestuurstrategieë. In samewerking met ander PhD-studente van die Woody Weeds-projek, stel ek die sleutelkomponente voor van navorsingsprojekte wat komplekse sosio-ekologiese onderwerpe, soos indringerspesies, te ondersoek deur inter- en transdissiplinêre benaderings. Transdissiplinêre benaderings behoort duidelike projekstruktuur te hê wat verskeie dissiplines inkorporeer deur middel van 'n multi-dissiplinêre span met gemeenskaplike navorsingsdoelwitte. Om navorsingsbevindings te kan integreer en uit te bou, moet data-insameling gesamentelik deur alle projekdeelnemers ontwerp word, sodat verskillende metodes vir dieselfde skaal of eksperimentele eenhede gebruik kan word. Dit is belangrik om bestuursopsies saam met belanghebbers te identifiseer, om die effekte en implementering van die bestuursopsies te toets, sowel as om transdissiplinêre opleiding aan te bied aan alle projekdeelnemers.

Ten einde die drywers van indringing deur uitheemse plante beter te integreer in die ontwikkeling van effektiewe bestuursopsies, sluit ek af met 'n bespreking van twee kritiese vrae: (i) Hoe kan 'n beter begrip van die eko-evolutionêre drywers van indringing ons help om die probleem beter te bestuur? (ii) Watter implikasies het insigte in die genetiese en ekologiese drywers van indringerspesies vir spesifieke beheermetodes, veral biologiese beheer?

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Figure 2.3. Hierarchical Bayesian clustering analyses of individuals of native (black labels) and non-native (red labels) areas of various *Prosopis* species: Argentina (Ar) = *P. chilensis*, *P. flexuosa*, *P. strombulifera*, *P. alba*, *P. nigra*, *P. torquata* and *P. vinalillo* and hybrids; Chile (Ch) = *P. chilensis* and *P. alba*; Mexico (Me) = *P. juliflora* and *P. laevigata*; Peru (Pe) = *P. pallida*; Australia (Au) = *P. pallida*, *P. glandulosa*, *P. velutina* and hybrids; Ethiopia (Et) = *P. juliflora*; Hawaii (Hw) = *P. pallida*; Kenya (Ke) = *P. juliflora* and *P. pallida*; South Africa (SA) = *Prosopis* spp.; Tanzania (Tz) = *P. juliflora*. Individuals were genotyped using seven nuclear microsatellite loci and clustered at three levels. a) Level 1: '*P. juliflora*' cluster in orange and 'other *Prosopis* species' cluster in blue; b) Level 2: only *P. juliflora* individuals and c) Level 2: '*P. pallida*' cluster in blue 'other *Prosopis* species' cluster in green; d) Level 3: individuals of 'other *Prosopis* species' cluster from Argentina, Chile, Mexico, Australia and South Africa. Vertical axes represent the assignment (q_{ik} values) of individual genomes to the inferred number of genetic clusters, in all cases $K=2$ (See Fig. S1).

Figure 2.4. Principal component analysis showing genetic structure among *Prosopis* species from different native and invaded areas. PCA was performed using Bruvo distances in *PolySat*

(Bruvo et al. 2004). PC1 and PC2 captured 63.6% and 11,0% of the variation, respectively. Individuals of tetraploid *P. juliflora* are separated from the rest of individuals of other species.

Figure 2.5. Pairwise F_{ST} (\pm 95% confidence interval) between native Mexico (Me) and invaded populations of *P. juliflora* in Ethiopia (Et), Kenya (Ke) and Tanzania (Tz); between invaded population of *P. juliflora*; between native populations of Peru (Pe) and introduced populations of Kenya (Ke) e invasive populations of Hawaii (Hw) of *P. pallida*.

Figures 3.1. Experimental design of reciprocal transplant experiment in Baringo, Kenya. Three plantation/invaser area combinations were included, each one consisting of one mixed original plantation where both *P. juliflora* and *P. pallida* are present, and one or two invaded areas between 4 and 6 km away from the plantation. Seedlings were planted in their site of origin or transplanted (indicated by 'T' in pot). The number of individuals from each collection site transplanted at all sites for each plantation/invaded combination is indicated. Around 150 individuals for each plantation/invaded combination and a total of 447 individuals for the three combinations.

Figure 3.3: Height (a), stem diameter (b) and number of stems (c) recorded for seedlings of invasive *P. juliflora* (black circles), plantation *P. juliflora* (black triangles), and plantation *P. pallida* (grey squares) growing in reciprocal transplant experiments in invaded and plantation sites. Separate linear models were used: one model included only invasive *P. juliflora* and plantation *P. juliflora*; second model included only plantation *P. juliflora* and plantation *P. pallida*. Bars represent mean \pm standard error.

Figure 3.4. Percentage germination (a), mean germination time (MGT, b) and percentage survival (c) of invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida* seedlings under greenhouse conditions. Values represent mean \pm standard error. Different letters above bars indicate significant differences ($p < 0.05$; Tukey's post hoc test).

Figure 3.5. RSR, root:shoot ratio (a), root length (b), stem length (c), total plant biomass (d), root biomass (e), leaf biomass (f), stem biomass (g) and number of leaves (h), of invasive *P. juliflora* (black boxes), plantation *P. juliflora* (grey boxes), and plantation *P. pallida* (light grey boxes) in reponse to different water and N availability treatments. Different letters above the boxplots indicate significant differences ($p < 0.05$; Tukey's post hoc test) among eco-morphotypes within each treatment.

Figure 3.6. Standardized major axis (SMA) regression relationships between total plant biomass and (a) root-shoot ratio (RSR), (b) stem length, (c) stem biomass, (d) root length, (e) root biomass and (f) leaf biomass in invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida*, in response to water and N availability treatments. Symbols represent the different greenhouse treatments. All traits were Ln-transformed so that relationships represent proportional changes. Different letters in brackets indicate significant differences in slopes or elevation ($p < 0.05$) of SMAs between eco-morphotypes. R^2 = Pearson correlation coefficients for evaluated relationships. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ^{ns}, not significant.

Figure 4.1. Location of the study areas in Baringo County, Kenya and Afar Region, Ethiopia.

Figure 4.2. a) STRUCTURE bar plots where vertical axes illustrate the proportional assignment (q_{ik} values) of individual genomes to the inferred two genetic clusters, cluster 1 in blue and cluster 2 in orange; and for all *P. juliflora*, *P. pallida* and triploids from plantations (Plant), neighbouring (Neig) and invaded sites (Inv) from Afar Region in Ethiopia (AF), Baringo County (BA), Mombasa (MO) and Taveta (TA) in Kenya. b) Principal component analysis showing genetic structure among *Prosopis* individuals from Kenya and Ethiopia. *Prosopis juliflora* (4x) is shown in blue, triploids in black and *P. pallida* (2x) in orange. PCA was performed using Bruvo distances in POLYSAT (Bruvo et al. 2004). PC1 and PC2 captured 65.9% and 8.57% of the variation, respectively.

Figure 4.3. a) STRUCTURE bar plots for *P. juliflora* individuals from plantations (Plant), neighbouring (Neig) and invaded sites (Inv) from Afar Region in Ethiopia (AF), Baringo County (BA) and Taveta (TA) in Kenya. Vertical axes represent the assignment (q_{ik} values) of individual genomes to the inferred number genetic clusters ($K = 2$). b) Principal component analysis showing genetic structure among *Prosopis* individuals from Kenya and Ethiopia. Colours represent assignment of individuals to the inferred genetic clusters: Cluster 1 = green; Cluster 2 = pink; admixed = black. PCA was performed using Bruvo distances in POLYSAT (Bruvo et al., 2004). PC1 and PC2 captured 27.8 and 22.1% of the variation, respectively.

Figure 4.2. Genetic diversity metrics of *Prosopis juliflora* found in plantations, sites neighbouring plantations and far-off invaded sites in Afar Region, Ethiopia (black boxes) and Baringo County, Kenya (grey boxes). *Prosopis pallida* individuals found in plantations in Kenya are also shown (white boxes). a) Allelic richness (A_R); b) Expected heterozygosity (H_E); c)

Observed heterozygosity (H_o); d) Inbreeding coefficient (F_{IS}). Boxplots depict the median value, interquartile ranges and outliers of each region. Different letters above the plots indicate significant differences (Kruskal-Wallis rank sum test; $P < 0.05$; Dunn's post hoc test) between the corresponding groups.

Figure 4.3. Comparison of genetic differentiation of *P. juliflora* populations in a) Baringo County, Kenya and b) Afar Region, Ethiopia. Pairwise F_{ST} between pairs of populations from invaded sites (Inv-Inv), invaded and neighbouring sites (Inv-Neig), invaded and plantation sites (Inv-Plant), plantations and neighbouring sites (Plant-Neig), and plantations sites (Plant-Plant). Different letters above the bars or boxplots indicate significant differences (Kruskal-Wallis rank sum test; $P < 0.05$; Dunn's post hoc test).

Figure 5.1. Hypothetical relationship between woody IAS cover and its positive (dashed line), negative (dotted line) and net effect (solid line) on ecosystem services.

Figure S2.1. Identification of the optimal number clusters (K) inferred by an hierarchical Bayesian clustering analyses with the software STRUCTURE. The level of clustering includes: a) Level 1, with all *Prosopis* individuals from 11 species; Level 2, with b) only *P. juliflora* individuals from Mexico, Ethiopia, Kenya and Tanzania, and c) all the rest of *Prosopis* species; d) Level 3, including only *Prosopis* species from Argentina, Australia, Chile, Mexico and South Africa. In all cases $K=2$ were identified as the optimal number of genetic clusters. Individuals were genotyped using seven nuclear microsatellites loci (see Material and Methods for parameters of the models).

Figure S2.2. Percentage of individuals assigned to genotype classes: pure first sp, pure second sp or mixed ancestry; by NEWHYBRIDS using seven nuclear microsatellites loci. The analysis was done on pairs of *Prosopis* species, and hybrids, identify morphologically from various native areas: Argentina (Ar), Chile (Ch) and Peru (Pe). *P. pallida* individuals from the native area of Peru (Pe) were compared with the introduced populations of Kenya (Ke) e invasive populations of Hawaii (Hw), respectively (see Material and Methods for parameters of the models).

Figure S4.1. Identification of the optimal number of clusters for *P. juliflora* in a) Baringo County, Kenya, and b) Afar Region, Ethiopia, separately, inferred by Bayesian clustering with

the software STRUCTURE. Vertical axes represent the assignment (q_{ik} values) of individual genomes to the inferred number genetic clusters ($K=2$). Two mayor genetic demes were identified in each area, including founder individuals from plantations (Plant), sites neighbouring plantations (Neig) and far-off invaded sites (Inv). Sample sites labels are indicated below each plot.

Figure S4.2. Identification of the optimal number clusters (K) for *P. juliflora* individuals from a) the invaded areas of Afar Region in Ethiopia, Baringo County and Taveta in Kenya, b) only Baringo County, Kenya, and c) only Afar Region, Ethiopia; inferred by Bayesian clustering with the software STRUCTURE. Data sets contain a total of a) 633 b) 424 and c) 194 individuals for seven nuclear microsatellites loci (see Material and Methods for parameters of the models).

Figure S4.3. Results of Mantel tests performed among all *P. juliflora* populations from (a) Baringo County, Kenya and (b) Afar Region, Ethiopia, testing for correlations between geographical distances and population genetic structure ($F_{ST} / 1 - F_{ST}$). Test statistic (R) are provided. All correlations were not significant at the significance level ($p < 0.05$).

CHAPTER 1 General Introduction

1.1 Introduction

Biological invasions are defined as the human-assisted arrival, subsequent establishment and spread of species into new geographic ranges (Richardson et al. 2000). Worldwide, the introduction of species has often led to controversy, as invasive alien species can have negative effects on biodiversity and human well-being and alter ecosystem functioning (Mack et al. 2000, McNeely 2006, Kumschick et al. 2012, Bekele et al. 2018), but can also provide benefits to humans (Choge et al. 2007, Shackleton et al. 2007). In order to develop effective management strategies, it is necessary to elucidate the drivers of the invasion process and to understand what determines invasiveness.

Understanding the drivers of successful invasions, and how to manage them best, requires proper taxonomic identification of invasive species. This aspect is crucial for biosecurity strategies to reduce the risk of new introductions and to have proper implementation of policies for the control and eradication of invasive species (Pyšek et al. 2013). Molecular studies have been increasingly applied to delimit species boundaries in cases where species belong to taxonomically complex groups, or where incomplete reproductive isolation, frequent hybridization and introgression occurs (Pyšek et al. 2013).

Mechanisms that allow introduced species to successfully colonize new areas include, for example, phenotypic plasticity, rapid post-introduction adaptation and release from natural enemies, among others (see Catford et al. 2009 for review). High levels of phenotypic plasticity would allow individuals to maintain high levels of performance under novel environmental contexts (Schlichting & Levin, 1986) and thereby facilitate invasive spread. There are good examples of introduced individuals showing higher trait differences in morphology and resource allocation under different environmental conditions, i.e. plasticity, compared to non-invasive congeneric species (Matzek 2011, Gallagher et al. 2015). When such comparisons are done between congeners co-occurring in similar habitats the evidence for a relationship between phenotypic plasticity and invasiveness is more evident (Lambdon & Hulme 2006).

Introduced individuals may also be exposed to novel selection pressures in their introduced ranges, leading to strong selection and therefore possibly rapid post-introduction evolution (Maron et al. 2004; Bossdorf et al. 2005). On the other hand, individuals may experience the relaxation of selection pressures from the species' native range when they are liberated from their specialist predators or parasites upon introduction (Blossey and Nötzold 1995). This may lead to rapid evolution, despite the fact that many invasive populations harbour low levels of standing genetic variation. Studies focussed on trait differences in ancestor-descendent comparisons of invasive and non-invasive native populations have been useful to detect such rapid post-introduction evolution (Keller and Taylor 2008, Guo et al. 2014, Stutz et al. 2018). Using this approach, rapid evolution during invasion is supported by trait divergence between introduced and invasive genotypes. One way in which rapid evolution may be assisted in genetically-depauparate populations is through hybridization between different species, which may create novel genotypes (Ellstrand and Schierenbeck 2000). On the other hand, non-adaptive evolutionary change in invasive species may also come from demographic chance events during range expansion (Schrieber 2016). These may shift genotype frequencies and may cause phenotypic differentiation related with spread ability, even in the absence of selection (Schrieber 2016). Alternatively, existing phenotypic differences in traits that affect dispersal in founder populations may show strong spatial structure when heritable, due to so-called spatial sorting (Shine et al. 2011). Specifically, spatial sorting during rapid range expansions will cause the accumulation of dispersive individuals at the invasion front, leaving less-dispersive individuals behind. This, in turn, will skew mating towards dispersive individuals at the invasion front, and therefore leading to shifts in traits (towards enhanced dispersal) at the invasion front. Such evolutionary change due to spatial sorting was observed in the invasive cane toad (*Rhinella marina*) in Australia (Shine et al. 2011).

The way a species responds to the new selection pressures in the new environment will depend, to some degree, on the level of standing genetic diversity present in introduced populations. Since the introduction of non-native species often involves founder events and subsequent genetic bottlenecks, opportunities for rapid evolution might be constrained (Kolbe et al. 2004, Shirk et al. 2014). Yet, many bottlenecked populations can, and often do, adapt, in what has been termed a 'genetic paradox' (Allendorf and Lundquist 2003). Some

introduced species, however, may replenish their genetic diversity following introduction through intra- or interspecific hybridization (Ellstrand and Schierenbeck 2000). Hybridization often leads to the immediate increase of genetic diversity (Stebbins 1959), the masking of deleterious alleles and fixed heterosis (te Beest et al. 2012) that usually increases performance and thus favours invasiveness. Other genetic attributes (like allopolyploid variation) enable the individuals to increase their genetic diversity as polyploid offspring from two different parental genomes (te Beest et al. 2012). Polyploidy has also been repeatedly linked to invasiveness. For example, polyploids generally show elevated levels of stress tolerance and higher growth vigour through increased plant size, seed size, flower size, niche breadth and phenotypic plasticity, among others (te Beest et al. 2012).

Research on the context-dependent determinants of invasive spread remains at the forefront of understanding the mechanisms that underlie successful biological invasions (Balkenhol et al. 2015, Cushman 2015). For management approaches, knowing the dispersal pathways that underlie invasion would help to explain the current distribution of the invasive populations, and could be used to predict and control their expansion into new habitats. However, such predictions would only be reliable if the extent of suitable habitat(s) (e.g. Le Roux et al. 2010) and the dispersal abilities of the species were to be considered together. Moreover, environmental conditions encountered in the new areas may or may not be conducive to effective dispersal (i.e., the dispersal that includes successful movement, survival, and reproduction of individuals), and are therefore critical to the successful spread of introduced species. Considering these factors, approaches that combine dispersal ability with the suitability of surrounding environments provide more realistic estimates of the potential range expansions of species.

The study of the causes and consequences of the global movement of plant and animal species has focussed mainly on biological components (e.g., Parker et al. 1999, Barney et al. 2013) and largely ignored their social dimensions. To fully appreciate the complexity of the problem, it would be essential to bring together various disciplines such as economics, ecology and sociology, and work together with the diverse perspectives of stakeholders under a transdisciplinary approach (Max-Neef 2005). Transdisciplinary research is defined here as “problem-oriented research involving cooperation among a wide range of stakeholders and academia to meet complex challenges of society” (Klein 2008, Klein et al. 2001) and addresses

problems that cannot be defined in any single disciplinary domain (Eigenbrode et al. 2007). While it is clear that the study of the causes and consequences of biological invasions involves social and ecological components, Vaz et al. (2017) found that, out of over 9,100 published studies in invasion science, most were monodisciplinary, and of those classified as interdisciplinary, 92% were ecological in nature; only 3.2 % could be confidently classified as social-ecological, and the involvement of stakeholders was not mentioned at all in this review. Therefore, to have a better understanding of the complex problems that involve the introduction, spread and effects of invasive alien species, we need more research, not only focussed on biology, but also considering the social dynamics of invasions. Such transdisciplinary approaches would enable the design of more effective management interventions.

The genus *Prosopis* (Fabaceae) provides excellent opportunities to study the drivers and mechanisms of successful biological invasions. Trees in this genus, commonly known as mesquite, are recognized as some of the world's worst invasive species (Shackleton et al. 2014). They have become naturalized and invasive in numerous countries, including Australia, Egypt, Eritrea, Hawaii, Malawi, Philippines, Senegal, and Sudan (Shackleton et al. 2014) and are declared as major invasive alien species in Australia, Ethiopia, India, Kenya and South Africa (van Klinken et al. 2006; van Wilgen et al. 2012). The history of global introductions of *Prosopis* species outside of their native areas has been characterized by multiple introductions to different localities (Pasiiecznik et al. 2001). *Prosopis* trees have been intentionally moved around the globe for many reasons, including stabilization of soils, fuel and wood supply, and fodder for livestock. *Prosopis* trees are now present in 103 countries outside of their native ranges and are considered invasive in 49 of these (Fig. 1.1) (Shackleton et al. 2014).

Prosopis belongs to the subfamily Mimosoideae in the Fabaceae family, and comprises 44 species (Burkart 1976), which are all from arid and semiarid zones of the Americas (40 native species), South West Asia (three native species) and North Africa (one native species). In the Americas, the genus is distributed from the south-west of the United States to Argentinean and Chilean Patagonia. South America appears to be the centre of diversification for the genus (31 species), with 11 species considered endemic to Argentina (Burkart 1976, Hunziker et al. 1986). *Prosopis* species have previously been grouped into five sections:

Prosopis and *Anonychium*, with species present in Africa and Asia; sections *Strombocarpa* and *Algarobia*, with species present in the Americas; and the monotypic *Monilicarpa* section, restricted to Argentina. The section *Algarobia* has also been divided into six series based on morphological traits (Burkart 1976), the validity of which has been questioned due to taxonomic difficulties, extensive inter-specific hybridization, and a probable polyphyletic origin (Bessega et al. 2006, Burghardt and Espert 2007).

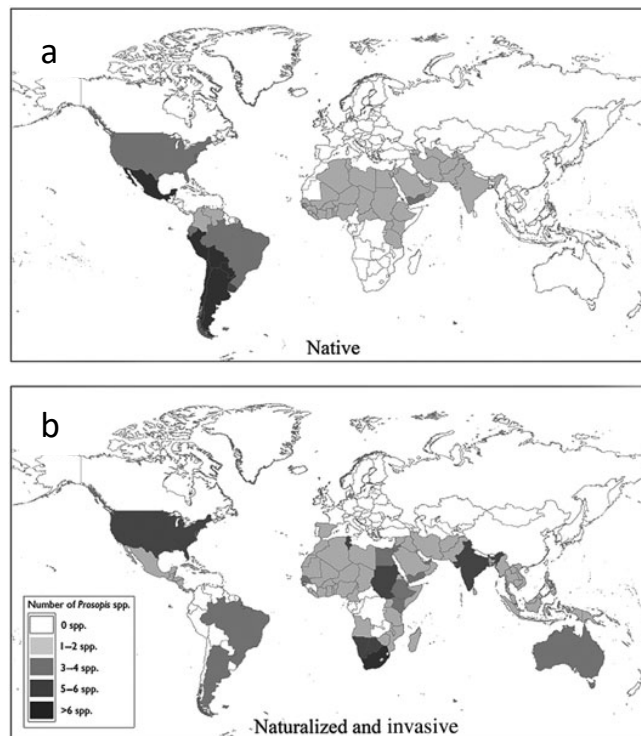


Figure 1.1. Global distribution of (a) native and (b) naturalized – invasive *Prosopis* species (modified from Shackleton et al. 2014). Number of species are indicated for each country.

1.2 Motivation, aims and thesis structure

Biological invasions, and aspects of ecology in general, are seriously understudied in developing regions such as Africa, Asia and South America (Pyšek et al. 2008), even for well-studied groups like woody species (Hulme et al. 2008). Various woody trees and shrubs were introduced to Africa during the first half of the 20th century and have since become invasive. *Prosopis* invasions in Eastern Africa (Kenya and Ethiopia) provide excellent opportunities for the examination of ecological and genetic attributes of invasiveness and drivers of invasion success that vary with context and taxon. Various species were introduced during the 1970s and 1980s to many parts of Eastern Africa and subsequently spread from the original plantations (Choge et al. 2002, Admasu 2008, Kebede and Coppock, 2015) leading to extensive invasions that have caused substantial social and economic conflicts (Bekele et al. 2018). The spread of *Prosopis* has been difficult to control. Moreover, different introduced species vary in their invasiveness and it is suspected that inter-species hybridization is occurring in some areas. In addition, accurate taxonomic identification of *Prosopis* species remains problematic and studies from many regions simply refer to the taxon as *Prosopis*. Importantly, there is no knowledge about the role of population genetic structure, intraspecific diversity and different mechanisms – such as plasticity, rapid post-introduction evolution and hybridization – in promoting the invasion of *Prosopis* in Eastern Africa.

The main goal of this PhD study was to understand the role of selected ecological-evolutionary drivers that influence the invasion of *Prosopis* species in Eastern Africa (Kenya and Ethiopia). To this end, I examined the patterns of genetic diversity of various *Prosopis* species from native and non-native areas. In addition, I investigated the relationship between species traits, genetic composition, habitat characteristics and invasion success. Lastly, a framework to integrate socio-economic and ecological data in transdisciplinary projects is proposed.

This thesis has been structured into six chapters with the following specific aims: Chapter 1 provides the general introduction and a review of the relevant literature. Chapter 2 aims to shed light on the taxonomic uncertainty that underlies *Prosopis* invasions globally, through a genetic study of various *Prosopis* species in their native and introduced ranges. Chapter 3 examines the contribution of phenotypic plasticity and rapid evolution to the

invasiveness of *P. juliflora* and *P. pallida* in Baringo County, Kenya. Chapter 4 examines the extent to which hybridization among *Prosopis* species and variation in ploidal levels contributed to their invasion success in Baringo County, Kenya and Afar Region, Ethiopia, and assesses the determinants of spread. Chapter 5 proposes a framework to integrate socio-economic and ecological data and to enable valid comparisons between these different disciplines in trans-disciplinary research. This chapter was a collaborative project between six PhD students involved in the first three-year phase of the Woody Weeds project of which this PhD thesis is part. Lastly, Chapter 6 provides a synthesis of the principal findings of this thesis and discusses how they could be used in the development of effective management options.

1.3 Study sites

The field research for this thesis was done in Afar Region, Ethiopia and Baringo County, Kenya. Both areas are part of Great Rift Valley of Eastern Africa and have characteristic types of arid and semi-arid habitats, with high agro-ecological and biological diversity that are vulnerable to invasion by woody alien species. These areas were selected because 1) they are representative of other areas highly invaded by *Prosopis*; 2) the original plantations of introduced *Prosopis* species are still present in the environment and identifiable; and 3) information on the most relevant ecological variables that determine the distribution of *Prosopis* is available (Shiferaw et al. 2018).

(a) Afar Region, Ethiopia

The Afar Region comprises the valley of the Awash River, which flows through the region to the Ethiopia-Djibouti border. The climate of the area is harsh. Temperatures can rise up to 40°C and precipitation range varies between 5 and 600mm annually, causing both droughts and floods (WAS 2000). *Prosopis* species, locally referred to as 'woyane', have invaded the riverine areas of the Awash river and southern part of the Afar Region (Fig. 1.2). In Afar Region the land is mainly used for livestock production and the spread of *Prosopis* has impacted negatively on the livelihoods of pastoralist people by reducing the amount of grass available for livestock (Rettberg 2010). *Prosopis* has been declared a noxious weed in Ethiopia and its cultivation is prohibited. The Ethiopian government developed a national strategy for the management of *P. juliflora* which focused on preventing further expansion into non-invaded areas, eradication in some areas and the restoration of invaded land, as well as the productive

use of *Prosopis* (National Strategy on *P. juliflora* Management, Ministry of Livestock and Fisheries, Federal Democratic Republic of Ethiopia, 2017).

(b) Baringo County, Kenya

The major watershed in this region includes Lake Baringo and Lake Bogoria. The land is principally used for livestock farming and crop agriculture. The climate of the area varies from humid in the highlands to arid in the lowlands. Mean annual temperatures ranges from 30 °C to 35 °C and mean annual precipitation ranges from 500mm to 1000mm per year (Meroni et al. 2014). Droughts, floods and landslides occur frequently. *Prosopis* species, locally called 'mathenge', have reduced the land available for grazing and have lowered the water table (Fig. 1.2). In 2006, after suffering heavy losses of cattle due to the impacts of *Prosopis*, the community sought compensation from the Government of Kenya by taking them to court, as the government was responsible for the introduction of *Prosopis*. The court ordered the government to compensate the community for the negative effects of *Prosopis*. While no national or regional plan to manage the invasion current exists (Choge and Muthike 2011), the utilization of *Prosopis* has been proposed as an effective strategy to control the spread of individuals (Choge et al. 2007, Shackleton et al. 2014).

1.4 Study species

The genus *Prosopis* belongs to the subfamily Mimosaceae in the Fabaceae, and includes 44 species (Burkart 1976), all of them distributed in arid and semiarid zones of the Americas (40 native species), South West Asia (three native species) and North Africa (one native species). In the Americas, the genus is distributed from the south-west of the United States to Patagonia in Argentina and Chile. South America appears to be the centre of diversification for the genus, being home to 31 species, with 11 species considered endemic to Argentina (Burkart 1976, Hunziker et al. 1986).

The taxonomy of the genus has been the subject of intense debate. *Prosopis* species have been split into five sections according to floral traits and vegetative differences in armature. The Sections *Anonychium* and *Molnicarpa* contain one species each, while the sections *Strombocarpa* and *Prosopis* contain nine and three species, respectively. The section *Algarobia* was divided into six series (Burkart 1976), but the validity of the sections has been

questioned due to taxonomic difficulties, frequent inter-specific hybridization, and a probable polyphyletic origin (Bessega et al. 2006, Burghardt and Espert 2007). Taxonomic uncertainty in *Prosopis* is exemplified by the cases of *P. juliflora* and *P. pallida*. Both species harbour different intra-specific morphological forms and they also have overlapping distributions in their native areas (Fosberg 1966, Burkart 1976, Diaz Celis 1995). Unsurprisingly, there have been numerous misidentifications in various native and non-native areas of these two species (Pasiiecznik et al. 2001). For this reason, Pasiiecznik et al. (2001) proposed the classification of a '*P. pallida* – *P. juliflora* complex' to deal with the taxonomic uncertainty.

Prosopis species are now present in more than 129 countries outside of their native ranges and have become naturalized or weedy in 122 of them (Shackleton et al. 2014). Intercontinental introductions of *Prosopis* in the 1800s and 1900s included movement to Australia, Hawaii, India, Philippines, South Africa, Sri Lanka, Senegal and Sudan (Pasiiecznik et al. 2001). Most of the introductions to African and Asian countries were aimed at restoring degraded land (Shackleton et al. 2014). The movement of *Prosopis* seeds has been uncoordinated, poorly documented, and lacking in taxonomic certainty, with introductions occurring from both native and non-native areas (Pasiiecznik et al. 2001). Shackleton et al. (2014) determined that 79% of introduced *Prosopis* species have become naturalized, while 38% have become invasive. *Prosopis* invasions are managed in 23 countries and mainly consists of mechanical and chemical control in developed countries, whereas in developing nations management strategies also include control through utilization (Shackleton et al. 2014). Biological control has been used in only a few countries, i.e. in Australia, and South Africa (Shackleton et al. 2014). Conflicts around the management of *Prosopis* invasion are mainly based on the tension between their perceived benefits and their harmful impacts (Shackleton et al. 2014).

Detailed information on study species: *Prosopis juliflora* and *P. pallida**(a) Prosopis juliflora (Sw.) DC.*

<i>Synonyms</i>	<i>Acacia cumanensis</i> Humb. & Bonpl. ex Willd.; <i>Algarobia juliflora</i> Sw.; <i>Mimosa juliflora</i> Sw.; <i>Mimosa salinarum</i> Vahl.; <i>Neltuma juliflora</i> (Sw.) Raf.; <i>Prosopis bracteolata</i> DC.; <i>Prosopis cumanensis</i> (Humb. et Bonpl. ex Willd.) Kunth. <i>Prosopis chilensis</i> (Mol.) Stuntz; <i>Prosopis chilensis</i> var. <i>glandulosa</i> Torr.; <i>Prosopis dominguensis</i> DC.; <i>Prosopis glandulosa</i> Torr.
<i>Common names</i>	Algarroba (Colombia, Brazil, Puerto Rico); algarrobo (Cuba, Colombia, Ecuador, Honduras, Peru); algarobeira, algarobia (Brazil); chácata, tsirisicua, tziritzecua (Tarasca language, Michoacán, Mexico); chachaca, chúcata (Michoacán, Mexico); espinheiro, spinho (Cabo Verde); inda-a (Cuicatleca language, Oaxaca, Mexico); jupala; katzimelk (Chihuahua, Mexico); mequite (Huichol language, Jalisco, Mexico); mezquite (Mexico, Puerto Rico); mezquite amarillo, mezquite blanco, mezquite Colorado, mezquite chino (Mexico); mizquitl (Náhuatl language, Mexico); t'ahi, majé, tai, taj, toji (Otomí language, Hidalgo, Mexico); uejoue (Tarahumara language, Chihuahua, Mexico); biia, yaga-bü (Zapoteca language, Oaxaca, Mexico); upala (Guarigia language, Chihuahua, Mexico); haas (Seri language, Sonora, Mexico).
<i>Species description</i>	Tree or shrubs between 3-8m high. Contains axillary spines, paired or solitary, not present on all branches, 0.5-5 cm long. Leaves present 1-3,4 pairs of pinnae and 10-16 pairs of glabrous leaf-left per pinnae. Racemes are 7-15 cm long, flowers are pale yellow when mature. Pods are 8-29cm long, flattened and straight or curved in the apex, endocarp segment up to 25 with oval and brown seeds (Burkart 1976, Ramírez 2015).
<i>Native range</i>	In Mexico occurs in arid lands of the Pacific coast from Baja California and Chihuahua until Oaxaca, and inland from Tamaulipas to Veracruz (Ramírez 2015). The distribution also extends through arid and semi-arid regions of Central and South America to Colombia (Pasicznik et al. 2001).
<i>Introduced range</i>	Present mostly in arid and semi-arid regions. The species has been introduced in 25 countries in Asia, 35 countries in Africa, two countries in North America, three countries in Central America and

the Caribbean, two countries in South America and three countries in Oceania (Figure 1.3).

Invasive characteristics The species fixes nitrogen through rhizobium symbiosis and has a high tolerance to drought and saline conditions, and allelopathy. It is considered the most invasive species in the genus. It is declared as invasive or noxious in Australia, Ethiopia, Kenya, Pakistan, South Africa, Sudan and some Asian countries. It forms impenetrable thickets preventing the movement of livestock to grazing and watering areas (Haregeweyn et al. 2013).

Human use Tree exudates (latex) have been used as gum and are believed to have medicinal properties while the resin is used in cellulose fibers, or as binding agent of medical products. The wood is used as firewood and for, charcoal production, construction and for making tools. Pods and seeds have high sugar and protein content and are used as fodder for animals. Individuals are planted for land restoration and for controlling soil erosion.

(a) Prosopis pallida (H. & B. ex Willd.) Kunth

<i>Synonyms</i>	<i>Acacia pallida</i> H. & B. ex Willd.; <i>Mimosa pallida</i> (Willd.) Poiret; <i>Prosopis affinis</i> (Sprengel) Ferreyra; <i>Prosopis juliflora</i> var. <i>horrida</i> (Kunth) Burkart; <i>Prosopis juliflora</i> var. <i>inermis</i> (Kunth) Burkart; <i>Prosopis limensis</i> Benth.; <i>Prosopis pallida</i> (H. & B. ex Willd.) H.B.K.; <i>Prosopis pallida</i> forma <i>decumbens</i> Ferreyra.
<i>Common names</i>	Algarrobo (Colombia, Ecuador); mesquite (Colombia, Hawaii – USA); Prosopis, algarobeira (Brazil); espinheiro (Cape Verde); anchipia guaiva, cuji, cuji negro, cuji yaque, manca-caballo, trupi, trupillo (Colombia); garawa (Djibouti); carobier (French Polynesia); kiawe (Hawaii).
<i>Species description</i>	Individual trees have a single stem up to 20m high (or can be shrubs depending on soil nutrient levels). Branches often have a zig-zag appearance, are glabrous or sometimes with scattered hairs. Spines can be present or absent, and when present, are paired, short (less than 4cm long) and divergent and axillary located. Hairy leaves with 2-4 pairs of pallid leaflets. Racemes are two or three times longer than leaves with a short and pubescent peduncle, florets with short pedicelled, yellow colour, with stamens 5-7 mm long, ovary stalked. Pods straight or curved, pale yellow to golden brown and glabrous, with parallel margins, of sweet taste; endocarp is segmented to 30 sections containing brown, oblong seeds (Burkart 1976).
<i>Native range</i>	From southern Colombia to Peru, distributed in arid and semi-arid lands along the Pacific coast (Pasiiecznik et al. 2001).
<i>Introduced range</i>	Present in many frost-free tropical regions. It has been introduced in three countries in Asia, six countries in Africa, one country in North America, two countries in Central America and the Caribbean, one country in South America and three countries in Oceania (Figure 1.3).
<i>Invasive characteristics</i>	The species fixes nitrogen through rhizobium symbiosis and is tolerant of drought and saline conditions. It has been declared a noxious weed in the United States and Australia, and as an invasive in Hawaii and in Australia's Western and Northern Territories and in Queensland. Invasive populations have been reported from Australia, Brazil, Cape Verde, Senegal, Mauritania and Djibouti.

Hybrids of *P. pallida* with other introduced *Prosopis* species as been observed in the Hawaiian Islands and Australia.

Human use

Wood is used for fuel, posts, poles and timber and with the main use being domestic consumption for firewood, as well as charcoal production for commercial use. Flowers are used as nectar sources for bee-farming and the production of honey and jam; its exudates (latex) have been used as gum. The pods are used as fodder for animals, to produce shampoos, alcoholic beverages, to make flour used in bakery, and as a source of sugar. Trees are also planted for land restoration and to control soil erosion.



Figure 1.2. Invaded areas and original plantations of *P. juliflora* in Afar Region, Ethiopia (a and c, respectively) and Baringo County, Kenya (b and d, respectively).

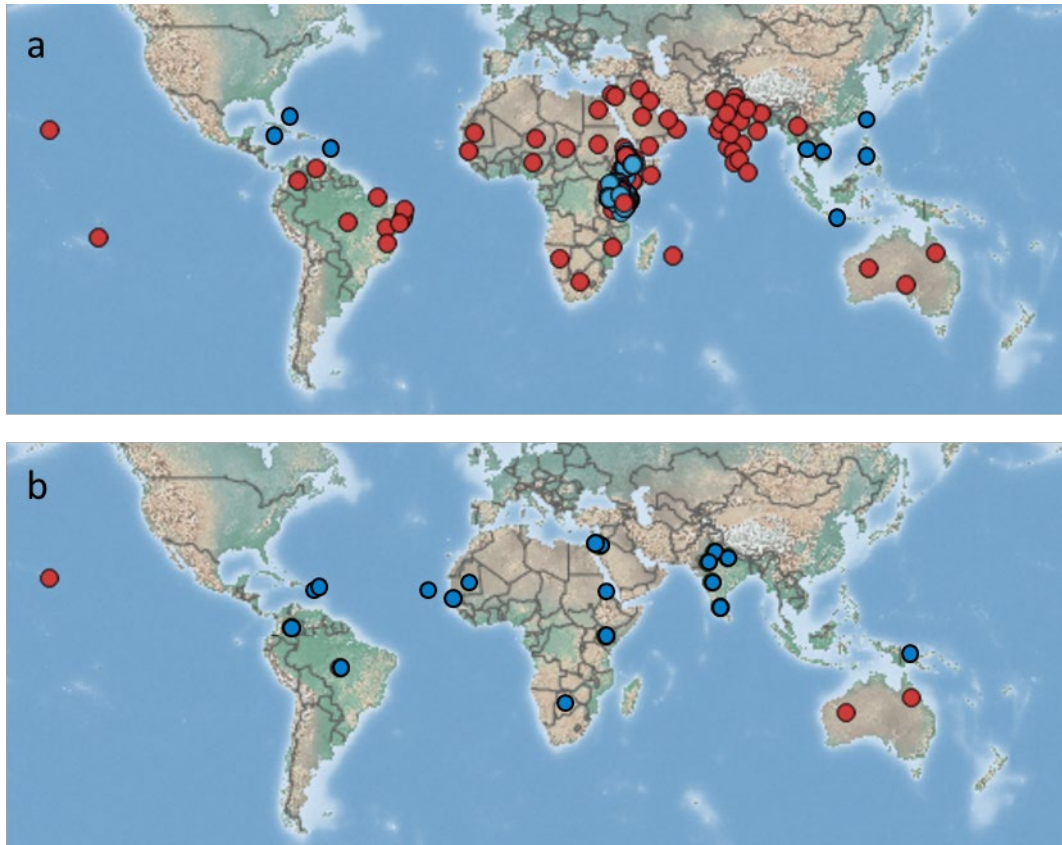


Figure 1.3. Records of invasive (red) and naturalized (blue) populations of (a) *P. juliflora* and (b) *P. pallida*. Sources: CABI Invasive Species Data and Natural Resources Conservation Services.

CHAPTER 2 Taxonomic uncertainty and genetic insights into the globally invasive tree genus *Prosopis*

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Abstract

Proper taxonomic identification of invasive alien species is crucial to detect new incursions, prevent or reduce the arrival of new invaders and implement management options such as biological control. Globally, the taxonomy of alien *Prosopis* species is problematic due to misidentification of initial introductions as well as hybridisation between the introduced species. Here, a global genetic analysis was carried out on diverse *Prosopis* species from both native and non-native populations to clarify the taxonomic placement of invasive populations, with a special focus on *Prosopis* invasions in Eastern Africa (Ethiopia, Kenya and Tanzania). DNA sequencing data from chloroplast markers confirmed high phylogenetic similarity (almost 100%) between all species analysed. Analysis based on seven nuclear microsatellites confirmed weak population genetic structure between *Prosopis* species at a global level. Inter-specific hybridization and incidences of polyploidy were recorded in both native and non-native areas. I found tetraploid *P. juliflora* to be highly differentiated from the rest of the (diploid) species within the genus. The population genetic analysis indicated that invasive genotypes of *P. juliflora* in Kenya and Ethiopia could have a similar native origin from Mexico, while Tanzanian introductions probably occurred from a different source. Genotypes of *P. pallida* in non-native areas of Kenya and Hawaii showed high similarity with native Peruvian *P. pallida* populations. Levels of introduced genetic diversity, relative to native areas, indicated that multiple introductions of *P. juliflora* and *P. pallida* occurred to Eastern Africa. The lack of genetic differentiation between some species from the native range supports the notion that hybridization between allopatric species may occur frequently when they are co-introduced into new ranges. Polyploidy is proposed as a significant mechanism that facilitates

reproductive isolation between species, and may explain the successful invasion of *Prosopis* in some areas, such as Eastern Africa.

Keywords: genetic diversity, hybridization, invasive alien species, microsatellites, polyploidy, *Prosopis*, taxonomic uncertainty, tree invasions

2.1 Introduction

Biological invasions result from human-aided movement of species across natural and historical barriers outside of their native ranges. As part of social and economic development in the era of globalization, the number of species being translocated, intentionally or accidentally, is ever increasing (Seebens et al. 2017). In many instances, these introductions have resulted in invasive populations, impacting on human well-being, biodiversity and ecosystem services (Pimentel et al. 2005, van Wilgen et al. 2011, Shackleton et al. 2014).

Sound taxonomic knowledge of invasive species is crucial to detect new invasions, to determine the potential sources and routes of introduction(s), to prevent or reduce the arrival of new invaders and to implement management options such as biological control (Le Roux and Wieczorek 2009). However, the taxonomy of many alien taxa remains problematic due to unresolved species relationships, uncertain geographic distributions and inter-specific hybridization, among other factors (Pyšek et al. 2013). For example, the invasive *Heracleum* species belong to a taxonomically complex group and in many non-native ranges several species have been introduced and have become invasive, making proper species identification difficult (Jahodová et al. 2007). In the United States, the aggressive spread of invasive saltceder (*Tamarix*) species consists of two morphologically similar species, and hybridization between these two species and a native species has led to multiple hybrids with different degrees of invasiveness (Gaskin and Schaal 2002). In such cases, morphological data and environmental requirements (i.e. eco-morphological approaches) are not sufficient to obtain conclusive species and/or hybrid identification. Complementing eco-morphological approaches with genetic information is therefore increasingly used to delimit species boundaries under these complex scenarios (Le Roux and Wieczorek 2009).

Comparative studies between invasive alien species of the same genus from different parts of the world provide opportunities to examine attributes of biological invasions at large

geographic scales and to understand the biogeographic and ecological factors that may be related with successful naturalization and spread. Such studies can also clarify genetic relationships and provide insights into the taxonomy and invasion history of species, i.e. knowing which species are invasive where. Various studies have examined genetic diversity and differentiation within invasive populations and between invasive and native populations. Comparisons of the levels of genetic diversity in native and invaded ranges have been used as a proxy for introduction histories (Sakai et al. 2001, Lavergne and Molofsky 2007, Le Roux et al. 2010), routes of dispersal within non-native ranges (e.g. Lachmuth et al. 2010), the role genetic constraints play in invasive performance (Dlugosch and Parker 2008) and, importantly, to resolve taxonomic uncertainties (Gaskin and Schaal 2002, Jahodová et al. 2007). The level of genetic diversity present in invasive populations may also be affected by historical range expansions and past demographic processes (Taylor and Keller 2007, Le Roux et al. 2011). In addition, species may replenish their genetic diversity following introduction through intra- or interspecific hybridization (Ellstrand and Schierenbeck 2000). Hybridization not only leads to the immediate increase of genetic diversity (Stebbins 1959), the masking of deleterious alleles and fixed heterosis (te Beest et al. 2012), but also further complicates taxonomic uncertainties in invasive lineages (Pyšek et al. 2013). In some instances, whole genome processes, such as polyploidization following hybridization, can also increase the genetic diversity of invasive species (te Beest et al. 2012). Numerous studies have shown a link between higher ploidal levels and invasiveness. Compared to diploids, polyploids often show higher levels of stress tolerance and growth vigour through increased plant size, seed size, flower size, niche breadth and phenotypic plasticity, among others, which may all contribute to higher invasiveness (see te Beest et al. 2012 and references therein). In the case of Fabaceae, more polyploids are widespread invasive species are than diploids (te Beest et al. 2012).

Trees in the genus *Prosopis* (Fabaceae), commonly known as mesquite, are recognized as some of the world's worst invasive species (Shackleton et al. 2014). They have become naturalized or invasive in numerous countries, including Australia, Egypt, Eritrea, Hawaii, Malawi, Philippines, Senegal, and Sudan (Shackleton et al. 2014) and are declared as major invasive species in Australia, Ethiopia, India, Kenya and South Africa (van Klinken et al. 2006, van Wilgen et al. 2012). Curiously, many *Prosopis* invasions, e.g. in Australia, East Africa and

South Africa, have been speculated to have resulted from hybrid species rather than pure species (Zimmerman 1991, van Klinken et al. 2006, Mazibuko 2012, Muturi 2012). For example, hybrids between *Prosopis juliflora* (Sw.) DC. and *Prosopis pallida* (Willd) Kunth or *Prosopis chilensis* (Molina) Stuntz have been reported to dominate invasive populations in some areas such as the Turkwel delta and Baringo County in Kenya (Muturi, 2012). In South Africa, numerous species were introduced, including *Prosopis glandulosa* Torr., *Prosopis pubescens* Benth., *Prosopis velutina* Wooton, *Prosopis laevigata* (Willd) M.C. Johnst, *Prosopis cineraria* (L.) Druce, among others (Poynton 2009). Extensive hybridization is thought to occur in South Africa and DNA sequencing data analyses were previously unable to identify 'pure' parental species (Mazibuko 2012). However, in the current national list of invasive species of South Africa, only *Prosopis glandulosa* var. *torreyana* (L.D. Benson) M.C. Johnst., *P. velutina*, and hybrids of these two species, are listed (National environmental management: biodiversity act, 2004: Alien and invasive species lists, 2016). In Australia, invasive populations have been identified as *P. glandulosa*, *P. velutina*, *P. juliflora*, *P. pallida*, and their hybrids (van Klinken et al. 2006). The most severe infestation within Australia is reported to represent a hybrid swarm between *P. pallida* x *P. velutina* x *P. glandulosa* var. *glandulosa* (van Klinken and Campbell 2001). In Hawaii, *P. pallida* and *P. juliflora* have become invasive and, based on morphological characters, hybrids between these species seems to be present in several locations (Gallaher and Merlin 2009). In Ethiopia, taxonomic identification of invasive *Prosopis* trees remains unclear and studies only refer to the taxon as *Prosopis*. In the Afar Region in Ethiopia, the aggressive spread of *Prosopis* has been mostly associated with *P. juliflora*, and hybrids have not been reported from the area (Wakie et al. 2014, Shiferaw et al. 2019).

Difficulties associated with correct species identification, complicated by supposedly extensive hybridization, may undermine the success of management strategies, especially biological control, applied in many countries. In addition to hybridization, taxonomic uncertainty in the group is exacerbated by the fact that diagnostic morphological traits between species are often lacking and because the native distribution of many species remain contentious (Pasiiecznik et al. 2001). Studies at large biogeographic scales, including both native and non-native regions, may provide valuable information about the genetic diversity of invasive *Prosopis* species, the occurrence of hybridization, and may help to clarify taxonomic uncertainty. This study aims to fill this research gap by performing a global genetic

analysis on numerous *Prosopis* species from both native and introduced populations. For this, phylogenetic relationships were inferred among selected *Prosopis* species. Then, the genetic diversity and genetic similarity were assessed between non-native and native populations around the world, with a special focus on *Prosopis* invasions in Eastern Africa (Ethiopia, Kenya and Tanzania).

2.2 Materials and methods

a) Sampling and DNA extraction

Samples of different *Prosopis* species were collected from various native and invaded areas around the world in 2016 (Table 2.1, Fig. 2.1). From the native area, we sampled populations of *P. juliflora* and *P. laevigata* from Mexico; *P. pallida* from Peru; *Prosopis alba* Griseb., *P. chilensis*, *Prosopis flexuosa* DC., *Prosopis nigra* Hieron., *Prosopis strombulifera* (Lam.) Benth., *Prosopis torcuata* (Lag.) DC. and *Prosopis vinalillo* Stuck. and putative hybrids, from Argentina; and *P. chilensis* and *P. alba* from Chile. These collections included two areas of that are centers radiation of the genus, the Argentine–Paraguayan–Chilean region and the Texan–Mexican region. *Prosopis laevigata*'s range extends across central Mexico, while *P. juliflora* occurs in Mexico along the Pacific coast from Baja California and Chihuahua until Oaxaca, and inland from Tamaulipas to Veracruz (Ramírez 2015). Other species in the genus are restricted to South America. *Prosopis pallida* is distributed in arid and semiarid areas of the Pacific coast from Colombia to Peru (Pasiiecznik et al. 2001, Palacios et al. 2012). *Prosopis alba* occurs in north and central Argentina and the north of Chile. *Prosopis chilensis* is commonly found in parts of northwestern Argentina, from the north to the center of Chile, southern Peru and Bolivia (Burkart 1976). *Prosopis flexuosa* is found in the arid regions of western Argentina and northern Chile (Alvarez and Villagra 2010). *Prosopis nigra* is found in the central areas of northern Argentina, in subtropic areas of Paraguay and Uruguay and arid zones of Bolivia. The endemic *P. torcuata* occurs only in northwestern Argentina. *Prosopis vinalillo* is found in northern Argentina, while *P. strombulifera* is found in areas of western Argentina and northern Chile (FAO 2000). From invaded areas, we sampled individuals of *P. pallida*, *P. glandulosa*, *P. velutina* and putative hybrids from Australia, *P. juliflora* from Ethiopia, *P. pallida* from Hawaii, *P. juliflora* and *P. pallida* from Kenya, various *Prosopis* species and putative hybrids from South Africa (hereafter referred to only as *Prosopis spp.*), and *P. juliflora*

from Tanzania. Species identity of individuals were determined using the key provided by Burkart (1976). *Prosopis vinalillo* belongs to the series Ruscifoliae; *P. pallida* belongs to the series Pallidae; *P. chilensis*, *P. juliflora*, *P. nigra*, *P. laevigata*, *P. flexuosa*, *P. glandulosa*, *P. alba* and *P. velutina* belong to the series Chilenses. One to 35 localities were sampled per country/region per species (Table 2.1, Fig. 2.1) and between 1-474 individuals were sampled per species per country/region ($n_{\text{total}}=1103$ individuals). Leaf material was air-dried and stored on silica gel until further use. Genomic DNA extractions from dried leaf tissue were performed following the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1990). All DNA extractions were diluted to a standard concentration of 50ng/ μl and 100 ng/ μl for microsatellite and DNA sequencing, respectively. Because *P. juliflora* is the only polyploid member of the genus (4x), the morphological classification of trees as this species was confirmed using flow cytometry analysis on a subset of the sampled individuals (n=63 for Kenya; n=10 for Ethiopia) (data not shown, but see Chapter 4).

Table 2.1. Species of *Prosopis* included in the study from various native and non-native regions.

Species	Country	N	Category
<i>P. alba</i>	Argentina	29	Native
<i>P. alba</i>	Chile	9	Native
<i>P. chilensis</i>	Argentina	19	Native
<i>P. chilensis</i>	Chile	24	Native
<i>P. flexuosa</i>	Argentina	9	Native
<i>P. glandulosa</i>	Australia	2	Invasive
<i>P. juliflora</i>	Mexico	20	Native
<i>P. juliflora</i>	Ethiopia	203	Invasive
<i>P. juliflora</i>	Kenya	474	Invasive
<i>P. juliflora</i>	Tanzania	50	Invasive
<i>P. laevigata</i>	Mexico	24	Native
<i>P. nigra</i>	Argentina	6	Native
<i>P. pallida</i>	Peru	14	Native
<i>P. pallida</i>	Australia	2	Invasive
<i>P. pallida</i>	Hawaii	15	Invasive
<i>P. pallida</i>	Kenya	50	Introduced
<i>P. strombulifera</i>	Argentina	1	Native
<i>P. torquata</i>	Argentina	1	Native
<i>P. velutina</i>	Australia	1	Invasive
<i>P. vinalillo</i>	Argentina	7	Invasive
<i>P. alba</i> x <i>P. chilensis</i>	Argentina	2	Native
<i>P. alba</i> x <i>P. nigra</i>	Argentina	3	Native
<i>P. alba</i> x <i>P. rustifolia</i>	Argentina	1	Native
<i>P. alba</i> x <i>P. vinalillo</i>	Argentina	1	Native
<i>P. chilensis</i> x <i>P. flexuosa</i>	Argentina	4	Native
<i>Hybrids</i>	Australia	4	Invasive
<i>Prosopis spp.</i>	South Africa	58	Invasive
<i>Prosopis spp.</i>	Argentina	35	Native
<i>Prosopis spp.</i>	Chile	35	Native

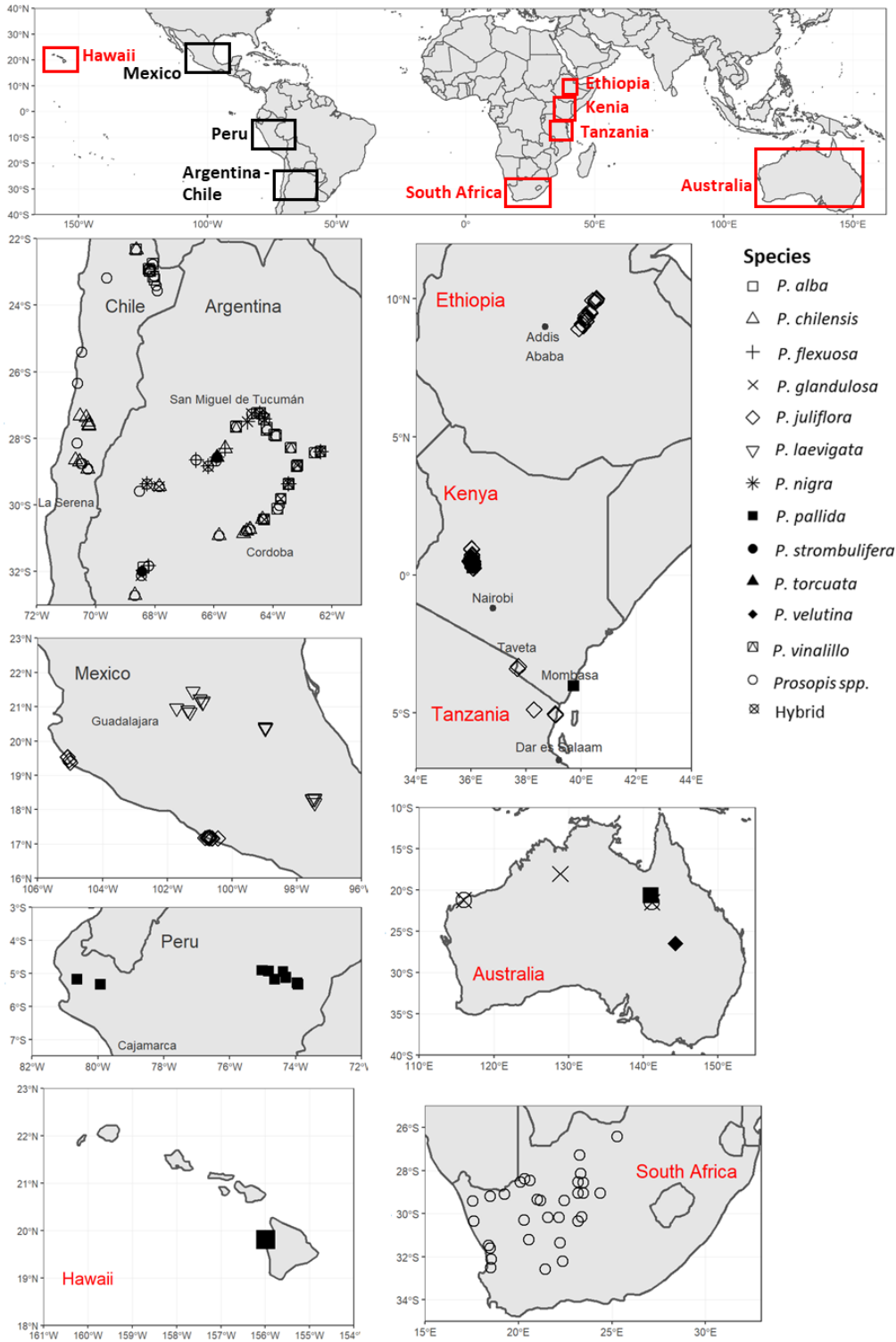


Figure 2.1. Sampling sites of various *Prosopis* species from native (black square) and non-native (red square) areas.

b) Phylogenetic analysis

We selected a representative individual of *P. chilensis* and *P. alba* from Chile; *P. flexuosa*, *P. vinalillo* and *P. nigra* from Argentina; *P. laevigata* and *P. juliflora* from Mexico; *P. pallida* from Peru; *P. juliflora* from Kenya, one unidentified individual from South Africa, and one putative hybrid from Australia for initial optimization of gene amplification and sequencing. The following genes were sequenced: nuclear (nDNA) internal transcribed spacer region (*ITS*) and external transcribed spacer region (ETS), and chloroplast (cpDNA) regions *rpl32* and *psbA* genes. For each gene region, polymerase chain reactions (PCRs) were performed in 30 μ l reaction volumes, each containing 3 μ l of genomic DNA, 0.6 μ l of each dNTP (AB gene; Southern Cross Biotechnologies, Cape Town, South Africa), 3 μ l of each primer, 10 X PCR reaction buffer, 25 mM MgCl₂ and 0.6 μ l of BSA (Promega), 0.6 μ l *Taq* DNA Polymerase (Super Therm JMR-801; Southern Cross Biotechnologies) was used for the regions ETS, *psbA* and *rpl32*; and 0.75 μ l *Taq* DNA Polymerase was used for *ITS* region. The ETS region was amplified using the primers described in Brown et al. (2008) and PCR cycling included an initial denaturation of 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 60 s, annealing at 60 °C for 60s, elongation at 72 °C for 2 min; and final extension at 72 °C for 10 min. For the *ITS* gene region we used the primers described by Sun et al. (1994) and PCR cycling that included an initial denaturation of 95 °C for 15 min, followed by 30 cycles at denaturation at 94 °C for 30 s, annealing at 64.7 °C for 30s, elongation at 72 °C for 20s; and final extension at 72 °C for 5 min. For the *rpl32* region we used the primers described by Shaw et al. (2007) and PCR cycling that included an initial denaturation of 95 °C for 2 min, followed by 30 cycles at denaturation at 95 °C for 30 s, annealing at 60 °C for 30s, elongation at 72 °C for 60s; and final extension at 72 °C for 10 min. For the *psbA* region we used the primers described by Sang et al. (1997) and PCR cycling that included an initial denaturation at 80 °C for 5 min, followed by 30 cycles at denaturation at 94 °C for 30 s, annealing at 60 °C for 30s, elongation at 72 °C for 60s; and final extension at 72 °C for 10 min. Sequence data were aligned and edited using *BioEdit* version 7.0.5.3 (Hall, 1999), and were manually edited.

c) Microsatellite genotyping

We tested cross-amplification success for 51 microsatellite loci previously developed and characterized in numerous *Prosopis* species (Table S2.1). These microsatellite primers were tested under different temperature conditions and we were able to obtain successful amplification for 30 loci. From these, 10 markers were selected considering their levels of polymorphism across *Prosopis* species, functional annotations in some instances, and similar amplification temperatures (Table S2.1). Amplification of these markers was performed by using multiplex PCR assays for which markers with non-overlapping amplicon size were combined. Each multiplex PCR reaction contained 1.5 µl of primer mix (2µM), 7.5 µl of KAPA2G Fast Multiplex Mix (Kapa Biosystem, Cape Town, South Africa), 4.5 µl purified H₂O and 1.5 µl of DNA in a total reaction volume of 15 µl. The concentration of each primer is provided in Table S2.2. The PCR cycle included 3 min of denaturation at 95° C, followed by 30 cycles of denaturation at 95° C for 15 s, annealing at 60° C for 30 s, elongation at 72° C for 25 min, and a final elongation at 72° C for 1 min. PCR reactions were performed in 96-well plates contained 92 samples plus five randomly selected replicate samples and two negative controls (H₂O). Amplified products were submitted for gel capillary electrophoresis at Stellenbosch University's Central Analytical Facility. Automated allele scoring was done using the GeneMarker version 2.6.4 software (SoftGenetics LLC, Pennsylvania, United States) and all alleles were manually checked. A total of 1107 samples were genotyped. The following three loci were excluded from subsequent analyses: I-P00930c as it was monomorphic; I-P07653 and GL23 as these showed extensive patterns of non-specific binding in numerous samples. Individual samples that failed to amplify at more than five loci were removed from all subsequent analyses, leaving a total of 1072 individuals. From these, 14 individuals not initially identified as *P. juliflora* (the only tetraploid species in the genus) presented more than two alleles at one or more loci (Table S2.3) and were excluded from subsequent analyses because their ploidy could not be determined.

d) Genetic diversity

Departures from Hardy-Weinberg equilibrium (HWE) were tested for all loci for all diploid species (i.e. excluding *P. juliflora*) using the R packages *adegenet* version 2.0.1 (Jombart 2008) and *pegas* version 0.11 (Excoffier et al. 1992) and significance tested using a permutation test

(10 000 permutations). The number of alleles per locus, observed heterozygosity (H_o), expected heterozygosity (H_E), and inbreeding coefficients (F_{IS}), were calculated for native and non-native areas of each *Prosopis* species. These metrics were estimated using the SPAGeDi version 1.5 software for polyploid *P. juliflora* (Hardy and Vekemans 2002) and the *diveRsity* R package version 1.9.90 (Keenan et al. 2013) for all diploid species. Lastly, allelic richness (A_R) and the number of private alleles were calculated for all *Prosopis* species from different native and non-native areas with the software ADZE version 1.0 (Szpiech et al. 2008). ADZE uses a rarefaction approach to calculate sample size-corrected estimates for these metrics. Due to the lower number of samples, were not included in this analysis each type of hybrid individuals from Australia and Argentina, and species with only one or two individuals from native and non-native areas. Since we wanted to compare the level of genetic diversity at species level individuals from native areas of Argentina and Chile that could not be confidently identified were not included in these analyses.

e) Genetic structure and hybridization

To identify the number of genetic clusters present in the overall dataset, Bayesian assignment tests were used as implemented in the software STRUCTURE version 2.3.4 (Pritchard et al., 2000). STRUCTURE uses Bayesian Monte–Carlo Markov chain sampling to identify the optimal number of genetic clusters for a given dataset by reducing departures from Hardy–Weinberg and linkage equilibrium expectations within genetic clusters. A hierarchical clustering approach was implemented including populations of various *Prosopis* species from native and invaded ranges. A number of genetic clusters (K) between $K = 1$ and $K = 12$ (number of species sampled), were tested and 10 independent models for each value of K were run. Each model consisted of 500,000 generations of which the first 100,000 were discarded as burnin. An admixture model with correlated allele frequencies was applied since this type of model is more robust for identifying the optimal number of clusters that captures the major structure of the data (François & Durand 2010) and enables the identification of hybrids among the species. STRUCTURE provides assignment values of the individuals to the different genetic clusters, calculated as the proportion (q_{ik}) of each genotype individuals sampled that is derived from each of the K clusters. Individuals with genomic assignment values under 60% ($q_{ik} > 0.6$) to a particular genetic cluster were not included in subsequent analyses. According to previous studies on ploidal variation in *Prosopis*, the only tetraploid species in the genus is

P. juliflora ($2n = 4x = 46$), with all other species being entirely diploid ($2n = 2x = 28$) (Trenchard et al., 2008). Considering this, the analysis was performed considering only polyploid individuals identified as *P. juliflora* and excluding individuals of other species that showed more than two alleles at loci ($n=14$; Table S2.3). Since the dataset includes individuals with different ploidy levels, an overall ploidy of $4x$ was specified. For analyses including polyploid individuals, the option *RECESSIVEALLELES* was set to one to account for allele copy ambiguity (Pritchard et al. 2010). For diploid-triploid individuals, a missing data symbol was added to complete the ploidy level, indicating that the individual is diploid-triploid at all the loci (Pritchard et al. 2010). For all these models, the optimal K value was estimated following the method described by Evanno et al. (2005) and STRUCTURE Harvester (Earl and VonHoldt 2012). CLUMPAK software (Kopelman et al. 2015) was used to graphically display of the results.

A principle component analysis (PCA) was also performed using the *PolySat* R package (Clark and Jasieniuk 2011). This analysis allows genetic analyses including individuals of mixed ploidy. A matrix of pairwise distances between individuals was generated using Bruvo distances (Bruvo et al 2004). This method was used because it incorporates distances between microsatellite alleles without information on allele copy number (Bruvo et al. 2004).

We tested the assignment of individuals to parents and hybrids of diploid *Prosopis* species using the software NewHybrids version 1.1beta (Anderson and Thompson 2002). This software identifies six genotypes classes (i.e. pure species 1, pure species 2, F1 hybrids, F2 hybrids, species 1 backcross and species 2 backcross) without information on the allele frequency of the parental species. The analysis was done on all possible pairwise combinations of *P. chilensis*, *P. alba*, *P. flexuosa*, *P. nigra*, and *P. vinalillo* and putative hybrids including only Argentinean individuals (i.e. excluding allopatric individuals of *P. chilensis* and *P. alba* from Chile), on *P. alba* and *P. chilensis* individuals from Chile, and between *P. pallida* individuals of Peru and *P. pallida* individuals of Hawaii and Kenya. Parameter setting included a burnin-period of 30 000 generations and 50 000 MCMC iterations. We used 'Jeffrey's like priors' and a posterior probability of 0.8 was used to assign individuals to the six genotypes classes. Individuals that could not be assigned to a genotype classes were considered of mixed ancestry.

f) Genetic differentiation

We tested the genetic differentiation between invasive and non-native populations of various *Prosopis* species. For *P. juliflora*, a matrix of pairwise genetic distances (F_{ST}) was calculated using the R package *Polysat* and for each pairwise differentiation statistic, a 95% confidence interval was calculated through bootstrap across loci. For diploid species, pairwise F_{ST} values were calculated following Weir (1996). For this, the FreeNA software (Chapuis and Estoup, 2007) was used to calculate corrected and uncorrected F_{ST} estimates since it applies an “excluding null alleles” (ENA) correction to account for the presence of null alleles. The 95% confidence intervals for F_{ST} values were obtained by 10 000 simulations. Species or hybrids with only one or two individuals from native and non-native areas, and individuals that could not be confidently identified to species level, were not included in these analyses. In addition, a hierarchical analysis of molecular variance (AMOVA) was performed using the *pegas* R package (Paradis 2010) between non-native and native *P. juliflora* and *P. pallida* populations of Ethiopia, Kenya and Tanzania. For *P. juliflora*, a matrix of pairwise distances between individuals was generated using Bruvo distances. In the case of *P. pallida*, Euclidian distance based on the allele frequencies was used to generate pairwise distances between individuals.

2.3 Results

a) Phylogenetic analysis

DNA sequencing data for the ETS, *psbA* and *rpl32* gene regions indicate extremely low sequence variability across species and regions. The exception was *P. tamarugo* compared to the rest of species, where we found 15 substitutions for the *psbA* region, 17 substitutions for the *rpl32* region and ~240 substitutions for the ETS region. For the rest of species included, all shared almost 100% DNA sequence similarity for these three gene regions sequenced. That is, when excluding *P. tamarugo*, we found only one substitution in the *psbA* region between *P. pallida* from Peru and all other species, one substitution in the *rpl32* region between *P. nigra* and *P. flexuosa* and all other species. For the ETS region, we found only four substitutions between *P. juliflora* from Kenya and all other species; *Prosopis spp.* from South Africa differed by three substitutions with all other species, while *Prosopis glandulosa* and the hybrids from Australia, and *P. pallida* from Peru had one substitution each when compared

with the rest of the species. The *ITS* region yielded low quality sequencing traces for all individuals, possibly due to multiple copies, and therefore the data were not analysed.

b) Genetic diversity

For diploid *Prosopis* species, 20 loci by species by country combinations (23.8%) did not meet the HWE expectations. All seven loci were polymorphic in the overall dataset. The average number of alleles per locus was 10.6 (ranging between 5-19 alleles). However, some markers were not polymorphic for some species in some regions (Table S2.4). *Prosopis pallida* individuals from Peru had one allele of locus Prsc9. *Prosopis juliflora* individuals from Mexico and *P. pallida* individuals from Peru and Hawaii had one allele at locus S-P1EPIV2. *Prosopis juliflora* individuals from Tanzania, *P. pallida* from Hawaii and *P. nigra* from Argentina also had one allele at locus I-P06639. *Prosopis pallida* individuals from Peru and Hawaii had one allele for locus S-P1DKSFA. Overall, *Prosopis* species from native areas in Argentina and Chile, *P. laevigata* from Mexico and *Prosopis* spp. from South Africa had higher levels of H_E and H_o compared to native and non-native individuals of *P. juliflora* and *P. pallida* (Table 2.2). Introduced Kenyan populations of *P. pallida* had similar H_E and H_o than native individuals from Peru. Invasive *P. pallida* individuals from Hawaii had lower H_E and H_o compared to native and other non-native populations of this species. In the case of *P. juliflora*, invasive populations from Kenya had higher H_E than the native populations from Mexico. *Prosopis juliflora* populations from the native range in Mexico had higher genetic diversity than invasive individuals from Tanzania. Inbreeding coefficients (F_{IS}) were higher in all *P. pallida* populations from native and non-native areas, *P. laevigata* individuals from Mexico and in *P. alba* individuals from Chile; compared to all other species and areas. Only *P. juliflora* individuals from Mexico had negative F_{IS} values (Table 2.2). Allelic richness (A_R) of most *Prosopis* species from native areas was higher than for *P. juliflora* from native Mexico and species from non-native areas, with the exception of invasive individuals from South Africa (Fig. 2.2). The number of private alleles was similar among species and regions (Fig. 2.2).

Table 2.2. Population genetic diversity indices for native, introduced and invasive populations of various *Prosopis* species. Statistics were calculated as mean values of each index over the seven loci analysed.

Species	Country	Category	H_E	H_o	F_{IS}
<i>P. alba</i>	Argentina	Native	0.70	0.65	0.07
	Chile	Native	0.64	0.49	0.23
<i>P. chilensis</i>	Argentina	Native	0.67	0.56	0.16
	Chile	Native	0.63	0.55	0.13
<i>P. flexuosa</i>	Argentina	Native	0.63	0.61	0.02
<i>P. juliflora</i>	Mexico	Native	0.31	0.44	-0.20
	Ethiopia	Invasive	0.35	0.42	0.08
	Kenya	Invasive	0.42	0.46	0.18
	Tanzania	Invasive	0.28	0.29	0.10
		All	0.41	0.44	0.19
<i>P. laevigata</i>	Mexico	Native	0.47	0.32	0.31
<i>P. nigra</i>	Argentina	Native	0.50	0.47	0.06
<i>P. pallida</i>	Peru	Native	0.39	0.30	0.24
	Hawaii	Invasive	0.28	0.20	0.28
	Kenya	Introduced	0.41	0.30	0.26
		All	0.36	0.27	0.26
<i>P. vinalillo</i>	Argentina	Native	0.62	0.58	0.06
<i>Prosopis spp.</i>	South Africa	Invasive	0.68	0.57	0.16

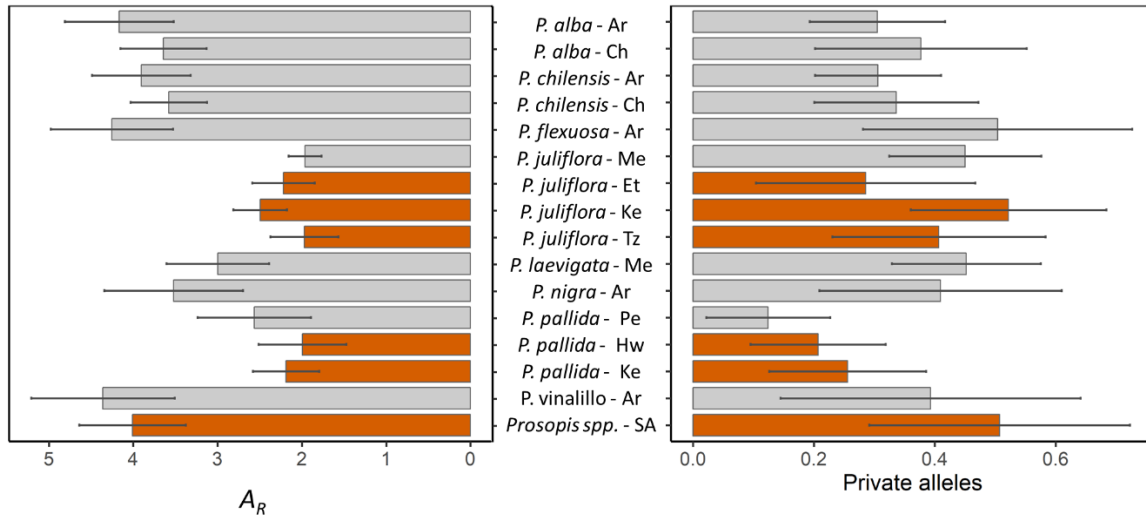


Figure 2.2. Allelic richness (A_R) and number of private alleles. (\pm 95% confidence interval) for various *Prosopis* species from native (grey bars) and non-native regions (red bars). Country codes: Argentina (Ar), Australia (Au), Chile (Ch), Ethiopia (Et), Hawaii (Hw), Kenya (Ke), Mexico (Me), South Africa (SA) and Tanzania (Tz).

c) Genetic structure and hybridization

Both Bayesian assignment tests and PCA analyses revealed that overall genetic structure followed ploidal variation, with tetraploid *P. juliflora* being highly differentiated from the rest of the diploid *Prosopis* species included here. A second level of hierarchical structure largely reflected series level relationships, showing genetic differences between *P. pallida* and all other species; while there was no clear genetic structure among *Prosopis* species from Argentina, Australia, Chile, Mexico and South Africa. In addition, both analyses identified admixed individuals of *P. juliflora*. Lastly, STRUCTURE, but not PCA analysis, showed some level of genetic structure between native populations of *P. juliflora* from Mexico and invasive populations from Ethiopia, Kenya and Tanzania. (Fig. 2.3, Fig. 2.4 and Fig. S2.1).

Assignment tests in NewHybrids were done between pairs of species per site (Fig. S2.2). This analysis was able to identify only three genotype classes: pure species 1, pure species 2 and mixed ancestry. That is, when analyses were done between pairs of species from Argentina: *P. alba* (n=27) - *P. chilensis* (n=19), *P. alba* - *P. flexuosa* (n=8), *P. alba* - *P. nigra* (n=6), *P. alba* - *P. vinalillo* (n=7), *P. chilensis* - *P. flexuosa*, *P. chilensis* - *P. nigra*, *P.*

chilensis - *P. vinalillo* and *P. nigra* - *P. vinalillo*; individuals morphologically identified as one of the two species were assigned as pure genotypes of the same species (between 57.1% to 100%), as pure genotypes of the other species (between 3.7% to 16.7%); or had mixed ancestry (between 5.3% to 42.9%), i.e. being hybrids. In contrast, when including pairs of the species *P. flexuosa* - *P. nigra* and *P. flexuosa* - *P. vinalillo*, the models were unable to assign individuals to any class. Interestingly, morphological hybrids (n=9) were genetically classified as being one of the pure parental species (66.7%) or had mixed ancestry (33.3%). Similarly, analyses based on *P. chilensis* (n=19) - *P. alba* (n=27) from Chile, classified individuals as pure genotypes of one of the two species (between 68.4% and 90.9%), as having mixed ancestry (between 10.5% and 9.1%), while some *P. chilensis* individuals were classified as pure *P. alba* (21.1%). Lastly, an analysis including *P. pallida* from Peru (n=12) and *P. pallida* from Hawaii (n=14), and between *P. pallida* from Peru and *P. pallida* from Kenya (n=50), classified all *P. pallida* individuals from Peru as pure *P. pallida* genotypes. For *P. pallida* from Hawaii, some individuals were classified as pure *P. pallida* genotypes from Peru (7.14%), while half of them represented a pure genotype different from the native Peruvian genotype, and 42.9% having mixed ancestry. For *P. pallida* from Kenya, almost all individuals were classified as pure Peruvian *P. pallida* genotypes (92.0%) and a few as having mixed ancestry (8.0%).

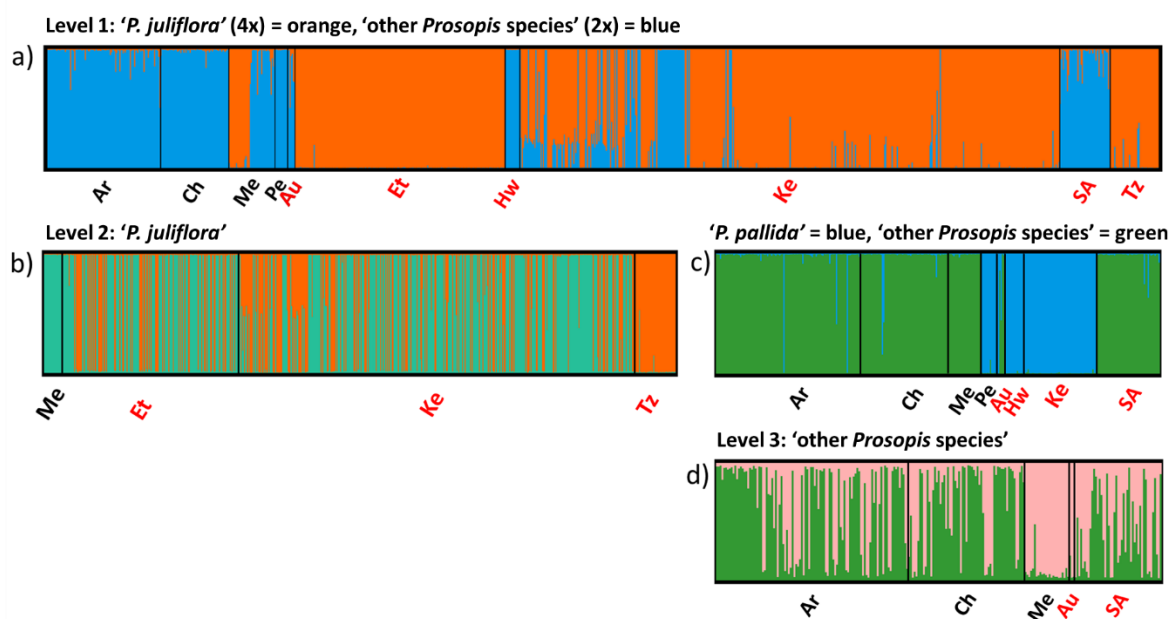


Figure 2.3. Hierarchical Bayesian clustering analyses of individuals of native (black labels) and non-native (red labels) areas of various *Prosopis* species: Argentina (Ar) = *P. chilensis*, *P. flexuosa*, *P. strombulifera*, *P. alba*, *P. nigra*, *P. torcuata* and *P. vinalillo* and hybrids; Chile (Ch) = *P. chilensis* and *P. alba*; Mexico (Me) = *P. juliflora* and *P. laevigata*; Peru (Pe) = *P. pallida*; Australia (Au) = *P. pallida*, *P. glandulosa*, *P. velutina* and hybrids; Ethiopia (Et) = *P. juliflora*; Hawaii (Hw) = *P. pallida*; Kenya (Ke) = *P. juliflora* and *P. pallida*; South Africa (SA) = *Prosopis spp.*; Tanzania (Tz) = *P. juliflora*. Individuals were genotyped using seven nuclear microsatellite loci and clustered at three levels. a) Level 1: '*P. juliflora*' cluster in orange and 'other *Prosopis* species' cluster in blue; b) Level 2: only *P. juliflora* individuals and c) Level 2: '*P. pallida*' cluster in blue 'other *Prosopis* species' cluster in green; d) Level 3: individuals of 'other *Prosopis* species' cluster from Argentina, Chile, Mexico, Australia and South Africa. Vertical axes represent the assignment (q_{ik} values) of individual genomes to the inferred number of genetic clusters, in all cases $K=2$ (See Fig. S1).

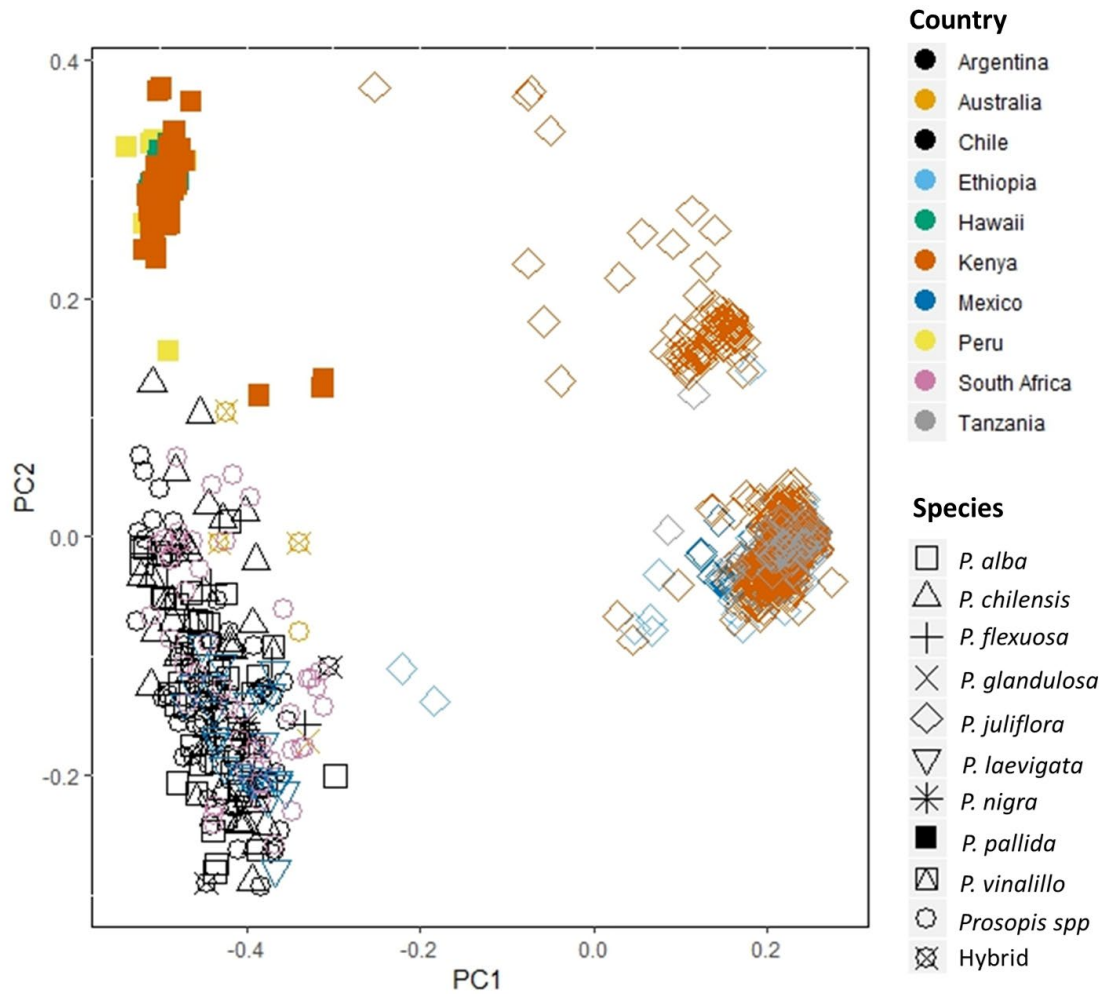


Figure 2.4. Principal component analysis showing genetic structure among *Prosopis* species from different native and invaded areas. PCA was performed using Bruvo distances in *PolySat* (Bruvo et al. 2004). PC1 and PC2 captured 63.6% and 11,0% of the variation, respectively. Individuals of tetraploid *P. juliflora* are clearly separated from all other species.

d) Genetic differentiation

Similar results were obtained with uncorrected and ENA-corrected pairwise F_{ST} estimates (Kruskal-Wallis chi-square = 0.1, $p = 0.75$), therefore, the uncorrected pairwise F_{ST} values (Table S2.5) with 95% confidence intervals are presented (Table S2.6). Overall, I found low genetic differentiation between *Prosopis* species in spite of allopatric distributions. For example, pairwise F_{ST} values between some sympatric *Prosopis* species from Chile and Argentina was similar than those between these species and *P. laevigata* from Mexico, *Prosopis spp.* individuals from South Africa, and hybrids from Australia. Nevertheless, the genetic distance between sympatric native populations of *P. chilensis* and *P. alba* from Chile was higher than that between the sympatric populations of the same species in Argentina.

With respect to Eastern Africa, the genetic distance between invasive Tanzanian and native Mexican populations of *P. juliflora* was higher than the genetic distances between the latter and invasive populations from Kenya and Ethiopia; and was also higher than the genetic distance between invasive populations from Kenya and Ethiopia (Fig. 2.5). The hierarchical AMOVA indicated considerable, but not significant, genetic variation among native and invasive *P. juliflora* populations (71.35%), while significant, and similar, genetic variation was found among invaded areas (12.32%) and within populations in invaded areas (16.33%) (Table 2.3). In the case of *P. pallida*, genetic distances were similar between all native populations from Peru and non-native (introduced and invasive) populations from Hawaii and Kenya (Fig. 2.5). There was also a substantial, but not significant, genetic variation between native and non-native (both introduced and invasive) populations of *P. pallida* (42.99%), while the genetic variation between introduced and invasive populations (28.48%) was significant and slightly lower, than the variation within populations (35.34%, Table 2.3).

Table 2.3. Hierarchical AMOVA partitioning of genetic variation for *P. juliflora* and *P. pallida* populations from various native, introduced and invasive populations.

Source of variation	d.f.	Sum of squares	Variance	Percent variation (%)	Fixation index
<i>P. juliflora</i>					
Native vs non-native populations	1		169.92	71.35	0.48
Among invasive populations	2		29.35	12.32	0.14*
Within populations	709		38.88	16.33	0.55
<i>P. pallida</i>					
Native vs non-native populations	1	35.79	21.88	42.99	0.15
Introduced vs invasive populations	1	14.98	16.76	28.48	0.21*
Within populations	73	429.08	20.21	34.35	0.01

*significant value, tested using 10 000 random permutations

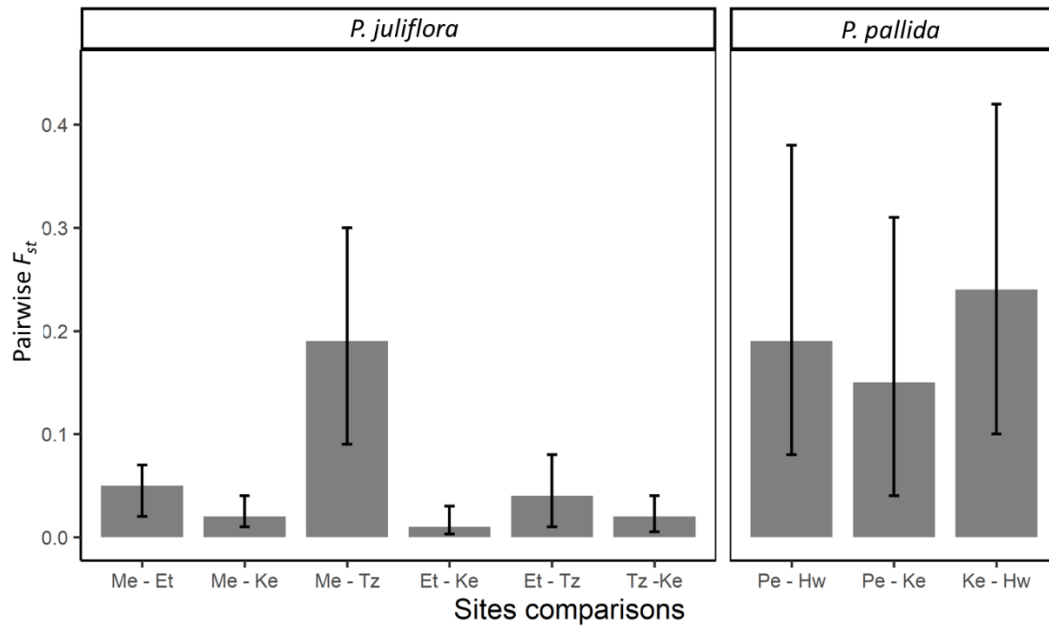


Figure 2.5. Pairwise F_{ST} (\pm 95% confidence interval) between native Mexico (Me) and invaded populations of *P. juliflora* in Ethiopia (Et), Kenya (Ke) and Tanzania (Tz); between invaded population of *P. juliflora*; between native populations of Peru (Pe) and introduced populations of Kenya (Ke) e invasive populations of Hawaii (Hw) of *P. pallida*.

2.4 Discussion

While numerous previous studies have reported on the genetic relationships between *Prosopis* species and population-level genetic variation (e.g. Ramírez et al. 1999, Saidman et al. 2000, Bessega et al. 2005, Bessega et al. 2006), our study is the first aimed at clarifying the taxonomic uncertainty of *Prosopis* invasions by examining patterns of genetic diversity, genetic similarity and phylogenetic relationships between various *Prosopis* species at a global scale, and between various introduced/invaded and native regions.

Uncertainty in Prosopis taxonomy

The taxonomy of *Prosopis* has been a topic of intense debate (Saidman and Vilardi 1987, Saidman et al. 2000, Pasiecznik et al. 2001). For example, low genetic variability among species has been postulated to blur species boundaries, with some authors considering *Algorobia* species to constitute a so-called 'syngameon', i.e. a hybrid swarm (Palacios and

Bravo 1981). Different varieties and ecotypes have been described for species within the Algarobia section in South America and it has been suggested that these are more closely related to each other than they are to species in other sections (Saidman et al. 2000). Our DNA sequencing data indicated that many *Prosopis* species within this section shared almost 100% genetic similarity for the three gene regions included here. While these markers may have insufficient resolution to differentiate closely related species, this finding does support incomplete reproductive isolation between these closely-related *Prosopis* species, suggesting a recent radiation of species (also see Catalano et al. 2008). This, in turn, may lead to frequent hybridization and introgression between species within the Algarobia section (Hunziker et al. 1986). In agreement with our finding that low genetic differentiation exists between *Prosopis* species in spite of allopatry, e.g. between *P. laevigata* from Mexico and the rest of South American species, previous comparisons between North and South American species of the Algarobia section yielded similar results. For example, Bessega et al. (2000) found that genetic differentiation between *P. rustifolia* and *P. flexuosa* from Argentina was similar to differentiation between these two species and *P. glandulosa* from North America. These researchers concluded that the extent of hybridization between species was not related to the genetic similarity among Algarobia species. Contrary to previous studies that have found high genetic similarity between *P. juliflora* from Colombia and two allopatric diploid species, *P. rustifolia* and *P. caldenia*, from Argentina (Saidman et al. 1997), our genetic analyses found *P. juliflora* to be highly differentiated from the rest of the species in the group (including species from different *Prosopis* sections such as *P. strombulifera* and *P. torquata*). Our data suggests that, in the case of *P. juliflora*, ploidal variation is an important mechanism that underlies reproductive isolation, and thus genetic differentiation, in the genus.

In invaded areas, taxonomic uncertainty in *Prosopis* is exemplified by populations of *P. juliflora* and *P. pallida* in Eastern Africa. Due to the different morphological forms of both species and their overlapping distributions in the native areas (Fosberg 1966, Burkart 1976, Diaz Celis 1995), misidentifications are common in both native and non-native areas (Pasiiecznik et al. 2001). Pasiiecznik et al. (2001) proposed the existence of a *P. pallida* – *P. juliflora* complex to deal with these issues. In Kenya, morphological data suggest the presence of intermediate morphotypes between *P. juliflora* and *P. pallida*, i.e. possible hybrids (W. Okellu, CABI, unpublished data). Our genetic results, together with previous work (Catalano

et al. 2008, Trenchard et al. 2008, Palacios et al. 2012), show distinct genetic differences between these two species, confirming that they are indeed two distinct taxa. In Kenya, while our genetic structure analysis identified some hybrid individuals, we failed to identify morphological hybrids during field sampling. Hybridization between these two species are not expected to persist in the environment because it is likely to result in sterile triploid offspring that will be unable to spread this cytotype through successive generations. Therefore, studies on ploidal level variation (e.g. flow cytometry analysis) within invasive populations would help to clarify the taxonomic identification of *P. juliflora* and the extent of hybridization with other diploid species in invaded ranges.

Taxonomic obscurity in *Prosopis* was further illustrated by our analysis of hybridization. Our genetic results showed that hybrids identified based on morphology from both native and invaded areas were either classified as such (i.e. mixed ancestry) or as pure parental species, while many of the species identified as morphologically pure had mixed ancestry. In addition, the difficulty in the assignment of individuals of the group *P. flexuosa* - *P. nigra* - *P. vinalillo* to any class, may reflect the fact that *P. vinalillo* diverged recently from *P. rustifolia* and is considered a non-stabilized hybrid involving sympatric populations of *P. alba*, *P. ruscifolia* and *P. nigra* (Ferreyra et al. 2004). A lack of congruency between the morphological and genetic data in the taxonomic classification of native *Prosopis* species has been consistently found in the native range (Saidman et al. 2000). Different morphological traits have been used to differentiate *Prosopis* ecotypes and subspecies, but they are largely plastic, i.e. dependent on the environmental conditions (Verga et al 2009), and therefore useless. In addition, Bessega et al. (2006) proposed that certain morphological characteristics in *Prosopis* may have evolved rapidly and recently under certain stress conditions. Therefore, the high phenotypic variation at intra-species level, rapid evolution in key traits, low genetic differentiation between species, and frequent hybridization all contribute to the complicated taxonomy of the genus *Prosopis*. Overall, our results support the hypothesis that *Prosopis* species of the *Algarobia* section constitute a syngameon, with *P. juliflora* being the only species that is differentiated from other species due to ploidy level differentiation.

Our study is the first attempt to clarify the taxonomic uncertainty of *Prosopis* invasions by examining patterns of genetic diversity, genetic similarity and phylogenetic relationships between various *Prosopis* species at a global scale, and between various introduced/invaded

and native regions. For this, sampling in the native areas was done within the two known centers of diversification of the genus, the Argentine–Paraguayan–Chilean region and the Texan–Mexican region. We aimed to include a high number of species, rather than sampling comprehensively across the distributions of only a few species. Sampling in some of the invaded areas were also somewhat limited in terms of the number of individuals samples and their geographical range. Including more individuals from certain species from across the target areas may have increased chances for finding higher genetic variability between and within species. Nonetheless, the low genetic variability we found among species is in agreement with previous studies (Saidman et al. 2000, Bessega et al. 2000). Greater sampling effort of some species and their putative hybrids may have helped in some cases to corroborate morphological identifications with genetic data (i.e. there would have been more hybrids assigned as having mixed ancestry). Considering the biological and socio-economic importance of *Prosopis* species, it is necessary to conduct complete genetic monitoring, morphological and physiological studies of native and non-native populations to disentangle the taxonomic obscurity in *Prosopis*. Future research should include genomic data, such as those generated through next-generation sequencing approaches, to provide more detailed insights into the genetics of *Prosopis* biogeography and invasiveness.

Hybridization, polyploidy and genetic diversity

In agreement with previous studies, we identified instances of hybridization between *Prosopis* species in both native (e.g. see Saidman et al. 2000) and invaded ranges (e.g. see Zimmerman 1991, van Klinken et al. 2006, Mazibuko 2012, Muturi 2012). The success of many plant invasions has been attributed to hybridization (Schierenbeck and Ellstrand 2009, Zalapa et al. 2010, Gaskin et al. 2012) and this may also be the case for some *Prosopis* invasions, but probably not for co-introduced species with different ploidy levels as in some areas of Eastern Africa. Moreover, the lack of genetic differentiation between some species in their native range suggest that reproductive isolation is incomplete and that admixture between allopatric species may occur frequently when they are co-introduced into new ranges as has been found in South Africa and Australia (Klinken et al. 2006, Mazibuko 2012). In the native area, reproductive isolation between *Prosopis* species appear to be low and incomplete (Hunzinger 1986, Earl 1998) and that hybridization between species are promoted by certain environmental conditions (Vega and Hernandez 2005) with some hybrids being mostly found

in disturbed areas (Verga 2005b). Considering this, interspecific hybridization between *Prosopis* species in the invaded area may not only be dependent on the genetic relatedness of species, but also whether certain habitat features facilitate co-occurrence and interbreeding. It may also be that only certain *Prosopis* genotypes, or hybrid combinations, are successful under particular environmental conditions, or that only hybrid genotypes are able to spread extensively into new environments. For example, Zenni *et al.* (2014) recently illustrated that admixture (i.e. intra-specific hybridization between previously isolated populations) and subsequent fixation of certain genotypes occurred multiple times, and independently, during the escape and invasive spread of the loblolly pine, *Pinus taeda* L., from plantations in Brazil. In Australia, *P. pallida* is widely distributed across the north of Australia from the east coast of Queensland through the Northern territory, to the west coast of Western Australia (CRC Weed Management Guide 2003). However, this species is not found in the cooler southern states, where *P. velutina* and hybrids between *P. velutina* and *P. glandulosa* var. *torreyana* seem to dominate (CRC Weed Management Guide 2003). While these patterns may reflect the initial introduction of particular species to particular areas, they may also be indicative of variation in climate preferences of different species and their hybrids. Therefore, studies are needed to evaluate whether different *Prosopis* species and their hybrids differ in their ecological requirements and tolerances.

We also identified *Prosopis* individuals that had more than two alleles at some loci in both native and non-native areas. These individuals were not classified as tetraploid *P. juliflora*, but rather as hybrids from Australia, *P. flexuosa* from Argentina, *P. laevigata* from Mexico and individuals from South Africa that could not be identify to species level but are putative hybrids. While polyploidy has been reported in *Prosopis* (Cherubini 1954, Hunziker *et al.* 1975, Burkart 1976), Trenchard *et al.* (2008) proposed that tetraploid *P. juliflora* is the only polyploid species in the genus. Our genetic results indicate that reproductive isolation seems to be incomplete among many *Prosopis* species. Pre-zygotic reproductive barriers, such as differences in flowering time or the use of different pollinators, are thought to be weak in *Prosopis*, while post-zygotic reproductive barriers such as pollen inviability may be more important (Palacio and Bravo 1981, Naranjo *et al.* 1984). Polyploidy would be an additional mechanism that facilitates immediate reproductive isolation between species. In addition, polyploidy plays an important role in restoring sexual reproduction following hybridization

and often leads to greater levels of stress tolerance, higher growth vigour through increased plant size, seed size, flower size, niche breadth and phenotypic plasticity, among others (te Beest et al. 2012). These beneficial effects of polyploidy may well explain the successful invasion of *P. juliflora*, in particular, in some regions like Eastern Africa. Surprisingly, few studies have investigated ploidy in *Prosopis*, and mostly from native areas (Cherubini 1954, Hunziker et al, 1975, Burkart 1976, Trenchard et al. 2008). Considering the important role that polyploidy may have on plant invasion success and reproductive isolation, we suggest that more effort should be focussed on the ploidal variation in the *Prosopis*, in both native and non-native populations.

We found genetic diversity in *Prosopis* to be high and similar among species and regions, with the exception of *P. pallida* and *P. juliflora*, both harbouring lower levels of genetic diversity compared to the rest of the species in the genus. The majority of species also showed homozygote excess, i.e. signs of inbreeding, with only *P. juliflora* individuals from native populations of Mexico displaying heterozygote excess. Previous studies have also found high genetic variability and levels of inbreeding for species in the *Algarobia* section in their native areas (Bessega et al. 2000), probably reflecting high levels of self-compatibility in the genus (Keys and Smith 1994, Bessega et al. 2000).

The historical diversification of *Prosopis* species and their biogeographic distribution is thought to be associated with long-distance dispersal events to the southern and western regions of South America from the centre of diversification of the genus in the Chaco ecoregion (Roig 1993). An alternative hypothesis postulates that the ancestor of *Prosopis* species was once broadly distributed across the Americas, followed by fragmentation during the Pleistocene (Bessega et al. 2000). Studies based on isozyme data (Bessega et al. 2000) and evidence from phylogenetic analyses (Bessega et al. 2006) suggest that the radiation of the genus included recolonizations, in both directions, between North and South America and recurrent vicariance and long-distance dispersal events. Dispersal of *Prosopis* in these native range areas may also pre-historically have been facilitated by humans, along with the movement of livestock and crops (McRostie et al. 2017). We found a similar number of private alleles among the species which is not in agreement with frequent long-distance gene flow between species in the native range. Previous work by Bessega et al. (2000), using isozyme analyses, found that most alleles were shared between species, leading them to conclude that

hybridization and introgression were important for the diversification of the genus, but that the high genetic similarity among species is not due to interspecific gene flow, and that instead colonization events involving large number of founders and high populations growth rates is a plausible explanation.

Invasion history of Prosopis

The introduction of *Prosopis* into many non-native areas is thought to have been characterized by multiple introductions, in some instances, from a small number of trees with low genetic variation (Pasiiecznik et al. 2001). Similar levels of genetic diversity in newly established populations and native populations may be indicative of multiple introductions or it may simply reflect a unique introduction from a source generated by admixture of multiple populations (Le Roux et al. 2011). This may well explain the similar levels of genetic diversity observed when comparing native Mexican *P. juliflora* with invasive Ethiopian individuals and native Peruvian *P. pallida* with introduced Kenyan individuals. In the case of *P. juliflora* in Kenya, invasive individuals had higher H_E than the native *P. juliflora* individuals from Mexico. Higher levels of genetic diversity among introduced individuals in comparison to native ones can be the result of multiple large-scale immigration events and cultivation, and can generate genetic novelties (Lavergne and Molofsky 2010, Thompson et al. 2012). In Kenya, *P. juliflora* trees were first introduced in 1973 to Mombasa (Johansson 1990). Later, demonstration plantations were established during the 1970s and 1980s in many parts of Kenya, including Baringo County, Tana River and Taveta (Johansson 1990, Otsamo and Maua 1993, Choge et al. 2002). In Ethiopia, *Prosopis* was first introduced in the early 1980s into the Afdem and Afar areas (Amibara and Gewane districts; Admasu 2008, Kebede and Coppock, 2015) with additional introductions between 1980s and 1990s (Kebede and Coppock 2015). Genetic novelty due to cultivation is likely to underlie *P. juliflora* invasion in Kenya, but makes it surprising that this would not to be the case of Ethiopia or Tanzania given the similar introduction histories.

The origin of *P. juliflora* and *P. pallida* individuals in Eastern Africa is unknown (Choge et al. 2011). Our results showed that in the case of *P. juliflora*, introduced genetic material seems to be similar for most non-native locations and similar to native Mexican *P. juliflora*, but different from Tanzanian populations. While Tanzanian genotypes were similar to some

individuals from Kenya and Ethiopia, the origin of these genotypes is probably not Mexican. In the case of *P. pallida* in Kenya, individuals were genetically similar to Peruvian individuals. Previous reports indicated that *P. pallida* in Hawaii may have been introduced from seeds of a single tree planted in France with presumably South American origin (Perry, 1998). This would explain the low genetic variation in *P. pallida* individuals from Hawaii compared to native Peruvian individuals. Even when our assignment analysis indicated that some individuals from Hawaii were not genetically similar to Peruvian genotypes, our genetic structure and genetic differentiation results support the proposition of a South American origin for *P. pallida* in Hawaii. Previous studies have showed that individuals from Hawaii are genetically similar to the ones from Australia (Panetta and Carstairs, 1989), giving support to the hypothesis that *P. pallida* arrived in Australia from some Pacific island, probably Hawaii (Panetta and Carstairs, 1989, Perry 1998), a notion supported by our genetic data. However, the low number of samples from Australia preclude confidence in these inferences.

Implications for management and regulation

Our findings also have important implications for the management of invasive *Prosopis* populations. Given the taxonomic uncertainty and frequent interbreeding between different species, regulations that consider only individual *Prosopis* species as invasive may not be effective. Rather, for successful management of *Prosopis* we strongly recommend that regulations consider the genus as a single group with high invasion risk and high current or potential negative impact (i.e. be declared as noxious weeds) rather than separate *Prosopis* species. This will help the implementation of national and sub-national strategies as well as the allocation of resources for their implementation. This will also contribute to evaluate the effectiveness of these managing practices and prevent the introduction of additional non-native species, in addition to those that are already included in national or regional lists. It will also facilitate the application of control strategies in areas where taxonomic uncertainty is problematic, for example, the removal of detected *Prosopis* plants of any non-native species should be prioritized and planting of any additional species be prohibited.

The high levels of hybridization between *Prosopis* species may also play a role in the limited success of biological control. In South Africa seed-feeding biological control agents have been introduced in an attempt to reduce the impacts of *Prosopis* invasions, but these

have had little impact to date (Zachariades et al. 2011), and the trees have continued to spread (Henderson and Wilson 2017). In addition, our results showed that populations from South Africa have the highest level of genetic diversity compared to the other non-native areas, likely as a consequence of the high number of species introduced and extensive hybridization among them (Mazibuko, 2012). Higher levels of genetic diversity would increase the capacity of populations to adapt to new conditions in non-native ranges and to expand their range (Sakai 2001, Lee 2002, Kolbe et al. 2004, Shirk et al. 2014). The current list of invasive species of South Africa, includes only *Prosopis glandulosa* var. *torreyana*, *P. velutina*, and hybrids of both species. Biological control agents that are pre-adapted to a particular species or eco-types may be less effective against hybrids (Goolsby et al. 2006). Given the substantial genetic similarity between the South African *Prosopis* spp. and species from Argentina and Chile, and *P. laevigata* from Mexico, it is strongly recommended that biological control in South Africa should be further investigated in future management strategies.

In Australia, out of four released biological control agents, a leaf-tying moth (an *Evippe* species) appears to have established stable populations. This agent is achieving high levels of defoliation of *Prosopis* trees in warm areas of Australia, causing reduced plant performance by lowering growth rates and seed production (van Klinken and Campbell 2001). However, there is low plant mortality and recruitment is still occurring, suggesting that the spread of *Prosopis* will continue (van Klinken and Campbell 2009, Pichancourt et al. 2012). It is not well-understood which ecological variables determine the current Australian distribution of different *Prosopis* species and their hybrids (van Klinken and Campbell 2001), information that is crucial to assess the effectiveness of biological control agents like *Evippe* moths under different environmental conditions (van Klinken and Campbell 2001). This is because intra- and interspecific variation that is environmentally induced may influence the response of plants to biological control agents. In addition, in areas like Australia, *Prosopis* invasions are still expanding and it is necessary to evaluate their habitat requirements in order to predict their future spread and the efficacy of biocontrol agents under new environmental conditions in these non-native areas.

Treating the genus *Prosopis* as a single taxonomic group for regulatory purposes not only has obvious management advantages, but also circumvent potential legal challenges to such regulations. Even under such a classification scheme we think that future research

should still aim to determine whether different taxa and their hybrids differ in invasiveness and their responses to different management practices.

2.5 Supporting information

Table S2.1. Details of the 51 microsatellites loci tested for amplification. From these, 10 markers were included in one multiplex PCR assay (in bold).

Locus name	Original study	Species developed for
Prb1	Alves et al. 2014	<i>P. rubriflora</i>
Prb2	Alves et al. 2014	<i>P. rubriflora</i>
Prb3	Alves et al. 2014	<i>P. rubriflora</i>
Prb4	Alves et al. 2014	<i>P. rubriflora</i>
Prb5	Alves et al. 2014	<i>P. rubriflora</i>
Prb6	Alves et al. 2014	<i>P. rubriflora</i>
Prb7	Alves et al. 2014	<i>P. rubriflora</i>
Prb8	Alves et al. 2014	<i>P. rubriflora</i>
Prb9	Alves et al. 2014	<i>P. rubriflora</i>
Prb10	Alves et al. 2014	<i>P. rubriflora</i>
Prsc1	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc2	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc3	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc4	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc5	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc6	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc7	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc8	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc9	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc10	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc11	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc12	Alves et al. 2014	<i>P. ruscifolia</i>
Prsc13	Alves et al. 2014	<i>P. ruscifolia</i>
GL6	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL8	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL9	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL12	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL15	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL16	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL18	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL21	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL23	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL24	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
GL26	Bessegga et al. 2013	<i>P. alba</i> - <i>P. chilensis</i>
Mo05	Mottura et al. 2005	<i>P. flexuosa</i> - <i>P. chilensis</i>
Mo07	Mottura et al. 2005	<i>P. flexuosa</i> - <i>P. chilensis</i>

Mo08	Mottura et al. 2005	<i>P. flexuosa</i> - <i>P. chilensis</i>
Mo09	Mottura et al. 2005	<i>P. flexuosa</i> - <i>P. chilensis</i>
Mo13	Mottura et al. 2005	<i>P. flexuosa</i> - <i>P. chilensis</i>
Mo16	Mottura et al. 2005	<i>P. flexuosa</i> - <i>P. chilensis</i>
I-P00930b*	Torales et al. 2003	<i>P. alba</i>
I-P00930c*	Torales et al. 2003	<i>P. alba</i>
I-P00930d*	Torales et al. 2003	<i>P. alba</i>
I-P03211*	Torales et al. 2003	<i>P. alba</i>
I-P03325a*	Torales et al. 2003	<i>P. alba</i>
I-P06286b*	Torales et al. 2003	<i>P. alba</i>
I-P06639*	Torales et al. 2003	<i>P. alba</i>
I-P07653*	Torales et al. 2003	<i>P. alba</i>
I-P10500*	Torales et al. 2003	<i>P. alba</i>
S-P1DKSFA*	Torales et al. 2003	<i>P. alba</i>
S-P1EPIV2*	Torales et al. 2003	<i>P. alba</i>

*Markers with functional annotations

Table S2.2. Volume of the 11 microsatellites primers included in one multiplex PCR assay. From these, seven were included in the study (indicated in bold). Different fluorescence dyes were used for different microsatellite primers.

Locus name	Primer volume
GL12	1
I-P06639	1
Prb4	1
Prsc7	1
Prsc9	0.5
S-P1DKSFA	0.5
S-P1EPIV2	1
GL23	2
I-P07653	0.3
I-P00930c	0.5
Prb8	2

Table S2.3. List of *Prosopis* individuals, from native and invaded areas, that had more than two alleles in at least one locus. These individuals were excluded from analyses. Non-native areas are indicated by asterisks (*).

Species	Country	Number of individuals
<i>P. flexuosa</i>	Argentina	1
<i>P. laevigata</i>	Mexico	1
Hybrid	Australia*	1
<i>Prosopis spp.</i>	Argentina	2
<i>Prosopis spp.</i>	South Africa*	9

Table S2.4. Number of alleles per microsatellites locus for different species of *Prosopis* from native and non-native areas. Non-native areas are indicated by asterisks (*).

Locus	<i>P. alba</i>		<i>P. chilensis</i>		<i>P. flexuosa</i>	<i>P. juliflora</i>				<i>P. laevigata</i>	<i>P. nigra</i>		<i>P. pallida</i>		<i>P. velutina</i>	<i>Prosopis spp.</i>
	Ar	Ch	Ar	Ch	Ar	Me	Et*	Ke*	Tz*	Me	Ar	Pe	Hw*	Ke*	Ar	SA*
GL12	13	8	9	8	7	2	4	8	4	6	7	5	2	6	10	13
I-P06639	4	3	4	4	4	3	2	5	1	3	1	2	1	4	3	4
Prb4	18	6	11	11	9	3	7	14	4	11	6	9	6	9	9	16
Prsc7	16	11	12	11	10	2	7	8	7	9	6	5	4	7	10	19
Prsc9	3	6	4	4	4	3	4	8	7	8	3	1	2	5	3	8
S-P1DKSFA	4	3	4	3	2	4	2	5	3	2	2	1	1	2	2	3
S-P1EPIV2	6	4	4	6	4	1	2	3	2	2	2	1	1	2	5	4
Overall	64	41	48	47	40	18	28	51	28	41	27	24	17	35	42	67

Ar=Argentina; Ch=Chile; Et=Ethiopia, Hw=Hawaii; Ke=Kenya, Me=Mexico, Pe=Peru; SA=South Africa; Tz=Tanzania.

Table S2.5. Pairwise F_{ST} values calculated for various *Prosopis* species for all investigated native and non-native populations. Non-native areas are indicated by asterisks (*).

		Argentina					Chile		Mexico	Peru	Hawaii*	Kenya*	Australia*
		<i>P. chilensis</i>	<i>P. alba</i>	<i>P. flexuosa</i>	<i>P. nigra</i>	<i>P. vinalillo</i>	<i>P. alba</i>	<i>P. chilensis</i>	<i>P. laevigata</i>	<i>P. pallida</i>	<i>P. pallida</i>	<i>P. pallida</i>	Hybrids
Argentina	<i>P. alba</i>	0.01	-	-	-	-	-	-	-	-	-	-	-
	<i>P. flexuosa</i>	0.11	0.08	-	-	-	-	-	-	-	-	-	-
	<i>P. nigra</i>	0.16	0.13	0.02	-	-	-	-	-	-	-	-	-
	<i>P. vinalillo</i>	0.07	0.05	-0.03	0.00	-	-	-	-	-	-	-	-
Chile	<i>P. alba</i>	0.06	0.06	0.10	0.12	0.07	-	-	-	-	-	-	-
	<i>P. chilensis</i>	0.04	0.04	0.11	0.14	0.09	0.10	-	-	-	-	-	-
Mexico	<i>P. laevigata</i>	0.21	0.17	0.15	0.10	0.13	0.18	0.21	-	-	-	-	-
Peru	<i>P. pallida</i>	0.28	0.25	0.32	0.40	0.32	0.36	0.29	0.44	-	-	-	-
Hawaii*	<i>P. pallida</i>	0.15	0.11	0.10	0.14	0.09	0.18	0.14	0.17	0.19	-	-	-
Kenya*	<i>P. pallida</i>	0.34	0.31	0.40	0.49	0.40	0.44	0.33	0.48	0.15	0.24	-	-
Australia*	Hybrids	0.31	0.29	0.34	0.40	0.33	0.41	0.29	0.43	0.11	0.17	0.07	-
South Africa*	<i>Prosopis spp.</i>	0.08	0.06	0.09	0.09	0.06	0.07	0.06	0.12	0.26	0.09	0.28	0.26

Table S2.6. 95% confidence interval of pairwise F_{ST} values (calculated on bootstrap resampling over loci) for various *Prosopis* species for all investigated native and non-native populations. Non-native areas are indicated by asterisks (*).

Populations		Argentina					Chile			Mexico	Peru	Hawaii*	Kenya*	Australia*
		<i>P. chilensis</i>	<i>P. alba</i>	<i>P. flexuosa</i>	<i>P. nigra</i>	<i>P. vinalillo</i>	<i>P. alba</i>	<i>P. chilensis</i>	<i>P. laevigata</i>	<i>P. pallida</i>	<i>P. pallida</i>	<i>P. pallida</i>	Hybrids	
Argentina	<i>P. alba</i>	0.01-0.03	-	-	-	-	-	-	-	-	-	-	-	
	<i>P. flexuosa</i>	0.03-0.22	0.003-0.18	-	-	-	-	-	-	-	-	-	-	
	<i>P. nigra</i>	0.05-0.29	0.02-0.25	0.01-0.05	-	-	-	-	-	-	-	-	-	
	<i>P. vinalillo</i>	0.02-0.15	0.002-0.12	0.06-0.01	0.03-0.03	-	-	-	-	-	-	-	-	
Chile	<i>P. alba</i>	0.02-0.10	0.02-0.10	0.02-0.20	0.02-0.23	0.02-0.13	-	-	-	-	-	-	-	
	<i>P. chilensis</i>	0.02-0.05	0.01-0.06	0.05-0.16	0.06-0.24	0.03-0.13	0.04-0.15	-	-	-	-	-	-	
Mexico	<i>P. laevigata</i>	0.08-0.34	0.07-0.29	0.01-0.29	0.001-0.20	0.03-0.23	0.08-0.30	0.10-0.31	-	-	-	-	-	
Peru	<i>P. pallida</i>	0.10-0.50	0.09-0.46	0.10-0.56	0.11-0.68	0.09-0.57	0.16-0.56	0.15-0.47	0.21-0.63	-	-	-	-	
Hawaii*	<i>P. pallida</i>	0.08-0.22	0.07-0.17	0.03-0.16	0.04-0.28	0.02-0.18	0.10-0.27	0.11-0.18	0.06-0.29	0.08-0.38	-	-	-	
Kenya*	<i>P. pallida</i>	0.19-0.51	0.17-0.47	0.19-0.60	0.21-0.72	0.19-0.61	0.27-0.59	0.19-0.48	0.25-0.64	0.04-0.31	0.10-0.42	-	-	
Australia*	Hybrids	0.17-0.45	0.17-0.42	0.16-0.53	0.15-0.63	0.12-0.53	0.24-0.57	0.16-0.41	0.22-0.60	0.02-0.22	0.07-0.33	0.02-0.12	-	
South Africa*	<i>Prosopis spp.</i>	0.05-0.10	0.04-0.08	0.02-0.19	0.03-0.13	0.01-0.12	0.04-0.10	0.03-0.09	0.08-0.17	0.13-0.41	0.07-0.12	0.16-0.41	0.15-0.36	

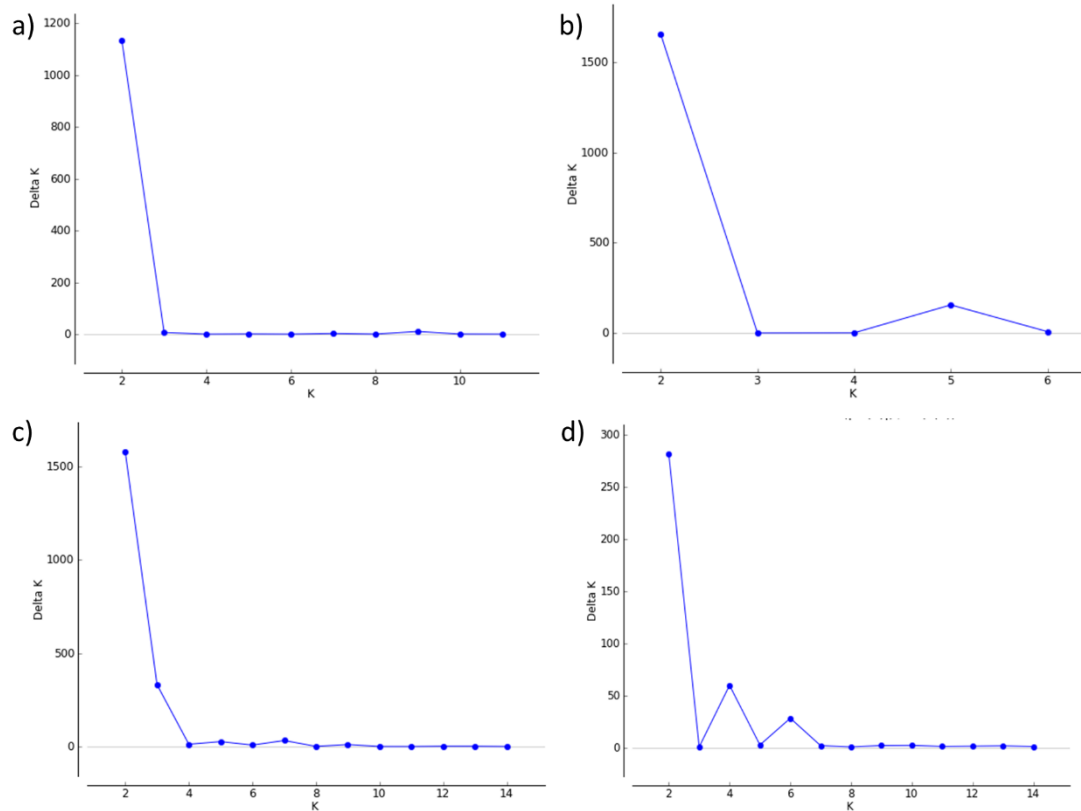


Figure S2.1. Identification of the optimal number clusters (K) inferred by an hierarchical Bayesian clustering analyses with the software STRUCTURE. The level of clustering includes: a) Level 1, with all *Prosopis* individuals from 11 species; Level 2, with b) only *P. juliflora* individuals from Mexico, Ethiopia, Kenya and Tanzania, and c) all the rest of *Prosopis* species; d) Level 3, including only *Prosopis* species from Argentina, Australia, Chile, Mexico and South Africa. In all cases $K=2$ were identified as the optimal number of genetic clusters. Individuals were genotyped using seven nuclear microsatellites loci (see Material and Methods for parameters of the models).

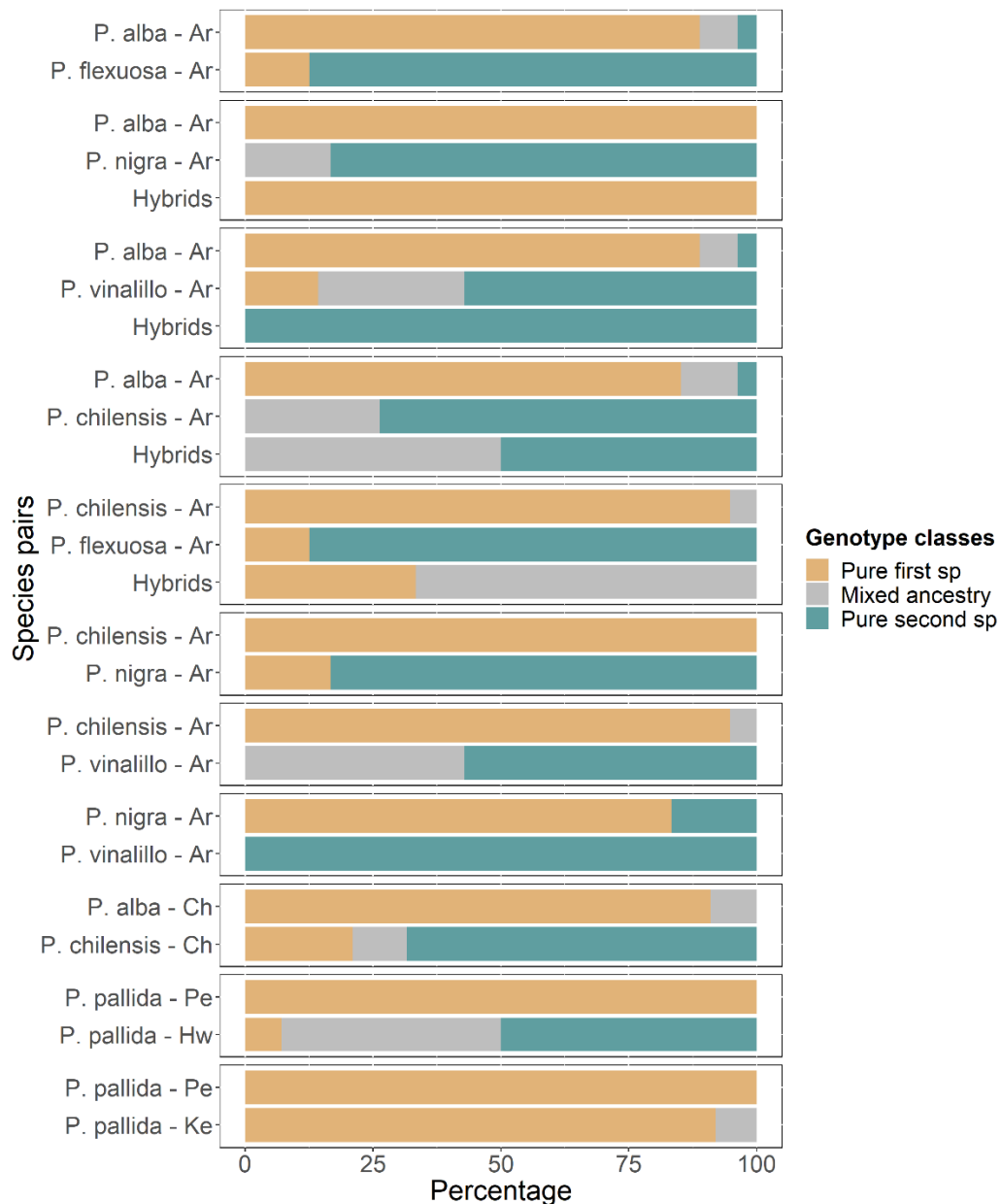


Figure S2.2. Percentage of individuals assigned to genotype classes: pure first sp, pure second sp or mixed ancestry; by NEWHYBRIDS using seven nuclear microsatellites loci. The analysis was done on pairs of *Prosopis* species, and hybrids, identify morphologically from various native areas: Argentina (Ar), Chile (Ch) and Peru (Pe). *P. pallida* individuals from the native area of Peru (Pe) were compared with the introduced populations of Kenya (Ke) e invasive populations of Hawaii (Hw), respectively (see Material and Methods for parameters of the models).

CHAPTER 3 The roles of rapid evolution and phenotypic plasticity in promoting invasiveness: insights from *Prosopis* invasions in Eastern Africa

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Abstract

Only a small proportion of introduced species become invasive. Two factors that can contribute to increased invasive potential are high phenotypic plasticity and rapid evolution in response to novel environmental conditions. We investigated the contribution of these two processes to the successful invasion of *Prosopis juliflora* in Kenya. We also compared the level of invasiveness of this species with the co-occurring, but non-invasive, *Prosopis pallida*. Seeds of the original founder individuals and surrounding invasive trees were used in a reciprocal transplant experiment with common garden plots located in the original plantations and in neighbouring invaded sites. We also grew these seed provenances in a greenhouse experiment, exposing them to different nitrogen and water availability conditions. We found higher plasticity in vegetative structures, higher seed production, germination, survival, and earlier maturity in offspring from invasive *Prosopis* individuals compared to offspring from original founder *P. juliflora* trees, indicative of rapid post-introduction evolution. We also recorded founder individuals of *P. juliflora* to differ in their plastic responses to resource availability in both above- and below-ground vegetative traits from *P. pallida* (which was only present in plantations). Taken together, this suggest that phenotypic plasticity promotes the naturalization of *Prosopis* in Baringo County. In synthesis, our study utilized a rare opportunity to investigate differences between founder and invasive populations of introduced trees. We found that both high levels of phenotypic plasticity and post-introduction evolution may have contributed to the phenomenal ecological success of *P. juliflora* invasions in Kenya. Levels of plasticity in key traits were absent from introduced but non-invasive *P. pallida*.

Keywords: common garden, environmental stress, invasiveness, phenotypic plasticity, plant traits, rapid evolution, tree invasions, woody invasive species

3.1 Introduction

What makes some species invasive and others not remains a central question in invasion ecology. Since Baker's (1965) proposition for the need to identify species characteristics that may promote invasiveness, much work has been done, and several studies have addressed whether particular traits are linked to the likelihood of a species becoming invasive (Goodwin et al. 1999, Pyšek and Richardson 2007, Rejmanek and Richardson 1996). Even when some species possess traits that predispose them to becoming invasive, novel conditions in the new range may impede invasion, at least initially. This, in part, may explain the so-called lag phase often experienced by introduced species, a period between the establishment of naturalized populations and the onset of aggressive spread. Lag phases may be caused by numerous factors, like limited availability of suitable environments, demographic processes linked to reproduction and spread, or time needed for local adaptations to accrue (Baker 1965, Crooks 2005).

Many ecological and evolutionary hypotheses have emerged to explain plant invasion success (see Catford et al. 2009 for review). From an ecological point of view, many of these hypotheses are formulated around phenotypic plasticity, broadly defined as the capability of an organism to display different phenotypes in response to different environmental conditions. That is, successful naturalization in novel environments by non-native species could be due to improved performance via a capacity for plastic responses (Davidson et al. 2011, Montesinos and Callaway 2018, Pyšek and Richardson 2006, Richards et al. 2006, Schlichting and Levin 1986). For example, in alligator weed (*Alternanthera philoxeroides*) phenotypic plasticity rather than local adaptation contributed to the species' invasion success in China (Geng et al. 2007). High levels of phenotypic plasticity may also play an important role during the initial phases of establishment, when tolerance to novel environmental conditions would be essential for survival and reproduction (Palacio-López and Gianoli 2011).

The evolutionary school of thought suggests that colonizing species may evolve in their new ranges, allowing them to become invasive (Bossdorf et al. 2005, Maron et al. 2007). Following their arrival, introduced populations often experience rapid evolution in response to novel selection pressures such as herbivory, mutualistic interactions, and altered abiotic conditions (Barrett et al. 2008, Prentis et al. 2008, Zenni et al. 2014). The recent surge in interest in the evolutionary dynamics of biological invasions is not surprising (Whitney and Gering 2015), given that biological invasions offer excellent opportunities to study evolutionary processes during ecological timescales and under 'natural laboratory' conditions. Many fascinating examples of such contemporary evolution exist. For example, using reciprocal transplant experiments in North America, Colautti and Barrett (2013) showed that latitudinal variation in, and trade-offs between, plant size and the timing of flowering of invasive purple loosestrife (*Lythrum salicaria*) resulted from local adaptation. Clinal divergence in these traits was linked to major effects on fitness (seed production) and, remarkably, evolved over just 50-100 years since introduction. In another example, invasive populations of *Ambrosia artemisiifolia* showed evidence of rapid, and repeated, local adaptation despite the recentness of this species' introduction to Australia (van Boheemen et al. 2018). This example shows that the adaptive potential of some invasive species is considerable and that evolution can occur quickly following introduction, even when genetic diversity is low.

Much effort has gone into identifying the traits involved in the transition of a species from being a successful colonizer (i.e. naturalized) to becoming invasive, and the mechanisms underlying their importance. It is clear that the combination of attributes that allow a species to become invasive depends on context (environmental and taxon), residence time, and introduction history, among others (Catford et al. 2009). In the case of plants, general characteristics such as plant size, flowering duration and specific leaf area have been related to invasion success (Gallagher et al. 2011), while traits related to invasiveness of woody plants include seed mass (Rejmanek and Richardson 1996), high germination levels (Moravcová et al. 2010), rapid seedling growth rate (Grotkopp and Rejmánek 2007), and age to maturity (Grotkopp et al. 2002, Rejmanek and Richardson 1996).

Evaluating the ecological and evolutionary mechanisms that underlie species invasiveness is usually difficult because the original founder individuals of invasive

populations do not exist anymore, or cannot be located, and because of biogeographic uncertainty on the native source regions of invasive populations. *Prosopis* invasions in Eastern Africa provide a unique opportunity in this regard because the original plantations of two species, *Prosopis juliflora* (Sw.) DC. and *P. pallida* (Willd.) Kunth, are still present in the field today. This makes it possible to examine the genotypes that acted as sources of the surrounding invasive individuals. To our knowledge, no such studies exist. Notably, the introduction of both species occurred at the same time and to the same areas (Chogee et al. 2002), so these species share similar residence times under similar local abiotic conditions. Despite this, only *P. juliflora* has become invasive (Chogee et al. 2002). This makes it possible to compare two closely-related non-native congeners in similar environments, and with similar introduction histories, to identify the reasons for differences in their invasiveness.

Here, we exploited the unique circumstances underlying *P. juliflora* and *P. pallida* invasions in Baringo County, Kenya, and conducted reciprocal transplant and greenhouse experiments to compare plant traits directly or indirectly associated with invasiveness. Species in the genus *Prosopis* are invasive in many areas worldwide and their widespread success has been linked to strong competition under low soil nitrogen (N) conditions (Pasiiecznik et al. 2001). Water conditions and higher soil humidity also influence *P. juliflora*'s growth and reproductive output (Alves 1981, Pasiiecznik et al. 2001). It is therefore conceivable that *Prosopis* species may overcome novel and harsh abiotic conditions because of high levels of phenotypic plasticity.

In this study, we investigated the contribution of two key processes during the invasion of *Prosopis* in Kenya. First, we wanted to determine whether invasiveness in *P. juliflora* was linked to rapid post-introduction evolution. Second, we aimed to evaluate differences in invasiveness between *P. juliflora* and *P. pallida* from plantations. To test this, we used *Prosopis* seeds collected at paired sites in founder plantations, and in invaded areas in a reciprocal transplant experiment. We also compared plastic responses of key traits directly or indirectly associated with invasiveness by growing seeds from the same paired sites under different water and N availability in a greenhouse experiment. For the first objective, we hypothesized that rapid post-introduction evolution would have occurred during invasion of *P. juliflora* and therefore, we predicted that invasive individuals would show an increase in mean performance for some traits compared to offspring from founder *P. juliflora* individuals,

independently of transplant site. For the greenhouse experiment, if phenotypic plasticity increases along the *P. juliflora* invasion continuum, we hypothesized that plastic responses in seedlings originating from invasive *P. juliflora* individuals would be higher than those originating from founder *P. juliflora* trees. That is, we expected steeper reaction norms in key performance traits for invasive genotypes compared to offspring from founder trees under different resource availability conditions. Alternatively, it is possible that plasticity is only important in the initial phases of establishment, and therefore, founder genotypes would be more plastic compared to invasive genotypes. The absence of variation in plasticity along the invasion gradient is also a possible outcome, since introduced individuals can be pre-adapted and display high fitness irrespective of site status. For the second objective, we hypothesize that founder *P. juliflora* will have a higher performance in a combination of traits absent from *P. pallida*, independently of transplant site and under low resource availability in the greenhouse experiment. This would indicate that higher invasiveness in *P. juliflora* compared to *P. pallida* may have enabled their aggressive spread.

3.2 Materials and Methods

a) Study species and study site

Our study site was located in Baringo County, Kenya, which is *ca.* 50 km north of the equator, at altitudes between 900 and 1200 m above sea level. The climate is semi-arid (Owen et al. 2004) and the average minimum and maximum temperatures are 20 °C and 30 °C, respectively (Kassilly, 2002). The area has two wet seasons and the mean annual rainfall is 635 mm (Kassilly, 2002). Two *Prosopis* species have been introduced to Baringo: *P. juliflora* and *P. pallida*. It is possible, from broad morphological differences in stem structure, leaf morphology and pod shape, to distinguish between these two species in the field (Burkart 1976, Pasiiecznik et al. 2001). Since hybridization between different *Prosopis* species occurs frequently (Hunziker et al. 1986, Trenchard et al. 2008), individuals with intermediate characteristics of the two parental species were considered hybrids. The morphological classification of trees as *P. juliflora*, *P. pallida*, or putative hybrids, was confirmed using genetic analysis (see Chapter 4).

We collected seeds from 55 healthy, mature mother trees, representing both founder (n=28) and invasive (n=27) genotypes for reciprocal transplant and greenhouse experiments.

Founder trees were from seven plantations. Invasive genotypes were from nine invaded sites, each characterized by the presence of mature trees and seedlings, indicating ongoing spread and reproduction. Each individual was labelled according to a combination of its origin (plantation or invasive) and species, i.e. invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida*, hereafter referred to as 'eco-morphotype'. We did not observe any putative hybrids, i.e. individuals with intermediate morphology of the two parental species; nor any *P. pallida* trees in the selected invaded areas (see results). From the majority of mother trees (n = 51), between five and 30 seed pods were collected per individual. The mean number of seeds/pod/individual (n=51 individuals), the percentage of undamaged seeds/pod/individual (n = 51 individuals), and seed size (mean weight, n = 50 individuals) were also determined. Seeds were classified as undamaged if they were not aborted and showed no signs of damage by seed-feeding insects or fungal infection.

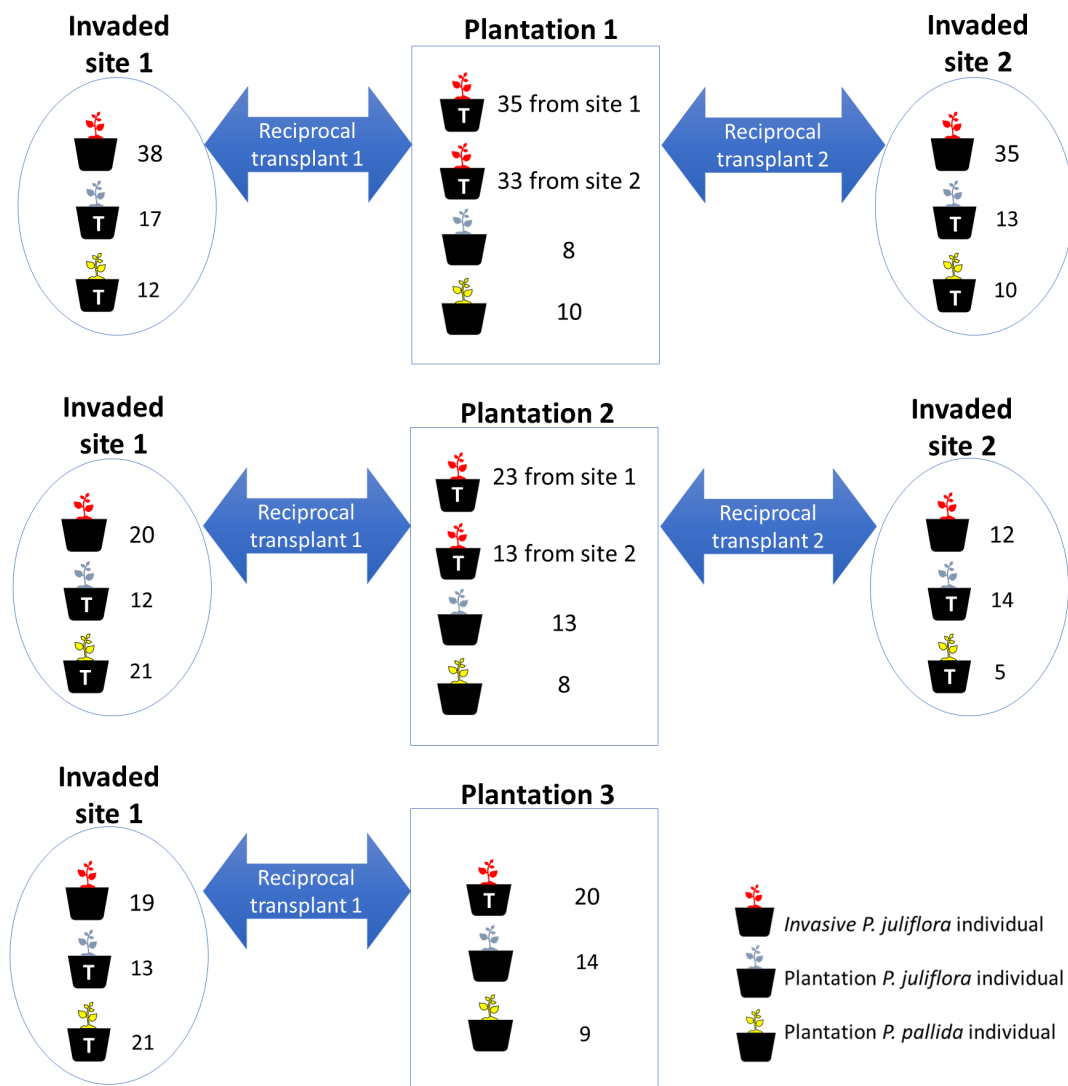
For the reciprocal transplant experiment, we used seeds from a subset of the sites, representing three paired mixed original plantation (i.e. where both *P. juliflora* and *P. pallida* were originally planted and are still present) and one or two surrounding invaded sites. One invaded site had to be eliminated because it was being used for farming at the time of transplanting. All these sites were located between 4 and 6 km away from the plantation (total of five invaded sites). The location of plantations and invaded areas was recorded with a handheld GPS (Table S3.1). Seeds from 38 *Prosopis* mother trees were used: 12 *P. pallida* and eight *P. juliflora* individuals from plantations, and 18 *P. juliflora* individuals from invaded sites (Table S3.1). The nutrient content (N, C, P, Ca, Mg, K and Na) and pH, as well as soil texture, was determined for these selected sites at the start of the experiment (Table S3.2).

For the common garden experiment, we used seeds from mother trees originating from seven plantation and seven invaded sites: eight *P. pallida* individuals from five plantations, 12 *P. juliflora* individuals from four plantations and 14 *P. juliflora* individuals from seven invaded sites. For this experiment, six invaded sites were between 4 and 6 km away from the plantations and one site was approximately 100 m from the plantation. Differences in seed collection per eco-morphotype/site were due to high variation of seed set and seed damage between individuals in each site or between sites.

b) Reciprocal transplant experiment

In July of 2016 seeds from each of the selected sites (i.e. different plantation and invaded sites) were germinated. Five seeds from the same mother were scarified by immersion in warm water (between 90 and 95 °C at the moment of immersion) for 12 hours and were then sown in pots containing soil from their local origin in a common garden and watered to soil capacity at regular intervals two or three times a week. Pots were rotated weekly until transplanting. Seedlings were randomly weeded after seedling emergence, leaving only one seedling per pot. Between November 14th, 2017 and November 21st, 2017, seedlings were transplanted in the field. As we wanted to test whether rapid evolution had enabled the spread of individuals from the initial plantations, transplants were done for each of the three plantation/invaded site combinations separately. That is, for each plantation/invaded site combination, transplants only involved seedlings from that specific combination, so that plantation seedlings were planted back in their putative plantation of origin and the surrounded invaded areas only. Similarly, invasive seedlings were planted in their invaded area of origin and the plantation that they surrounded only. Seven maternal lines of plantation *P. pallida* were replicated between one and seven times in the plantation and in one of the invaded sites, and five maternal lines were replicated between one to seven times in one of the sites. Similarly, seven maternal lines of plantation *P. juliflora* were replicated between one to 11 times in the plantation and in one of the invaded sites, and one maternal line was present twice in one of the sites. Lastly, for invasive *P. juliflora*, 16 maternal lines were replicated between three to 22 times in both the plantation and the invaded site while two maternal lines were present only once in one of the sites. Between 11 and 36 individuals from each collection site were transplanted at all sites for each plantation/invaded combination. Between 13-30 individuals of plantation *P. pallida*, and 20-27 individuals of plantation *P. juliflora* and 33-71 individuals of invasive *P. juliflora* were transplanted at all sites for each plantation/invaded combination (Fig. 3.1). This resulted in a mean of around 150 individuals for each plantation/invaded combination (total of 447 individuals for the three combinations). Sites were cleared before the transplant and seedlings were randomly planted in each area in the corners of a grid with 1 m side length to avoid competition. A wire mesh fence was set up to protect transplanted seedlings from browsing and trampling until the end of the experiment.

Following one year and three months of growth in the field, we recorded the number of stems, stem diameter (summed basal diameter of all stems for multi-stemmed individuals), and height (the distance between the top of photosynthetic tissue and the ground) for each individual. *Prosopis* individuals in invaded areas have been recorded to flower 1-2 years after germination (Pasiiecznik et al. 2001 and ref. herein), therefore, we also recorded any sign of flowering, age at maturity, and number of inflorescences and pods per individual.



Figures 3.1. Experimental design of reciprocal transplant experiment in Baringo, Kenya. Three plantation/invader area combinations were included, each one consisting of one mixed original plantation where both *P. juliflora* and *P. pallida* are present, and one or two invaded areas between 4 and 6 km away from the plantation. Seedlings were planted in their site of origin or transplanted (indicated by 'T' in pot). The number of individuals from each collection site transplanted at all sites for each plantation/invaded combination is indicated. Around 150 individuals for each plantation/invaded combination and a total of 447 individuals for the three combinations.

c) *Greenhouse experiment*

A greenhouse experiment was conducted at Stellenbosch University, South Africa, between June and November 2017. Before planting, seeds were scarified as explained above. Five seeds from the same mother tree were sown in 30 cm-deep pots filled with a 2:3 silica sand-vermiculate mixture. Each eco-morphotype (*P. juliflora* or *P. pallida* from plantations and *P. juliflora* from invaded areas) was replicated between 14-26 times for each treatment, leading to a total of 249 individual treatment combinations. A total of seven maternal lines of plantation *P. pallida* were replicated between one and four times in each treatment. In the case of plantation *P. juliflora*, 11 maternal lines were replicated once in each treatment. Similarly, 11 maternal lines of invasive *P. juliflora* were replicated between one and three times in each treatment. Sown pots were completely randomized for each treatment and rotated weekly until harvesting. Seedling emergence above the soil surface was recorded for each individual pot and seedlings were randomly weeded out 15–20 days after seedling emergence, leaving only one seedling per pot. Immediately after emergence, pots were inoculated weekly for three weeks with a generalist rhizobium strain isolated from Australian *Acacia* trees (see Le Roux et al., 2018 for details). After four weeks of growth, four different treatments were applied: high water/high N, high water/low N, low water/high N, low water/low N. Treatments representing low and high water availability were watered once per week to around 100% and 20% of soil capacity, respectively. For this, soil water hold capacity at around 100% was evaluated as the moisture holding capacity at soil saturation once per week (soil moisture content that will remain in soil water drainage) and then 20% of soil water hold capacity was calculated. Since only a portion of the total soil water is readily available for plant use, it was possible to keep moisture availability to the plants relatively constant. Treatments with low and high N availability were supplied with a quarter-strength Long Ashton nutrient solution (Smith et al. 1983) containing either 1 mM or 5 mM NH_4NO_3 as N source, respectively. We chose the low N availability to mimic the lowest concentration of N found in soil samples from the selected invaded areas. The level of N used in the high availability treatment was similar to the soil nutrient level in riverine forest invaded by *Prosopis spp.* and characterized by well-drained and productive soils (Muturi 2012).

We recorded the mean germination time (MGT; the time in days between planting and seedling emergence) and percentage of germination (the proportion of emerging

seedlings). Harvesting of seedlings took place after 22 weeks of growth, at which time seedling survival was also recorded. During harvesting, seedlings were carefully removed from pots and the roots washed. We measured air dried root and stem lengths and estimated root:shoot ratios (RSR). Roots, leaves, and shoots were then placed into separate envelopes and oven-dried at 50 °C for five days. These were weighed with an analytical balance and total plant biomass was calculated as sum of dry leaf, stem and root biomass.

d) Statistical analysis

We performed all analyses in RStudio (R Core Team, 2015). Differences in seed size, number of seeds per pods and percentage of undamaged seeds per pods between eco-morphotypes of mother trees were analysed by fitting a linear mixed-effect models (LMMs) using the *nlme* package (Pinheiro et al. 2017). For this, mother tree was nested in site as random factors and eco-morphotype (invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida*) was included as fixed effect. The models were fitted by restricted maximum likelihood estimation (REML).

For the reciprocal transplant experiment, we assessed the effect of transplant site (plantation or invaded) and the level of invasion of *P. juliflora* (i.e. invasive *P. juliflora* - plantation *P. juliflora*) on height and stem diameter (log-transformed) in the reciprocal transplant experiment by using LMMs in the *nlme* package. For number of stems, we fitted generalized linear mixed models (GLMMs) with Poisson distribution and logit link function in the *lme4* package (Bates et al. 2017). This allow us to test for significant interaction effect between the two factors, which would indicate local adaptation (e.g. see Kawecki and Elbert 2014). Instead, rapid evolution would be supported by a significant level of invasion effect. A second model was fitted using as fixed effects: transplant site and founder genotypes of both species (i.e. plantation *P. juliflora* - plantation *P. pallida*). As for the first analysis, for height (square-root transformed) and stem diameter (log-transformed) LMMs were fitted using the *nlme* package. A GLMMs with Poisson distribution and logit link function in the *lme4* package was used for number of stems. In all models, mother tree nested in site was included as random factor, we used REML to fit the models and interactions were removed when they were not significant. In addition, to evaluate possible maternal effects on these traits (log-transformed seed numbers and seed size of mother trees were significantly related, $p < 0.05$; $R^2 = 0.11$), we ran all models including mean seed size of mother trees as a covariate. In all

cases, the effect of seed size was not significant and therefore we only report on model results without seed mass. Lastly, a Kruskal-Wallis test was performed to compare the chemical soil properties of plantation and invaded sites.

For the greenhouse experiment, percentage germination and MGT were compared between between eco-morphotypes by using GLMMs with Poisson distribution and logit link function. A GLMMs with binomial distribution and logit link function was used for probability of survival. For these traits, we included mother tree and site as random factors and seed size value of mother trees as a covariable. Root length, RSR, stem length, root biomass, stem biomass, and total plant biomass were first log-transformed to satisfy model assumptions and then analysed using LMMs. Number of leaves were analysed by using GLMMs with Poisson distribution and logit link function. Mother tree was included as a random variable in the models, with treatment (high water/high N, high water/low N, low water/ high N, low water/ low N and eco-morphotype as fixed explanatory variables. All the models were fitted using REML. We included interactions between treatment and eco-morphotype and removed them from models when they were not significant.

For all the analyses, the significance of the terms of the fixed factors and their interaction was tested by conditional F-tests for the LMMs (Faraway 2016), and for the GLMMs, by using a likelihood-ratio χ^2 analysis of variance (Pinheiro and Bates 2000), with a significant P-value of < 0.05. Significant effects were then evaluated with Tukey's HSD post hoc tests by performing multiple comparisons with the 'glht' function of the 'multcomp' R package for all measured parameters (Hothorn et al. 2016).

To account for the ontogenetic effect exhibited in each phenotypic trait recorded in our greenhouse experiment, the relationship between Ln(total plant biomass) and each Ln-transformed trait was evaluated (i.e. analyzed allometrically) using standardized major axis (SMA) regression (Sokal and Rohlf 1995). With this, we aim to estimate differences in plasticity in biomass allocation. This method was preferred over traditional linear regression because it includes the variability of both variables. SMA slopes (i.e. ratio between traits) were tested for significant differences among eco-morphotypes along the gradient of water-nutrient availability conditions. When eco-morphotypes had similar slopes, differences among SMA intercepts were evaluated. From an allometric viewpoint, different slopes or different

elevations indicate that allocation is affected by the gradient of environment factors tested, indicating differential plasticity among eco-morphotypes for that particular trait, where higher slopes equal higher levels of plasticity. SMA regressions and tests were implemented using the SMATR package in R (Warton and Ormerod 2007).

3.3 Results

a) Reproductive output of mother trees

Broad morphological traits and origin allowed us to identify three distinct eco-morphotypes: invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida*. We found a significant difference in the number of seeds produced per pod ($\chi^2 = 14.16$; $P < 0.001$; Fig. 3.2a) and in the percentage of undamaged seeds per pod ($\chi^2 = 11.46$; $P < 0.001$; Fig. 3.3b) between eco-morphotypes, while seed size was marginally affected by eco-morphotypes ($\chi^2 = 3.38$; $P = 0.047$; Fig. 3.2c). Compared to invasive *P. juliflora*, both *P. juliflora* and *P. pallida* plantation trees produced less seeds per pod (Tukey HSD: both $P < 0.001$). The percentage of undamaged seeds did not differ between invasive and plantation *P. juliflora* (Tukey HSD: $P = 0.93$), but both had higher percentage than *P. pallida* (Tukey HSD: both $P < 0.001$). Invasive trees of *P. juliflora* produce seeds of similar size to plantation trees (Tukey HSD: $P = 0.08$), but larger than the seeds of plantation *P. pallida* trees (Tukey HSD: $P < 0.05$).

b) Reciprocal transplant experiment

When only including invasive and plantation *P. juliflora* in the analysis (i.e. for testing for local adaptation, Table 3.1), transplant site remained significant with seedlings growing in plantations being taller (Fig. 3.3a) and having larger stem diameters (Fig. 3.3b) compared to seedlings from invaded areas. Number of stems was also significantly different with invasive genotypes of *P. juliflora* having more stems than plantation genotypes (Fig. 3.3c). No level of invasion *P. juliflora* x transplant site interactions were found for stem diameter, height or number of stems. When only including invasive-non-invasive founder genotype (i.e. for testing for species level differences between founders *P. juliflora* – *P. pallida*, Table 3.1), seedlings growing in plantations were taller compared to seedlings from invaded areas (Fig. 3.3a). Plantation *P. pallida* were taller (Fig. 3.3a), but seedlings had smaller stem diameters (Fig. 3.2b) and produced a lower number of stems (Fig. 3.3c) than plantation *P. juliflora*. At

the time of the data collection (one and a half years after the seedlings were transplanted), seven invasive *P. juliflora* reached reproductive maturity (first production of flowers and/or presence of pods) while neither plantation *P. juliflora* nor *P. pallida* individuals showed any signs of reproduction. These seven individuals had between two and nine flowers and between one and 17 pods.

No significant differences ($P < 0.05$; Kruskal Wallis test) were detected for any of the soil chemistry characteristics between invaded and plantation sites (Table S3.2). With respect to the physical soil characteristics, all sites had fine-textured soils, ranging from clay to silty clay.

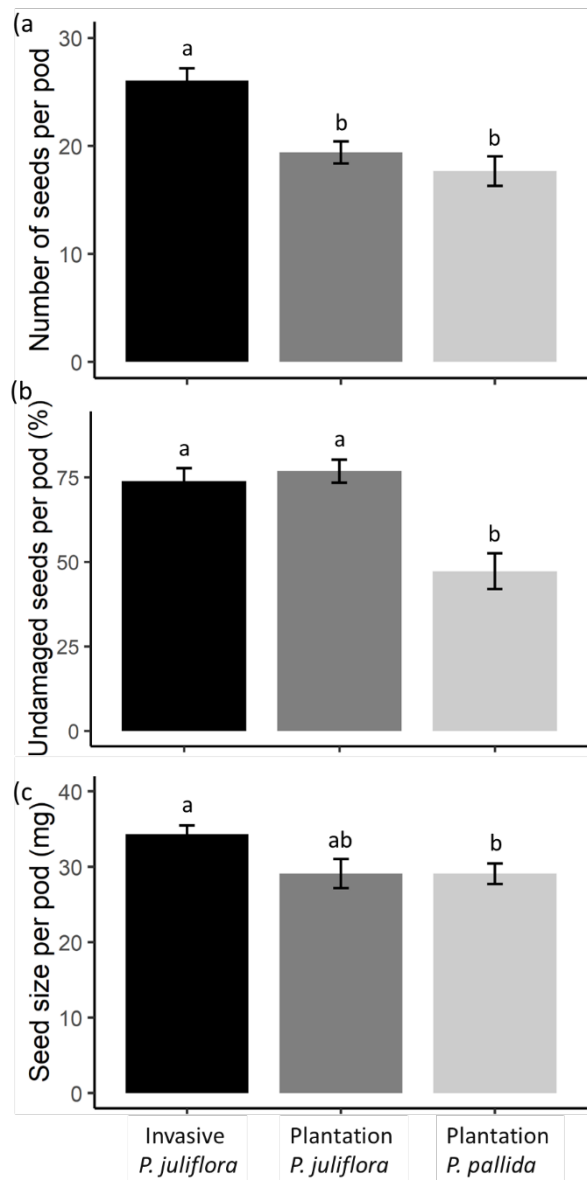


Figure 3.2. Seed production per pod (a) undamaged seeds per pod (b) and seed size per pod (c) collected from mother trees of invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida*; of which the seeds were used in greenhouse experiments. Values represent mean \pm standard error. Different letters above the plots indicate significant differences ($p < 0.05$; Tukey's post hoc test).

Table 3.1. Summary of mixed-effect models fitted for different trait data from the reciprocal transplant experiment. A first model was done using as fixed factors: transplant site (invaded and plantation) and the level of invasion of *P. juliflora* (invasive *P. juliflora* and plantation *P. juliflora*); while the second model had transplant site and founder genotypes (plantation *P. juliflora* and plantation *P. pallida*) as fixed factors. All interactions of fixed factors were not significant (see Material and Methods for model details).

Trait	N	Transplant site		Level of invasion <i>P. juliflora</i>	
		F-value $-\chi^2$	p-value	F-value $-\chi^2$	p-value
Height	317	45.12	<0.001	0.38	0.56
Log (stem diameter)	317	8.67	<0.01	0.87	0.39
Number of stems	317	0.18	0.67	4.25	<0.05
				Founder genotypes	
				F-value $-\chi^2$	p-value
Log (height)	167	15.98	<0.01	5.04	<0.05
Log (stem diameter)	167	3.19	0.08	7.91	<0.05
Number of stems	167	0.26	0.61	13.71	<0.001

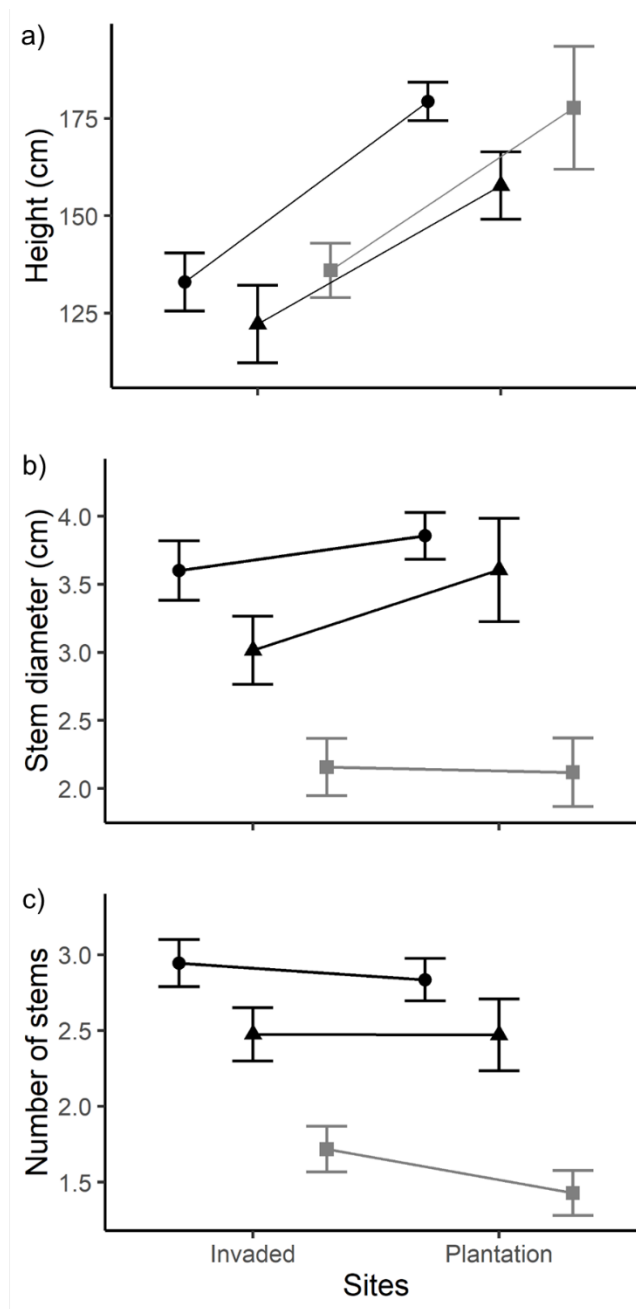


Figure 3.3: Height (a), stem diameter (b) and number of stems (c) recorded for seedlings of invasive *P. juliflora* (black circles), plantation *P. juliflora* (black triangles), and plantation *P. pallida* (grey squares) growing in reciprocal transplant experiments in invaded and plantation sites. Separate linear models were used: one model included only invasive *P. juliflora* and plantation *P. juliflora*; second model included only plantation *P. juliflora* and plantation *P. pallida*. Bars represent mean \pm standard error.

c) Greenhouse experiment

Eco-morphotypes differed significantly in germination ($\chi^2 = 7.67$; $P = 0.022$; Fig. 3.4a), MGT ($\chi^2 = 20.20$; $P < 0.001$; Fig. 3.4b) and survival ($\chi^2 = 15.52$; $P < 0.001$; Fig. 3.4c). Invasive *P. juliflora* seeds germinated better than plantation *P. juliflora* seeds (Tukey HSD: $P < 0.05$), but had a similar mean germination time (Tukey HSD: $P = 0.10$). In comparison with *P. pallida* seeds, invasive *P. juliflora* and plantation *P. juliflora* seeds had similar germination (Tukey HSD: both $P > 0.35$), but seedlings took more time to emerge (Tukey HSD: both $P < 0.001$). Seedlings of invasive *P. juliflora* survived better than those from plantation *P. juliflora* (Tukey HSD: $P < 0.05$) and those from *P. pallida* (Tukey HSD: $P < 0.01$), while survival of plantation *P. pallida* and *P. juliflora* seedlings did not differ (Tukey HSD: $P = 0.62$).

Overall, the performance of individuals was significantly affected by the different water and N availability treatments (Table 3.2). Different eco-morphotypes showed variation in their plastic responses (significant eco-morphotype x treatment effect) for root length, stem length, leaf biomass, stem biomass and number of leaves (Table 3.2). Variation in plasticity between eco-morphotypes was supported by differences in slopes or elevations in SMA regressions for a number of traits (Fig. 3.6). *Prosopis pallida* seedlings had lower RSR than *P. juliflora* from plantations independent of treatment (Tukey HSD: $P < 0.0001$; Table 3.2; Fig. 3.5a) and, under high water availability, longer stems (Tukey HSD: high water/high N, $P < 0.01$; Tukey HSD: high water/low N, $P < 0.05$; Fig. 3.5c). This variation in plasticity was confirmed by the differences in SMA slopes of RSR and in elevation of SMA relationships of stem length and stem biomass (Fig. 3.6a–c, respectively). Under high water/high N conditions, invasive *P. juliflora* seedlings had longer stems (Tukey HSD: $P < 0.01$), higher leaf biomass (Tukey HSD: $P < 0.05$) and stem biomass (Tukey HSD: $P < 0.05$) than plantation *P. juliflora* (Fig. 3.5c, f, g, respectively). After controlling for ontogenetic effect, we found increased plasticity in RSR, stem length, and stem biomass for invasive *P. juliflora* compared to plantation *P. juliflora*, as shown by significant higher slopes in SMA relationships for each trait (Fig. 3.6a, b, and c, respectively) while no differences in plasticity were observed for leaf biomass (Fig. 3.5f). *Prosopis pallida* seedlings did not have a significant ratio between total plant biomass and root length (Fig. 3.6d).

Table 3.2. Summary of mixed-effect models fitted for different phenotypic trait data from the greenhouse experiment. For all models, individual was included as a random effect (not showed) and treatment (high water/high N, high water/low N, low water/ high N, low water/ low N), eco-morphotype (invasive *P. juliflora*, *P. juliflora* plantations, *P. pallida* plantations), and their interaction as fixed effects. RSR = root:shoot ratio. Treatment x eco-morphotype interactions were remove from models when they were not significant.

Trait	n	Treatment		Eco-morphotype		Treatment x Eco-morphotype	
		F-value	p-value	F-value	p-value	F-value	p-value
RSR	226	59.47	<0.0001	13.42	<0.0001	-	-
Root length	226	8.17	<0.0001	0.72	0.49	3.44	<0.01
Stem length	241	112.64	<0.0001	15.27	<0.0001	2.49	<0.05
Roots biomass	127	27.13	<0.0001	1.11	0.34	-	-
Leaf biomass	251	128.86	<0.0001	0.51	0.61	2.58	<0.05
Stem biomass	243	130.66	<0.0001	0.75	0.48	2.74	<0.05
Total plant biomass	122	58.51	<0.0001	1.24	0.31	-	-
		χ^2	p-value	χ^2	p-value	χ^2	p-value
Number of leaves	237	359.89	<0.0001	0.32	0.85	21.50	<0.01

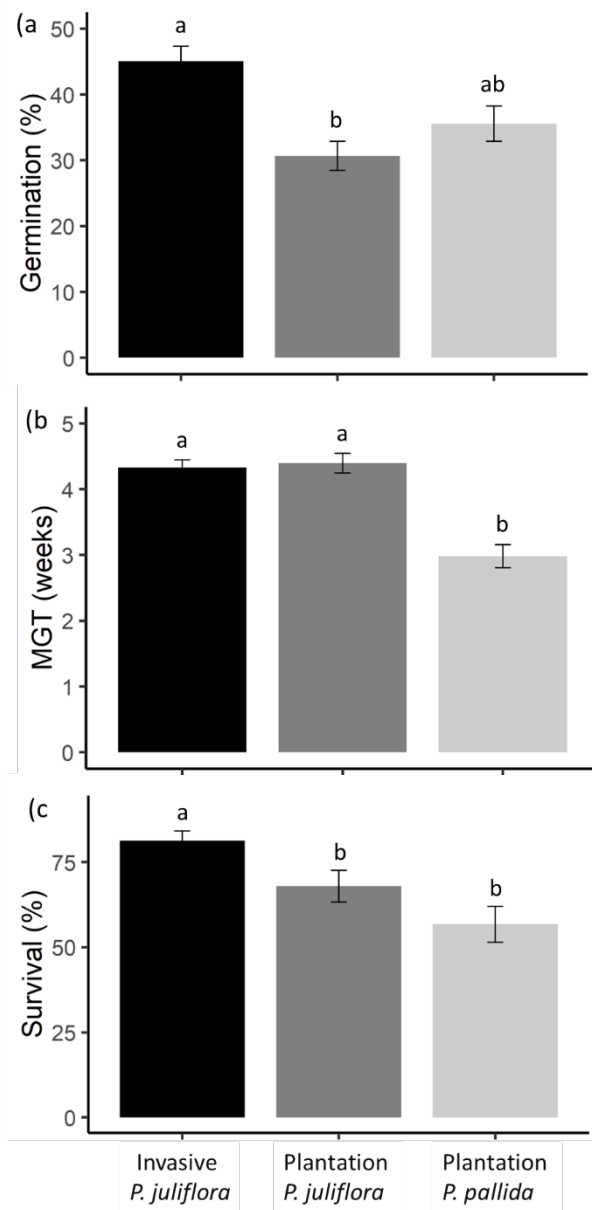


Figure 3.4. Percentage germination (a), mean germination time (MGT, b) and percentage survival (c) of invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida* seedlings under greenhouse conditions. Values represent mean \pm standard error. Different letters above bars indicate significant differences ($p < 0.05$; Tukey's post hoc test).

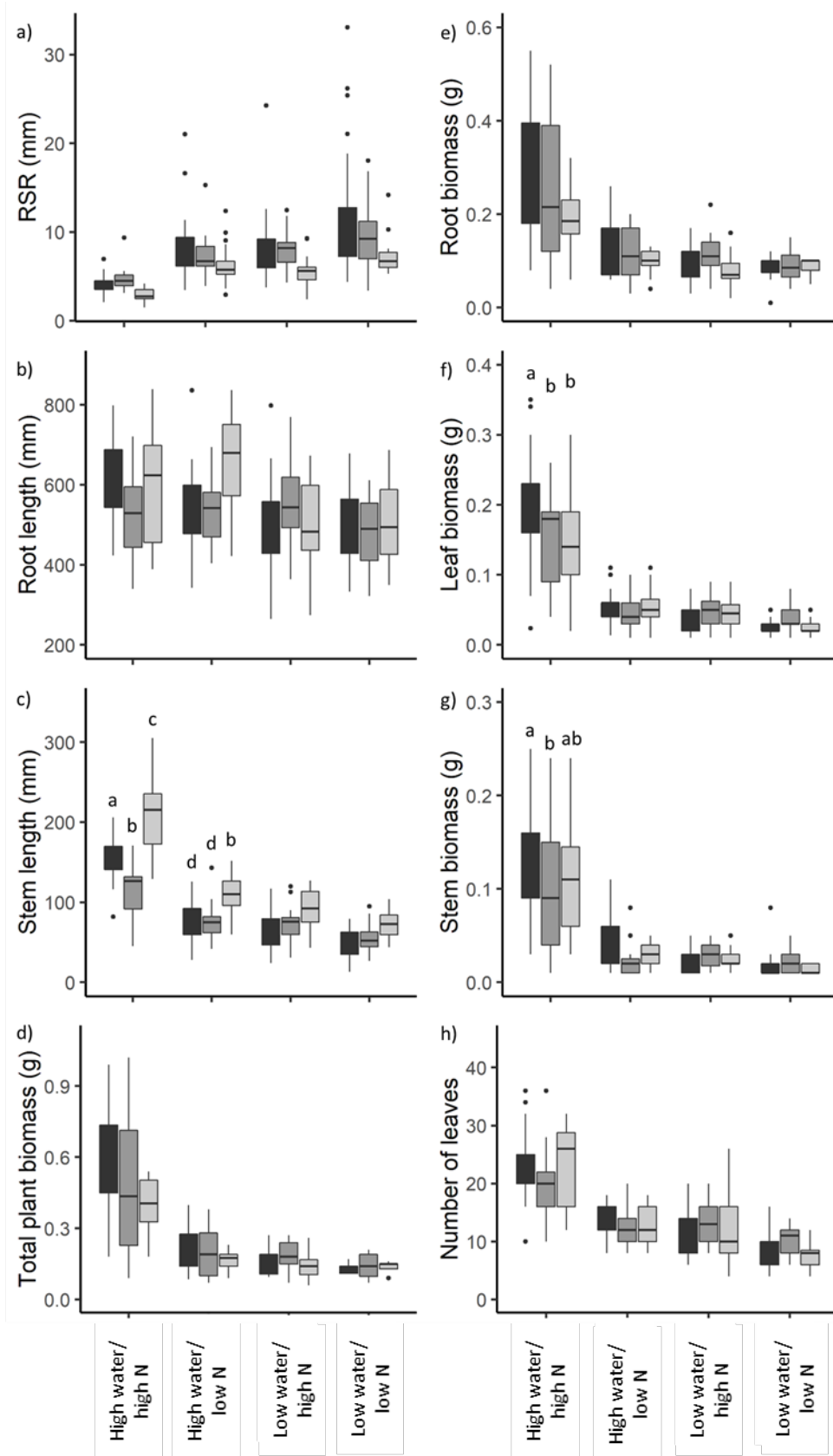


Figure 3.5. RSR, root:shoot ratio (a), root length (b), stem length (c), total plant biomass (d), root biomass (e), leaf biomass (f), stem biomass (g) and number of leaves (h), of invasive *P. juliflora* (black boxes), plantation *P. juliflora* (grey boxes), and plantation *P. pallida* (light grey boxes) in response to different water and N availability treatments. Different letters above the boxplots indicate significant differences ($p < 0.05$; Tukey's post hoc test) among eco-morphotypes within each treatment.

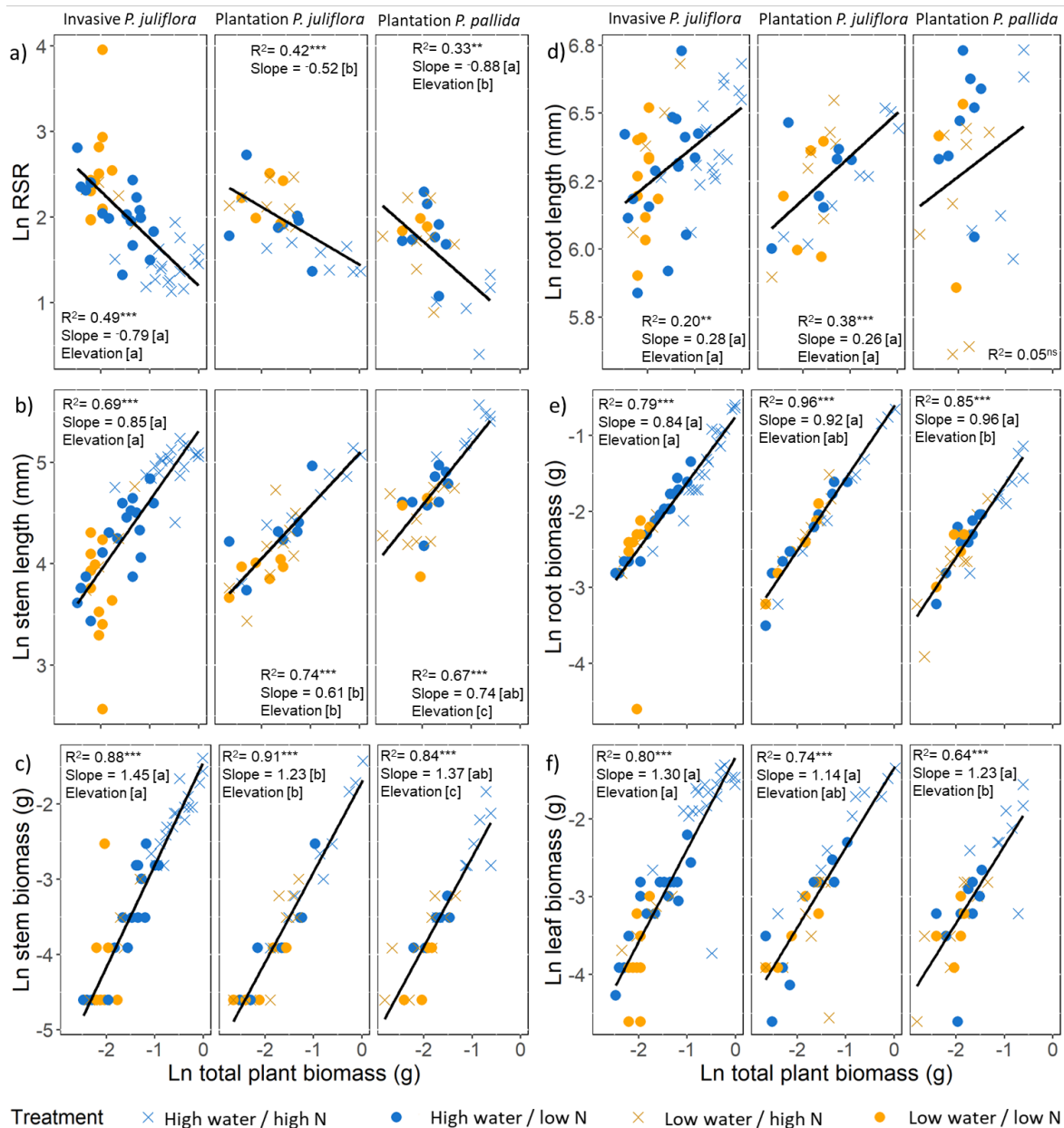


Figure 3.6. Standardized major axis (SMA) regression relationships between total plant biomass and (a) root-shoot ratio (RSR), (b) stem length, (c) stem biomass, (d) root length, (e) root biomass and (f) leaf biomass in invasive *P. juliflora*, plantation *P. juliflora*, and plantation *P. pallida*, in response to water and N availability treatments. Symbols represent the different greenhouse treatments. All traits were Ln-transformed so that relationships represent proportional changes. Different letters in brackets indicate significant differences in slopes or elevation ($p < 0.05$) of SMAs between eco-morphotypes. R^2 = Pearson correlation coefficients for evaluated relationships. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; *, $P < 0.001$; ns, not significant.**

3.4 Discussion

This study is the first to evaluate the roles of rapid post-introduction evolution and phenotypic plasticity during biological invasion by comparing invasive genotypes to their original founders. We also compared the introduced genotypes of two congeneric species, which are aggressive invaders in many parts of the world (Gallaher and Merlin 2010), share the same introduction history and are found in close proximity in the study region, but that differed in their invasiveness in Kenya. Molecular studies revealed that hybridization between the two species is very rare in the study region (Chapter 2). Therefore, compared to traditional studies that contrast populations from the native and invaded ranges, our study utilized a unique opportunity to make inferences about the mechanisms and processes that underlie invasion success. We found that both phenotypic plasticity in key performance traits and rapid post-introduction evolution in response to novel abiotic conditions, are likely to have facilitated invasion of *P. juliflora* in Eastern Africa.

Phenotypic plasticity and invasiveness

We found seedlings of founder individuals of *P. pallida* and *P. juliflora* to differ in their plastic responses to resource availability in traits related with above – below vegetative development (e.g. RSR and stem length); they also differed in seed damage, seedling establishment (e.g. MGT), height, stem diameter and number of stems. Phenotypic plasticity is often linked to invasion success. This is because individuals with higher levels of phenotypic plasticity are better equipped to survive and reproduce under heterogenous environmental conditions (Richards et al. 2006). In agreement with our results, a recent meta-analysis found invasive species to be more plastic in RSR, root biomass and plant biomass, among others, compared to non-invasive congeners (Davidson et al. 2011). However, phenotypic plasticity does not always facilitate invasions (Palacio-López and Gianoli 2011) or it may only be important during the initial stages of invasions (Bossdorf et al. 2005, Lande 2015) as plastic responses may not always translate into fitness advantages (Davidson et al. 2011, but see Pichancourt and van Klinken, 2012). Our results indicate that phenotypic plasticity is a key mechanism for the colonization of *Prosopis* species.

Evidence for rapid post-introduction evolution

Results from both the reciprocal transplant and the common garden experiment suggest that strategies in trait–environment conditions not only differ between invasive and non-invasive naturalized *Prosopis* species, but also between the founder and the invasive genotypes within *P. juliflora*. That is, we found that invasive *P. juliflora* genotypes displayed higher number of stems, earlier maturity (we observed the onset of maturity in invasive *P. juliflora* after only 17 months, but not in plantations), higher seed production per pod, and they germinate and survive more, than plantation *P. juliflora* individuals. Moreover, invasive *P. juliflora* genotypes are more plastic (different and higher reaction norm slopes) than plantation individuals for both above-ground vegetative structures (stem length and stem biomass) and above-below ground allocation strategies (i.e. RSR). Many successful invaders have short generation times, an attribute that often distinguishes them from non-invasive congeners (e.g. herbaceous plants, Schlaepfer et al. 2010; pines, Grotkopp et al. 2002). In addition, the extend of naturalization and spread often increases with seed production (Correia et al. 2016). The fact that invasive *P. juliflora* increased growth in response to high resource availability, compared to the plantation individuals, is also in agreement with the hypothesis that strong response to high nutrient availability is common in invasive plants (Catford et al. 2009). Overall, these differences may have contributed to the ability of the species to transition from being a successful colonizer to becoming an aggressive invader.

While our research provides evidence for rapid post-introduction evolution in invasive *P. juliflora* genotypes, we found little support that evolution in invasive *Prosopis* individuals has led to local adaptation. Local adaptation would imply a specific form of transplant site x eco-morphotype interaction in our model which includes the two *P. juliflora* eco-morphotypes only (e.g. see Kawecki and Ebert 2004). In each habitat, the local eco-morphotype is expected to show higher fitness than the eco-morphotype from the other habitat. It is maybe unsurprising that we did not find such an interaction, as one would not expect founder genotypes to be locally adapted, since they find themselves in a new environment purely as a result of human agency, having been planted there.

Differential maternal effects could be an alternative explanation for the difference in performance between *Prosopis* eco-morphotypes. Responses in germination traits (e.g.

germination success and germination time) and performance of offspring can be strongly affected by environmental conditions, but also by maternal effects (Donohue et al. 2010, Galloway 2005). The influence of maternal effects can be reduced by using seeds from maternal lines grown under controlled conditions (e.g., Ågren and Schemske 2012, Hierro et al. 2009), but this is difficult to achieve for species with long juvenile periods, like mesquite trees. Maternal effects linked to number of seeds produced (Pichancourt and van Klinken, 2012) and seed size has been repeatedly documented (Roach and Wulff 1987) with larger seeds tending to store more nutrients, which may influence various early-growth traits such as germination, survival and size of seedlings. However, seed number and size were positively correlated and the fact that the results of height, stem diameter, number of stems and survival between *P. juliflora* individuals grown in plantations and those grown in invaded areas did not differ when average seed weight was incorporated as a covariable, suggests that variable provisioning of nutrients does not explain the observed differences in survival and growth between these two eco-morphotypes.

Overall, our study provides strong support for the hypothesis of post-introduction evolution during invasion. Considering that our results showed that *P. juliflora* becomes reproductive after 17 months, this evolutionary change may have occurred over as little as 26 generations. Compared to previous studies (e.g., Caño et al. 2008, Henery et al. 2010), our results are based on a comparison of the founder and the invasive genotypes, providing important evidence in support of the proposition that rapid evolution and plastic responses, in concert, contribute to increased invasiveness. In addition, admixture between founding *P. juliflora* genotypes may have increased novel heritable variation in levels of phenotypic plasticity for selection to act upon, i.e. evolving increased levels of plasticity, and therefore, probably post-introduction evolutionary change in plasticity. Alternatively, differential phenotypic plasticity in traits that affect dispersal abilities, either directly through the movement of seed, or indirectly through the increased survival of dispersed individuals, may have influenced range expansion, leading to spatial sorting (Shine et al. 2011). Spatial sorting during rapid range expansions can cause an accumulation of dispersive (fitter) genotypes at the leading-edge compared to core range. While empirical evidence for spatial sorting during plant invasions remains scant, examples from animal invasions illustrate that this process can facilitate rapid microevolutionary change through processes like non-assortative mating (Shine et al. 2011).

Coping with environmental variation in the invaded range

Both *P. pallida* and *P. juliflora* share similar native range geographic distributions, physiological responses and environmental tolerances (Pasiiecznik et al. 2001 and references therein). Morphologically, they are also very similar, which is why some considered them to be a species complex (Pasiiecznik et al. 2001). In Baringo County, both species share similar introduction histories and this, coupled with both species' invasiveness elsewhere in the world, makes it surprising that only one species appears to be invasive in Kenya.

Previous studies have showed that larger size and fecundity to be positively related to invasiveness (Leger and Rice 2007, Catford et al. 2009). In general, seeds of *Prosopis* species tend to have high dormancy levels and older seeds are capable of germination without treatment if they are viable and conditions are favourable (Pasiiecznik and Felker 1992). While little is known about the soil seed bank of these species, *P. velutina* seeds have been found to remain viable between two and 10 years (Glendening and Paulsen 1995), and more than 50% viability in pods stored over 10-15 years has been found in some *Prosopis* species (Pasiiecznik and Felker 1992). Multi-stemmed plants are successful in many habitats. Stem numbers have been linked to higher growth rates and faster seed production. Moreover, multiple stems also enhance survival and growth if one stem dies (Götmark et al. 2016). We found invasive *Prosopis* individuals to grow larger (larger stem diameter) and to have less damaged seeds than the less invasive plants, possibly contributing to differences in invasiveness between *P. juliflora* and *P. pallida* in Kenya.

Seedlings of all eco-morphotypes grew better (taller plants and larger stem diameters of *P. juliflora* individuals) in plantations compared with invaded areas, indicating that environmental conditions in the invaded areas are possibly harsher than plantation conditions. As these differences were not be related to physiochemical soil characteristics, it is likely that they are due to a non-random selection of plantation sites. Under these conditions it is thus likely that the invasion success of *P. juliflora* in Baringo is, besides its higher reproductive output, also due to the ability of plantation *P. juliflora* to outperform plantation *P. pallida*. Many hypotheses have been proposed in invasion ecology to explain invasion success. For example, non-native *Prosopis* species may have been released from their natural enemies in invaded areas, enabling the invader to allocate resources away from

enemy defence and towards growth and performance (i.e. Evolution of increased competitive ability hypothesis, Blossey & Notzgold, 1995). Alternatively, *Prosopis* could have taken advantage of an increase in resource availability (e.g. high water or nitrogen availability) to colonize and establish (i.e. Increased resource availability hypothesis, Sher and Hyatt, 1999). Further work is needed to test these hypotheses, and others, to better understand the evolutionary drivers underlying *Prosopis* invasions in East Africa.

3.5 Conclusion

We provide compelling evidence that the interactions between species traits and abiotic environmental conditions can facilitate rapid evolution and make the difference between a species being a successful colonizer or an aggressive invader. The inter- and intraspecific comparisons in our study were made possible by the fact that founder and invasive genotypes of two introduced plant species still co-occurred in the field – a rare opportunity. While this allowed us to directly compare founder genotypes and genotypes spreading away from the plantations, our experimental design does not exclude other possible explanations for the observed differences in the field and the common garden experiment, and their contribution to invasiveness, such as maternal effects, propagule pressure, or biotic interactions, among others. Further studies are needed to evaluate how plasticity and rapid evolution can affect these trends, as well as the management strategies against *Prosopis* invasions, like the efficiency of biological control.

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3.6 Supporting information

Table S3.1. Plantations and invaded sites included in the reciprocal transplant experiment in Baringo, Kenya. For each site the species of *Prosopis* trees from which seeds were used in the experiment and the number of mother trees for each species (N) are shown.

Site	Latitude	Longitude	Species	N
Plantation 1	0.546	36.04	<i>P. pallida</i>	5
			<i>P. juliflora</i>	3
Plantation 2	0.463	36.01	<i>P. pallida</i>	3
			<i>P. juliflora</i>	3
Plantation 3	0.470	35.99	<i>P. pallida</i>	4
			<i>P. juliflora</i>	2
Invaded site1-1	0.588	36.01	<i>P. juliflora</i>	2
Invaded site1-2	0.500	36.04	<i>P. juliflora</i>	3
Invaded site2-1	0.410	36.02	<i>P. juliflora</i>	3
Invaded site2-2	0.489	36.05	<i>P. juliflora</i>	7
Invaded site3-1	0.427	36.03	<i>P. juliflora</i>	3

1 **Table S3.2. Selected chemical and texture soil characteristics of sites included in the reciprocal transplant experiment. Three plantations and**
 2 **one or two surrounding invaded sites were selected. Transplants were done for each of the plantation/invaded area combinations separately.**

3

Characteristic	Plantation 1	Plantation 2	Plantation 3	Invaded site 1-1	Invaded site 1-2	Invaded site 2-1	Invaded site 2-2	Invaded site 3-1
N (% weight)*	0.05	0.08	0.06	0.05	0.04	0.05	0.07	0.05
Organic carbon (% weigh)*	0.24	0.56	0.40	0.35	0.24	0.24	0.49	0.31
Phosphorus (ppm) *	100	170	180	170	170	40	170	30
pH*	8.1	7.3	7.4	8.4	8.6	7.2	7.2	6.0
Calcium (me%)*	41.5	30.8	26.1	47.3	40.6	30.1	33.6	24.6
Magnesium (me%)*	4.9	4.6	4.0	6.9	4.5	5.5	6.5	2.5
Potassium (me%)*	1.3	1.6	4.7	4.7	3.5	1.9	1.3	0.6
Sodium (me%)*	2.2	1.4	2.0	2.9	2.4	2.0	1.9	1.9
Sand (% weigh)	38	38	14	20	10	26	20	6
Silt (% weigh)	10	34	56	48	56	30	48	58
Clay (% weigh)	52	28	30	32	34	44	32	36
Texture Class	C	CL	SiCL	SiCL	SiCL	C	SiCL	SiCL

4 Texture class: C, clay; CL, clay loam; SiCL, silty clay. *, indicate no significant differences ($P > 0.05$; Kruskal Wallis test)
 5 detected for the soil characteristic between invaded and plantation sites.

6 **Table S3.3. Summary of mixed-effect models fitted for different trait data from the**
 7 **reciprocal transplant experiment. For all models, individuals and sites were included as**
 8 **random effects (not shown) while transplanted site (plantation or invaded area) and eco-**
 9 **morphotypes: invasive *P. juliflora*, plantation *P. juliflora*, plantation *P. pallida*; were**
 10 **included as fixed effects.**

11

Trait	N	Transplant site		Eco-morphotype	
		F-value	p-value	F-value	p-value
Height	391	50.64	<0.0001	1.70	0.20
Log (stem diameter)	391	9.92	<0.01	6.93	<0.01
		χ^2	p-value	χ^2	p-value
Number of stems	391	0.47	0.49	34.17	<0.0001

12

CHAPTER 4 **Following the footsteps of invasion: genetic comparisons between founder and invasive *Prosopis* trees in Eastern Africa**

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- Candidate Journals: Biological Invasions, Scientific Reports

Abstract

Invasive species face unique challenges during rapid range expansions related to propagule pressure, founder population sizes, and thus also genetic diversity. We investigated the eco-evolutionary processes during the invasion of *Prosopis juliflora* and *P. pallida* in Kenya (Baringo County) and Ethiopia (Afar Region). First, we compared founder (plantation) and invasive genotypes from invasive *Prosopis* populations from both countries to evaluate 1) whether hybridization or different ploidal levels are associated with invasiveness and 2) how much genetic diversity and differentiation characterize these invasions. As a proxy for dispersal, we also used landscape resistance modelling to infer how various landscape variables may influence gene flow. In Baringo County, despite the similar residence time and introduction history of *P. pallida* and *P. juliflora* into the same environments and probable inter-species hybridization between these species, only *P. juliflora* individuals have invaded the region; indicating that the success of *Prosopis* invasion is not attributed to hybridization but potentially due to the higher ploidy of *P. juliflora*. In addition, invasive *P. juliflora* genotypes had lower genetic diversity than founders, and higher gene flow between themselves than with founder genotypes. This may indicate contemporary genetic change during invasive spread, whereby consecutive founder effects during range expansion from plantations caused genetic erosion. In Afar, founder and invasive genotypes consisted exclusively of *P. juliflora* that were genetically similar, probably due to high levels of ongoing gene flow. Thus, the successful spread in Afar Region is characterized by extensive gene flow

from “source” plantations that homogenizes standing genetic diversity across the invasion. In both Kenya and Ethiopia, our landscape resistance modelling results suggest that dispersal was not hampered by geographic distance, nor by any of the landscape variables included (i.e. bioclimatic conditions, distance to roads, rivers and villages), at least at the spatial scales of this study, indicating frequent long-distance dispersal. Thus, by using a rare opportunity to investigate founder and invasive genotypes, our study showed that despite similar introduction histories, different demographic processes may be operating in these two regions, giving important insights into the site-specific dynamics of the invasion process.

Keywords: demographic stochasticity, invasiveness, microsatellite, polyploidy, *Prosopis*, tree invasions, woody invasive species

4.1 Introduction

Increased movement of humans and goods around the world has facilitated the transportation of species into new geographic ranges. A significant number of these have become invasive, causing substantial ecological, social, and economic impacts (Pimentel et al. 2005, van Wilgen et al. 2011, Shackleton et al. 2014). Research on the drivers and determinants of invasiveness, including the ability to establish and expand in their novel ranges, is important for predicting and managing invasive species and to reduce their negative impact.

Invasive alien species face unique eco-evolutionary challenges during rapid range expansions since most introductions are characterized by founder events and subsequent genetic bottlenecks, and thus reduced genetic variation (Henry et al. 2009). However, invasive spread is often preceded by periods of relatively small population sizes due to so-called lag phases. These periods during which spread is minimal is thought to reflect the time needed for introduced populations to replenish genetic diversity, overcome demographic processes that negatively affect populations growth such as allee effects, among others (Zenger et al. 2003, Bousset et al 2004). There is growing evidence suggesting that some non-native species undergo rapid evolution in the new range before becoming invasive (Maron et al. 2007, Williams et al. 2016, Ochocki and Miller 2017, Weiss-Lehman et al. 2017, van Boheemen et al. 2019). This may be the result of novel selection pressures found in the new environment

and/or the relaxation of selection pressure from the species' native range. For example, environmental conditions, like climate, may differ substantially between a species' non-native and historical ranges, leading to strong selection. On the other hand, species may experience relaxed selection when they are liberated from their specialist predators or parasites upon introduction, i.e. historical selection pressures are either eliminated or dramatically reduced (Blossey and Nötzold 1995). How non-native species respond to novel selection regimes is dependent on the amount of standing genetic diversity present in introduced populations. Despite this 'genetic paradox', many bottlenecked populations can, and do, evolve rapidly following their introduction into new environments (Prentis et al. 2008). Inter- and intraspecific hybridization may also increase novel heritable genetic variation, often associated with increased levels of invasiveness (Ellstrand and Schierenbeck 2000). In addition, invasive alien species may also experience rearrangements of genetic diversity among populations during rapid range expansions (Henry et al. 2009). Other genetic attributes, like ploidal variation, have also been repeatedly linked to invasiveness. For example, polyploids generally show greater levels of stress tolerance, higher growth vigour through increased plant size, seed size, flower size, niche breadth and phenotypic plasticity, among others (te Beest et al. 2012).

Dispersal is an important attribute of the invasion process and is expected to leave a genetic imprint in the form of spatio-temporal distribution of genotypes, as modulated by gene flow (Zeller et al., 2012). Strategies for successful dispersal vary greatly between taxa and habitats and therefore, the study of the context-dependent determinants of spread remains at the forefront of understanding the mechanisms that underlie successful biological invasions (Balkenhol et al. 2015, Cushman 2015). While many studies have been helpful in elucidating the underlying processes and patterns associated with dispersal (e.g. Lenormand 2002, Bridle et al. 2010), there is a need for broader research approaches that combine genetics and spatial ecology and that integrates information on the genetic structure and gene flow of introduced species (as a proxy for dispersal) with their probability of establishment under novel spatial and habitat conditions. For example, knowing the factors affecting dispersal rates may be of limited management value, especially in heterogeneous habitats, as range expansion ultimately depends on the availability of habitat(s) that will allow establishment (Le Roux et al. 2010). Similarly, suitable habitats alone would not guarantee

the establishment of a particular species if limitations in dispersal hinder propagules from reaching these habitats. Under these circumstances, approaches combining dispersal ability with the suitability of the environment provide more realistic estimates of the potential range expansions of species.

Prosopis invasions in Eastern Africa are good system to examine the context- and taxon-dependencies of ecological and genetic attributes to invasiveness because the founder trees of two species, *Prosopis juliflora* (Sw.) DC. and *Prosopis pallida* (Willd.), are still present in the original plantations today (Choge et al. 2002, Swallow and Mwangi 2009, Shiferaw et al. 2019). This makes it possible to examine the genotypes that acted as sources of one of the most widespread invasions in the region, providing a unique opportunity to use genetic approaches to evaluate the processes underlying invasion. To our knowledge, no such studies exist. Notably, in Baringo County, Kenya, *P. juliflora* and *P. pallida*, were introduced in similar numbers, at the same time, and to the same areas; thus sharing the same residence time and propagule pressure under similar local abiotic conditions (Choge et al. 2002). Despite this, only *P. juliflora* has become invasive. We found that differences in plastic responses between founder and invasive genotypes of *P. juliflora* in Baringo County, and post-introduction evolution may have enabled the transition from successful naturalization to aggressive spread in this species (Chapter 3). Interestingly, these levels of plasticity were absent from introduced but non-invasive *P. pallida*. It is therefore conceivable that different stages of invasion, i.e. founder vs spreading populations - will be comprised of different genotypes, which may be indicative of contemporary genetic change during the invasion, whether through neutral and stochastic (i.e. founder events) or deterministic (i.e. selection) processes.

Successful hybridization between different *Prosopis* species has been repeatedly described, particularly from non-native areas (Pasiiecznik et al. 2001, Chapter 2). In Kenya, morphotypes that are intermediate between *P. juliflora* and *P. pallida*, i.e. possible hybrids, have been observed (W. Okellu, CABI, unpublished data). In Ethiopia, taxonomic identification of invasive trees remains unclear and studies only refer to the taxon as *Prosopis* or *P. juliflora*. In the Afar Region in Ethiopia, and unlike in Kenya, hybrids have not been observed in the area (Wakie et al. 2014, Shiferaw et al. 2019). Previous genetic studies in both Kenya and Ethiopia found some levels of hybridization in *P. juliflora* individuals in Kenya (Chapter 2), however, genetic studies including founder and invasive genotypes are necessary to

evaluated whether hybridization is contributing to the invasiveness of *Prosopis* in both countries. In addition, according to previous studies on ploidal variation in *Prosopis* (including samples from Ethiopia and Kenya), the only tetraploid species in the genus is *P. juliflora* ($2n = 4x = 46$), with all other species in the genus being entirely diploid ($2n = 2x = 28$) (Trenchard et al. 2008). While it has not been tested, the success of *P. juliflora* could be attributed to higher ploidy level.

Furthermore, in both countries, the most relevant environmental conditions that explain the current distribution of *Prosopis* have been identified, including elevation, rivers, roads and climatic variables related to temperature and precipitation (Rima, et al. *in prep.*; Shiferaw et al. 2019). This provides the opportunity to combine resistance models (spatial hypotheses about how landscape features influence gene flow) and environmental variables that are ecologically relevant for the occurrence and spread of *Prosopis* species.

Here, we investigated the role of selected ecological-evolutionary drivers underlying the invasion success of *Prosopis* species in Eastern Africa (Ethiopia and Kenya) by (i) examining whether different stages of invasion – founder vs invasive *Prosopis* individuals - are dominated by different genotypes, which may be indicative of contemporary genetic change between founder and invasive *Prosopis* populations, and (ii) assessing the determinants of dispersal of *Prosopis* in both countries. For the first objective, we evaluated whether hybridization or different ploidal levels are associated with invasiveness and examined the genetic diversity and differentiation of individuals along different stages of invasion. For the second objective, we evaluated how different environmental conditions known to influence the occurrence of *Prosopis* in Eastern Africa may influence gene flow, and thus dispersal, between *Prosopis* populations.

4.2 Methods

a) Study sites and study species

This study was carried out in two areas in the Great Rift Valley of Eastern Africa, the Baringo County in Kenya and Afar Region in Ethiopia (Fig. 4.1). Baringo County is located in western Kenya just north of the equator. The Afar Region is one of the main pastoral regions in Eastern Africa and is located in the north-eastern part of Ethiopia. The major watershed in the Afar

Region is the Awash River Basin. The area is characterized by a fragmented landscape with presence of dry shrubs, acacia woodland, bushland, grassland and wooded grassland (MoA 1997).

In Kenya, *P. juliflora* and *P. pallida* trees sourced from Brazil and Hawaii were first introduced in 1973 to Mombasa (Johansson 1990), with later introductions during the 1970s and 1980s to many parts of Kenya, including Baringo County, Tana River and Taveta (Johansson 1990, Otsamo and Maua 1993, Choge et al. 2002). During this time, demonstration plantations of *Prosopis* species were established using seed sourced from commercial suppliers of unknown origin (Choge et al. 2002). In the Afar Region of Ethiopia, *Prosopis* was first introduced in the early 1980s into the Afdem and Afar areas (Amibara and Gewane districts; Admasu 2008, Kebede and Coppock, 2015) with further introductions between 1980s and 1990s as shade trees in villages, crops fields and to control erosion (Kebede and Coppock 2015). *Prosopis* has since become invasive in both countries causing substantial social and economic conflicts (Swallow and Mwangi 2009, Kebede and Coppock 2015, Bekele et al. 2018).

Prosopis trees are primarily insect-pollinated and are generally assumed to be self-incompatible, but limited self-compatibility has been observed in *P. juliflora* (Pasiiecznik et al. 2001). Seed dispersal is mainly by animals, with both livestock and wildlife being important vectors. Natural dispersal of *Prosopis* propagules also occurs along rivers (Muturi 2012), while human-assisted spread is mostly associated with the presence of settlements and roads (Pasiiecznik et al. 2001, Muturi 2012).

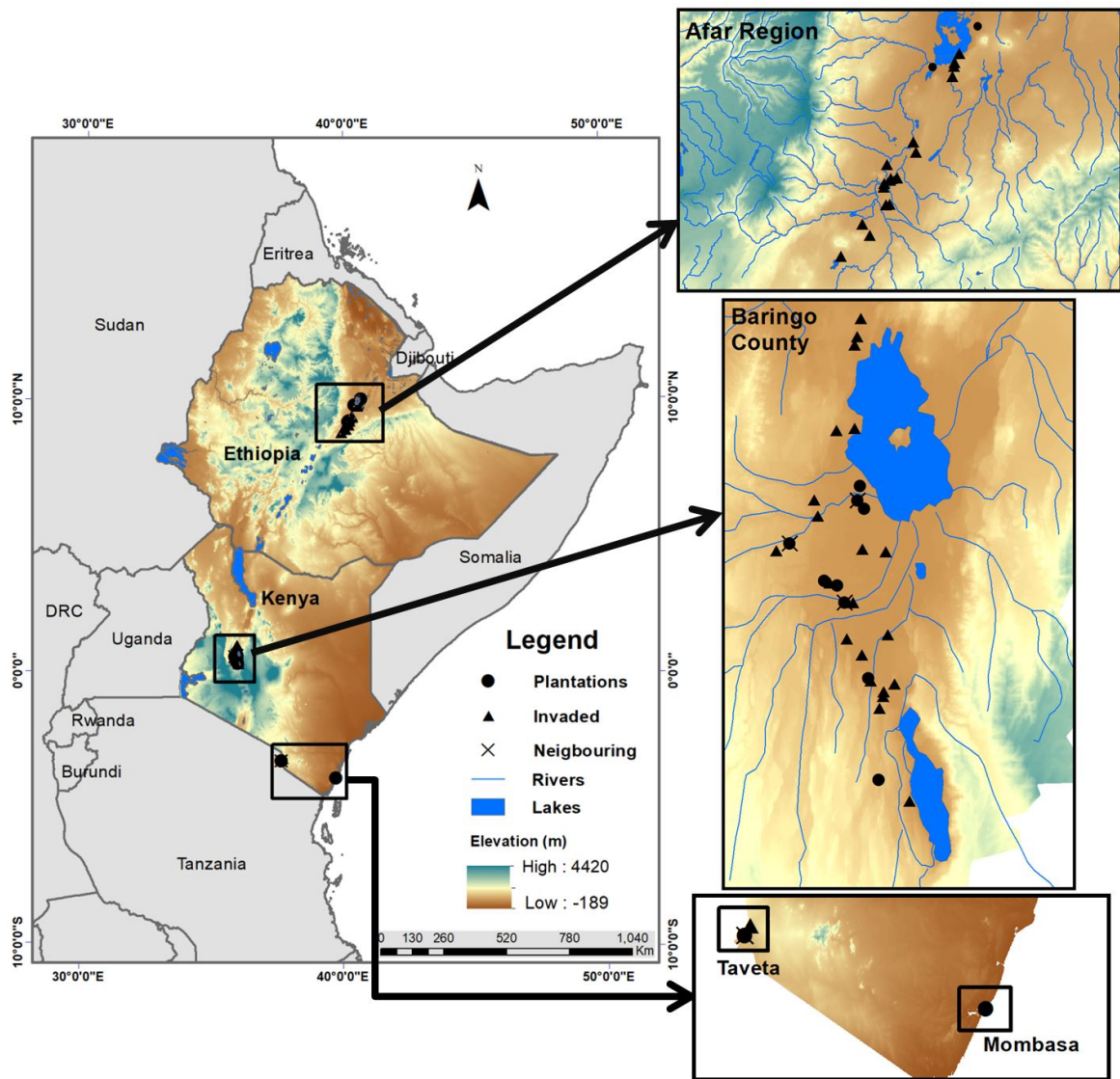


Figure 4.1. Location of the study areas in Baringo County, Kenya and Afar Region, Ethiopia.

b) Samples collection and DNA extraction

Prosopis leaf material was collected from 43 sites in Baringo County, Kenya (Fig. 4.1a). These sites were selected representing three stages of invasion: original plantations, sites neighbouring these plantations, which may include early generations or hybrids; and invaded sites found away from plantations. We sampled individuals from eight plantations, three neighbouring sites, and 32 invaded areas (Table S4.1). Neighbouring samples consisted of trees located at a distance of less than 100m from plantations, whereas trees from invaded areas were located at a distance more than 300m from plantations. The latter followed the classification of Richardson *et al.*, (2004) to include 'reproductive offspring' established at a distance of more than 100 m from adults. In addition, we also included accessions from one plantation in Mombasa, one plantation in Taveta, one site neighbouring the Taveta plantation, and one invaded area in Taveta. The Mombasa plantation is located in the south-eastern coast of Kenya and was the first plantation of *P. pallida* in the country, whereas Taveta plantation is also situated in south-eastern Kenya, at the border with Tanzania. Between one and 49 founder trees of *P. pallida* and *P. juliflora* were sampled from each plantation (some plantations only had one founder tree left). At sites neighbouring plantations and in invaded areas, between one and 25 trees were randomly chosen and their morphotype recorded as either *P. juliflora*, *P. pallida* or putative hybrid. Identification of these morphotypes was based on broad morphological differences in stem structure, leaf morphology, pod shape, and the presence or absence of thorns (Burkart 1976, Pasiecznik *et al.* 2001). Individuals with intermediate characteristics of the two parental species were classified as putative hybrids. In the Afar Region in Ethiopia, trees were sampled from 22 sites, including five plantations and 17 invaded areas (Table S4.1; Fig. 4.1). Between eight and 20 trees were sampled from each plantation and between three and 15 individuals from invaded areas. All the individuals were morphologically identified as *P. juliflora* (see results section), therefore, no individuals were sampled around plantations.

In total, we sampled 740 individuals (203 from Ethiopia and 537 from Kenya) between September 2016 and March 2017 (Table S4.1). The geographic location of each collection site was recorded using a handheld GPS. Leaf material was stored on silica gel until DNA extraction. Genomic DNA was extracted from dried leaf tissue using the cetyltrimethylammonium bromide protocol (Doyle and Doyle 1990). All DNA extractions were

diluted to a final concentration of 50 ng/ μ L and stored at -20 °C until further analysis. Individuals of three sites in Kenya (KEN42, KEN43 and KEN44; n=4) were not genotyped and samples were only included in flow cytometry analyses (see below). For simplicity throughout this manuscript, the term populations will be used to refer to the group of individuals sampled in each site.

According to Shiferaw et al. (2019), the most important variables related to the spread of *Prosopis* in Afar Region are: elevation, distance from rivers and distance from roads. Following Rima et al. (in prep), the most important variables correlated with the spread of *Prosopis* in Baringo County are elevation, precipitation in the wettest month, mean temperature of driest quarter and maximum temperature of the warmest month. Considering this, invaded sites were selected for sampling across both Baringo County and Afar Region to incorporate variation in these spread correlates, by including areas at different elevations, distances from villages, roads and riverbanks.

c) Genotyping

Individuals were genotyped at seven microsatellite markers selected based on successful PCR amplification in *P. juliflora* and *P. pallida* (see Chapter 2). The amplification of these markers was performed by using one multiplex PCR assay for which markers with non-overlapping amplicon size were combined. Multiplex PCR reaction contained 1.5 μ L of primer mix (2 μ M), 7.5 μ L of KAPA2G Fast Multiplex Mix (Kapa Biosystem, Cape Town, South Africa), 4.5 μ L purified H₂O and 1.5 μ L of DNA making a total of 15 μ L of volume solution. Volume of each primer is provided in Table S4.2. PCR cycle included 3 minutes of denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds, elongation at 72 °C for 25 minutes, and a final elongation at 72 °C for 1 minute. PCR reactions were performed in 96-well plates contained 92 samples plus five randomly selected replicate samples and two negative control (H₂O). Amplified products were submitted for gel capillary electrophoretic separation at the Central Analytical Facility, Stellenbosch University, Stellenbosch, South Africa. GeneMarker software (version 2.6.4; SoftGenetics LLC, Pennsylvania, United States) was used for automated genotype scoring, which was then manually checked. Out of 734 samples, 17 samples that failed to amplify at more than five loci were excluded from the analysis.

d) Evaluating allopolyploidy or autopolyploidy origin of P. juliflora

According to previous studies on ploidal variation in *Prosopis* (including samples from Ethiopia and Kenya), the only tetraploid species in the genus is *P. juliflora* ($2n = 4x = 46$), with all other species in the genus being entirely diploid ($2n = 2x = 28$) (Trenchard et al. 2008). Following Soltis and Soltis (2000), strict allopolyploids are characterized by the presence of fixed heterozygosity. We therefore used the proportion of fixed heterozygosity per locus to evaluate whether tetraploid *P. juliflora* individuals represent autopolyploids or allopolyploids. We also evaluated inbreeding co-efficients (F_{IS}) since fixed heterozygosity will lead to negative F_{IS} values (Meirmans and Van Tienderen 2013). Observed heterozygosity (H_o) and F_{IS} values were calculated using SPAGeDi version 1.5 (Hardy and Vekemans 2002) for both *P. juliflora* and *P. pallida*.

e) Polyploidy, hybridization and population genetic structure

Because of differences in ploidy between *P. juliflora* (4x) and *P. pallida* (2x), F1 hybrids between these two species are expected to be triploid (3x). We therefore estimated genome sizes of *Prosopis* individuals using flow cytometry analysis for a subset of our sampled individuals. For this analysis we included 63 individuals from Baringo County, representing both plantations ($n = 25$) and invaded areas ($n = 28$), and 10 individuals from the Afar Region, representing plantations ($n = 6$) and invaded areas ($n = 4$). These samples represented morphotypes of both *P. pallida* ($n = 12$) and *P. juliflora* ($n = 51$) (Table S4.3). During the field survey, no putative hybrids were detected based on morphology (see results). For flow cytometry, the method of Temsch et al. (2010) was followed, using 1 cm³ of mature leaf sample per individual and *Solanum pseudocapsicum* L. as internal standard. A Pearson's Chi-squared test was performed to evaluate the association between the cytotype of individuals from both Baringo County and the Afar Region, and their frequency in invaded areas.

Population genetic structure and the presence of hybrids was estimated using the STRUCTURE v2.3.4 software (Pritchard et al. 2000). STRUCTURE uses Bayesian Monte-Carlo Markov chain sampling to identify the optimal number of genetic clusters for a given dataset by reducing departures from Hardy-Weinberg and linkage equilibrium expectations within genetic clusters. For a first 'overall' STRUCTURE analysis, two genetic clusters, corresponding

to the number of species sampled (*P. juliflora* and *P. pallida*), were tested and 10 independent models for each value of K (number of genetic clusters = 2) were run. Each model consisted of 500,000 generations of which the first 100,000 were discarded as burnin. Due to the probable presence of hybrids, an admixture model with correlated allele frequencies was specified. For datasets including individuals with different ploidy levels, STRUCTURE requires an overall ploidy to be specified. In this case, an overall ploidy of 4x was used. Following Pritchard et al. (2010) for analyses including polyploid individuals, the option *RECESSIVEALLELES* was set to one to account for allele copy ambiguity and for diploids-triploids individuals, a missing data symbol was added to complete the ploidy level. This indicates that the individual is diploid-triploids at all the loci. STRUCTURE provide assignment values for each individual to the different genetic clusters tested, calculated as the proportion (q_{ik}) of each individual genotype assigned to each of the optimal number of genetic clusters. These assignment values were used to determine the presence of hybrids, with individuals having similar membership to both genetic clusters being classified as F1 hybrids. We expected all putative F1 hybrids to be sterile due to their triploid genomes, and therefore that assignment values (q_{ik}) to each genetic cluster to be close to 0.5.

A second STRUCTURE analysis was run that included only *P. juliflora* individuals from Kenya and Ethiopia. For this '*P. juliflora*-only' analysis, triploid individuals identified by flow cytometry analyses (see results section), hybrids identified by the first STRUCTURE analysis (see above and results section), and *Prosopis pallida* trees (identified by morphology, flow cytometry and results from the overall STRUCTURE analysis; see results section) were excluded. For this analysis we ran models with similar parameters as described above for the 'overall' analysis but specifying K values ranging between one to 20. The optimal K value was estimated according to the method of by Evanno et al. (2005), using the program STRUCTURE Harvester software (Earl and VonHoldt 2012). CLUMPAK software (Kopelman et al. 2015) was used to graphically display of the results.

In addition, two separate principal components analyses (PCAs) were performed, one with the 'overall' and one with the '*P. juliflora*-only' datasets (see above) using the *PolySat* R package (Clark and Jasieniuk 2011). This package allows for the inclusion of microsatellite data of any ploidal level, including populations with mixed ploidy levels. For this, a matrix of pairwise distances between individuals was generated using Bruvo distances (Bruvo et al.

2004). This method was preferred because it incorporates distances between microsatellite alleles without information on allele copy number (Bruvo et al. 2004).

To assess the presence of sub-genetic clusters in Baringo County and Afar Region separately, STRUCTURE analyses were performed using the same dataset as for the '*P. juliflora*-only' analysis. For this 'study area-only' analysis, separate STRUCTURE runs included individuals from either Kenya or Ethiopia only. Parameters of these models were similar as described above. *K* values ranged between one to 36 for Baringo County and between one to 21 for Afar Region, corresponding to the number of locations sampled in each country. The optimal *K* value was as estimated according to the method of Evanno et al. (2005) using the STRUCTURE Harvester software (Earl and VonHoldt 2012).

f) Genetic analysis of stages of invasion

Allelic richness (A_R), expected heterozygosity (H_E ; corrected for sample size, Nei, 1978), observed heterozygosity (H_o) and inbreeding coefficients (F_{IS}), were calculated for each population from Baringo County and the Afar Region, using SPAGeDi version 1.5 (Hardy and Vekemans 2002). These metrics were compared across stages of invasion for *P. juliflora* individuals in Baringo County (i.e. plantations, neighbouring areas and invasive populations) and the Afar Region (plantations and invasive populations), and for *P. pallida* from plantations in Baringo County using Kruskal-Wallis tests. Dunn tests were used for multiple *post hoc* comparisons.

To examine patterns of gene flow between different stages of invasion in both areas, pairwise fixation indices (F_{ST}) were calculated between *P. juliflora* populations in Baringo County and the Afar Region separately in the *PolySat* R package. For this, allele frequencies were estimated using *deSilvaFreq* function. This method considers "allelic phenotypes" instead of genotypes to estimate allele frequencies, assuming random mating and either disomic or polysomic inheritance without double reduction (De Silva et al. 2005). For partial heterozygous genotypes, this approach assumes that all alleles have an equal chance of having more than one copy. It also enables the inclusion of selfing rates in the analysis. A selfing rate of 0.04 was specified in the analysis (Sareen and Yadav 1987). Sites where only a single individual was collected were not included in these analyses. We then tested for pairwise genetic differences between all pairs of populations from the same and different

stages of invasion in Baringo County (plantations, neighbouring areas and invasive populations) and the Afar Region (plantations and invasive populations) separately by using Kruskal-Wallis tests and Dunn tests for *post hoc* multiple comparisons. In addition, to test the significance of the stages of invasion in both areas, an analysis of molecular variance (AMOVA) was performed using pairwise distances between individuals generated with Bruvo distances (Bruvo et al. 2004). For this, the significance of stages of invasion was tested in Baringo County and the Afar Region separately, with the *pegas* R package (Paradis 2010) at three levels: among stages of invasion, among populations and within populations.

g) Landscape genetic analysis

Pairwise fixation indices (F_{ST}) between populations were calculated using the *PolySat* R package and *deSilvaFreq* function implemented in the R package. This was done for *P. juliflora* individuals from Baringo County and the Afar Region separately. Only plantation and invasive populations were included and neighbouring sites and those with only one individual sampled were excluded.

As a proxy for dispersal, we used landscape resistance modelling to infer how various landscape variables may influence gene flow. Geographic distances between populations were calculated from GPS coordinates with the *pointDistance* function in the *raster* R package (Hijmans and van Etten 2014). The influence of various landscape variables and geographic distance on gene flow (pairwise F_{ST} values) between *Prosopis* populations in both regions was tested. For each site were selected different variables know to influence the presence of *Prosopis*. For the Afar Region, elevation, mean precipitation, and distance to roads, rivers and villages were included. For Baringo County, elevation, precipitation in the wettest month (Bioclim 13), and distance to roads and rivers were included in the analyses (see Table 4.1). Individual environmental variables were gathered from the same sources used previously to investigate the ecological attributes underlying current *Prosopis* distributions in both areas (Rima, et al. *in prep.*; Shiferaw et al. 2019). The spatial extent, projections and spatial resolution (30m) was the same for both areas. To create resistance surfaces the cell values of the raster layers were used as resistance values. We considered that higher distances to roads, rivers, villages, higher altitudes and higher temperatures would affect dispersal negatively. In contrast, high precipitation will facilitate dispersal. Least-cost path approaches

were used for modelling ecological connectivity throughout the landscape for the variables considered. Least-cost path models correlate genetic distances with ecological distances along the shortest, single suitable path between locations (i.e. path with the lower resistance values) (Vignieri 2005). The *Costdistance* function within the *gdistance* R package was used to calculate the least-cost distance between points (van Etten 2017). With this methodology and the landscape variables described above, the ecological distance between populations were calculated. Finally, to assess the determinants of dispersal of *Prosopis* in both countries, Mantel tests were carried out with all populations from each country to assess correlation between linearized pairwise F_{ST} values (i.e. $F_{ST}/1 - F_{ST}$) and geographic and ecological distances respectively ln-transformed to conform to Mantel test assumptions.

Table 4.1. Landscape variables included in the study.

Country	Landscape variable abbreviation	Description	Source
Baringo District	Elevation	Shuttle Radar Topography Mission digital elevation model (30 m spatial resolution)	United States Geological Survey (USGS)
	Precipitation	the wet season (July)	Derived from worldclim
	DistRoad	Distances derived from road network data	Calculated from ILRI GIS Services
	DistRiver	Distances derived from data on watercourses	Calculated from ILRI GIS Services
Afar Region	Elevation	Shuttle Radar Topography Mission digital elevation model (30 m spatial resolution)	United States Geological Survey (USGS)
	Precipitation	Mean annual rainfall	Ethiopian National Meteorol. Agency
	DistRoad	Distances derived from road network data	Ethiopian Road Authority
	DistRiver	Distances derived from data on watercourses	Calculated from EthioGIS
	DistVillage	Distances derived from settlement data	Calculated from EthioGIS and Central Statistical Agency

4.3 Results

a) *Evaluating allopolyploidy or autopolyploidy origin of P. juliflora*

Morphological identification of trees suggested that only *P. juliflora* individuals were present in invaded areas in both Kenya and Ethiopia. Intermediate morphotypes, i.e. putative hybrids, appeared to be present in plantations, but absent from all invaded areas. Morphologically, *P. pallida* was differentiated from *P. juliflora* by having a single stem with branches that have a zig-zag appearance and are glabrous or with scattered hairs. Leaves of *P. pallida* were hairy, with two to four pairs of pallid leaflets. Also, *P. pallida* individuals had no thorns and their pods were straight or curved, having a pale yellow to golden brown colour, glabrous. *Prosopis pallida* pods are sweeter than those from *P. juliflora*. *Prosopis pallida* was only recorded in plantations and in one case in an area right next to a plantation in Baringo County (Table S4.1).

For tetraploid *P. juliflora* individuals of both countries, negative F_{IS} values were obtained for two loci (Prb4 and I-P06639), indicating an excess of heterozygotes while an excess of homozygotes was indicated by five loci with positive F_{IS} values (IV2, Gl12, Prsc7, Prsc9 and S-P1DKSFA; Table 4.2). In the diploid *P. pallida* individuals from Kenya, negative values of F_{IS} were found in four loci (IV2, Gl12, Prsc9 and S-P1DKSFA) whereas positive values of F_{IS} were found for three loci (Prb4, Prsc9 and I-P06639; Table 4.2).

Table 4.2. Observed heterozygosity (H_o) and inbreeding coefficient (F_{IS}) for individual loci of the tetraploid *P. juliflora* and the diploid *P. pallida*.

Locus	H_o		F_{IS}	
	<i>Prosopis juliflora</i>	<i>Prosopis pallida</i>	<i>Prosopis juliflora</i>	<i>Prosopis pallida</i>
Prb4	0.97	0.43	-0.30	0.28
S-P1EPIIV2*	0.11	0.05	0.52	-0.08
Gl12	0.56	0.12	0.28	-0.31
Prsc7	0.42	0.21	0.49	0.64
Prsc9	0.43	0.50	0.16	-0.24
I-P06639*	0.64	0.02	-0.05	0.81
S-P1DKSFA*	0.04	0.07	0.35	-0.33
Mean	0.45	0.20	0.21	0.11

*Markers with functional annotations

b) Polypoidy, hybridization and population genetic structure

As expected, flow cytometry analysis revealed that individuals in Baringo County morphologically identified as *P. juliflora* to be mostly tetraploid (n=34), but also occasionally triploid (n=13). Moreover, all morphotypes identified as *P. pallida* were diploid (n=12). In the Afar Region, individuals morphologically identified as *P. juliflora* were mostly tetraploids, although diploid (n=3) and triploid (n=4) individuals were identified in both plantations (n=3) and invaded areas (n=4) (Table S4.3). A significant association between cytotype and their frequency in invaded areas in both Baringo County and the Afar Region was found ($X^2=8.81$, $df=2$, $P<0.05$), with tetraploid *P. juliflora* individuals dominating in invaded areas compared to diploid *P. pallida* and triploid individuals.

According to the 'overall' STRUCTURE analysis, '*P. pallida*' cluster (orange cluster in Fig. 4.4) included individuals identified morphologically as *P. pallida* and those identified as diploid through flow cytometry analysis (88.5% of individuals, q_{ik} values ≥ 0.99). '*P. juliflora*' cluster included individuals identified morphologically as this species and tetraploids individuals identified through flow cytometry analysis (86.2% of individuals, q_{ik} values ≥ 0.99). Only diploid individuals from Ethiopia and one diploid tree from Kenya were assigned to the

tetraploid '*P. juliflora*' genetic cluster (blue cluster in Fig. 4.4). With respect to the triploid individuals, seven were assigned to the tetraploid '*P. juliflora*' cluster (q_{ik} values ≥ 0.99), one was assigned to the '*P. pallida*' cluster (q_{ik} value ≥ 0.99) and two showed some level of admixture (q_{ik} values ranging from 0.76 to 0.56 to '*P. juliflora*' cluster). Hybrids individuals (i.e. showing some level of admixture; mean $q_{ik} = 0.61$ to '*P. juliflora*' cluster) were only identified in Kenya (n=76), most of them from plantations (58 individuals) and the rest from neighbouring (two individuals) and invaded areas (16 individuals, Fig. 4.4). These STRUCTURE results were corroborated by the PCA analysis based on pairwise distances between individuals performed using Bruvo distances (Fig. 4.4).

The results of the '*P. juliflora*-only' STRUCTURE analysis in both countries identified two genetic clusters (Fig. 4.5, Fig. S4.2). Multivariate analyses, however, appeared to identify more clusters (Fig. 4.5). Groupings/clusters identified by these approaches did not reveal any patterns related to geography (i.e. country specific) or stages of invasion (i.e. plantation vs invaded areas). The results of the 'study area-only' STRUCTURE analysis, including putative *P. juliflora* individuals from Baringo County and Afar Region separately, identified two main genetic clusters in each country (hereafter referred to as 'blue' and 'white' clusters, Fig. S4.1, Fig. S4.2). In Baringo County, most of the invasive genotypes were assigned to the 'blue' genetic cluster (70.5%; $X^2=219.03$, $df=2$, $P<0.001$) in contrast, the assignment of founder individuals to both genetic clusters was similar ($X^2=11.88$, $df=2$, $P=0.12$). Similarly, in Afar Region, the majority of invasive genotypes were assigned to the 'blue' genetic cluster (63.9%; $X^2=9.97$, $df=1$, $P<0.01$), while founder genotypes were assigned in the equal proportion to both genetic clusters ($X^2=3.06$, $df=1$, $P=0.08$).

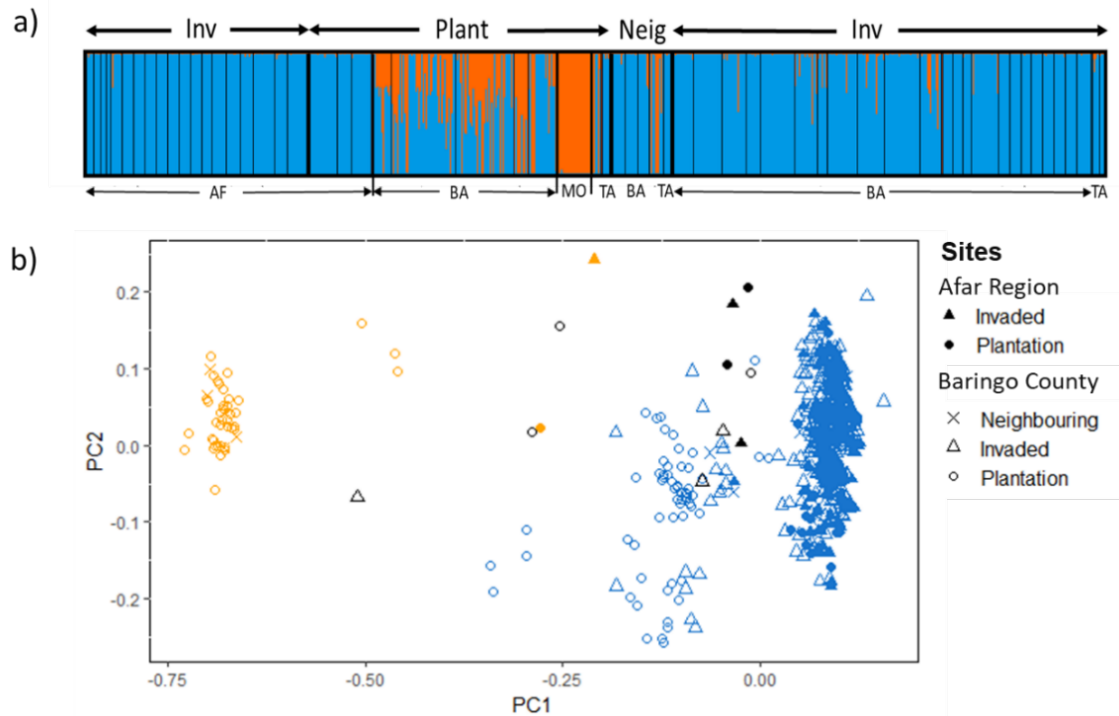


Figure 4.2. a) STRUCTURE bar plots where vertical axes illustrate the proportional assignment (q_{ik} values) of individual genomes to the inferred two genetic clusters, cluster 1 in blue and cluster 2 in orange; and for all *P. juliflora*, *P. pallida* and triploids from plantations (Plant), neighbouring (Neig) and invaded sites (Inv) from Afar Region in Ethiopia (AF), Baringo County (BA), Mombasa (MO) and Taveta (TA) in Kenya. b) Principal component analysis showing genetic structure among *Prosopis* individuals from Kenya and Ethiopia. *Prosopis juliflora* (4x) is shown in blue, triploids in black and *P. pallida* (2x) in orange. PCA was performed using Bruvo distances in POLYSAT (Bruvo et al. 2004). PC1 and PC2 captured 65.9% and 8.57% of the variation, respectively.

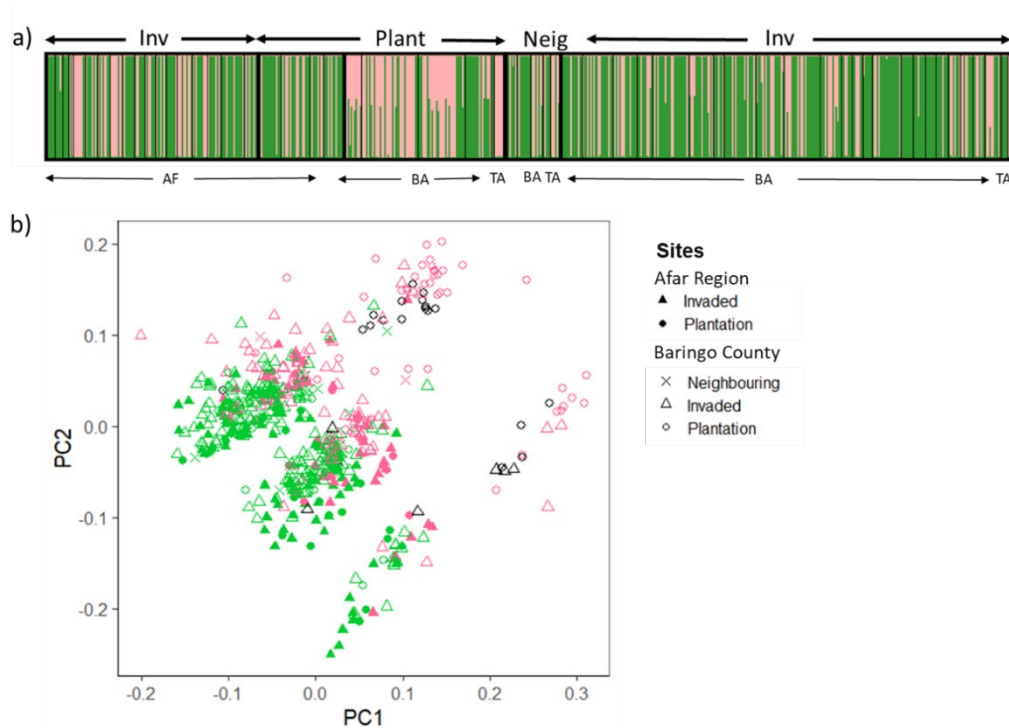


Figure 4.3. a) STRUCTURE bar plots for *P. juliflora* individuals from plantations (Plant), neighbouring (Neig) and invaded sites (Inv) from Afar Region in Ethiopia (AF), Baringo County (BA) and Taveta (TA) in Kenya. Vertical axes represent the assignment (q_{ik} values) of individual genomes to the inferred number genetic clusters ($K = 2$). b) Principal component analysis showing genetic structure among *Prosopis* individuals from Kenya and Ethiopia. Colours represent assignment of individuals to the inferred genetic clusters: Cluster 1 = green; Cluster 2 = pink; admixed = black. PCA was performed using Bruvo distances in POLYSAT (Bruvo et al., 2004). PC1 and PC2 captured 27.8 and 22.1% of the variation, respectively.

c) *Genetic analysis of stages of invasion*

Overall, low allelic richness was found for both species and in both countries (Table 4.3). In Baringo County in Kenya, values of A_R , H_e and H_o were higher in founder *P. juliflora* than founder *P. pallida*. In addition, founders *P. juliflora* had higher values of A_R , H_e and H_o than those from neighbouring and far-off invaded sites (Fig. 4.2a, b, c, respectively). The latter two also differed in levels of H_o and F_{IS} (Fig. 4.2). In the Afar Region, founder *P. juliflora* had similar A_R , H_e , H_o and F_{IS} than those from invaded sites. In comparison founder *P. juliflora* from Baringo County, founder individuals in the Afar Region had lower values of A_R , H_e , H_o and F_{IS} . Invasive *P. juliflora* individuals from Baringo County had similar values of A_R , H_e , H_o and F_{IS} than those from the Afar Region (Fig. 4.2).

For Baringo County, pairwise F_{ST} values between pairs of populations from plantations and invaded sites were higher than F_{ST} values between pairs of populations from invaded sites while for the Afar Region, the opposite pattern was found ($P < 0.05$ both; Fig. 4.3). AMOVA results showed a similar genetic variation among the levels of invasion in Baringo County (10.68%) compared to the genetic variation among populations (14.88%), while most of the genetic variation resided within populations (74.44%; Table 4.4). For the Afar Region, a low genetic variation was found between invasion levels (8.83%), at population level the genetic of variation was higher (12.92%) while most of the genetic variation was present within populations (78.66%; Table 4.4).

Table 4.3. Population genetic diversity indices for *Prosopis* individuals from plantations, areas neighbouring plantations and invaded sites far away from plantations in Baringo County, Kenya and Afar Region, Ethiopia. Statistics were calculated as mean values of each index over the seven loci analysed.

Sites	Species	Stages of invasion	A_R	H_E	H_o	F_{IS}
Baringo County	<i>P. juliflora</i>	Plantation	3.94	0.46	0.51	0.18
	<i>P. juliflora</i>	Neighbouring	3.81	0.37	0.40	0.21
	<i>P. juliflora</i>	Invaded	3.64	0.39	0.46	0.07
	<i>P. juliflora</i>	All	3.91	0.42	0.47	0.14
	<i>P. pallida</i>	Plantation	1.60	0.29	0.19	0.36
	<i>P. pallida</i>	Neighbouring	1.21	0.13	0.04	0.80
	<i>P. pallida</i>	All	1.60	0.28	0.17	0.42
Afar Region	<i>P. juliflora</i>	Plantation	2.84	0.34	0.41	0.05
	<i>P. juliflora</i>	Invaded	3.44	0.36	0.43	0.09
	<i>P. juliflora</i>	All	3.32	0.35	0.42	0.08

Table 4.4. Hierarchical AMOVA partitioning of genetic variation for *P. juliflora* populations from different stages of invasion (plantations, areas neighbouring plantations and invaded sites far away from plantations) in Baringo County, Kenya and Afar Region, Ethiopia.

Source of variation	d.f.	Sum of squares	Variance	Percent variation (%)	Fixation index
Baringo County					
Among stages of invasion	2	1.24	13.12	10.68	0.19***
Among populations	28	1.52	18.28	14.88	0.09***
Within populations	391	8.99	91.47	74.44	0.27
Afar Region					
Between stages of invasion	1	0.03	8.53	8.42	-0.02
Among populations	20	1.03	13.08	12.92	0.19*
Within populations	172	2.97	79.67	78.66	0.17

Statistical significance: *, $P < 0.05$; ***, $P < 0.001$. Testing was done using 10 000 random permutations

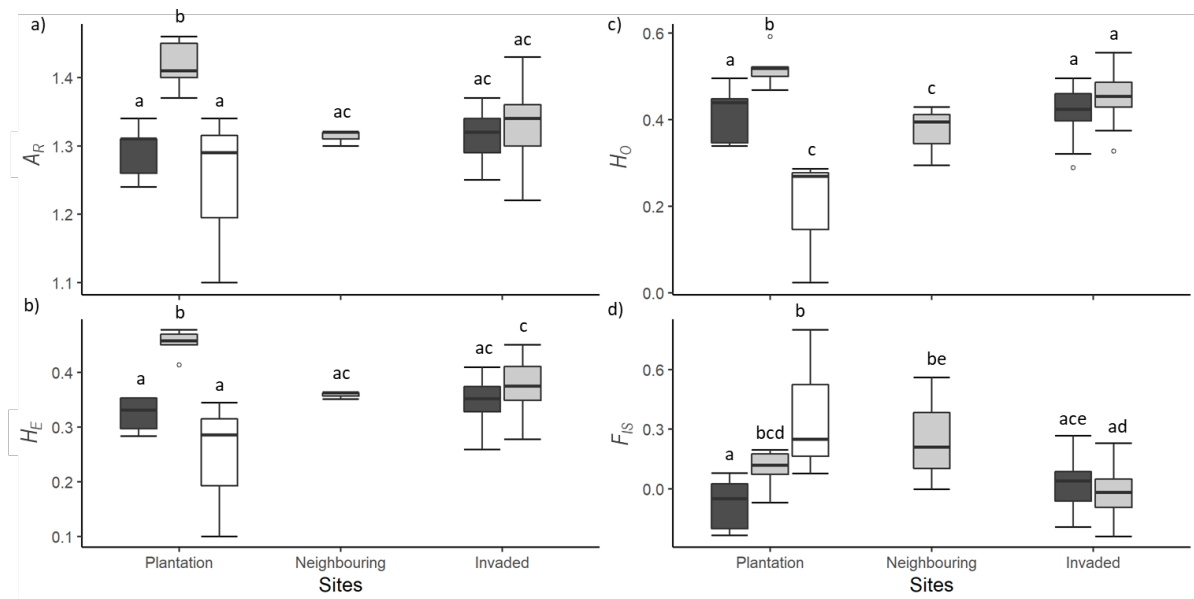


Figure 4.2. Genetic diversity metrics of *Prosopis juliflora* found in plantations, sites neighbouring plantations and far-off invaded sites in Afar Region, Ethiopia (black boxes) and Baringo County, Kenya (grey boxes). *Prosopis pallida* individuals found in plantations in Kenya are also show (white boxes). a) Allelic richness (A_R); b) Expected heterozygosity (H_E); c) Observed heterozygosity (H_O); d) Inbreeding coefficient (F_{IS}). Boxplots depict the median value, interquartile ranges and outliers of each region. Different letters above the plots indicate significant differences (Kruskal-Wallis rank sum test; $P < 0.05$; Dunn's post hoc test) between the corresponding groups.

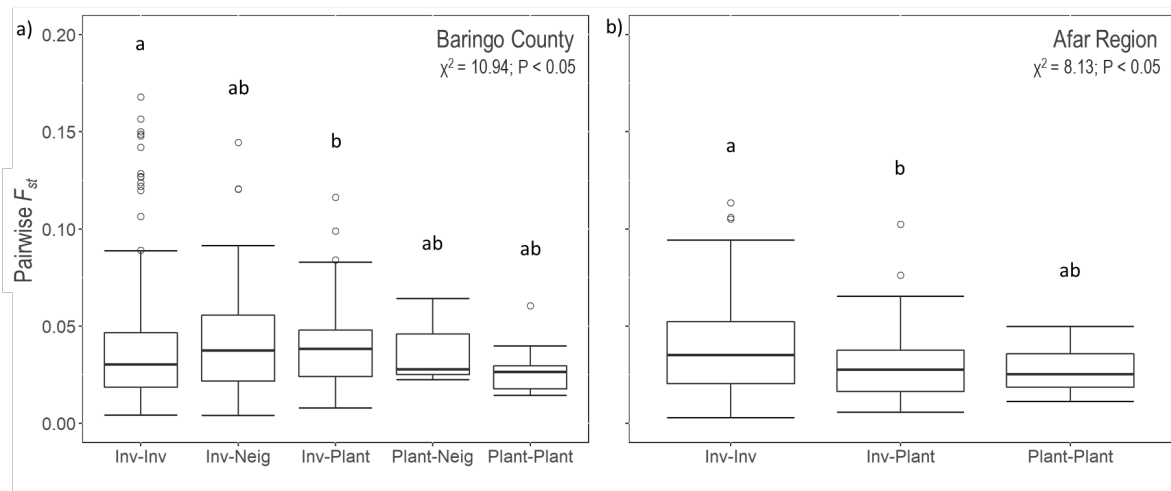


Figure 4.3. Comparison of genetic differentiation of *P. juliflora* populations in a) Baringo County, Kenya and b) Afar Region, Ethiopia. Pairwise F_{ST} between pairs of populations from invaded sites (Inv-Inv), invaded and neighbouring sites (Inv-Neig), invaded and plantation sites (Inv-Plant), plantations and neighbouring sites (Plant-Neig), and plantations sites (Plant-Plant). Different letters above the bars or boxplots indicate significant differences (Kruskal-Wallis rank sum test; $P < 0.05$; Dunn's post hoc test).

d) Effect of landscape variables on dispersal

Pairwise F_{ST} values between populations were not related to the geographical distance between them, neither in Baringo County nor in the Afar Region (Table 4.5). For both countries, the landscape genetic approaches indicated no significant relationships between pairwise F_{ST} values and any of the pairwise ecological distances based on the landscape variables considered (Table 4.5, Fig. S4.3).

Table 4.5. Results of Mantel tests performed among all *P. juliflora* populations from Baringo County, Kenya and Afar Region, Ethiopia, testing for correlations between various biotic and abiotic factors and population genetic structure. Test statistic (R) and the significance level (p) are provided.

Region	Tested relation	Mantel test	
		R	p
Baringo			
County	F_{ST} x geographic distance	-0.094	0.69
	F_{ST} x elevation	-0.003	0.49
	F_{ST} x precipitation wet season	-0.096	0.68
	F_{ST} x DistRoad	-0.006	0.52
	F_{ST} x DistRiver	-0.080	0.65
Afar			
Region	F_{ST} x geographic distance	0.015	0.46
	F_{ST} x elevation	0.008	0.48
	F_{ST} x precipitation	0.010	0.49
	F_{ST} x DistRoad	0.033	0.46
	F_{ST} x DistRiver	0.120	0.72
	F_{ST} x DistVillage	0.150	0.23

4.4 Discussion

Despite similar introduction histories of *P. juliflora* and *P. pallida* in Baringo County in Kenya, i.e. similar residence times in the same environments, and evidence for hybridization, only *P. juliflora* have become invasive and widespread in the region. In contrast to other invaded parts of the world dominated by *Prosopis* hybrids (i.e. Australia and South Africa), *P. juliflora* is the main invading species in Eastern Africa. In addition, genetic comparisons between founder and invasive genotypes presented with a unique opportunity to study these *Prosopis* populations in Baringo County and Afar Region. This approach showed that, despite similar introduction histories, different demographic processes may be operating in these two regions, giving important insights into the site-specific dynamics of invasion processes.

Polyploidy, hybridization and invasiveness

We did find evidence for the occurrence of hybridization between *P. juliflora* and *P. pallida* in Kenya, however, many of these hybrids were founder trees and only very few hybrids were present in invasive populations. This indicates that hybrid individuals were planted or probably originated during the initial cultivation of *P. juliflora* and *P. pallida* in Eastern Africa. While the success of many plant invasions has been attributed to hybridization (Schierenbeck and Ellstrand 2009, Zalapa et al. 2010, Gaskin et al. 2012), this has clearly not been the case for *Prosopis* invasions in Eastern Africa. The low level of invasive hybrids should not be surprising given the difference in ploidal levels between *P. juliflora* and *P. pallida*. Closely related allopatric species with different ploidal levels can often co-exist as reproductive isolation is reinforced through hybrid sterility (Petit et al. 2004). In the case of *P. juliflora* and *P. pallida* most hybrids were, as expected, triploid, and thus presumed to be sterile. In contrast, *Prosopis* invasions in places like Australia and South Africa are dominated by hybrid swarms (Van Klinken et al. 2006, Mazibuko 2012), probably as a consequence of the introduction, and subsequent hybridization, of numerous diploid *Prosopis* species.

We found tetraploid *P. juliflora* to have negative F_{IS} values in only two of the seven loci we genotyped, whereas four loci had negative F_{IS} values in diploid *P. pallida*. These findings suggest an autopolyploid origin of *P. juliflora*. Polyploidy is an important evolutionary process in flowering plants in general, with one third of all Angiosperms being descendants of polyploids (Wright 1962). Polyploidy has also been repeatedly linked to plant invasiveness

(Pandit et al. 2011, te Beest et al. 2012) with higher ploidal levels often being correlated with higher invasiveness (Nagy et al. 2017). This is, partly, due to the effects of genome doubling on gene expression that can lead to novel phenotypes and the creation of adaptive variation (te Beest et al. 2012). In addition, the assignment of some triploid and diploid individuals from plantations and invaded areas to '*P. juliflora*' cluster, may indicate that genome reduction is occurring in *P. juliflora*. This could also be the case for *P. pallida* x *P. juliflora* hybrids, since DNA elimination occurs more frequently in allopolyploids than in autopolyploid (Parisod et al. 2010). Genome reduction can occur within a few generations and is often associated with phenotypic changes that may increase invasiveness (Lavergne et al. 2010). Overall, the beneficial effects of polyploidy may well explain the successful invasion of *P. juliflora* in Eastern Africa.

Genetic insights from different invasion stages

In Baringo County, invasive and recently established *P. juliflora* individuals near plantations were genetically less diverse than founder trees from plantations. In contrast, no differences in genetic diversity were found between invasive individuals and founder trees in the Afar Region. A previous study found similar levels of genetic diversity in native Mexican *P. juliflora* populations and invasive Ethiopian populations, while Kenyan populations had higher genetic diversity than the Mexican populations (Chapter 2). In theory, it is likely that invasive populations of any species may include genotypes of differential fitness so that, in particular habitats, some genotypes may produce a disproportionate fraction of offspring (Richardson and Pyšek 2006, Theoharides and Dukes 2007, Zenni et al. 2014). As a consequence, one might hypothesize that different invasion stages (i.e. introduction, naturalization and spread) could be dominated by different genotypes which may be indicative of contemporary genetic change due to selective pressures encountered during the invasion process. An alternative hypothesis is that dispersal from initially introduced populations often involves consecutive founder events which can erode the genetic diversity along the direction of dispersal (Austerlitz et al. 1997, Dlugosch and Parker 2008), thereby reducing adaptive potential (Baker and Stebbins 1965). Our '*P. juliflora*-only' analyses did not identify genetic structuring of *P. juliflora* individuals according to different stages of invasion. However, the 'study area-only' analysis showed that invasive individuals in Baringo were mostly assigned to one of the two identified genetic clusters. In addition, the higher standing genetic diversity present in

founder individuals in Baringo County may provide higher adaptive potential during invasion compared to the lower standing genetic diversity of founder individuals in the Afar Region. We previously showed that rapid evolution in the invasive range in Baringo County may have increased the invasiveness in *P. juliflora*, which supports the first hypothesis (Chapter 3). However, we cannot dismiss the possible role of demographic stochasticity (i.e. consecutive founder events) in explaining these results with 100% certainty.

The link between genetic diversity within and among populations and patterns of gene flow are intuitive. In Baringo County, pairwise F_{ST} values suggest that the gene flow between plantations and invaded sites is lower than the gene flow between different invaded sites, while the opposite pattern was found in the Afar Region. Often small outlying colonizing populations may rely on gene flow from “source” populations for their successful establishment and further spread, in what has been termed “genetic rescue”. This could be the case for the invasion process of *Prosopis* in the Afar Region but not in Baringo County. In small founder populations gene flow may increase genetic diversity for selection to act upon, as it has been demonstrated in plants (e. g. Sexton et al. 2011). In our case, the gene flow between founding *P. juliflora* individuals in Afar Region and the invasive individuals may have led to the homogenization of the standing genetic diversity.

Effect of landscape variables on dispersal

Frequently, dispersal may cause pattern of isolation-by-distance (Wright 1943), whereby populations are more genetically differentiated when separated by larger geographical distances compared to shorter distances. However, dispersal can also be modulated by the influence of various biotic and abiotic factors on gene flow (Zeller et al. 2012). This suggests that dispersal can be higher between populations under similar environmental conditions, in what has been termed isolation-by-environment (Wang and Bradburd 2014). Evidence of isolation-by-distance and isolation-by-environment have been found across environmental gradients in numerous species (see Sexton et al. 2012 and reference therein). In our study, genetic divergence between populations of *P. juliflora* in the Afar Region and Baringo County was low and not correlated with geographic distance. In addition, we found no constraints to dispersal of *Prosopis* in both areas due to any of the landscape variables/barriers analysed, at least at the spatial scales we investigated. Globally *Prosopis* species are frequently dispersed

by livestock where they have been introduced outside of their native ranges (Pasiiecznik et al. 2001) and, therefore, potentially over substantial distances. Considering the relatively small spatial scales of both our study areas (Baringo County covers an area of 1,015 km² while Afar Region covers an area of 270,000 km²; Bekele et al. 2018) such frequent long-distance dispersal may explain the absence of any patterns of isolation-by-resistance. Therefore, even when variables like temperature and altitude are important to predict the presence of *Prosopis* in invaded areas of Eastern Africa, they may have no impact on dispersal over small spatial scales. Future studies on the effect of these and other landscape variables (i.e. soil nutrients, pastoralist routes or flooding risks) on the dispersal of *Prosopis* are necessary.

Implications for management

Our results showed that in both Afar Region and Baringo County dispersal of *P. juliflora* is not restricted by climatic conditions, distance to roads, rivers and villages at the spatial scales we analyzed. Considering this, successful management of *Prosopis* requires strategies to prevent the production and dispersal of seeds into currently uninvaded areas, in particular along roads and livestock migratory routes which could facilitate further long-distance dispersal and spread. In Baringo County, control of *Prosopis* through utilization (i.e. firewood, pods as fodder for animals) has been largely encouraged and implemented by local and national government authorities in some areas (Choge 2002), however, this strategy does not prevent the production and dispersal of seeds and probably explain why control of the spread of *Prosopis* in this region has been unsuccessful.

Biocontrol has been proposed as a safe, economic and effective way to reduce the spread of *Prosopis* (van Wilgen et al. 2012). In South Africa, seed-feeding biological control agents have been introduced in an attempt to reduce the fecundity of *Prosopis* trees, but these have had little impact to date (Zachariades et al. 2011), and the trees have continued to spread (Henderson and Wilson 2017). The relative ineffectiveness of biological control may be because seed-feeding agents have not reduced seed production drastically, or because only these types of agents, and not more destructive ones, have been used. However, in South Africa, almost all invasive *Prosopis* trees are hybrids (Mazibuko, 2012) and biological control agents that are pre-adapted to a particular species may be less effective against hybrids (Goolsby et al. 2006). In Australia, a leaf-tying agent is causing high levels of defoliation on

Prosopis trees in the warmest region and reducing the performance of trees by reducing plant growth rates and seed production. However, *Prosopis* recruitment is still occurring, suggesting that the invasive population would continue expanding in areas invaded by the hybrid swarm *P. pallida* x *P. velutina* x *P. glandulosa* var. *glandulosa* (van Klinken and Campbell 2009, Pichancourt et al. 2012). Hybridization may affect resistance to biological control because hybrid vigour may translate into resistance against herbivores used as biocontrol agents. For example, in *Fallopia* species, levels of herbivory resistance against a potential biological control agent varied depending on species identity or whether individuals were hybrids (Krebs et al. 2011). Similarly, in the invasive salt cedar trees (genus *Tamarix*), levels of hybridization and introgression between species resulted in variation in tolerance to herbivory and resistance to a biological control agent (Williams et al. 2014). Unlike in many parts of the world, invasive *Prosopis* in Eastern Africa are not hybrids, which could increase the chances of finding an effective biological control agent that is specific to only *P. juliflora* since the co-evolutionary history between host plants and control agents has not been diluted/alterd through hybridization. In addition, due to the different ploidy levels of introduced *Prosopis* species in East Africa, hybrids would not be able to form stable populations. All this would contribute to the potential higher effectiveness of biological control agents in Eastern Africa than in places like South Africa where hybridization between different *Prosopis* species is commonplace. Given the substantial negative impacts of *Prosopis* invasions in Eastern Africa, we strongly recommend that biological control be investigated as a potential management strategy.

4.5 Conclusion

We used a unique opportunity to assess both the original founder and invasive genotypes of *Prosopis* trees in Eastern Africa to investigate the genetic and eco-evolutionary factors that may explain their phenomenal ecological success in this region. We found a loss of genetic diversity following introduction and spread in Baringo County but not in Afar. This might indicate that *Prosopis* in Baringo County were subject to selective forces during the invasion process, as found previously (Chapter 3). We also found, in contrast to other parts of the world where *Prosopis* invasions are dominated hybrids, that *P. juliflora* is the main invading species in Eastern Africa. Thus, by using *Prosopis* invasion as a model system, and by

including the invasive and introduced founder genotypes that acted as the source of the invasion, our study provides valuable insights about the site-specific dynamics of the invasive success in plants at local scales. This is important when management plans are needed to contain and reduce impact as is the case of *Prosopis* invasion in Eastern Africa. Our results also provide important information that can be used to maximize control efforts in *Prosopis* and supports the use of biological control as part of management strategies.

4.6 Supporting information

Table S4.1. Sample sites of *Prosopis* individuals from Kenya and Ethiopia included in the study. For each sample site the following is indicated: the locality, ID sample site; site category, i.e. plantation, areas neighbouring plantations, and invaded sites distant from plantations; the *Prosopis* species found in each sample site and category; the number of individuals of each species (N); and the location in decimal degrees. Neighbouring sites have the same ID and coordinates as their plantations. Neighbouring sites include trees located at a distance of less than 100m from the plantations.

Country	Locality	ID	Category	Species	N	Latitude	Longitude
Kenya	Baringo County	KEN1	Plantation	<i>P. pallida</i>	1	0.370	36.043
	Baringo County	KEN2	Plantation	<i>P. juliflora</i>	13	0.446	36.019
	Baringo County	KEN2	Neighbouring	<i>P. juliflora</i>	9		
	Baringo County	KEN3	Plantation	<i>P. pallida</i>	5	0.549	36.032
	Baringo County	KEN3	Plantation	<i>P. juliflora</i>	44	0.549	36.032
	Baringo County	KEN3	Neighbouring	<i>P. juliflora</i>	9		
	Baringo County	KEN4	Plantation	<i>P. pallida</i>	6	0.506	35.964
	Baringo County	KEN4	Plantation	<i>P. juliflora</i>	36	0.506	35.964
	Baringo County	KEN4	Neighbouring	<i>P. pallida</i>	4		
	Baringo County	KEN4	Neighbouring	<i>P. juliflora</i>	6		
	Baringo County	KEN5	Plantation	<i>P. pallida</i>	10	0.268	36.054
	Baringo County	KEN6	Plantation	<i>P. pallida</i>	3	0.268	36.054
	Baringo County	KEN6	Plantation	<i>P. juliflora</i>	19	0.468	35.999
	Baringo County	KEN7	Plantation	<i>P. pallida</i>	1	0.541	36.039
	Baringo County	KEN8	Plantation	<i>P. pallida</i>	3	0.464	36.012
	Baringo County	KEN8	Plantation	<i>P. juliflora</i>	3	0.464	36.012
	Baringo County	KEN9	Plantation	<i>P. pallida</i>	1	0.564	36.035
	Baringo County	KEN10	Invaded	<i>P. juliflora</i>	10	0.367	36.045
	Baringo County	KEN11	Invaded	<i>P. juliflora</i>	15	0.446	36.027
	Baringo County	KEN12	Invaded	<i>P. juliflora</i>	20		
	Baringo County	KEN13	Invaded	<i>P. juliflora</i>	17	0.340	36.054
	Baringo County	KEN14	Invaded	<i>P. juliflora</i>	11	0.364	36.069
	Baringo County	KEN15	Invaded	<i>P. juliflora</i>	25	0.414	36.062
	Baringo County	KEN16	Invaded	<i>P. juliflora</i>	16	0.352	36.058
	Baringo County	KEN17	Invaded	<i>P. juliflora</i>	7	0.356	36.058
	Baringo County	KEN18	Invaded	<i>P. juliflora</i>	21	0.393	36.036
	Baringo County	KEN19	Invaded	<i>P. juliflora</i>	20	0.466	36.004
	Baringo County	KEN20	Invaded	<i>P. juliflora</i>	25		
	Baringo County	KEN21	Invaded	<i>P. juliflora</i>	16	0.498	36.060
	Baringo County	KEN22	Invaded	<i>P. juliflora</i>	8	0.550	35.988
	Baringo County	KEN23	Invaded	<i>P. juliflora</i>	8	0.534	35.992

Baringo County	KEN24	Invaded	<i>P. juliflora</i>	8	0.948	36.014	
Baringo County	KEN25	Invaded	<i>P. juliflora</i>	21	0.949	36.012	
Baringo County	KEN26	Invaded	<i>P. juliflora</i>	8	0.938	36.020	
Baringo County	KEN27	Invaded	<i>P. juliflora</i>	8	0.733	36.035	
Baringo County	KEN28	Invaded	<i>P. juliflora</i>	7	0.714	36.032	
Baringo County	KEN29	Invaded	<i>P. juliflora</i>	8	0.706	36.029	
Baringo County	KEN30	Invaded	<i>P. juliflora</i>	8	0.620	36.011	
Baringo County	KEN31	Invaded	<i>P. juliflora</i>	8	0.622	36.029	
Baringo County	KEN32	Invaded	<i>P. juliflora</i>	8	0.499	35.950	
Baringo County	KEN33	Invaded	<i>P. juliflora</i>	10	0.246	36.084	
Baringo County	KEN34	Invaded	<i>P. juliflora</i>	1	0.427	36.030	
Baringo County	KEN35	Invaded	<i>P. juliflora</i>	4	0.489	36.054	
Baringo County	KEN36	Invaded	<i>P. juliflora</i>	1	0.410	36.021	
Baringo County	KEN37	Invaded	<i>P. juliflora</i>	1	0.500	36.037	
Mombasa	KEN38	Plantation	<i>P. pallida</i>	23	-4.018	39.721	
Taveta	KEN39	Plantation	<i>P. juliflora</i>	6	-3.394	37.677	
Taveta	KEN39	Neighbouring	<i>P. juliflora</i>	6			
Taveta	KEN40	Invaded	<i>P. juliflora</i>	3	-3.314	37.718	
Baringo County	KEN41	Invaded	<i>P. juliflora</i>	2	0.588	36.010	
Baringo County*	KEN42	Invaded	<i>P. juliflora</i>	1	0.556	35.990	
Baringo County*	KEN43	Invaded	<i>P. juliflora</i>	2	0.515	35.998	
Baringo County*	KEN44	Invaded	<i>P. juliflora</i>	1	0.417	36.059	
Ethiopia	Afar Region	ETH1	Plantation	<i>P. juliflora</i>	15	10.159	40.662
Afar Region	ETH2	Plantation	<i>P. juliflora</i>	8	9.940	40.413	
Afar Region	ETH3	Plantation	<i>P. juliflora</i>	20	9.318	40.177	
Afar Region	ETH4	Plantation	<i>P. juliflora</i>	10	9.318	40.180	
Afar Region	ETH5	Plantation	<i>P. juliflora</i>	15	9.327	40.208	
Afar Region	ETH6	Invaded	<i>P. juliflora</i>	5	9.337	40.215	
Afar Region	ETH7	Invaded	<i>P. juliflora</i>	5	9.194	40.174	
Afar Region	ETH8	Invaded	<i>P. juliflora</i>	5	9.193	40.152	
Afar Region	ETH9	Invaded	<i>P. juliflora</i>	3	9.477	40.318	
Afar Region	ETH10	Invaded	<i>P. juliflora</i>	8	8.913	39.905	
Afar Region	ETH11	Invaded	<i>P. juliflora</i>	8	9.025	40.064	
Afar Region	ETH12	Invaded	<i>P. juliflora</i>	8	9.089	40.024	
Afar Region	ETH13	Invaded	<i>P. juliflora</i>	8	9.288	40.142	
Afar Region	ETH14	Invaded	<i>P. juliflora</i>	8	9.309	40.146	
Afar Region	ETH15	Invaded	<i>P. juliflora</i>	8	9.328	40.177	
Afar Region	ETH16	Invaded	<i>P. juliflora</i>	8	9.412	40.159	
Afar Region	ETH17	Invaded	<i>P. juliflora</i>	8	9.335	40.181	
Afar Region	ETH18	Invaded	<i>P. juliflora</i>	10	9.533	40.303	
Afar Region	ETH19	Invaded	<i>P. juliflora</i>	10	9.890	40.520	
Afar Region	ETH20	Invaded	<i>P. juliflora</i>	9	9.945	40.530	
Afar Region	ETH21	Invaded	<i>P. juliflora</i>	15	10.015	40.558	
Afar Region	ETH22	Invaded	<i>P. juliflora</i>	9	9.965	40.534	

*samples included in flow cytometry analysis only

Table S4.2 Volume of the 10 microsatellites primers included in one multiplex PCR assay. From these, seven were included in the study (in bold).

Locus name	Primer volume
Prsc9	0.5
Prsc7	1
Prb8	2
S-P1DKSFA	0.5
Prb4	1
I-P07653	0.3
I-P06639	1
I-P00930c	0.5
S-P1EPIV2	1
GL23	2
GL12	1

Table S4.3. Flow cytometry results for *Prosopis* individuals from Baringo County, Kenya (KEN) and Afar Region, Ethiopia (ETH). For each individual is indicated the ID sample site; species morphological identification, site category, i.e. plantation, areas neighbouring plantations, and invaded sites distant from plantations; cytotype and rel gen size values. Nuclear content of diploids had a mean genome size of $2c = 0.90$ pg (SE=0.026), while those for triploids and tetraploids were $3c = 1.36$ pg (SE 0.018) and $4c = 1.81$ pg (SE=0.021), respectively.

ID	Putative species	Category	Cytotype	Rel gen size (pg)
KEN1	<i>P. pallida</i>	Plantation	2x	1.007
KEN3	<i>P. pallida</i>	Plantation	2x	1.015
KEN3	<i>P. pallida</i>	Plantation	2x	0.989
KEN3	<i>P. pallida</i>	Plantation	2x	0.818
KEN3	<i>P. pallida</i>	Plantation	2x	0.733
KEN3	<i>P. juliflora</i>	Plantation	4x	1.849
KEN3	<i>P. juliflora</i>	Plantation	4x	1.957
KEN3	<i>P. juliflora</i>	Plantation	4x	1.942
KEN4	<i>P. juliflora</i>	Plantation	4x	1.649
KEN4	<i>P. juliflora</i>	Plantation	4x	1.621
KEN4	<i>P. juliflora</i>	Plantation	4x	1.805
KEN4	<i>P. juliflora</i>	Plantation	3x	1.387
KEN4	<i>P. juliflora</i>	Plantation	3x	1.270
KEN6	<i>P. pallida</i>	Plantation	2x	0.950
KEN6	<i>P. juliflora</i>	Plantation	4x	1.916
KEN6	<i>P. juliflora</i>	Plantation	4x	1.812
KEN6	<i>P. pallida</i>	Plantation	2x	0.963
KEN6	<i>P. pallida</i>	Plantation	2x	1.005
KEN6	<i>P. pallida</i>	Plantation	2x	0.994
KEN8	<i>P. pallida</i>	Plantation	2x	0.865
KEN8	<i>P. pallida</i>	Plantation	2x	0.847
KEN8	<i>P. juliflora</i>	Plantation	3x	1.333
KEN8	<i>P. juliflora</i>	Plantation	4x	1.686
KEN8	<i>P. juliflora</i>	Plantation	4x	1.947
KEN9	<i>P. pallida</i>	Plantation	2x	0.997
KEN10	<i>P. juliflora</i>	Invaded area	3x	1.434
KEN20	<i>P. juliflora</i>	Invaded area	4x	1.618
KEN20	<i>P. juliflora</i>	Invaded area	2x	0.950
KEN21	<i>P. juliflora</i>	Invaded area	4x	1.667
KEN21	<i>P. juliflora</i>	Invaded area	3x	1.370
KEN21	<i>P. juliflora</i>	Invaded area	4x	1.709
KEN23	<i>P. juliflora</i>	Invaded area	4x	1.561
KEN25	<i>P. juliflora</i>	Invaded area	4x	1.818
KEN34	<i>P. juliflora</i>	Invaded area	4x	1.691

KEN34	<i>P. juliflora</i>	Invaded area	4x	1.649
KEN35	<i>P. juliflora</i>	Invaded area	3x	1.256
KEN35	<i>P. juliflora</i>	Invaded area	4x	1.926
KEN35	<i>P. juliflora</i>	Invaded area	3x	1.427
KEN35	<i>P. juliflora</i>	Invaded area	4x	1.854
KEN35	<i>P. juliflora</i>	Invaded area	4x	1.856
KEN35	<i>P. juliflora</i>	Invaded area	4x	1.989
KEN36	<i>P. juliflora</i>	Invaded area	4x	1.911
KEN36	<i>P. juliflora</i>	Invaded area	4x	1.872
KEN36	<i>P. juliflora</i>	Invaded area	4x	1.898
KEN37	<i>P. juliflora</i>	Invaded area	4x	1.784
KEN37	<i>P. juliflora</i>	Invaded area	3x	1.346
KEN41	<i>P. juliflora</i>	Invaded area	4x	1.600
KEN41	<i>P. juliflora</i>	Invaded area	4x	1.916
KEN42	<i>P. juliflora</i>	Invaded area	3x	1.385
KEN43	<i>P. juliflora</i>	Invaded area	4x	1.864
KEN43	<i>P. juliflora</i>	Invaded area	4x	1.600
KEN44	<i>P. juliflora</i>	Invaded area	4x	1.911
KEN44	<i>P. juliflora</i>	Invaded area	4x	1.916
ETH1	<i>P. juliflora</i>	Plantation	4x	1.771
ETH1	<i>P. juliflora</i>	Plantation	4x	1.854
ETH3	<i>P. juliflora</i>	Plantation	3x	1.473
ETH4	<i>P. juliflora</i>	Plantation	3x	1.287
ETH5	<i>P. juliflora</i>	Plantation	4x	1.792
ETH5	<i>P. juliflora</i>	Plantation	2x	0.722
ETH14	<i>P. juliflora</i>	Invaded area	3x	1.383
ETH15	<i>P. juliflora</i>	Invaded area	2x	0.844
ETH16	<i>P. juliflora</i>	Invaded area	3x	1.370
ETH17	<i>P. juliflora</i>	Invaded area	2x	0.756

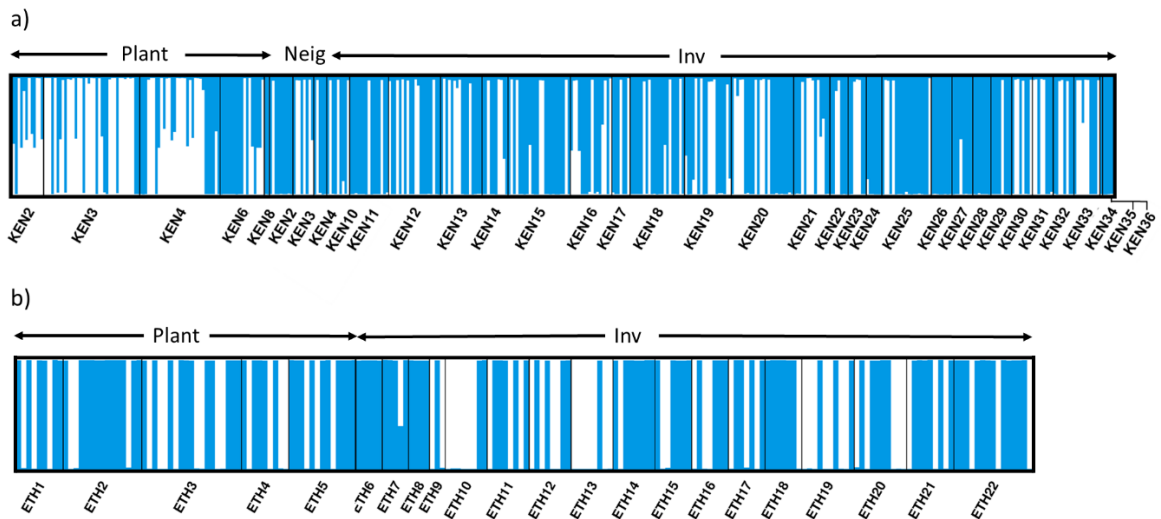


Figure S4.1. Identification of the optimal number of clusters for *P. juliflora* in a) Baringo County, Kenya, and b) Afar Region, Ethiopia, separately, inferred by Bayesian clustering with the software STRUCTURE. Vertical axes represent the assignment (q_{ik} values) of individual genomes to the inferred number genetic clusters ($K=2$). Two mayor genetic demes were identified in each area, including founder individuals from plantations (Plant), sites neighbouring plantations (Neig) and far-off invaded sites (Inv). Sample sites labels are indicated below each plot.

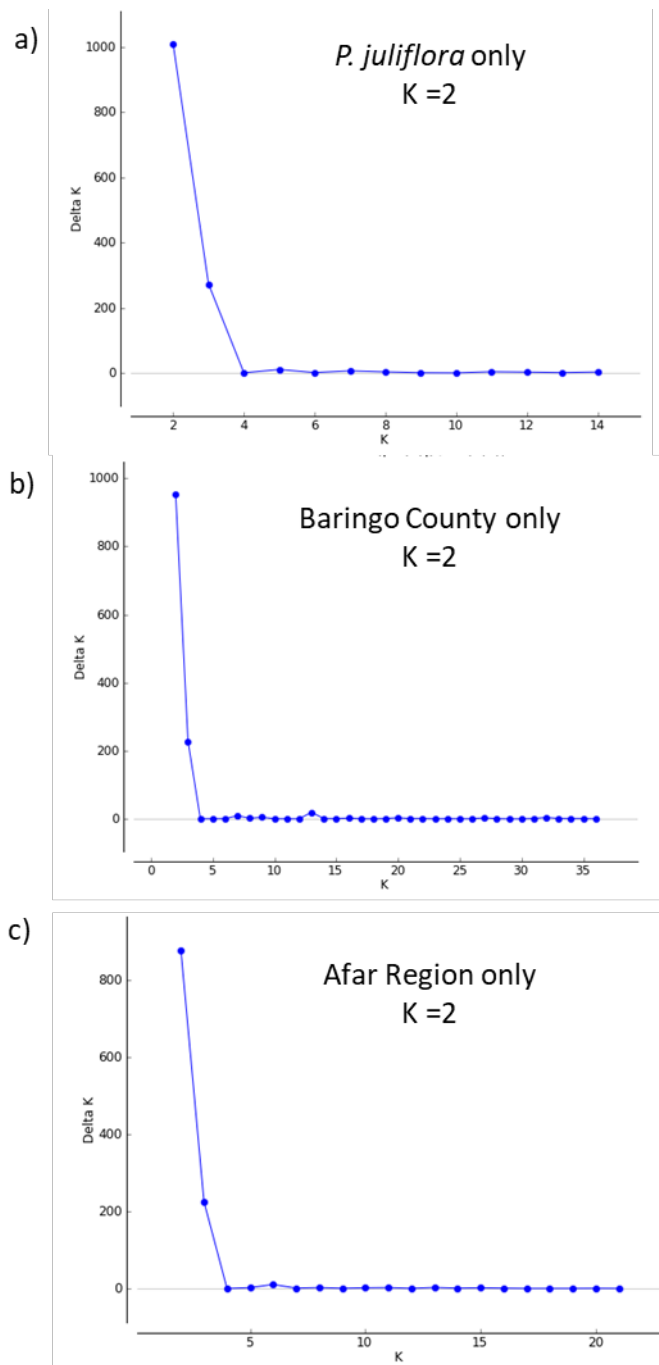


Figure S4.2. Identification of the optimal number clusters (K) for *P. juliflora* individuals from a) the invaded areas of Afar Region in Ethiopia, Baringo County and Taveta in Kenya, b) only Baringo County, Kenya, and c) only Afar Region, Ethiopia; inferred by Bayesian clustering with the software STRUCTURE. Data sets contain a total of a) 633 b) 424 and c) 194 individuals for seven nuclear microsatellites loci (see Material and Methods for parameters of the models).

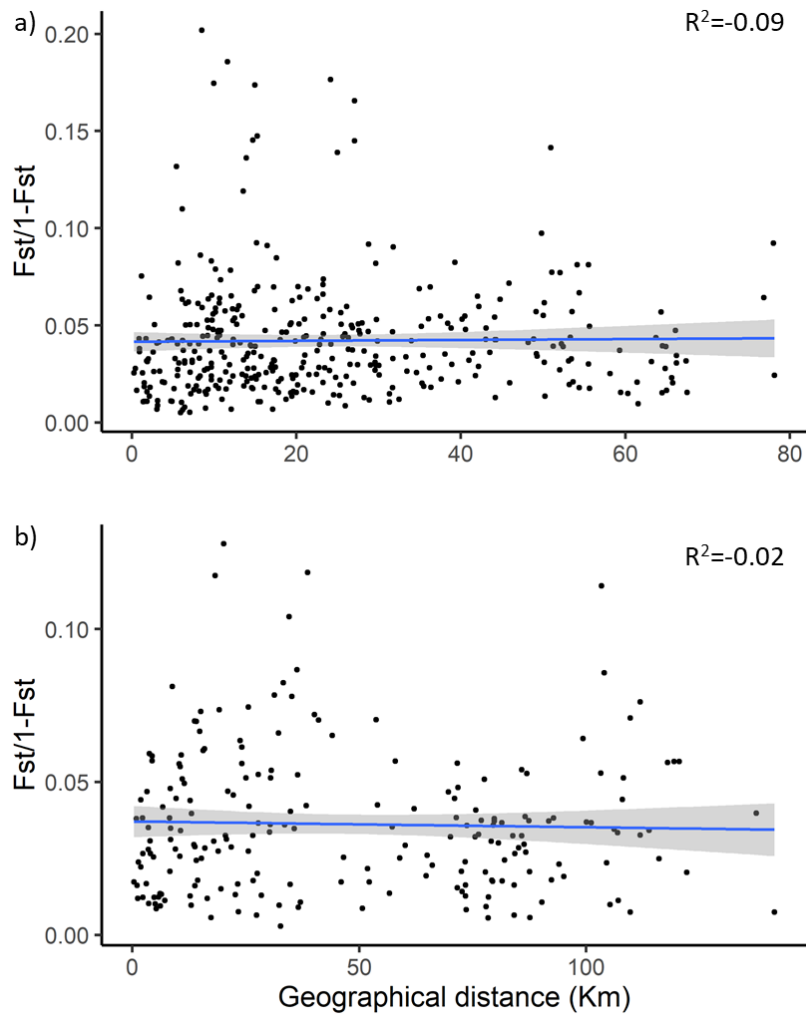


Figure S4.3. Results of Mantel tests performed among all *P. juliflora* populations from (a) Baringo County, Kenya and (b) Afar Region, Ethiopia, testing for correlations between geographical distances and population genetic structure ($F_{ST} / 1 - F_{ST}$). Test statistic (R) are provided. All correlations were not significant at the significance level ($p < 0.05$).

CHAPTER 5 Key components of social-ecological system research designed for impact

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Abstract

Understanding the effects of global change on the environment and human well-being, and delivering tools for management and policy are major challenges, as they require transdisciplinary research in complex social-ecological systems. We emphasize the inclusion of six components of transdisciplinary research that should increase the likelihood of relevant and effective solutions to complex problems. Drawing on experiences in a project on invasive alien trees, we argue that projects addressing social-ecological problems need a clear structure that transcends disciplines and that gathers data using different methodologies in the same experimental units to allow for integration and upscaling of findings. Furthermore, there should be a clear intent to identify management options with stakeholders, estimate their effects and test their implementation. Such projects may need to overcome potential misunderstandings among partners with different scientific or cultural backgrounds and train scientists in collaborative research, but they offer opportunities to better address complex social-ecological challenges.

Key words: Transdisciplinary, environmental change, ecosystem services, collaborative research, human well-being

5.1 Introduction

Human activities have changed the Earth's systems in ways that will be detectable for millennia to come (Waters et al. 2016). People promote global environmental change in many ways, including changing land use, nitrogen deposition, atmospheric CO₂ accumulation, and through the global redistribution of plant and animal species (Sala et al. 2000). These global environmental changes negatively affect the environment by threatening biodiversity, food

production and water resources as well as increasing the risks of natural hazards (Steffen 2005).

Global environmental change phenomena and their underlying causes are highly complex and are often referred to as 'wicked problems', i.e., they have no clear definition, there is no easy solution, and they are human-caused (Waddock 2013, Woodford et al. 2016). The complexity of understanding global environmental change is exacerbated by the fact that the determinants are dynamic, differ over space and time, and interact with each other (Li et al 1996, Vitousek et al. 1997). Moreover, ecosystem responses to global environmental change often unfold slowly so that early warning signals of an approaching tipping point, which would be followed by a rapid shift towards another regime with consequences for human society, may be missed or ignored (Hughes et al 2012). These factors add complexity, making it difficult to understand the temporal and spatial effects of global change on the environment and human well-being, and therefore complicating the task of formulating effective policy and management responses. Clearly, addressing the conservation and development challenges of the 21st century will need to be based on a better understanding of the interactions between ecosystems and human societies.

Social-ecological systems (SES) research promises to support this understanding. SES research demands the bridging of historically disjoint and isolated fields, such as economics, ecology and sociology, and has to be embedded in the diverse perspectives of multiple stakeholders to produce transdisciplinary results (Max-Neef 2005). Transdisciplinary research defined here as “problem-oriented research involving cooperation among a wide range of stakeholders and academia to meet complex challenges of society” (Klein 2008, Klein et al. 2001), addresses problems that cannot be defined in any single disciplinary domain (Eigenbrode et al. 2007). It is thus a precondition to achieve (1) a better understanding of the multiple, often interrelated ecological and social drivers underlying SES, (2) clarification of social conflicts, interests, values, perceptions, and attitudes associated with human-caused problems in SES, and (3) improved strategies for management and policy (Vaz et al. 2017).

Over the past decades, a variety of frameworks have been developed to improve the understanding of human–environment relationships (Scholz et al. 2011) and to develop sustainable solutions (Fischer et al. 2015). Yet, despite the considerable advances in concepts that could be used to better understand SES (Liu et al. 2007, Binder et al. 2013), practice still lags behind theory (Fischer et al. 2015). For example, Vaz et al. (2017) found that, out of over

9,100 published studies focussing on biological invasions, 51% were monodisciplinary, and of those classified as interdisciplinary, 92% were ecological in nature; only 3.2% could be confidently classified as social-ecological, and the involvement of stakeholders was not mentioned at all in this review. Based on those results, it is apparent that many studies on the introduction, spread and effects of invasive alien species (IAS) focus on the biological component (e.g., Parker et al. 1999, Barney et al. 2013) and largely ignore the social dimensions.

Given the dynamic behaviour of the determinants of global environmental change and the complexity of their social-ecological consequences, research projects that aim to improve the understanding of SES and to develop sustainable management solutions must consider multiple states and transitions of SES. Such projects must include research that advances an understanding of how specific cases or general patterns in SES can affect ecosystem health and human well-being, as well as how the effects could be managed or mitigated. However, it is not a trivial task to design research projects to achieve these goals (Fischer et al. 2015). In this paper, we outline six components that we consider essential for social-ecological research projects if they are to produce a better understanding of complex problems that would result in the design of effective management interventions (Table 5.1). We outline why we consider these six components relevant for addressing complex problems in SES and illustrate them using examples from a transdisciplinary research project on woody IAS in Eastern Africa (Box 1). We argue that projects that incorporate the six components will be more likely to successfully address and find potential ways to mitigate complex social-ecological problems. We also discuss the challenges that underlie such transdisciplinary research efforts using a SES approach and suggest ways to deal with them.

Table 5.1. Elements of six proposed essential components of social-ecological systems research, with expected improvements over current practice.

Component of research	Required changes to current research practices	Expected improvements to research outputs
Structure the project around a multidisciplinary team with common goals	Agree on an overarching goal and co-design the goals of component projects	Improved communication and overarching sense of purpose among research participants
	Define and agree on a common vocabulary	
	Define flexible shared outputs (“boundary objects”)	
	Explore the potential interactions between biophysical and social processes	
Establish meaningful relationships with important stakeholders	Co-design of project goals with a range of potential end-users	Improved likelihood of implementation of research recommendations
	Identify relevant and realistic management options	
Co-design data collection across disciplines to ensure effective integration	Identify common ground between participating disciplines and plan for integration of data	Ability to link ecological and socio-economic findings in a statistically robust way
	Collect data for component projects at common localities and scales	
Ensure that data are compatible across multiple scales	Select local research sites so as to cover geographic variation at a landscape scale	Improved ability to upscale local research findings to scales relevant to management and policy formulation
	Include sufficient local research sites to allow for extrapolation to higher scales	
Include transdisciplinarity in the training of project participants	Plan for, and allocate resources to joint training of participants in transdisciplinarity	Enhancement of progress towards goals by avoiding misunderstanding
	Include training workshops with practitioners experienced in	

Actively consider the implementation of research results	conducting transdisciplinary projects Plan for the dissemination of research results in a format suitable for uptake by stakeholders Establish demonstration sites where solutions are implemented	Improved likelihood of implementation of research recommendations
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5.2 Key components of successful transdisciplinary social-ecological projects

We identified six key components based on our experience gained in the design and execution of an ongoing transdisciplinary research project investigating the ecology, impacts and management of woody IAS in Eastern Africa (Box 1). Each of these is described below, using examples from our experience to illustrate the points made. However, we believe that the relevance of these components extends beyond projects addressing IAS and will be useful to a wider community of researchers who seek to develop viable solutions to today's complex environmental problems.

a) Structuring a project around a multidisciplinary team with common goals

Transdisciplinary projects require inputs from a wide range of participants with different backgrounds, and it would therefore be necessary to focus the project on a shared high-level goal with shared outputs that jointly utilize findings from different disciplines.

Transdisciplinary projects are complex and involve scientists from different technical backgrounds, as well as stakeholders. Transdisciplinary research necessitates a team that is balanced between disciplines, includes people with proven transdisciplinary research skills, and acknowledges and deals with differences in organizational structure and functioning of the participating institutions (Norris et al. 2016). As a result of differences in background, participants may not have known each other prior to the project start, few people may have an overview or understanding of all the aspects of the project, and differences in vocabularies used in the disciplines may render discussions difficult (Klein 2008). It may be necessary to agree on a common vocabulary in the project from the very beginning, to avoid miscommunication. This can be particularly problematic when studying biological invasions, as terminology is confusing (Colautti and MacIsaac 2004) despite attempts from biologists at standardization (Richardson et al. 2011).

The overarching goal of any transdisciplinary project should be made clear at the very beginning, along with the expectations among team members, but it is also essential to allow for flexibility as the project moves on (Turner et al. 2016). There should be strong co-design of project goals, objectives, and methodologies, which together define the framework of the project. The involvement of non-scientific stakeholders from the start of the project can be

valuable when defining goals and objectives (see next section). It is also important to define boundary objects from the research topic. Boundary objects are defined as joint outputs that are flexible enough to be adaptable to different viewpoints and robust enough to be employed by several actors, while maintaining identity across them (Star and Griesemer 1989). Boundary objects form the basis of transdisciplinary research and help to re-integrate research findings into science and society (Lang et al. 2012).

Apart from agreeing on a common goal and terminology, the project should be structured in a way that allows for clear flows across and within different spatial scales of the SES, as they interact with each other (Table 5.2), and across project phases. An initial phase of integrative transdisciplinary collaboration and co-design, involving scientists and other stakeholders, may be followed by a more mono-disciplinary phase when single disciplines collect data (KFPE 2014). The transdisciplinary activities within the project will then increase again when mono-disciplinary research data are jointly analysed, interpreted, translated into management options and integrated in both the scientific community and society (Lang et al. 2012; KFPE 2014). Hence, social-ecological projects may include a research phase to collect site-specific data on biophysical and socio-economic processes and to assess how they interact (Reyers et al. 2013). The relative importance of the various factors of SES is often context-dependent (Liu et al. 2007) and replication across sites or case study areas is important to assess this variation, and to take it into account when proceeding to developing or implementing management measures.

A complex transdisciplinary research project benefits from a clear structure, for example, by dividing the research thematically and temporally into work packages with specific aims and well-defined tasks and responsibilities. Problems of trust and legitimacy among project partners may arise if there are no clearly established and defined obligations and responsibilities for individual team members (Lang et al. 2012, Turner et al. 2016). A potential way to solve this could be to appoint a leader for each work package, who would coordinate the work and ensure communication and linkage between different work packages. In this way, the division of labour is clear and tasks and progress can be discussed and agreed upon during regular project meetings attended by all team members. Progress within work packages may be reviewed through regular updates that are shared by the relevant leaders.

Table 5.2. Examples of the socio-economic and ecological effects of invasive alien species, and management implications arising from these across spatial scales and disciplines, though interactions between scales and disciplines are likely.

Scale	Effects		Management implications
	Socio-economic	Ecological	
Local	<ul style="list-style-type: none"> • Reductions in crop production • Source of firewood/charcoal • Jobs • Cultural services • Human allergies/diseases 	<ul style="list-style-type: none"> • Changes in vegetation composition • Changes in soil nutrient status • Microclimate 	<ul style="list-style-type: none"> • Control & restoration measures • Sustainable land management strategies
Regional	<ul style="list-style-type: none"> • Disruptions to systems of Pastoralism • Ethnic conflicts • Negative effects on Tourism 	<ul style="list-style-type: none"> • Ecosystem integrity • Water resources • Biodiversity 	<ul style="list-style-type: none"> • Early detection/rapid response • Protection of high-value areas • Prevention of spread
National	<ul style="list-style-type: none"> • Food security • Gross domestic product • Institutional settings 	<ul style="list-style-type: none"> • Carbon storage 	<ul style="list-style-type: none"> • International agreements • National management strategies / policies • Biological control

b) Establishing relationships with important stakeholders

Society can play, through stakeholder participation, a crucial role in the formulation of project goals, especially in complex social-ecological studies. Involvement of stakeholders is necessary both to define realistic goals and to achieve them.

While the level of stakeholder involvement varies during project execution (Herweg et al. 2010), stakeholder engagement throughout the project is essential for co-designing the project and creating a sense of ownership (Gadgil et al. 2003, Reed et al. 2010, Mauser et al. 2013). Researchers stand to learn a great deal from stakeholder knowledge of local conditions, and this learning should be incorporated in the initial phases of any transdisciplinary project. Stakeholder workshops provide a useful way in which to define local problems and to define or refine research goals (Davis and Wagner 2003). Stakeholder engagement can also help to establish co-management (Plummer and Fitzgibbon, 2004), as seen in the case of American mink management in Northeast Scotland (Bryce et al. 2011). Stakeholder workshops should be held regularly during the project, and a wide spectrum of relevant stakeholders should be invited, from affected local communities to national and international policy makers. During these workshops, stakeholders should be brought up-to-date with the project, and should be enabled to provide input.

One possible approach can be to have several structured workshops at the start of the project, each involving people with different interests, e.g. local (e.g. community members), regional (e.g., NGO's), and national (e.g., government officials) stakeholders. These meetings should aim to build trust, gather opinions and research ideas, discuss the research goals and thematic areas, and identify stakeholders who are willing to participate in the implementation phase. Stakeholders should be directly involved in the process of selecting and testing management options. This can be facilitated by creating local implementation groups, consisting of representatives of key stakeholder groups, and by assisting them in decision-making and implementation processes (see below).

c) Co-designing data collection across disciplines

Our ability to link findings from social and ecological studies is often impeded by the fact that data were collected at different scales, at different localities, or to answer different questions. Projects that seek to develop an understanding of, and solutions to, complex environmental problems will have to collect data that can be integrated across disciplines in a statistically robust way.

While multiple frameworks overarching both ecological and socio-economic disciplines have been developed, most do not treat both disciplines equally (Binder et al. 2013). Over the past 20 years, the concept of ecosystem services (ES; Costanza et al. 1997) has become central to many areas of ecological research and policy development. Most ecological data that document environmental change (e.g., species richness, soil nutrients, organismal physiology) can be linked to ecosystem services, and this has resulted in most research presenting an eco-centric perspective on social-ecological systems. Other frameworks have been promoted in research and politics to address the needs from the society's perspective, for example the 'Sustainable Rural Livelihood Framework' (SRL) (Scoones 1998) that aims to understand the dynamics of human livelihoods, especially of the rural poor, from a sociocentric perspective. When integrating disciplines, it is important to understand how widely-used frameworks from different disciplines link to each other, as was done by O'Rourke et al. (2016). For example, the natural capital in the SRL framework is closely related to the provisioning ES, providing a link between the frameworks. Finding these links is a way of finding common ground between different frameworks and disciplines, and common ground can support integration that creates a better overview of the whole SES.

Research projects dealing with SES should carefully consider how to integrate ecological and socio-economic data from the start. This is particularly important, because of the potentially very different, discipline-specific ways of data collection. For example, detailed socio-economic data are often gathered by means of face-to-face interviews at the household level, whereas ecological data are obtained from single plots or sampling points. It is important to co-design data collection across disciplines in a way that they can be analysed using a common study unit; otherwise integrating results from multiple disciplines would not

be easily achieved. Hence, attention should be paid to how the results from the various disciplines will be integrated at the planning of interdisciplinary studies.

One way to facilitate integration and comparison of social, economic, and ecological disciplines is to collect information using a blocked study design. Data from different disciplines may be collected from replicated points in the same geographic area and integrated at a common, larger scale that allows some level of replication. Replication at, for example, the level of the smallest administrative unit will then allow for the linkage of ecological and socio-economic indicators and the statistical assessment of patterns among them. This requires all ecological and socio-economic data to be collected together with their precise locations, which can be a point (e.g., the centre of a plot or of a household) or an area (e.g., a village).

Given the dynamic and complex nature of problems in SES it is often difficult to disentangle causation for particular response variables. One way to circumvent this problem would be to collect data along a cline/gradient of predictors of problems associated with SES. In the case the Woody Weeds project (Box 1), rural stakeholder communities were selected along an IAS cover gradient, and cover was used in the analysis as predictor variable. Working along a cover gradient is preferable to a simple comparison between invaded and non-invaded sites, as invader effects are often density-dependent (Shackleton et al. 2007) and can be non-linear (e.g., Gooden et al. 2009). Working along a gradient is also preferable from a mitigation and management perspective as it is often not possible to completely reverse the effects of large-scale environmental change (van Wilgen et al. 2012). With a gradient approach, invasion levels can be determined at which negative effects are minimized or at least considerably reduced.

The integration of social and ecological variables is of vital importance, as transdisciplinary research does not merely involve the comparison of research results from different disciplines, but integrating them so as to identify interactions between them that eventually bring about positive change in society (Reyers et al. 2013). Hence, integration allows assessment of perceived and actual biophysical effects on ecosystem services, their effects on human well-being, as well as the valuation by stakeholders and their motivation to adjust land management or policies.

d) Ensuring data compatibility across multiple scales

Complex environmental problems manifest themselves in different ways, and at different scales, depending on the perspective of the researcher or stakeholder, and finding sustainable solutions for them requires actions that take these scale issues into account.

Local-scale and short-term research is often not sufficient to address complex problems (Berkes and Folke 1998). Research focusing on one spatial scale can provide a snapshot of specific processes that influence changes in SES, but integration of the multiple scales at which social-ecological problems manifest themselves is necessary to fully assess the complexity of the problem and its effects on the environment and human well-being (Table 5.2). Upscaling may also help researchers to understand large-scale social-ecological interactions (Fischer et al. 2015), as well as enable them to make predictions of the future extent and effects of these interactions. Furthermore, effects may be either positive or negative, depending on the spatial scale at which they are assessed. For example, invasive trees in the genus *Prosopis* may increase income for local charcoal producers, but may threaten downstream water security through the excessive water use (Dzikiti et al. 2013).

An ability to link and upscale ecological and socio-economic information would require collection of data together with details of their location. Thus, for all collected variables, the geographic location (e.g. a point or an area in space) of any data collected must be defined. This may be relatively easy with biophysical measurements taken in field plots. However, care should be taken when the location of data collection and the affected area are not the same, such as may occur during household interviews. Moreover, it is important that enough data are collected, and from a sufficiently large variety of relevant locations, to be able to make meaningful extrapolations.

e) Transdisciplinarity training for project participants

The growth in the magnitude and extent of complex environmental problems over the past few decades means that modern researchers will have to be able to understand, and deal with, complexity if they are to make a difference.

The emergence of complex social-ecological problems requires transdisciplinary research, yet most university education focuses on a single discipline (McWilliam et al. 2008) and many researchers have limited experience working with colleagues from different disciplines. Hence, it would be important to train researchers to work cooperatively across disciplines and to engage effectively in transdisciplinary communication (Norris et al. 2016), since transdisciplinary work is also a process of mutual learning (Hirsch-Hadorn et al. 2006). While it is clear that not every researcher will become an expert in all disciplines relevant to the topic he or she is addressing, all should recognize, appreciate, and understand differences in research methodologies, analyses, and interpretation of the data collected by colleagues working in other disciplines if they are to be effective.

Scientists, both junior and senior, working on transdisciplinary projects may encounter differences in communication and value systems among project partners and stakeholders with different societal and cultural backgrounds. Failure to recognise and address differences in fundamental assumptions and values concerning the scientific process and/or communication can impede progress in a project (e.g. Campbell 2005, Lele and Norgaard 2005). Other potential differences may have their origin in different disciplinary vocabularies (Klein 2008), study designs, data collection approaches, and analytical methods (Spangenberg 2011). The allocation of resources or project components to address such differences should be addressed during the project planning phase, and attention should be given to these differences and potential friction or conflict throughout the project.

To stimulate smooth cooperation, workshops and training sessions for the entire project team should be considered. In the case of our East African project, for example, we held regular compulsory workshops for all post-graduate students, in which participants interacted with a view to exchanging ideas and ensuring that information could be combined to address higher-level questions. Many transdisciplinary challenges are social and philosophical by nature and structured communication sessions may help to reveal and explore key philosophical assumptions among the team members. This awareness can significantly facilitate cooperation in interdisciplinary or transdisciplinary projects (Eigenbrode et al. 2007). A lecture series about aspects of various disciplines as part of regular project meetings can also help to promote a common understanding of the issues at hand, facilitate discussion and coordination of data collection, and harmonise research methodologies to ensure cross-compatibility.

On a more practical level, collaboration and mutual understanding may especially be promoted through joint field work or practical training sessions. For example, workshops where postgraduate students in the project jointly analyse data collected in separate studies or co-author the resulting manuscripts can significantly improve the respective understanding of the used methodologies and may lead to new ideas about the use or analysis of the data. Finally, good personal relationships can be very important to successful, lasting collaboration and social time or team-building exercises may be useful components of project meetings (Cheruvilil et al. 2014).

f) Implementation of research results

The likelihood of research recommendations being heeded by target end-users can be substantially improved if this aspect is actively considered while the project is being planned and executed.

As outlined above, planning the implementation components starts together with the planning of the other project components. In SES research it is important that applied research not only focuses on how effects can potentially be mitigated, for example by developing biological control against invasive species (Sheppard et al. 2006) or investing in green energy to reduce CO₂ outputs (Boyle 1997), but also looks at how results can be implemented. At the local level, stability of SES can be achieved through the development and adoption of sustainable land management (SLM) strategies to combat land degradation (Hurni et al. 2006), but the implications for ecosystem health and food security can be upscaled for consideration at regional or even national levels (Table 5.2).

Scientific knowledge from own research or from the literature should be processed in a way that it can be used by stakeholders in decision-making processes, e.g. in assisting stakeholder groups in prioritizing management options. In a structured decision-making process to prioritize management options, Proctor and Drechsler (2006) proposed that, on the basis of their own and of the latest scientific evidence, stakeholders should identify ecological, social and economic criteria, assess how these criteria are affected by the management options, and reflect on trade-offs and synergies, while integrating multiple social-ecological dimensions. This would engage both researchers and stakeholders in transformation processes through co-creation of knowledge and a joint responsibility for

implementation action, and are hence a paradigm of transdisciplinary research (Schwilch et al. 2012). The description of possible management scenarios and their likely effects on ecological and socio-economic criteria flowing from the research should be disseminated in a user-friendly way to keep the newly gained knowledge easily accessible and comprehensible to the target audience (Lang et al. 2012).

The likelihood of adoption of interventions by stakeholders at any scale of a SES is largely driven by motivation. While SES research is highly complex by nature, interactions with stakeholders during the inception phase may already hint at the key factors that may affect their motivation to consider not only short-term but also long-term consequences of management decisions and the potential uptake of new recommendations. These key factors should be considered during the entire project duration and taken seriously. They are particularly important during the development of effective problem mitigation and management strategies and ensure that developed management practices are adopted by stakeholders. In the end they are the implementing bodies, be it on local, regional, or national scale. In South Africa, ecologists seeking to convince government that woody IAS should be controlled to protect water resources, had to also include the social aspect of potential job creation for the rural poor when it became apparent that this was more important to stakeholders than the biophysical aspects around water and biodiversity (van Wilgen and Wannenburg 2016).

5.3 Synthesis

If researchers are to develop effective solutions to complex environmental problems, transdisciplinary approaches will be needed. Despite the fact that the interlinked nature of SES has led to significant advances in sustainability science in recent years, these have been more at a theoretical and conceptual level than in terms of solving complex problems (Fischer et al. 2015). We propose that, in order to address specific social-ecological problems, future projects should be structured around the basic principles of transdisciplinarity. We believe that the components of social-ecological research outlined above will improve the understanding of determinants of SES and increase the chances of achieving sustainable management options. Over time, the analysis of SES in different ecological and socio-economic settings may potentially lead to the identification of a relatively small set of socio-economic and ecological drivers of SES in specific societal and ecological settings, thus

potentially reducing complexity and increasing the chances of successfully implementing solutions. For example, Scholes (2009) argued that the majority of instances of dryland degradation in southern Africa are due to a relatively small set of distinct biophysical mechanisms, interacting with a similarly small set of human system contexts, thereby creating specific degradation syndromes. Should the sustainability or instability of specific SES be shown to be based on a limited set of biophysical and socio-economic factors, it would significantly improve the likelihood of finding sustainable management solutions, but more research is needed to verify the existence of such patterns.

It is challenging to design, conduct, and successfully implement a research project that (a) aims to understand both the determinants of change in a SES and the effects of this change and (b) develop feasible solutions to sustainability problems. Longer-term commitments by funding agencies, and strong political support, would be needed to allow researchers to develop, test, and implement potential solutions to complex social-ecological problems. As described in Shackleton et al. (2017), this can be achieved by reducing risks in investment, getting buy-in and funding from multiple sectors through strategic planning and prioritisation, and by building stronger collaborations. We therefore suggest that donor organizations consider extending the duration of funding schemes that are directed to addressing and developing solutions for complex problems, as has recently been done by the Swiss Programme for Research on Global Issues for Development (<http://www.r4d.ch/>).

Though we draw from examples on invasive alien trees in Eastern Africa, we feel that the proposed components of a research project to address and solve complex problems are applicable to other SES in other parts of the world. We are hopeful that the proposed components outlined here will stimulate the designing and funding of projects that combine research and practice in order to more effectively address the major challenges of the 21st century, such as those highlighted in the Sustainable Development Goals (Griggs et al. 2013).

Box 5.1. The Woody Weeds project

The transdisciplinary "Woody Weeds" project (Woody Invasive Alien Species in Eastern Africa: Assessing and mitigating their negative impacts on ecosystem services and rural livelihoods - <http://woodyweeds.org/>) was initiated in January 2015 and is designed to last 6 years. The Woody Weeds project's main goal is to help to mitigate the negative effects of woody IAS on biodiversity, ecosystem services, and human well-being Ethiopia, Kenya, and Tanzania. Both research and policies that effectively address IAS management are lacking in the region (Pysek et al. 2008), though invasions are widespread (Witt 2010).

The project team includes some 30 researchers with backgrounds in ecology, invasive species management, forestry, genetics, economics, sustainable development, GIS, remote sensing, and natural resource governance. The project aims to train up to 20 post-graduate students to doctoral or master level. The project is structured in three major components: (1) understanding the local effects of woody IAS, (2) upscaling results to higher spatial scales, and (3) developing and implementing management strategies. The central hypotheses in the Woody Weeds project are (1) that the effects of woody IAS are dependent on the abundance of the invader, and (2) that woody IAS, which were often deliberately introduced, can have both positive and negative socio-economic and environmental effects, and that the net effect changes with invader abundance. We hypothesised that the positive benefits would outweigh the negative impacts when the abundance of the invader is low, but as the abundance and range of the woody IAS increases (i.e. when the species become invasive), the net effect becomes increasingly negative (Fig. 5.1).

From the initiation of the project there have been regular meetings with a wide range of stakeholders, including local communities, managers, and policy makers in each of the study countries, as well as scientists and international organisations, aimed at informing stakeholders about the project and its outcomes, but also at involving stakeholders in priority setting within the project.

Research is currently conducted in case study areas in each country to assess the magnitude and direction (positive or negative) of effects of woody IAS on biodiversity, ecosystem services, and human livelihoods, along a gradient of woody IAS abundance, using both small-scale plots for ecological measurements and household interviews for socio-economical assessments. These data are then compared on the "community" level, the

smallest administrative unit, and that comparison will allow us to upscale effects to regional level by using remote sensing and modelling approaches that link existing woody IAS cover to social-ecological outcomes. Spatial models can then be used to predict the potential invasion potential and impacts at the national level.

Results from the first two components will be used in the implementation phase and will provide inputs into the development of management options and sustainable land management strategies. Management options will be implemented in local implementation groups that include researchers and local stakeholders. All of the results gathered will be disseminated in user-friendly leaflets, posters, and other materials to the public and via scientific articles and presentations to the scientific community. Finally, the aim is to influence policy makers through policy briefs, multimedia materials, and a final stakeholder workshop.

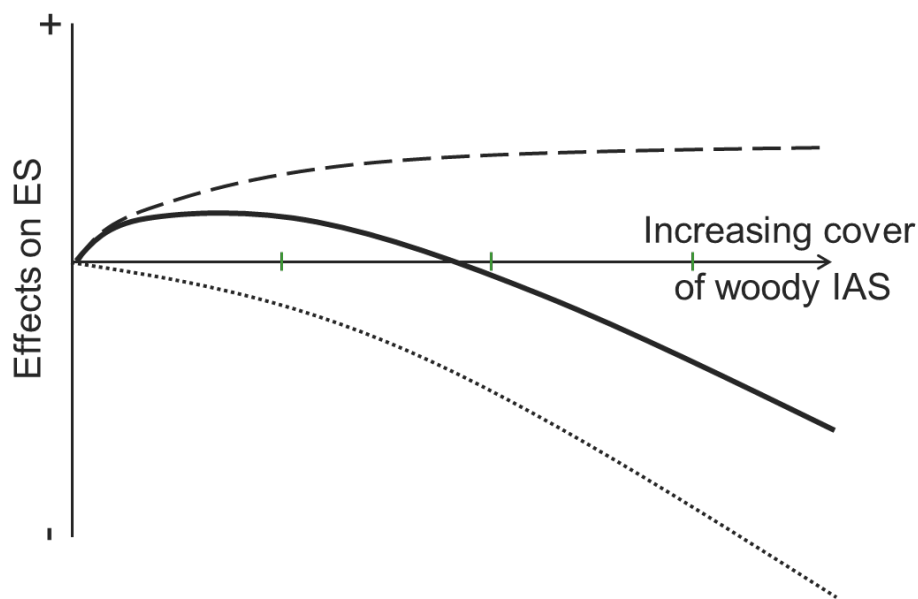


Figure 5.1. Hypothetical relationship between woody IAS cover and its positive (dashed line), negative (dotted line) and net effect (solid line) on ecosystem services.

CHAPTER 6 Conclusions

Woody invasive alien species provide good model systems to study the drivers of plant invasion related to invasiveness and invasibility (Richardson and Rejmánek, 2004, Simberloff et al. 2010). To develop effective management strategies it is crucial to understand what determines invasiveness and the mechanisms that allow introduced species to successfully colonize new areas.

Prosopis species in Eastern Africa represent an excellent case for the study of the drivers of plant invasion. *Prosopis* species have been widely introduced to many parts of Africa mainly for the rehabilitation of degraded landscapes, in many instances becoming invasive, with negative impacts on biodiversity and human well-being (Shackleton et al. 2014, Bekele et al. 2018). To investigate the drivers of plant introduction and invasion, *Prosopis* invasions in Eastern Africa were used as case studies to answer the following questions: *Who are the invaders?* and *What underlies their phenomenal ecological success as invasive species?* Answering the first question would require taxonomic knowledge that is not only crucial to reduce the risk of new introductions, but also for implementing management options such as biological control. The second question requires information about the mechanisms of invasion, such as rapid post-invasion adaptation and phenotypic plasticity. To answer these questions, Chapter 2 includes a global perspective on the invasive tree genus *Prosopis*, while Chapter 3 and Chapter 4 focus on the *Prosopis* invasion in Eastern Africa. The specific study areas in Eastern Africa provided a rare opportunity because the founder trees of the two originally introduced *Prosopis* species, *P. juliflora* and *P. pallida*, were still present in the original plantations (Choge et al. 2002, Swallow and Mwangi 2009, Shiferaw et al. 2019).

To determine *who the invaders are*, I tackled a significant and age-old problem: how to identify *Prosopis* species and determine the taxonomic relationships between them, both in their native and invaded ranges. The results of Chapter 2 suggest a lack of genetic differentiation and indicate that reproductive isolation is incomplete between various *Prosopis* species, and indicated that hybridization between allopatric diploid species may occur frequently when co-introduced into new ranges. Hybrids identified based on morphology were recorded in both native and invaded areas. However, genetic analysis

showed a lack of congruency between morphological and genetic data that complicates taxonomic identification. We also found polyploid individuals in native and non-native areas. In addition, this study provided clarity as to the taxonomic identity of *Prosopis* species in Eastern Africa. The results in Chapter 2 confirmed the presence of *P. juliflora* in Ethiopia, Kenya and Tanzania. I also identified instances of hybridization in Kenya's Baringo County. Moreover, in Chapter 3 I also found that, despite the similar residence time and introduction history of *P. pallida* and *P. juliflora* into the same environments, and the occurrence of hybridization, only *P. juliflora* individuals became invasive in the region. Results from Chapter 2 also provided important evidence regarding the origin of introduced individuals in Eastern Africa, indicating that *P. juliflora* invaders in Kenya and Ethiopia could have a similar Mexican origin, while the origin for Tanzanian individuals are not Mexican. *Prosopis pallida* individuals in Hawaii and Kenya shared a similar Peruvian origin.

To address *what underlies invasion success of Prosopis*, the results reported in Chapter 2 confirm that hybridization between introduced species in non-native regions may contribute to invasiveness, but stress the need to evaluate whether different *Prosopis* genotypes (parental or hybrid type) have differences in ecological tolerance in invaded areas, and how this can be associated with invasiveness. Additionally, the results showed that in the case of *P. juliflora*, ploidal variation may be an important mechanism for genetic differentiation from the rest of the species in the genus. Similarly, strong reproductive barriers have been proposed to explain the absence of gene flow between diploid and hexaploids individuals of *Aster amellus* (Münzbergová et al. 2012). I propose that, since reproductive isolation seems to be poor in *Prosopis*, polyploidy could be an additional mechanism that facilitates immediate reproductive isolation between species (te Beest et al. 2012). In addition, polyploids have been frequently found to be more invasive than their diploid congeners (e.g. in the Fabaceae; te Beest et al. 2012). Polyploidy may therefore also explain the successful invasion of *P. juliflora* in some areas, such as Eastern Africa. In many cases, the success of plant invasions has also been attributed to hybridization (Schierenbeck and Ellstrand 2009, Zalapa et al. 2010, Gaskin et al. 2012). For *Prosopis* this is the case in Australia and South Africa (Klinken et al. 2006, Mazibuko 2012), probably due to the introduction of various diploid *Prosopis* species, and subsequent hybridization.

In Chapter 4, by including both founder and invasive genotypes from Kenya's Baringo County and Ethiopia's Afar Region, I found that the success of *P. juliflora* invasion was not related to hybridization but is likely due to the higher ploidal levels of the species. In Chapter 3, the differences in traits such as time until germination, stem diameter, level of undamaged seed between founders *P. juliflora* and *P. pallida* are in line with the hypothesis on the role of polyploidy on invasion success. The results also showed that invasion dynamics seem to be different in each country. In the Afar Region, the successful spread may have been promoted by continuous (and ongoing) gene flow from "source" plantations leading to homogenization of the standing genetic diversity across the invasion. In contrast, in the Baringo County, selective pressures or stochastic events may have occurred during invasion, resulting in invasive genotypes with lower genetic diversity than their founder ancestors. The former is supported by data reported in Chapter 3, which provides evidence to suggest that high levels of phenotypic plasticity and rapid post-introduction evolution may have contributed to the ecological success of invasive *P. juliflora* in Kenya. These levels of plasticity in key traits were absent from the non-invasive *P. pallida*, and may explain why this species has not become invasive. The results in Chapter 4 also showed that the dispersal of *P. juliflora* in Afar Region and Baringo County are not limited by geographic distance, nor by any of the landscape variables analysed at the spatial scales of this study (i.e. bioclimatic conditions, distance to roads, rivers and villages), indicating frequent long-distance dispersal.

The role of polyploidy and hybridization in evolutionary responses is broadly recognized (Mable 2013, Van de Peer et al. 2017), however, how this relationship is linked with invasiveness has not yet been conclusively established (Mable 2013). For example, the invasive genus *Hieracium*, represents a case of an extremely complex taxon due to recent speciation and polyploidy of various species, while only certain species hybridise freely and are invasive (Trewick et al. 2004, Loomis and Fishman 2009). Because of hybridization and polyploidy, species delimitation in the group is contentious with hundred of species, subspecies and types, as well as the misidentification of introduced species, having been reported (Wilson et al. 2006). Novel hybrid genetic combinations and their increased genetic diversity may be important factors underlying the invasiveness of some *Hieracium* taxa (Loomis and Fishman 2009). However, for some species, such as *Hieracium aurantiacum*,

high levels of plasticity seem more important than sexual recombination and genetic diversity in facilitating invasiveness of this species (Loomis and Fishman 2009).

Generally, polyploids are more frequently found in disturbed and high stress environments than diploids (Van de Peer et al. 2017), but the precise mechanisms underlying these trends are not well understood (Mable 2013). Studies on *Glycine* species indicate that allopolyploids have higher light stress tolerance compared to their diploid progenitors (Coate et al. 2013). Similarly, diploid and hexaploid cytotypes of *Solidago altissima* showed differential response to water availability and temperature through adaptive plasticity of different traits (Zlonis and Etterson 2019). As for *Prosopis*, it remains to be determined whether hybridization, polyploidy or both contribute to invasiveness of *S. altissima*. In the case of *Prosopis*, experiments testing the performance of autopolyploid and allopolyploids individuals under simulated environmental conditions, involving common garden and reciprocal transplant experiments are needed, and would provide valuable insight in this regard.

Recently, van Wilgen et al. (2012) proposed that management of woody invasive alien species should be based on plans that prioritize species and areas, and that biological control should be used wherever possible. A range of approaches have been implemented to control the spread and densification of *Prosopis* invasions, including manual, chemical and biological control, combined with managed utilisation (Choge 2002, Wise et al. 2012, Shackleton et al. 2014). When evaluating how the findings of this thesis could be used for the development of effective management plans, I aimed to address two principal questions: (i) How does an improved understanding of the eco-evolutionary drivers of invasiveness help us to better manage the problem? and (ii) What implications do the greater understanding of genetic and ecological drivers have for the use of particular control methods such as biological control?

Overall, the results showed that accurate identification of *Prosopis* trees to species level can be challenging, given taxonomic uncertainties, and it becomes more difficult when different species are co-introduced to the same environment, often leading to hybridization. Based on my findings, I strongly recommend that country- and region-level regulation of *Prosopis* should consider all of the species of the genus as invasive, rather than treating species separately in national or regional lists. In contrast, the accurate identification of

species and hybrid morphotypes should be regarded as important factors in experimental studies aimed at understanding the processes underlying successful invasion. Accurate taxonomic identification would be needed for an adequate understanding of the site-specific dynamics of invasive populations and, certainly, for studies of the effectiveness of management practices such as biological control. For example, management interventions in the areas of contact between parental and hybrid species could be more effective than generalized strategies (Le Roux and Wieczorek 2009).

This study also indicated that in Eastern Africa, *Prosopis* invasion may be facilitated by a combination of cytogenetic (i.e. polyploidy), plastic and rapid post-introduction evolutionary mechanisms. The results also highlight the context-dependency of invasion dynamics as illustrated by *Prosopis* invasions in the Afar Region and Baringo County. In Baringo County, analyses suggested contemporary genetic change due to selective pressures encountered during the invasion process. Best management practice often suggests that a focus of control efforts on areas of low invasion density (such as the leading edge of an invasion) would slow or reverse invasions and restore ecosystem functions more effectively than focussing on dense invasions (such as the core areas of invasion) (van Wilgen et al. 2000). This would be even more important in cases where adaptive forces operate at species level and at small scales, as the adaptive process would also be retarded. In contrast to Eastern Africa, in areas like South Africa and Australia, *Prosopis* invasiveness would be promoted by inter-species hybridization of the introduced diploid species (Zimmerman 1991, van Klinken et al. 2006, Mazibuko 2012). Further research would be needed to evaluate the contributions of mechanisms such as rapid evolution and phenotypic plasticity to invasion success in these areas. For example, in South Africa, I found high levels of genetic diversity that may increase the capacity of invasive populations to adapt to new conditions and expand their range (Sakai 2001, Lee 2002, Kolbe et al. 2004, Shirk et al. 2014). Lastly, my results have also suggested that dispersal is not affected by climatic conditions or distance to roads, rivers and villages in Eastern Africa. Management efforts should therefore focus on reducing the seed production and the dispersal of seeds into non-invaded areas. It is probable that attempts to control *Prosopis* spread through utilization in some areas such as Baringo County (Choge 2002) have not been successful because they do not prevent the production and dispersal of seeds. In fact, they may even promote the spread of seeds, because people would be tempted to plant

Prosopis in areas where it does not yet occur in order to benefit from utilization, even though the net benefit would be negative (e.g., Wise et al. 2012).

Biological control has been proposed as a long-term management strategy because it is a safe, relatively inexpensive, and sustainable, and could possibly be effective at reducing the spread of *Prosopis* invasions (van Wilgen et al. 2012). However, biological control agents have had variable results in controlling *Prosopis* in South Africa and Australia (van Klinken and Campbell 2009, Zachariades et al. 2011, Pichancourt et al. 2012). In these areas, the high levels of hybridization between *Prosopis* species may also play a role in the limited success of biological control, since biological control agents that are pre-adapted to a particular species or eco-types may be less effective against hybrids (Goolsby et al. 2006). In invaded areas where instances of interspecific hybridization exist, such as Hawaii and South Africa, studies are needed to evaluate different biological control agents considering the different *Prosopis* taxa, hybrid morphotypes and environmental conditions (van Klinken and Campbell 2001). Thus, the use of biological control should consider taxonomic uncertainty in *Prosopis*, since targets in invaded areas may be misidentified if only using morphology for species identification. My results showed that *P. juliflora* is genetically highly differentiated from other *Prosopis* species. A practical consequence of this is that biological control agents that have been tested against invasive *Prosopis* species in Australia and South Africa could have different performances on *P. juliflora* invasion in Eastern Africa. Overall, the results of this thesis provide important information that can be used to inform efforts in the use of biological control as part of management strategies in *Prosopis*.

This thesis formed part of a larger transdisciplinary project on *Prosopis* invasion which aims to synthesize the knowledge from different disciplines and apply it to developing solutions. To effectively integrate this new knowledge for the development of effective management plans, it would be necessary to adopt a transdisciplinary approach. For this, five essential components that should be included in a transdisciplinary research project were proposed in Chapter 5. These included a clear structure that transcends the disciplines that are present in a multidisciplinary team with common goals. To allow for integration and upscaling of findings, there was a co-design of data collection using different methodologies in the same experimental units/scales. It was important to have the clear intention to identify

management options with stakeholders, estimate their effects and test their implementation, as well as transdisciplinary training for project participants.

The study of the genetics of *Prosopis* invasion bring opportunities to resolve taxonomic uncertainty, to investigate the drivers, spread, pathways and evolutionary change of invasive alien species, identifying gaps in existing knowledge of plant introduction and invasion (e.g. dispersal, hybridization, plasticity), preventing future events of biological invasions and managing invasive alien species and the efficacy of risk assessment as a tool for managing such species and their impacts on environments.

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