Genetic risk and the transition to cultivation in Cape endemic crops—The example of honeybush (*Cyclopia*)?

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A B S T R A C T

Many endemic Cape plant species are commercially valuable, but information required to manage the resources is often lacking. Here I consider the potential genetic risk that the transition to cultivation may pose for Cape endemic plants and use honeybush – which is based on the members of the Cape endemic genus *Cyclopia* – as an example. The honeybush industry is expanding, in part driven by the transition from wild harvesting to cultivation. This change offers substantial environmental and economic benefits but may pose risks to wild populations through genetic contamination. I discuss (1) the importance of maintaining genetic diversity and structure of wild populations, (2) the levels of genetic structuring we might expect within the members of the genus *Cyclopia*, (3) the potential threats to genetic diversity, (4) suggestions for minimising genetic contamination of wild populations by cultivated plantations, and (5) why these issues may be important for the honeybush industry.

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1. Prelude: hybridisation and genetic contamination in the Cape Floristic Region?

At present, there are very few examples in the scientific literature of hybridisation in the Cape. This suggests that hybridisation is exceptionally rare in the Cape. However, this absence of evidence should not suggest that hybridisation is absent. This absence has likely been driven by a range of factors such as the challenges involved in studying hybridisation in non-model organisms and an ecological setting where generations are commonly non-overlapping – separated by fire events – in many species. Personal communications with expert botanists suggest that hybrids do occur in the wild across a range of lineages in the Cape; examples include members of the Orchidaceae (S. Johnson; University of KwaZulu-Natal), *Agapanthus* (T. Rebelo; South African National Biodiversity Institute), *Disa* (P. Linder, University of Zurich), *Psoralea* (M. Muasya, University of Cape Town), *Aspalathus* (C. Stirton, University of Cape Town), and many instances

within the Proteaceae (T. Rebelo). Coupled with the dearth of studies of hybridisation in the wild, there are no examples of genetic contamination of local populations by non-local species or genotypes, including cultivars. This topic has only gained international attention in recent years (e.g., Laikre et al., 2010). My objectives here are to highlight the potential of genetic contamination of Cape lineages and the urgent need to understand the consequences for Cape biodiversity.

2. Introduction

The Cape Floristic Region (CFR) of South Africa is well-positioned to grow and develop industries based on indigenous plant products given the region's exceptionally high species diversity (Manning and Goldblatt, 2012) and wide range of traditional and commercial plant uses (van Wyk, 2008; Joubert et al., 2008a). Initially, these industries can be, and are being, fuelled by collecting material from wild populations. However, a transition from wild harvesting to an agriculture-

based setting is necessary when demand outstrips supply (Cunningham and Davis, 1997). Different aspects of this transition may establish new threats or boons to conserving Cape biodiversity (Schippmann et al., 2002). As highlighted by van Wyk (2008), one aspect rarely considered during domestication is the underlying genetic diversity and structuring of species and populations. Here I will explore this further in the light of the expanding Honeybush Tea industry.

The South African Honeybush Tea industry is undergoing rapid expansion in response to high market demand (Joubert et al., 2011; Morokolo, 2011). Honeybush tea is made from members of genus *Cyclopia* (Joubert et al., 2011), which is endemic to the fynbos of the CFR; there are six species with commercial value: *Cyclopia genistoides*, *Cyclopia intermedia*, *Cyclopia longifolia*, *Cyclopia maculata*, *Cyclopia sessiflora*, and *Cyclopia subternata*. At present, the majority of honeybush tea is gathered from wild populations, and many species are listed as declining or threatened directly due to harvesting activities (Joubert et al., 2011; Morokolo, 2011; Vlok and Raimondo, 2011). Honeybush gained popularity, in part, due to the demand of consumers world-wide for health-promoting foods and ingredients, especially antioxidants (Joubert et al., 2008b,c; 2011) and, more recently, anti-obesity agents (Dudhia et al., 2013). There is concern that increased value and demand has led, or will lead, to a surge in poverty-driven and/or inexperienced harvesters overexploiting the populations on private land and in protected areas. In response to this, the industry is expanding by growing honeybush plants in agricultural plantations or, to a much lesser extent, by bolstering wild populations. This expansion is to be welcomed since it will likely provide relief for wild populations as well as substantial opportunities for employment in economically depressed rural areas. However, expansion of the industry has a range of potential pitfalls (Schippmann et al., 2002). From the perspective of phylogeography – the study of genetic lineages in space – one of the primary problems in managing the transition from wild harvesting to managed plantations is the absence of systematic exploration of the geographic structuring or levels of genetic diversity of populations or species. Closely linked to genetic differences between populations is the possibility of gene flow between cultivated plants and their wild relatives (inter- and intra-species hybridisation). Very little is known about the genetic diversity and potential hybridisation within *Cyclopia*. Schutte (1997) provides chromosome numbers for a limited number of species based on assessments of single individuals. Further sampling within species is required as there may be mixed ploidy levels; for example, the widespread Cape species, *Oxalis obtuse*, has six cytotypes occurring in the wild (Krejčíková et al., 2013). In addition, predicting the likelihood of hybridisation based only on ploidy levels is not straightforward (Petit et al., 1999; Soltis and Soltis, 1999). Within *Cyclopia*, fertile hybrids have been obtained from hybridisation experiments, although crossings in general had a very low success rate (Hannes de Lange, unpublished data summarised in the South African Honeybush Tea Association Newsletter No. 14, 8–15). Without understanding the genetic diversity or hybridisation potential, how can the honeybush tea industry – and other Cape flora-based industries – proceed in accord with precautionary principles?

3. Why is maintaining genetic diversity and structure important?

'Genetic diversity' or 'plant genetic resources' (in biological or agricultural jargon, respectively) has a substantial current or future economic value, especially for the agricultural industry. This diversity is not only a fundamental component of biodiversity (Noss, 1990) but also provides the building blocks for plant breeders to develop new cultivars. The loss of genetic resources important for agriculture (and biodiversity) has been identified as a global issue in the Millennium Ecosystem Assessment (2005) and has been driven largely by the intensification of agricultural farming systems coupled with the homogenising effects of plant breeding and globalisation. This loss lowers the resilience and adaptability of domesticated and wild populations and

species. In a review of the direct value of genetic diversity, Hammer and Teklu (2008) provide numerous examples of modern varieties of crop species that have been improved through breeding with wild relatives or populations. These examples include the following: resistance to 32 major tomato diseases incorporated through crossing domesticated tomato with wild species; a wild potato species used as the major source of genes responsible for developing resistance to potato late blight; and dramatic increases in yield achieved using wild landraces and wild species in rice breeding. Thus, genetic diversity is the cornerstone for populations and species to survive environmental changes, such as disease or climate change, both in the wild and in cultivation.

There are two immediate reasons why maintaining the genetic diversity and structure of honeybush species is important. First, honeybush tea is a 'green product' with discerning environmentally conscious consumers, and, as with Rooibos tea, the industry is well-placed to promote environmentally friendly practices. Maintaining the genetic integrity of the raw resource demonstrates that the industry is minimally invasive in using this indigenous herbal product that is becoming an icon in the South African bioeconomy (e.g., by avoiding genetic swamping which may influence the taste of local species or populations, or cause a breakdown of intrinsic or extrinsic local adaptations). Second, and arguably more importantly, wild populations may house unsampled and potentially valuable genetic traits. For example, the Cape is predicted to undergo significant anthropogenically induced climate change (Altwegg et al., 2014). Phenotypic resilience and the ability to adapt to changing conditions will be crucial to avoid local population extinctions. The transition of species from the wild to cultivation usually involves a reduction in genetic diversity of cultivated populations. As an example, cultivated populations of the medicinal plant, *Scutellaria baicalensis* (Chinese skullcap; Lamiaceae), have lost a substantial proportion of rare alleles present in wild populations (Yuan et al., 2010). Such losses are likely to be the case in many honeybush plantations (e.g., plants have been made from cuttings from a few individuals, or seeds are repeatedly sourced from a single seed lot). Losing genetic diversity also involves losing phenotypic plasticity and adaptation potential. Maintaining the genetic diversity of *Cyclopia* is an important part of biodiversity conservation and will give the industry the best possible chance of weathering environmental or biological challenges, such as the climate change storm that is simmering on the horizon.

4. What levels of phylogeographic structuring can we expect to find in honeybush?

The Cape is a unique and exceptional biodiversity hotspot (Myers et al., 2000; Manning and Goldblatt, 2012), and this is, for the most part, driven by the vast number of species that have small geographic ranges (Cowling and Lombard, 2002; Manning and Goldblatt, 2012). This suggests that evolutionary divergence can take place at small spatial scales and that populations can rapidly diverge from one another and adapt to local environmental conditions (Klak et al., 2004; Ellis et al., 2006; Prunier and Holsinger, 2010). This will lead to phylogeographic structuring—meaning that populations are isolated and their genetic lineages develop a strong association with geography. This is born out in a number of phylogeographic studies that examine widely distributed taxa along the dissected landscape of the southern African coastal lowlands. For example, phylogeographic structuring of populations has been found within the high-elevation Cape plant species, *Tetraria triangularis* (Boeckeler) C.B. Clarke (Cyperaceae) (Britton et al., 2014). *Berkheya cuneata* (Thunb.) Willd. (Asteraceae) – a succulent karoo endemic – has genetically distinct populations within the different sub-basins of the Gouritz catchment (Potts et al., 2013a) within the CFR. In the Albany Subtropical Thicket, which adjoins the Cape Floristic Region, two plant species, *Nymania capensis* (Thunb.) Lindb. (Meliaceae) and *Pappea capensis* Eckl. & Zeyh. (Sapindaceae), have chloroplast lineages that are restricted to primary drainage basins (Potts et al., 2013b); such patterns in the chloroplast likely arose due to

restricted seed dispersal between basins. This pattern also extends to nuclear DNA, a proxy for pollen flow, of *Nymania capensis* (Potts et al., 2014). These patterns of genetic isolation between populations are not only restricted to plants. Highly vagile cicadas (Cicadidae) display phylogeographic structuring associated with drainage basins (Price et al., 2010), and chameleons of the genus *Bradypodion* are structured in relation to Cape vegetation types (Tolley et al., 2006). Such structuring suggests that populations are commonly genetically isolated and are likely to develop high levels of local adaptation (Carlson et al., 2011).

Certainly not all species show strong phylogeographic structuring. *Elytropappus rhinocerotis* (L.f.) Less. (Asteraceae) has no obvious genetic breaks (Bergh et al., 2007). *Schotia afra* (L.) Thunb. (Fabaceae) can be considered to comprise a single large meta-population that spans over 800 km across eight primary drainage basins (Potts et al., 2013b). However, both of these species have adaptations for long-distance dispersal; *E. rhinocerotis* has minute seeds with a parachute pappus that can be wind-dispersed and travel long distances in the atmosphere, and *S. afra* pods are eaten by elephants, which are ideal dispersal agents due to long gut retention times and migratory behaviour.

The members of *Cyclopia* have no such adaptations; indeed, their moderately sized seeds are dispersed short distances by ants (Schutte et al., 1995; Schutte, 1997). Therefore, we can expect these species to exhibit phylogeographic structuring, at least of the chloroplast genome inherited in seeds, where populations have unique genetic compositions and local adaptations. Motsa et al. (2017-in this issue) observed significant differences in phenophases (flowering and fruiting) of plants grown from cuttings of *C. subternata* and *C. genistoides* that were sourced from different areas and grown in a common garden for two years. This was most pronounced in the *C. genistoides* plants, where flowering appears out of phase across genotypes sampled from different areas, which is suggestive of possible phenophase-related local adaptations.

5. Are wild populations at risk of genetic contamination?

Cape species, such as honeybush, may be under threat of genetic erosion and the loss of genetic diversity by a range of factors (Fig. 1), such as loss of habitat or overexploitation of wild populations. An often overlooked potential factor driving genetic erosion is the transition of a species into the 'green revolution' agricultural setting (Wood and Lenne, 1997), specifically the widespread dissemination of, and focus on, 'high-yielding' varieties. The Rooibos industry, based on *Aspalathus linearis* (Burm. f.) Dahlg., offers a prime example. The commercial cultivation of Rooibos is predominantly based on genetic material sourced from a single population (the 'Nortier' shrub-type from the Pakhuis area of the northern Cederberg) (Hawkins et al., 2011). This cultivar is now widely grown across the Cape. However, the species is exceptionally diverse, with populations that are morphologically, chemically, ecologically and genetically varied (Dahlgren, 1968, 1988; Van Der Bank

et al., 1995; van Heerden et al., 2003; Malgas et al., 2010; Hawkins et al., 2011). Thus, although the overall population size of *A. linearis* has increased dramatically in cultivation, the agricultural transition has to be viewed as a genetic bottleneck for the species. In addition, wild populations may also be at risk of genetic erosion whenever they occur close to cultivated plantations (e.g., Laikre et al., 2010) (Fig. 1)—this is a significant and unmonitored threat as farmers in some regions have used the presence of wild populations to decide where to cultivate this often difficult-to-grow species (RM Cowling, personal communication, 2013).

Laikre et al. (2010) reviewed the risks of compromising the genetic diversity of local populations via translocations (Fig. 1). Even if local and non-local populations share a gene pool, the gene frequency of local populations may be altered by the gene frequency of the non-local population. Thus, if the gene pool of the non-local population is shallower or biased relative to the local population, this can lead to a homogenising effect and loss of genetic diversity within and between wild populations. This problem could also arise if locally sourced seeds selected for extensive propagation only represent a subset of the genetic diversity in the local population. Alternatively, non-local populations may contain entirely different genetic lineages, and these would then imprint upon, or entirely replace, local gene pools (genetic swamping).

Thus, the transition from wild harvesting to active cultivation may place wild species and populations of *Cyclopia* at 'genetic risk'; i.e., the potential negative impacts generated from gene flow – genetic contamination – between non-local plantations and wild populations (Laikre et al., 2010; Byrne and Stone, 2011). Gene flow and hybridisation between species or populations of *Cyclopia* has not been investigated. In general, the consequences of genetic contamination are nearly impossible to predict or evaluate: hybrids may have increased vigour (which might also be eventually lost over generations), reproduction may be reduced, or hybrids may have lowered fitness (Levin et al., 1996; Rieseberg and Carney, 1998; Byrne et al., 2011). In addition, predicting the outcome of genetic contamination is further complicated as impacts may change in space (in response to local contexts) and time (over generations or in response to extreme events). Nonetheless, the outcome of these processes may lead to a breakdown of intrinsic or extrinsic genetic adaptations, which could reduce the overall fitness of the local population, and ultimately, in extreme cases, genetic or demographic extinction (Laikre et al., 2010). Thus, planting of non-local provenances or improved material within the range can affect local gene pools to varying, and usually unpredictable, degrees (Potts et al., 2003).

Byrne et al. (2011) have developed a protocol for assessing the risk of genetic contamination in wild populations during the revegetation of degraded landscapes, and this protocol is applied to the case of *Cyclopia* below. Both revegetation and agricultural cropping share similar concerns regarding genetic contamination as they usually involve large numbers of plants within plantations of non-local lineages that may grow near local wild populations. This assessment is based on
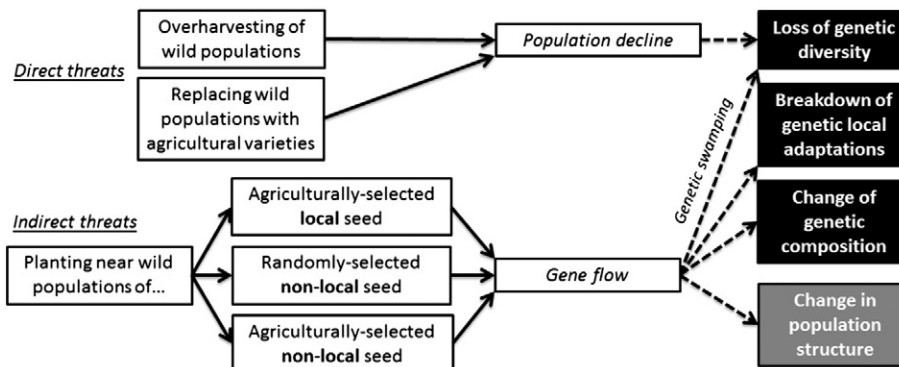

Fig. 1. The primary human-mediated pathways that can affect genetic diversity and structure between (grey box) and within (black boxes) populations of a wild plant species (Adapted from Laikre et al., 2010). Direct threats have a predictable effect on genetic diversity, whereas indirect threats have far less predictable outcomes.

three separate criteria that cover aspects of taxonomy, biology, and geography. These criteria attempt to rule out potential genetic risk.

The taxonomic criterion suggests that the likelihood of genetic contamination is low when there is a single species with no phylogeographic structuring in the region of interest (i.e., a single large metapopulation); this is not the case in *Cyclopia* as there are many species within the Cape, and there is good reason to suspect that there may be phylogeographic structuring within species and across species.

The biological criterion would rule out genetic risk if there was no overlap in flowering time within the genus and between populations or if there were known incompatibilities in the mating system. The majority of species, and thus populations, within *Cyclopia* overlap at some point in their flowering times during October to December (Manning and Goldblatt, 2012). Motsa et al. (2017-in this issue) demonstrated that flowering in *C. subternata* and *C. genistoides* is somewhat out of sync (Aug–Sept and Sept–Oct, respectively), but there is still a period of overlap during September for most of the examined genotypes. Whether there are any incompatibilities in the mating system remains unexplored.

The last criterion, the geographic distribution of plantations in relation to wild populations, incorporates the size of local populations in relation to plantations, the conservation importance of wild populations, and aspects of pollen dispersal. Given the area and density of plants in a plantation, it is more than likely that this will exceed any of the local populations within the vicinity of the plantation. This would increase the likelihood of genetic contamination of local populations. All local populations of honeybush can be considered of conservation importance for two reasons: many of the species are listed as critically endangered (Red List of South African Plants: Vlok and Raimondo, 2011), and local populations may house unique genetic diversity. Lastly, the distance and pattern of pollen dispersal is unknown for *Cyclopia*, and cannot be inferred from a similar species due to lack of information. Byrne et al. (2011) suggest that high genetic risk should be assumed until further information is available and plantations should be situated 'significantly' distant to wild populations. However, what is the 'significant' distance that will prevent genetic contamination?

The sweetly scented and rigid flowers shared between all members of *Cyclopia* are likely adapted to pollination by xylocopid bees (Schutte, 1997). Short-term monitoring of *Xylocopa flavorufa* in Kenya suggests that foraging flight distances were usually between 200 and 1000 m, and rare long-distance foraging flights (maximum observed was ~6 km) can occur (Pasquet et al., 2008). Homing tests revealed that xylocopid bees have a potential flight range of around 10 km (Pasquet et al., 2008). Usually long-distance foraging, say > 4 km, could be considered rare given the trade-offs between energy expenditure and pollen gathering. However, pollinators may travel far further than expected for the higher pollinator rewards offered by the high concentration of flowering plants in plantations (Pasquet et al., 2008); this has also been observed in other pollinators such as bumblebees (Batra, 1993; Cresswell and Osborne, 2004). The dense synchronised flowering in plantations will attract flying pollinators, such as xylocopid bees, from farther away than smaller and sparse patches of wild populations. Thus, as a preliminary guideline, a 'significant' distance to prevent gene movement between wild populations and plantations of non-local origin is likely to be in the region of 10 km or more.

6. How can the transition to cultivation be managed until the, admittedly usually slow, process of research is complete?

If the aim is to maintain the genetic integrity of Cape species and populations entering domestication, such as members of the genus *Cyclopia*, then there are three precautionary principles that can be recommended as guidelines until the scientific facts have been established. First, more than one species (or population) should not be grown together in the same plantation to avoid potential problems of interspecific hybridisation. Second, cultivated stands should be planted away

from wild populations to reduce lineage mixing or genetic swamping. The distance between cultivated stand and wild populations to safely avoid genetic contamination requires further research, but a preliminary guideline may be further than 10 km. Third, if stands are situated near wild populations, these should be grown from locally sourced seeds. Defining the radius of this local sourcing is a difficult task. However, tentative general rules may include that seeds or seedlings should not be moved (1) over watersheds of primary and secondary catchments (catchment maps and Google Earth files are available from the Department of Water and Sanitation's website: https://www.dwa.gov.za) or between mountain ranges, which have have restricted gene flow in other plant species along the southern coastal lowlands (Potts et al., 2013a; Potts et al., 2013b; Britton et al., 2014), (2) drastically along altitudinal gradients (i.e., uplands to lowlands) as these represent major environmental shifts (Bradshaw and Cowling, 2014), and (3) more than 20 km from the source population to maintain local genotypes and frequencies. The last point is possibly an extreme use of the precautionary principle as there is little relevant information to provide suitable guidance. Applying these principles, which ensure that plantations are far enough away to protect wild populations or limit the dispersal of material, will require extensive mapping of honeybush populations, as well as stakeholder engagement to assess issues of practicality. To conserve the genetic resources stored in wild populations of Cape plant species, including the members of the genus *Cyclopia*, we need to understand their phylogeographies (i.e., population genetic structure), local adaptations, potentials for hybridisation, the agents of gene dispersal, and agent behaviour. Such research should be prioritised for any Cape species that are, or may become, commercially important where a shift to cultivation will be required to meet growing demand.

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