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Contrasting changes in biotic interactions of orchid populations subject to conservation introduction vs. conventional translocation in tropical China

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ABSTRACT

Conservation introduction (CI), i.e. moving a species out of its current natural range for conservation purposes, is a highly controversial and rarely attempted conservation measure. One particular concern with these conservation actions is whether species with obligate symbiotic relationships will be able to form these associations in the recipient locations. This study takes advantage of a massive conservation translocation of orchids at the Yachang National Nature Reserve in southwestern China. We compared the mycorrhizal associations of four of these targeted species. Two of the species (*Cymbidium bicolor* Lindl. and *Geodorum eulophioides* Schltr.) had narrow geographic ranges and were subjected to CI because the movement had placed them out of their natural ranges. While the other two species (*Paphiopedilum dianthum*, Tang and Wang, *and Paphiopedilum hirsutissimum*, (Lindl. ex Hook. f.) Pfitzer) had wide geographic ranges and were not moved outside of them. This movement can be considered a conventional translocation. Using DNA techniques, we identified orchid mycorrhizal fungi (OMF) associated with translocated and natural populations of these species. Our results indicated that translocated populations of both kinds (CI and conventional translocation) were able to establish new relationships with root fungi, including known OMF groups. Wide-ranging species associated with a greater number (24 OTUs) and more diverse groups of fungi (Shannon diversity (H') = 0.93) than narrow range species (15 OTUs, H' = 0.62). However, translocated populations of wide-ranging species shared fewer fungi with their natural populations than those of narrow-ranging species. This research provides the first evaluation of how critical biotic interactions may change following conservation introduction compared to that of conventional translocation, and shed light upon the feasibility of conservation introduction.

1. Introduction

Conservation introduction (sensu IUCN (2013), hereafter referred to as CI), also known as assisted colonization or managed relocation, is the movement of a species by humans to higher latitudes or elevations beyond their current native range to habitats predicted to be suitable under future climatic conditions (McLachlan et al., 2007; Hunter, 2007). CI as a viable strategy to mitigate biodiversity losses as a result of climate change remains a highly debated and rarely attempted conservation action (Hoegh-Guldberg et al., 2008; Minteer and Collins, 2010; Hewitt et al., 2011). Because of the scarcity of such controversial initiatives, only a few known examples of CI currently exist (Liu et al., 2015), and an even smaller portion of these CIs have been evaluated (Willis et al., 2009; Liu et al., 2012). Central arguments about CI include whether endangered species have the flexibility in their physiological and ecological requirements to survive in the recipient community or whether moving species out of their natural range under any circumstances is a good idea (Reichard et al., 2012), as some species introductions have led to disastrous consequences, a prime example being invasive species (Ricciardi and Simberloff, 2009).

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While the debate continues, it is important to take advantage of special circumstances in which actions such as relocating species to out-of-range sites have occurred. Studies of these cases can provide empirical data on the distributional requirements, both abiotic and biotic of the selected species and help to build a decision-making framework that can identify which species may be fit for CI (Liu et al., 2010; Lunt et al., 2013). One major factor to consider for CI assessments is the possible changes in the mutualistic interactions of the translocated populations. The ability to form beneficial biotic interactions in a new range is essential to the establishment of many species in a new environment (Richardson et al., 2000; Mitchell et al., 2006; Pemberton and Liu, 2008). This suggests that the degree of flexibility in biotic interactions (generalization) of a species can determine whether it will survive or not following a CI. Yet, highly specialized species may still be targeted for assisted colonization because of their intrinsic value, the unique ecosystem services they provide, or because they exhibit low ecological redundancy (Lunt et al., 2013).

Orchids have previously been identified as candidates for testing the CI strategy (see examples from Keel et al. (2011), Liu et al. (2010), Lunt et al. (2013)). Orchids can serve as model organisms to test the feasibility of moving species with a spectrum of biotic specificity because of their strong dependency on biotic interactions, including their associations with mycorrhizal fungi that are necessary for seed germination and also in later stages in life (Rasmussen, 1995; Taylor, 2004; McCormick et al., 2006). In 2006, thousands of wild orchids belonging to 29 species, and 16 genera, were moved to higher elevation sites in anticipation of the completion of the Longtan Reservoir near the Yachang National Orchid Reserve (hereafter Yachang Reserve) in Guangxi Province, southwestern China (Liu et al., 2012). With an estimated 44 genera, and 140 species in the Yachang Reserve along, this region is considered a hotspot of orchid diversity (Cribb et al., 2003; Liu et al., 2010). The impacted area consists of a 20 km stretch along the Hongshui River, where all of the low-lying areas below 400 m were to be completely inundated. The translocation recipient site in the Yachang Reserve was located < 30 km from the source sites but was 600 m higher in elevation and was 3.6 °C cooler in mean annual temperature (Liu et al., 2012).

Here we examined the mycorrhizal associations of four of these translocated species; *Cymbidium bicolor* Lindl., *Geodorum eulophioides* Schltr., *Paphiopedilum dianthum* Tang and Wang, *and Paphiopedilum hirsutissimum* (Lindl. ex Hook. f.) Pfitzer. The first two species have naturally narrow elevational ranges and are found only at lower elevations. The subsequent upward translocation of the narrow range species *C. bicolor* and *G. eulophioides*, to elevations > 1000 m above sea level (a.s.l.), exposed these species to conditions well outside their natural range (conservation introduction). In contrast, both *Paphiopedilum* species have wide elevational ranges and can be found naturally at both low and high elevations. The translocations of these two species in the Yachang Reserve were within their natural range and therefore the realm of conventional translocation.

We aimed to evaluate how mycorrhizal communities vary between natural and translocated populations and between populations subjected to CI vs. conventional translocation, both of which are critical to evaluate the feasibility of CI for orchid species and other plants that depend on similar biotic interactions. We used DNA sequencing techniques and fungal specific PCR primers to identify potential mycorrhizal root fungi. Phylogenetic analysis was used to compare the mycorrhizal communities of conspecific populations that remained in their natural range to the individuals that were translocated. We also estimated the overall richness and diversity of mycorrhizal fungi among the different orchid populations. With the notion that wide-ranging orchid species may be associated with a wide array of fungal partners, we formulated our working hypotheses as follows: 1) elevational wide-ranging species will associate with more diverse mycorrhizal fungi than narrow-ranging species; 2a) translocated populations of elevational wide-ranging species will associate with different groups of fungi than those within their natural sites; and 2b) translocated populations of elevational narrow-ranging species (the CI populations) will associate with the same groups of fungi as those in natural sites, due to the expected high level of specificity. We acknowledge that association with relatively few but wide-ranging species of mycorrhizal fungi could also enable orchids to be wide spread but for simplicity we will put this notion aside for now and take it into account in the discussion section. These hypotheses will address three major questions regarding the movement of orchid species as a conservation action; 1) can orchids form mycorrhizal associations beyond their natural elevation range? If so, 2) will translocated populations associate with similar groups of fungi in their natural and recipient sites and 3), are changes in fungal associations seen in CI populations (of elevational narrow-ranging species) similar to those conventionally translocated populations (of elevational wide-ranging species)?

2. Methods

2.1. Study species

Narrow-ranging, low elevation species (growing at a range of 350–700 m a.s.l.): *C. bicolor* and *G. eulophioides*. *Cymbidium bicolor* can be seen naturally growing on trees and limestone cliffs in southern China, Vietnam, Peninsular Malaysia, Borneo, Sulawesi, Java, Sumatra and the Philippines (Liu et al., 2012). *Geodorum eulophioides* is an extremely rare terrestrial orchid with only two disjoint occurrences globally, one near Yachang Reserve (Liu, 2010), and the other in central Myanmar (Tanaka et al., 2011). At Yachang, only two small populations consisting of a few dozen adult individuals remain and each is confined to a single hillside.

Wide-ranging elevation species (growing at a range of 350–1100 m a.s.l. in southwestern China): *P. dianthum* and *P. hirsutissimum*. *Paphiopedilum dianthum* are predominantly lithophytic, but in some instances, they can also be found growing epiphytically. This species is considered endangered by the IUCN Redlist and has a very restricted distribution in China, Laos and Vietnam (Liu et al., 2010). *Paphiopedilum hirsutissimum* is a relatively wide spread species and occurs on shaded cliffs, or in limestone forests. It can be found in Guangxi, Guizhou, and Yunnan of China, and in India, Laos, Thailand, and Vietnam.

2.2. Study sites

The Yachang Reserve, located in northwest Guangxi Province, China (Fig. 1) consists of numerous hills of limestone and non-limestone substrates and steep limestone outcrops, the highest of which are ca. 800 m a.s.l. in elevation. It has a variety of vegetation types, largely depending upon elevation. It is known for its great diversity of terrestrial and lithophytic orchids, with > 140 species in 44 genera (Shi et al., 2007; Liu et al., 2010). Here orchids occur in both monotypic and mixed-species mosaics that can dominate the understory community. The translocation site at the Yachang Reserve, the “orchid garden,” was located at ~1000 m in elevation in a semi-deciduous limestone subtropical forest. Other sampled natural populations were all located within Guangxi Province (Appendix A.1) and included Dingshu Village (hereafter Dingshu), Mulun Nature Reserve (hereafter Mulun), Jingxi County rural areas (hereafter Jingxi), and Bangliang Natural Reserve (hereafter Bangliang).

2.3. Field sampling

All of the field sampling occurred in June 2014. At the Yachang Reserve, fieldwork was conducted on 6/19–6/21. There were two different sampling sites within Yachang: the “orchid garden,” which is in the interior reserve and naturally rich in orchids, including natural populations of *P. hirsutissimum* and *P. dianthum*, and “Laya,” a cliff-side