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Long-distance dispersal and recolonization of a fire-destroyed niche by a mite-associated fungus

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ABSTRACT

The Fynbos Biome in the Core Cape Subregion of South Africa is prone to recurrent fires that can clear vast areas of vegetation. Between periods of fire, ophiostomatoid fungi colonize the fruiting structures of serotinous *Protea* species through arthropod-mediated dispersal. Using microsatellite markers, this study considered the process whereby a *Protea*-associated ophiostomatoid fungus, *Knoxdaviesia proteae*, recolonizes a burnt area. The genetic diversity, composition and structure of fungal populations from young *P. repens* plants in a recently burnt area were compared to populations from the adjacent, unburnt *Protea* population. The only difference between *K. proteae* populations from the two areas was found in the number of private alleles, which was significantly higher in the unburnt population. The population structure, although weak, indicated that most *K. proteae* individuals from recently burnt areas originated from the unburnt population. However, individuals from unsampled source populations were also detected. This, together with the lack of isolation-by-distance across the landscape, suggested that long-distance dispersal is important for *K. proteae* to recolonize burnt areas. Similarly, the high level of gene flow and low differentiation observed between two distantly separated *K. proteae* populations also supported the existence of long-distance dispersal. The genetic cohesiveness of populations over long distances and the genetic diversity within populations could be attributed to frequent multiple fungal migration events mediated primarily by arthropods but, potentially, also by birds.

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Abbreviations; CCR, Core Cape Subregion; IBD, isolation-by-distance; MSN, Minimum Spanning Network

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Introduction

The Fynbos Biome of the Core Cape Subregion (CCR) in South Africa is an unique vegetation type dominated by woody shrubs growing in nutrient-poor soils (Cowling & Richardson 1995). The area is characterized by a Mediterranean-type climate with short, wet winters and long, dry summers (Cowling 1992; Day *et al.* 1979). Recurrent summer-fires (approximately every 10–15 y) often clear vast areas (more than 4000 ha) of nearly all the above-ground fynbos biomass (Day *et al.* 1979; Kruger *et al.* 2000; Southey 2009) and most plants either re-sprout or recruit from seeds stored in the soil (Keeley 1995; Wilgen *et al.* 1992). In the case of serotinous *Protea* species, mature plants are killed by fire, but their survival is governed by the seeds released from above-ground seed-storage structures (infructescences) that form after flowers mature (Rebelo 1995). New *Protea* recruits take *ca.* four years to reach maturity and flower for the first time (Le Maitre & Midgley 1992).

Between fires, *Protea* infructescences are colonized by numerous organisms such as insects (Coetzee & Giliomee 1985; Roets *et al.* 2006b), mites (Theron *et al.* 2012) and fungi (Lee *et al.* 2003, 2005), including ophiostomatoid fungi (Roets *et al.* 2005, 2013). *Protea*-associated ophiostomatoid fungi represent a polyphyletic assemblage (Wingfield *et al.* 1999) that is characterized by occupation of infructescences and arthropod-mediated dispersal (Roets *et al.* 2013). The long ostiolar necks of the perithcia and the production of sticky spores make these fungi ideally suited for dispersal by arthropods rather than air currents (Cassar & Blackwell 1996). Interestingly, these apparently native ophiostomatoid fungi are not associated with disease symptoms and they have no known adverse effects on growth or reproduction on their hosts (Marais 1996; Roets *et al.* 2013).

Knoxdaviesia proteae was the first *Protea*-associated ophiostomatoid fungus to be discovered (Wingfield *et al.* 1988). It was isolated from the infructescences of the common sugarbush, *Protea repens*, an indigenous fynbos species that is the only known host of this fungus (Roets *et al.* 2009b). Since the discovery of *K. proteae*, 11 additional ophiostomatoid fungi associated with serotinous *Protea* species have been identified, bringing the current total of species known in this niche to 12 (De Beer *et al.* 2013). This extraordinary *Protea*-ophiostomatoid fungus association is not restricted to the CCR, but has also been noted in other areas of South Africa and in Zambia (Crous *et al.* 2012; Marais & Wingfield 2001; Roets *et al.* 2010, 2013).

Like many organisms capable of colonizing *Protea* infructescences, the *Protea*-associated ophiostomatoid fungi seem to be specialists of this niche and have not been recorded from any other habitat, including other parts of *Protea* plants, soil and leaf and twig litter (Lee *et al.* 2005; Marincowitz *et al.* 2008). Therefore, recolonization of post-fire, newly formed infructescences by these organisms can take place only via dispersal from unburnt areas. This form of dispersal is more easily achieved for winged-groups like insects, than for the mites and ophiostomatoid fungi. However, many of the mites from this niche are phoretic on beetles that pollinate *Protea* species (Roets *et al.* 2009a). Using these *Protea*-specialist beetles as vectors, mites could easily recolonize infructescences, presumably over long distances. Some of these phoretic mites also have mutualistic associations with the *Protea*-associated ophiostomatoid fungi and a few even have specialized spore carrying structures for the fungi on which they feed (Roets *et al.* 2007). While mites appear to be primarily responsible for spore capture and dispersal on a single *P. repens* plant, beetles carry mites to facilitate long-distance dispersal (Aylward *et al.* 2014b; Roets *et al.* 2009a).

Previous research has shown that gene flow facilitated by mites and beetles is sufficient to maintain a panmictic *K. proteae* population in a *P. repens* stand covering approximately three square kilometres (Aylward *et al.* 2014b). Gene flow between fungi in these plants thus exceeds genetic drift, preventing the fungal population from becoming structured based on individual *Protea* plants. The role of beetles in facilitating between-plant dispersal of mites and the ophiostomatoid fungi they carry, therefore, seems to dominate *K. proteae* movement within a *P. repens* stand.

Flight mill studies on bark beetles have shown that most beetles achieve at least an hour of uninterrupted flight (Atkins 1961; Forsse & Solbreck 1985) and can reach a speed of 2 m s^{-1} in still air (Byers 1996). Mark-recapture studies with the southern pine beetle, *Dendroctonus frontalis* Zimm., showed that a third of the released individuals dispersed more than one kilometre, leading the authors to conclude that ‘beetles are capable of dispersing quite far’ (Turchin & Thoeny 1993). These results suggest that the dispersal kernel (probability distribution) of beetles is typically a fat-tailed one in which long-distance dispersal events are more common (Klein *et al.* 2006). The geographic distance over which the *Protea*-beetle vectors are capable of supplying sufficient *K. proteae* migrants to maintain panmixia therefore becomes intriguing.

Recolonization of large areas of burnt fynbos by ophiostomatoid fungi presents unique opportunities to study the dispersal patterns of these *Protea*-associated fungi. Fire essentially creates a clean slate so that the origin of fungal inoculants in young *Protea* hosts may be established. Most likely, fungal inoculants enter young plants via short-to medium-distance dispersal from neighbouring *Protea* plants that escaped the fire. In this case, burnt areas will have ophiostomatoid populations that represent a subset of the ophiostomatoid fungi in neighbouring unburnt areas. Young *Protea* plants closest to the source populations may also receive more inoculants than plants further away, producing patterns of isolation-by-distance (IBD). Beetles may, however, be able to facilitate sufficient between-plant dispersal that such patterns do not appear. Additionally, beetles may have the ability to transport ophiostomatoid fungi from more distant source populations into the recently burnt areas (Roets *et al.* 2009a), adding greater genetic diversity and potentially novel genetic information to the population.

In this study, we used microsatellite markers specific to *K. proteae* (Aylward *et al.* 2014a) to compare fungal isolates sampled from a recently burnt fynbos area to isolates from neighbouring unburnt areas. The aim was to establish whether adjacent, mature *K. proteae* populations act as the source of inoculants for new *P. repens* stands and whether infructescences can be colonized over long distances, in the first year of