TOOLS TO INVESTIGATE AN EXTRAORDINARY PROTEA-FUNGUS-MITE RELATIONSHIP

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A unique symbiosis between *Protea* plants, ophiostomatoid fungi and mites exists in the Cape Floristic Region (CFR). The *Protea*-associated fungi are of the genera *Ophiostoma* and *Knoxdavesia* and are related to ophiostomatoid conifer pathogens. The pathogenic species are notorious to the forestry industry and are known for their tendency to use arthropods as vectors for dispersal. In the CFR. Ophiostomatoid fungi occur in the flower heads (infructescences) of serotinous *Protea* species and exploit the plant's natural pollinators (mites and beetles) for dispersal.

Infructescence on a young *Protea repens* plant.

One of the proteas harbouring these fungi is the iconic *P. repens*, also known as the Common Sugarbush. With its widespread distribution as well as biological and economic significance, this species is ideal for studying the role of ophiostomatoid fungi in this unique environment. The dispersal ability of the fungi is specifically intriguing, since it determines how closely individuals residing in different *Protea* trees or different *Protea* populations will be related and how new *Protea* plants in a burnt fynbos area are recolonised.

In order to study the dispersal of *K. proteae* occurring in *P. repens*, we developed microsatellite markers. Microsatellites are regions in an organism's DNA that contains a motif of 1-6 base pairs repeated several times. Variation of the number of repeats in different individuals provides information regarding their relatedness. The DNA sequence information required to develop the markers was obtained from the ex-type strain of *K. proteae* by using pyrosequencing (a high-throughput sequencing method). We first enriched the genome for microsatellite sequences using the Interspersed Simple Sequence Repeat-Polymerase Chain Reaction (ISSR-PCR) technique and sent these enriched regions for Roche 454 sequencing at Inqaba Biotec in Pretoria.

In silico ("performed on a computer") methods were used to identify microsatellite regions from the sequence data and design primers for PCR amplification. After testing 23 potential microsatellite markers on 10 *K. proteae* individuals, 12 markers amplified successfully and

showed polymorphism in this species. Half of these also amplified in the closest relative of *K. proteae*, *K. capensis* – also a *Protea*-associate. These markers are the tools needed to track the movement of *K. proteae* over the landscape of the CFR and may be able to provide the same information for *K. capensis*.

At the moment, the 12 microsatellite markers are being applied to investigate two populations of *K. proteae*. The population in Gouritz, close to Mosselbay, is being used to study fine-scale dispersal patterns, whereas the population in the recently burnt Franschoek area enables us to investigate colonisation of new *P. repens* infructescences. This study will generate novel information concerning the dispersal ability of *Protea*-associated ophiostomatoid fungi. The application of these markers to *K. capensis* will also allow ecological comparisons between these two ophiostomatoids.