A molecular assessment of the key biological processes of dispersal and mating in Banksias: implications for conservation

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Abstract

The conservation and management of biodiversity should be based not only on numbers, but also on a clear understanding of key biological processes in the target species. These long-term studies on Banksia hookeriana *(Proteaceae) demonstrate that molecular markers provide unique opportunities to understanding key processes such as mating and the dispersal of pollen and seed. Using a population allocation approach, it was found that both the numbers (ca. 6.8%) and distance (up to 2.5 km) of seed dispersal between discrete populations was much higher than expected. Using paternity assignment, it was also found that as many as 10% of all seeds were sired by plants outside of the local population. These results highlight the importance of inter-population dynamics in this species. It was also found that near random pollinator movements among plants within the population of* B. hookeriana *by highly mobile nectar-feeding birds, and possibly selection against the products of consanguineous matings, influence a more or less panmictic pattern of realized pollen dispersal and very high multiple paternity. These pollen dispersal processes act to sustain high genetic variation within naturally fragmented populations. These results highlight the importance of not only the correct pollinators, but also population size and metapopulation dynamics for the conservation of key processes, all perhaps critical considerations in reintroductions and translocations of other rare Banksias and other species pollinated by highly mobile nectar-feeding birds.*

The conservation and management of biodiversity should be based not only on numbers, but also on a clear understanding of biological processes in the target species that have shaped and sustained genetic diversity.

Information regarding the key biological processes, such as the extent of dispersal within and among populations is therefore critical to determine whether a species requires translocation of individuals to prevent inbreeding and loss of genetic diversity. Gene flow, migration and colonization are fundamental processes in metapopulation dynamics. Migrants create gene flow, colonize empty habitats and counteract genetic differentiation through local adaptation. In the ongoing process of habitat fragmentation gene flow will become even more important for the long-term survival of species. An understanding of the spatial and temporal dynamics of dispersal with respect to extant landscape features can be helpful in predicting demographic and genetic responses of species to naturally occurring or human-mediated population subdivisions (Sork *et al.,* 1999, Cain *et al.*, 2000).

Our long-term studies on *Banksia hookeriana* (Proteaceae) demonstrate that molecular markers provide powerful opportunities to understanding key processes such as mating and the dispersal of pollen and seed, which are critical to manage as part of species conservation and recovery. *Banksia hookeriana* Meissner (Proteaceae) is a fire-killed shrub up to 2.5 m tall that is endemic to the upper slopes and crests of deep sand dunes of the Eneabba sandplain, southwestern Australia, about 300 km north of Perth (George, 1981; Taylor & Hopper, 1988; Lamont *et al.*, 1989). *B. hookeriana* exists in at least part of its range as a metapopulation in which individual populations on dune crests are geographically separated by uninhabitable intervening swales of metres to kilometres in width as a consequence of the undulating landscape within which it occurs. Genetic methods provide a broadly applicable way to best quantify long distance dispersal (Ouborg *et al.,* 1999; Cain *et al.,* 2000), stimulated in part by the powerful new statistical approaches that utilize highly polymorphic molecular markers have been developed recently (Rannala & Mountain, 1997; Waser & Strobeck, 1998; Cornuet *et al*., 1999).

Assignment tests, which were based on multilocus genetic data (AFLP, amplified fragment length polymorphisms) and used both individual genotypes and population level allele frequencies, were conducted on 221 individuals from 18 populations allocated each of 177 (80.1%) to a single genetic population. Of these, 6 (2.7%) were identified as genetic outliers from the population from which they were sampled and were allocated to another population within the metapopulation sample (Fig. 1). A further 9 individuals (4.1%) were allocated to outside of the metapopulation sample. Therefore, 6.8% of all individuals sampled were statistically allocated based on genotype to populations other than that from which they were sampled. For the 6 individuals assigned within the metapopulation to a known population other than that from which they were sampled, the geographic distance between source and sink averaged 2.0 km, with a range of 1.6 km to > 2.5 km (Fig. 1). Exact distances between source and sink populations for the 9 individuals allocated to outside of the metapopulation area are unknown, but the minimum distance from each sink population and the edge of the metapopulation area averaged 1 km. This molecular approach sheds light on a previously intractable topic of long distance seed dispersal, and contrasts markedly with direct observations of primary seed dispersal in *B. hookeriana* following fire, which shows a typically leptokurtic distribution, with 75% of all known dispersal events within 15 m and a detectable tail extending to only 36 m. This was assessed directly by moving 11 adult plants to a newly burnt location, torching cones to stimulate the release of seeds and subsequently monitoring the distance of seedlings from source plants.

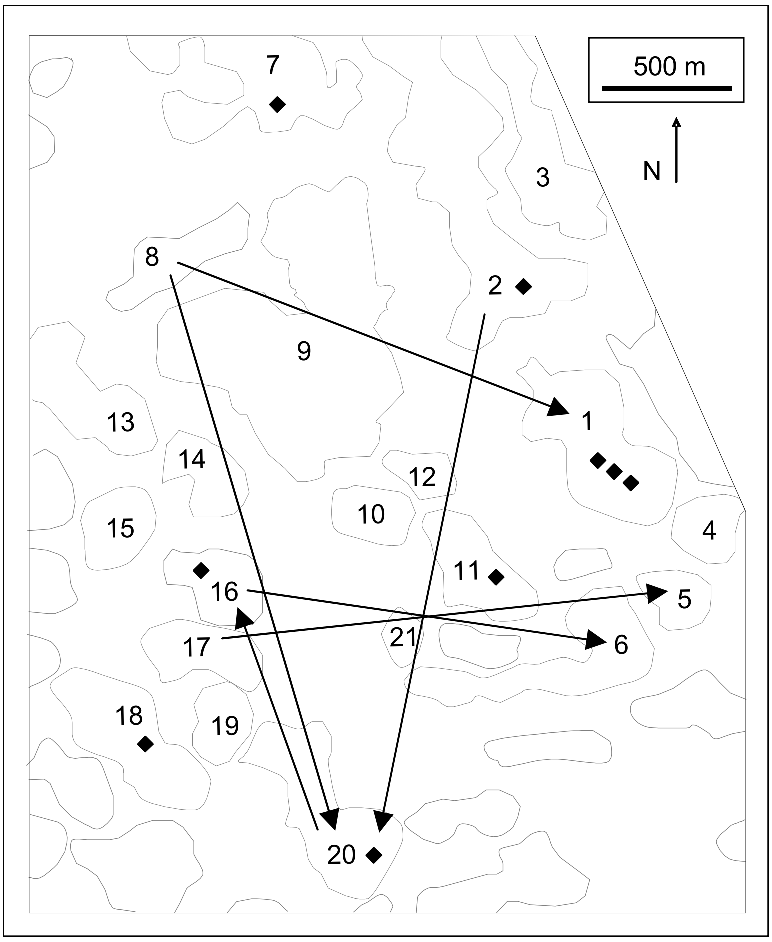

Figure 1. Seed dispersal events between populations of Banksia hookeriana*, inferred from the statistical allocation of individual genotypes to genetic populations based on AFLP data. Diamonds indicate the genetic allocation of an individual to outside of the metapopulation sample, suggesting a seed dispersal event from outside the study area*

Our molecular results have indicated that seed dispersal can extend to at least several kilometers. Wind vortices, a common feature observed after fire (Hammill *et al.,* 1998), together with reduced obstacles to wind dispersal after fire (Lamont, 1985), may facilitate this long-distance seed dispersal in *B. hookeriana*. Another possible long-distance dispersal vector is granivorous birds. Black cockatoo (*Calyptorhynchus latirostris*) are common in the area, and they seek out Banksias after fire to feed on the seeds that are made more readily accessible by the fire-triggered rupture of the protective woody follicles and removal of camouflaging foliage. Multiple fires during the juvenile period cause local extinction – a phenomenon common to species killed by the fore (Benson, 1985). Additionally, increasing development of the region since the 1960's – including farming, infrastructure and population – are adding ignition sources and increasing the frequency of fire (Bell *et al,*. 1987), so that increased fire frequency threatens the survival of fire-killed woody species such as *B. hookeriana.* Consequently, the capacity of the species to naturally recolonise sites lost to local extinction needs to be understood for conservation and management purposes.

Using Amplified Fragment Length Polymorphism (AFLP), the realized pollen dispersal within a population of *Banksia hookeriana* was also investigated. Realized pollen dispersal was assessed in a population with 112 individuals using 148 polymorphic AFLP markers and a likelihood assignment approach to paternity for 274 seeds from 5 families. Realized pollen dispersal demonstrated a significant departure from nearest-neighbour pollination and a distribution that was not significantly different from the spatial distribution of plants for 4 of the 5 families (Fig 2), and best described by a two-parameter Weibull function with scale 29.9 m and shape 1.9. Overall, only 8% of all realized pollen dispersal events occurred between plants in the shortest distance class (up to 10 m), while most (32%) realized pollen dispersal events occurred between plants separated by between 10 and 20 m. Pollen dispersal frequency then declined exponentially for distance classes beyond 20 m, up to a local maximum of 77 m (Fig. 3). With adjustments for deceased local plants that may have contributed to past mating, it is estimated that 9% of offspring were sired by pollen originating from outside the local population. Comparison with the earlier direct estimates of seed flow of 7% indicates a pollen: seed flow ratio near unity, which is at least an order of magnitude lower than indirect estimates for most other species.

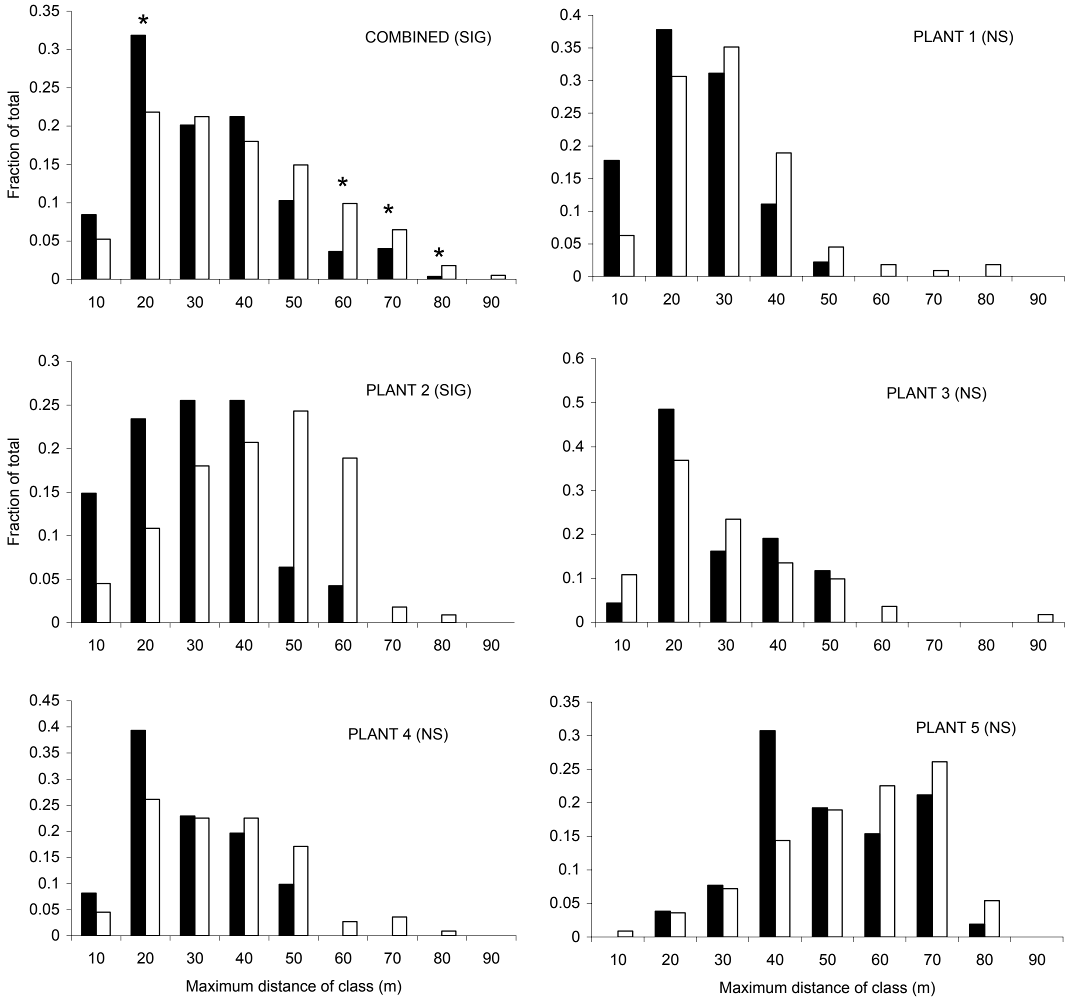

*Figure 2. Fractions of realized pollen dispersal (solid bars) and plant pairs (unfilled bars) for each distance class for the combined data set across all five maternal plants, and for each plant (1 to 5) from a population of Banksia hookeriana. "SIG" indicates a significant departure ($P < 0.001$) between the two distributions, "NS" indicates no significant departure ($P > 0.05$) between the two distributions, as determined by Kolmogorov-Smirnov two-sample tests. * indicates significant difference (t-test, $P < 0.05$) between fraction of realized pollen dispersal and plant pairs within a distance class for the combined data set.*

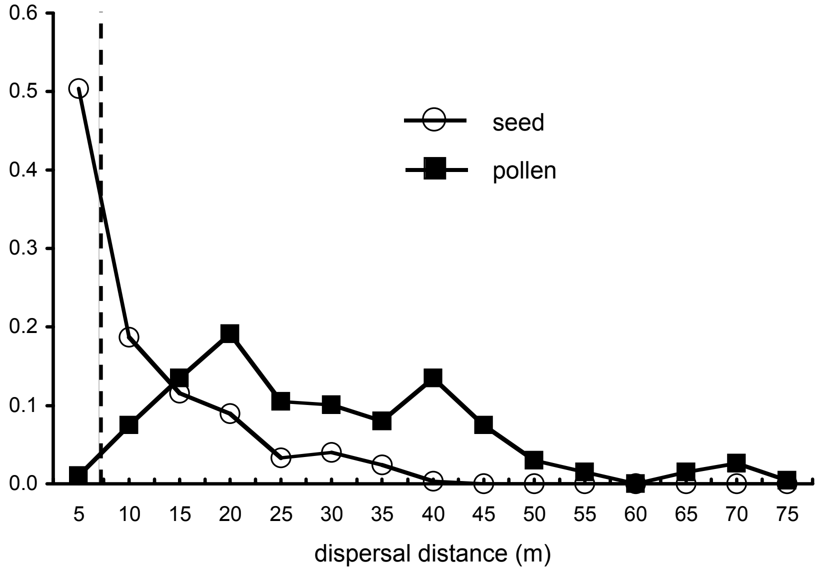

Figure 3. Realized seed- and pollen- dispersal in Banksia hookeriana, showing the percentage of dispersal events for each 5 m distance class. Vertical dashed line indicates the longest distance at which positive genetic structure was detected by spatial autocorrelation analysis of a population of 112 plants.

We suggest that near random pollinator movements among plants within the population by highly mobile nectar-feeding birds, together with extensive movements between populations, and possibly selection against the products of consanguineous matings, influence a near panmictic pattern of realized pollen dispersal. These results highlight the importance of not only the correct pollinators, but also the importance of population size and metapopulation dynamics for the conservation of key processes, all perhaps critical considerations in reintroductions and translocations of other rare Banksias and other species pollinated by nectar-feeding birds. With 15% of the Australian temperate flora pollinated by nectarivorous birds, these results have broad and novel evolutionary significance for many other Gondwanan plant species.

The results indicate that extensive pollen dispersal and metapopulation dynamics play a key role in influencing the genetic structure and genetic variation within and among naturally fragmented populations of nectarivorous bird pollinated plants. Such key evolutionary processes determine the response of a species to changes in the biotic and physical environment and, thus, population viability under current and future conditions.

Acknowledgements

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