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POLLINATION ECOLOGY OF NEW ZEALAND ORCHIDS

A thesis presented in partial fulfilment of the requirements for the degree of
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“It will be tragic if the remaining natural areas of the world are filled with ageing plants silent as graveyards with no butterfly or sunbird pollinators working their flowers or large birds eating their fruits” (Bond 1995).

To Angélica

ABSTRACT

The New Zealand orchid flora comprises twenty-five genera and at least 100 species occurring throughout the country. Although the number of endemic species is high (69%) only four genera are endemic to New Zealand. The main physical threats to orchid survival in New Zealand are habitat destruction, modification and fragmentation. The effect of the disruption of interactions with their pollinators has never been considered. This study concentrates on this mutualistic interaction, by assessing the breeding system, pollination syndromes and pollinator-dependence of four widespread terrestrial (*Gastrodia cunninghamii*, *Thelymitra longifolia*, *Pterostylis alobula* and *P. patens*) and four widespread epiphytic orchids (*Earina autumnalis*, *E. aestivalis*, *E. mucronata* and *Winika cunninghamii*) occurring in the southern portion of the North Island.

In order to determine the breeding system and the presence of self-incompatibility, hand-pollination treatments were conducted in all eight orchid species during the flowering seasons of 2001 and 2002. Pollen grains and ovules numbers, pollen:ovule ratio and presence of floral scent glands were assessed. In those nectariferous species (*E. autumnalis*, *E. aestivalis*, *E. mucronata* and *W. cunninghamii*), the nectar standing crop was determined using the anthrone colorimetric assay for total carbohydrates. The activity of pollinator was observed both in the field and in captivity. Insects observed foraging in these orchids were identified and ranked according to their likely pollination effectiveness. Finally, measurements of pollination success and pollinia removal and deposition were used to assess whether fruit-set is pollen limited in these species and explore the effect contrasting rewarding strategies (nectar v/s deception) has on the pollination success of these orchids.

Pollination treatments in three terrestrial (*T. longifolia*, *P. alobula* and *P. patens*) and two epiphytic (*E. autumnalis* and *E. mucronata*) orchids confirmed the absence of genetic incompatibility. Despite these five orchids being self-compatible, their reproduction relies on contrasting reproductive strategies. *T. longifolia* is predominantly self-pollinated, whereas *Pterostylis* and *Earina* species are incapable of autonomous selfing and completely dependent on pollinators.

The epiphytic species *E. aestivalis* and *W. cunninghamii* are partially self-incompatible and also completely dependent on pollinators. Agamospermy is likely to occur in *G. cunninghamii* but not involved in seed-production in any of the remaining seven orchids. Both terrestrial and epiphytic species showed a positive reaction to neutral red except *E. autumnalis*. This indicated the presence of scent glands, mainly located around the column, lip and sepal tips. Pollen:ovule ratios calculated for these species ranged from 20: 1 in *E. mucronata* and *E. aestivalis* to 320: 1 in *P. alobula*.

Of the four terrestrial orchids studied, insect visitation was observed only in *P. alobula*. This orchid is pollinated by male fungus gnats of the genus *Zygomia* (Diptera: Mycetophilidae). Pollination by sexual deception is likely to occur in species of this genus. Numerous insects were recorded visiting the nectariferous epiphytic orchids (3 orders, 13 families). Insects considered as “probable pollinator” were *Eristalis tenax* (Diptera: Syrphidae) for *Earina autumnalis*, *Dilophus nigrostigmus* (Diptera: Bibionidae) for *E. mucronata*, and *Melangyna novaezealandiae* (Diptera: Syrphidae), *Calliphora quadrimaculata* (Diptera: Calliphoridae), the Ichneumonid wasp *Aucklandella* sp. (Hymenoptera: Ichneumonidae), *Hylaeus* sp. (Hymenoptera: Colletidae) and an unidentified weevil (Coleoptera: Curculionidae) for *E. aestivalis*. In *W. cunninghamii* the species *Apis mellifera* and the native syrphid flies *Helophilus antipodus* and *M. novaezealandiae* were considered as “probable pollinators”.

Levels of natural fruit-set were similarly low in rewarding and non-rewarding species fluctuating from 4.3% (*P. alobula*) to 40% (*P. patens*). Fruiting in these orchids is pollen limited, as supplementary hand-pollinations increased fruit set above 40% in all species except *P. patens*. The degree of pollen limitation varied from 0.32 (*P. patens*) and 0.94 (*P. alobula* and *E. mucronata*). Pollen limitation in these orchids may be caused by the simplicity of their flowers, the poor efficiency of their pollinators in depositing pollinia and the use of species-specific pollination systems (e.g. *Pterostylis*).

The survival capability and conservation requirements of these orchids are discussed in the light of the specific reproductive requirements revealed by this study.

RESUMEN

La familia Orchidaceae en Nueva Zelanda consta de 25 géneros y más de 100 especies distribuidas por todo el país. El número de edemismos es alto pero solo a nivel de especies (69%), solo cuatro géneros son endémicos. Aunque las principales amenazas físicas a la sobrevivencia de estas orquídeas ya han sido identificadas (destrucción, modificación y fragmentación del habitat), el efecto que la ruptura de interacciones mutualísticas pueda tener en su sobrevivencia nunca ha sido considerado. Este estudio pretende evaluar la relación mutualística planta-polinizador en cuatro especies nativas de orquídeas terrestres (*Gastrodia cunninghamii*, *Thelymitra longifolia*, *Pterostylis alobula* and *P. patens*) y cuatro epífitas (*E. autumnalis*, *E. mucronata*, *E. aestivalis* and *W. cunninghamii*) creciendo en la parte sur del la Isla Norte de Nueva Zelanda, estudiando su sistema reproductivo, síndromes de polinización y la dependencia de éstas especies a su polinizador.

Durante el periodo de floración del año 2001 y 2002 se realizaron tratamientos de polinización manual en las ocho especies a fin de determinar su sistema reproductivo y la presencia de barreras de auto-incompatibilidad. También se evaluó la relación polen/ovulo, el número de granos de polen y ovulos por flor, y finalmente la presencia de glándulas productoras de aroma. En las especies nectaríferas (*E. autumnalis*, *E. mucronata*, *E. aestivalis* and *W. cunninghamii*) se midió la cantidad de carbohidratos totales utilizando el método colorimétrico de antrona. La actividad de los polinizadores se observó tanto en terreno como en el laboratorio. Los insectos observados fueron identificados y clasificados de acuerdo a su eficiencia polinizadora. Finalmente, a fin de determinar si el éxito reproductivo esta limitado por la disponibilidad de polen se midió el éxito de la polinización, la remoción y deposición de polinia en las poblaciones estudiadas.

Los tratamientos de polinización demostraron que al menos tres de las orquídeas terrestres (*T. longifolia*, *P. alobula* y *P. patens*) y dos de las epífitas (*E. autumnalis* y *E. mucronata*) son autocompatibles. A pesar de que estas cinco especies carecen de barreras de auto-incompatibilidad, su reproducción depende en diferentes estrategias reproductivas. *T. longifolia*, por ejemplo es autogama, mientras que las especies de los

géneros *Pterostylis* and *Earina* son xenógamas obligadas y dependen completamente su polinizador para formar frutos.

Por el contrario las epífitas *E. aestivalis* y *W. cunninghamii* son parcialmente incompatibles y altamente dependiente de sus polinizadores. La agamosperma al parecer solo esta involucrada en la formación de frutos de la especie *G. cunninghamii*. La tinción de rojo neutro presentó una reacción positiva en todas las especies, excepto en *E. autumnalis*. Estas orquídeas presentan glándulas productoras de aroma ubicadas principalmente alrededor de la columna, labelo y ápice de los sépalos. Los valores de la relación polen/ovulo fluctuó entre 20: 1 y 320: 1 en *E. aestivalis*, *E. mucronata* y *P. alobula*, respectivamente.

De todas las especies terrestres, sólo en *P. alobula* se observó la visita de insectos. Esta orquídea es polinizada por mosquitos machos del genero *Zygomyia* (Diptera: Mycetophilidae). Es posible que la atracción de estos insectos sea mediada por un sistema de atracción por engaño sexual. Por otra parte, las orquídeas epífitas son frecuentadas por numerosos insectos (3 ordenes, 13 familias). Los insectos considerados como “probables polinizadores” fueron *Eristalis tenax* (Diptera: Syrphidae) en *Earina autumnalis*, *Dilophus nigrostigmus* (Diptera: Bibionidae) en *E. mucronata*, y las especies *Melangyna novaezealandiae* (Diptera: Syrphidae), *Calliphora quadrimaculata* (Diptera: Calliphoridae), *Aucklandella* sp. (Hymenoptera: Ichneumonidae), *Hylaeus* sp. (Hymenoptera: Colletidae) y un gorgojo sin identificar (Coleoptera: Curculionidae) en *E. aestivalis*. Para la orquídea *W. cunninghamii* la abeja común (*Apis mellifera*) y los sírfidos *Helophilus antipodus* y *M. novaezealandiae* se consideraron como sus “probables polinizadores”.

Los niveles de fructificación bajo condiciones naturales de polinización fueron bajos, independiente de la presencia o ausencia de nectar. La fructificación fluctuó entre el 4.4% en *P. alobula* y el 40% en *P. patens*. El bajo éxito reproductivo de estas especies se debe a la limitación en la disponibilidad de polen, esto se confirma al observar el aumento de la fructificación tras polinizaciones manuales sobre el 40% en todas las especies, excepto *P. apatens*. La intesidad en la limitación de polen fluctuó entre 0.32 (*P. patens*) y 0.94 (*P. alobula* y *E. mucronata*).

La limitación en la disponibilidad de polen en estas especies es producto de la simpleza de sus estructuras florales, la baja eficiencia en la deposición de polinia por parte de los insectos y el uso de elaborados de sistemas de polinización (e.g. *Pterostylis*).

Finalmente, se discute la conservación y capacidad de sobrevivencia de estas especies a la luz de sus requerimientos reproductivos.

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