

Does Height Off the Ground Affect Bird Visitation and Fruit Set in the Pollen-Limited Mistletoe *Peraxilla tetrapetala* (Loranthaceae)?

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ABSTRACT

For pollination studies of forest species it is sometimes only possible to work on those flowers nearest to the ground. We test whether using low flowers introduces bias, by measuring height effects on bird visitation and fruit set in one mistletoe species pollinated by bellbirds in New Zealand. At this site, previous studies have shown fruit set near the ground to be pollen limited. We measured fruit set on 32 mistletoes at different heights in 11 host trees. Mistletoe fruit set varied significantly among host trees but did not vary with height. Although bellbirds generally forage preferentially in the upper part of the forest, mistletoe flowers appear to be attractive enough to ensure adequate visitation and fruit set at all heights.

Key words: *Anthornis melanura*; bellbirds; height effects; New Zealand; pollen limitation; pollination; sampling bias; vertical stratification.

THE VERTICAL DIMENSION OF FORESTS presents a sampling problem for pollination and fruit dispersal studies because of the difficulty of access to at least some of the flowers or fruits. To study some plants, access to the forest canopy is essential, which makes sampling expensive and difficult while, in others, flowers may be dispersed throughout all tiers of the forest, allowing easy access to a subset that is near the ground. However, studying this subset may introduce a significant sampling bias, because flowers are not sampled from the full range of habitat (Barker & Pinard 2001). This kind of bias could be serious where there are strong vertical gradients in the pollinator or frugivore community composition, species richness, or density, such as typically found in tropical and subtropical forests (Pearson 1971, Roubik 1993, Bernard 2001, Devries & Walla 2001, Kalko & Handley 2001, Lim & Engstrom 2001, Walther 2002). Sampling flowers within easy reach of the ground samples from only one end of these gradients.

Surprisingly, despite this being a common problem, this bias appears to be rarely tested. Few studies provide quantitative measurements of pollination service at different strata within forests (Carpenter 1976, Frankie & Coville 1979, Roubik *et al.* 1982, Hingston & Potts 2005), and none of these were designed to explicitly test the importance of the height gradient.

Here, we test whether there is any trend from the forest floor to the canopy in pollination service to a pollen-limited New Zealand mistletoe, *Peraxilla tetrapetala* (L.f.) Tiegh. (Loranthaceae). The Loranthaceae is a largely tropical family, though some species including *P. tetrapetala*, extend into the temperate southern hemisphere (Barlow 1983). *Peraxilla tetrapetala* is visited by birds, with a similar pollination system to that of the tropical Loranthaceae (Ladley & Kelly 1995, Ladley *et al.* 1997, Renner 1998). This mistle-

toe parasitizes mountain beech, *Nothofagus solandri* var. *cliffortioides* (Nothofagaceae), and can occur from the top of the canopy (around 20 m at our study site) to < 1 m from the ground (Norton *et al.* 1997). *Peraxilla tetrapetala* relies on native honeyeater birds (family Meliphagidae) and two species of native solitary bees to open and pollinate its flowers (Kelly *et al.* 1996, Ladley *et al.* 1997). Flower buds are 'explosive'—once ripe, they must be opened by birds or bees in order to be pollinated and set seed. Birds are more effective at opening flowers (Robertson *et al.* 2005) and are the main focus of this paper. The visitation rate by pollinators is the main determinant of fruit set rates (Robertson *et al.* 1999, Kelly *et al.* 2004, Robertson *et al.* 2005). At our study site at Craigieburn, we have shown over ten seasons that *P. tetrapetala* is consistently strongly pollen limited with natural fruit set usually < 50 percent (Kelly *et al.* 2004). However, most of those data were obtained from plants within 3 m of the ground, and thus may be biased. Indeed, observations at Craigieburn of the primary bird pollinator, the bellbird (*Anthornis melanura*, Meliphagidae), showed that throughout the year, only 9 percent of bellbird feeding observations were on or near the ground (Murphy & Kelly 2003). This preference for the upper and middle canopy layers is also found at other sites in all four bird species that are most important for pollination and dispersal in New Zealand (Kelly *et al.* 2006); bellbirds, tuis (*Prosthemadera novaeseelandiae*, Meliphagidae), silvereyes (*Zosterops lateralis*, Zosteropidae), and New Zealand pigeons (*Hemiphaga novaeseelandiae*, Columbidae; Rasch & Craig 1988, O'Donnell & Dilks 1994). If birds tend to forage higher in the canopy, low plants might be more likely to be pollen limited than higher plants, and therefore the levels of pollen limitation we have previously reported may be unrepresentative of the mistletoe population as a whole.

To test this hypothesis, we (1) recorded the height distribution of all *P. tetrapetala* plants found within randomly chosen belt transects; (2) observed the rate of visitation by birds to mistletoes as a

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function of height; and (3) measured flower visitation and fruit production across the natural height range of mistletoes using climbing ropes to access the canopy. From these data, we compared the performance of plants in the usually-sampled lower stratum to those higher in the canopy, and tested whether there was any relationship between height of the mistletoe and the rates of flower visitation or fruit set.

Peraxilla tetrapetala is one of six endemic loranthaceous mistletoe species in New Zealand. *Peraxilla tetrapetala* and another loranthaceous mistletoe species *Alepis flavida* are both locally abundant and are the only common native bird-pollinated plants at our study site in Craigieburn Forest Park, inland Canterbury (43°9'08" S, 171°42'30" E, 950 m asl). The site carries old-growth pure forest of mountain beech (Kelly *et al.* 2004, 2005).

To estimate the height distribution of the *P. tetrapetala* population at Craigieburn, in 2000 we searched 18 randomly placed 100 m × 5 m transects (Kelly *et al.* 2005) totaling 0.9 ha of forest. The mountain beech canopy at Craigieburn is 10–20 m high with an open understorey, and *P. tetrapetala* grows as compact balls, which contrast with the mountain beech foliage. In addition, we surveyed during the flowering season (January) when its red flowers make *P. tetrapetala* conspicuous. These features make it easier to spot mistletoes, although smaller or nonflowering plants were probably sometimes overlooked. For every mistletoe sighted within the transects, the height off the ground was measured using metal tapes or, for higher plants, estimated.

Bird visitation rates to mistletoe plants were measured by direct observation of groups of mistletoe plants. In January over three years (2000, 2001, and 2002) at the Craigieburn study site, and over the latter two years in the adjacent Cheeseman catchment (Kelly *et al.* 2005), we selected locations with unobstructed views of one or more flowering *P. tetrapetala* plants. Observations were made from a distance (usually > 10 m) that did not appear to affect bird activity. Plants at each location were observed, usually for a total of 90–110 min (range. 50–150) in 10-min periods over 3–5 d. For each avian flower visitor, the bird species and duration of visit in seconds were recorded. Most visits (> 90%) were by bellbirds, with occasional silvereyes and chaffinches (*Fringilla coelebs*). Here, we present data for all bird species combined, but the conclusions are the same if only bellbirds are counted (analysis not shown). The total number of available flowers including unopened buds and open flowers on each mistletoe was counted, or for large plants, estimated independently by two observers and the mean taken, and its height recorded. In total over the three years, we recorded visitation to 179 mistletoes, over 294 plant-hours (= 1 h of observation on one plant), during which time we noted 197 bird visitation-minutes. The visitation rate was expressed as seconds of bird visit/mistletoe flower/h (the number of seconds that birds visited a plant divided by the number of available flowers divided by the number of observation hours per plant), and was $\log(x + 1)$ transformed before analysis.

In January 1998, 11 host trees were selected as vertical transects on the basis that each contained between two and five *P. tetrapetala* mistletoes, which could be reached using a single standard climbing rope and harness. This gave a total of 36 mistletoes and included plants up to 15.7 m off the ground. On each mistletoe, several

flowering branches were tagged and the number of flowers and buds were recorded. Flowers attacked by the lepidopteran flower predator *Zelleria maculata* were excluded from all analyses as they usually fail to set seed (Robertson *et al.* 1999). Flower buds must be ripe (*i.e.*, containing dehisced anthers, a receptive stigma, and nectar) before they can be opened, and ripe buds can be identified by color. Flowers and buds were recorded separately by stage of development (unripe, ripe but unopened, opened). We calculated the percentage of unopened ripe buds (*i.e.*, ripe flowers that had not yet been opened by a flower visitor). The percentage of unopened buds is inversely related to the flower visitation rate, because buds do not self-open (Ladley & Kelly 1995, Kelly *et al.* 2005). We also recorded the total number of flowers to determine the potential for fruit set. On one plant, we could not get close enough to determine the percentage of unopened ripe buds and it was excluded from analyses that included this variable. For more details of these methods see Ladley *et al.* (1997) and Robertson *et al.* (2005).

Two months after flowering, the number of developing single-seeded fruits on the tagged branches was counted to calculate fruit set. Fruit set could not be determined on four plants (one had died, and three had lost their branch tags), so they were excluded from the analysis, leaving 32 mistletoes with a maximum height of 13.4 m. As a consequence, one host had only a single usable mistletoe in it; this record was still included in the analysis.

The analysis used binomial generalized linear models (GLMs) run in S-Plus version 4.5 (Mathsoft, Inc., Seattle, WA, U.S.A.). For the vertical transects, testing both fruit set and percentage of unopened buds, host tree was entered first as a block effect, then height. We also tested whether the mean percentage of unopened buds per plant predicted mean fruit set rate, as expected if unopened buds are an inverse proxy for visitation rate and visitation determines fruit set.

At the same time as the vertical transects were established, we similarly measured 57 permanently tagged mistletoes within 3.1 m of the ground, which have been previously shown to be pollen limited (Robertson *et al.* 1999, Kelly *et al.* 2004, Robertson *et al.* 2005). Each of these plants was on a separate host tree, widely scattered throughout the study area. Fruit set on these low-growing plants was compared to those in the vertical transects, using a binomial GLM with two groups (low-growing vs. those in vertical transects).

Along the 18 horizontal transects covering 0.9 ha, we found 184 *P. tetrapetala* plants. The plants ranged in height off the ground from 0.5 to 16.0 m, with an average height of 6.3 m ± 0.23 SE; Fig. 1A). Notably, only 21 percent of these mistletoe plants were within the range (< 3 m) easily reached by stepladders. Therefore, most mistletoes at Craigieburn were out of reach of sampling from the ground.

The 179 plants at which we recorded bird visitation rates ranged from 0.4 to 15 m in height with an identical mean to the horizontal transects (6.3 m ± 0.25 SE.). Visitation rate varied significantly among years ($F_{2,173} = 19.3$, $P < 0.001$; means: 0.107, 0.028, and 0.011 sec/flower/h in 2000–2003, respectively), and also varied with height ($F_{1,173} = 5.5$, $P = 0.020$). The effect of height was positive, but relatively weak (Fig. 1B), with only a small increase in visitation rates to higher plants and only 2 percent of the

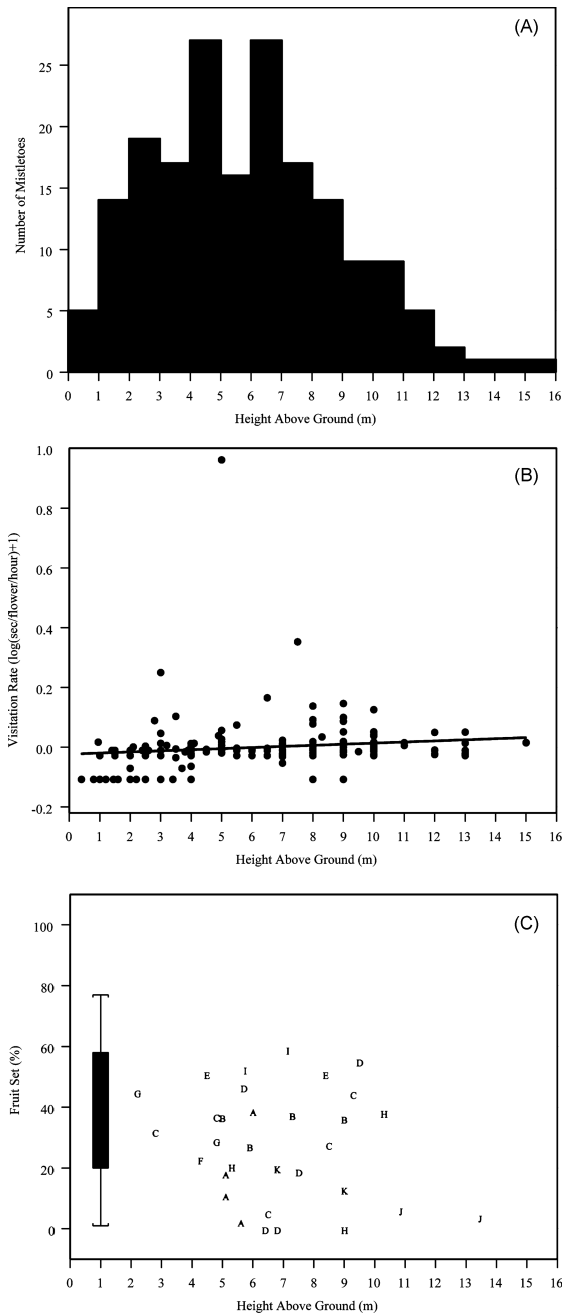


FIGURE 1. *Penaxilla tetrapetala* height distribution and pollination success in Craigieburn Forest Park. (A) The height distribution of all 184 *P. tetrapetala* mistletoes located within eighteen 100 m × 5 m randomly located transects (total area searched = 0.9 ha). (B) The visitation rate by birds (seconds of visit/flower/h) to 179 *P. tetrapetala* plants at Craigieburn in relation to height in 2000–2003. To show the height effect, we first removed variation among years using a GLM, then ran another GLM, which fitted height as a predictor plotted here with the line of best fit. (C) Fruit set as a function of height in 1998. The box plot shows the range of fruit set values obtained from 57 tagged plants used for our normal pollination treatments (all located within 3.1 m of the ground), while letters mark the 32 plants located up the vertical transects accessed by climbing ropes. Shared letters indicate plants that are located on the same host.

deviance explained. The interaction between year and height was significant ($F_{2,173} = 5.8$, $P = 0.003$), with the slope of the height effect being shallower in the second and third years than in the first.

In all, the vertical transects used 11 host trees (A–K) and included 32 mistletoes with an average of 2.9 mistletoes per host (range 1–5). Mistletoe height ranged from 2.1 to 13.4 m with a mean of $6.7 \text{ m} \pm 0.43 \text{ SE}$. Fruit set for these plants varied significantly among host trees ($F_{10,20} = 3.7$, $P = 0.006$), but not with height ($F_{1,20} = 0.91$, $P = 0.35$; Fig. 1C). Percentage of unopened buds (an inverse measure of flower visitation) showed the same pattern: significant effect of host tree ($F_{10,19} = 2.8$, $P = 0.026$), but no effect of height ($F_{1,19} = 0.007$, $P = 0.93$). If flower visitation rates are the main determinant of fruit set rates, then there should be an inverse relationship between percentage of unopened buds and percentage of fruit set; this relationship was present, albeit only marginally significant ($F_{1,29} = 3.8$, $P = 0.062$). All this suggests that flower opening rates (a proxy for visitation), and fruit set rates, do not vary significantly with mistletoe height at Craigieburn.

The 57 low-growing plants (< 3.1 m height) had higher mean fruit set (38.4%; Fig. 1C) than the 32 vertical transect plants (27.8%; binomial GLM, $F_{1,86} = 5.2$, $P = 0.025$), rather than the lower fruit set expected if pollinators visit higher plants more often. This higher fruit set was probably due to nonrandom mistletoe selection (the 57 plants were selected for pollination studies on the basis of being healthy and having sufficient flowers).

Our study system has several advantages for testing whether vertical position in a forest influenced the pollination service provided to flowers: mistletoes flowered over the full range of heights from canopy down nearly to ground level; the forest is monospecific and relatively uniform; and the mistletoes are consistently pollen limited, so fruit set is very responsive to level of pollination. Earlier observations of bellbird behavior by Murphy and Kelly (2003) at our site and by others elsewhere (Rasch & Craig 1988, O'Donnell & Dilks 1994) showed that bellbirds feed less often close to the ground, so we hypothesized that low-growing mistletoes may be more pollen limited than those higher in the trees. In fact, we found no evidence that plant height affected pollen limitation in *P. tetrapetala*. Visitation rate estimates differed: direct observations showed a weak trend for more bird visitation to higher mistletoe flowers, but flower opening rates showed no such trend. Fruit set also showed no trend with height through the full range of heights in this forest.

Two factors probably contributed to the lack of a height effect in this system. First, the large nectar rewards in *P. tetrapetala* (Ladley *et al.* 1997) are highly attractive to bellbirds when available (Murphy & Kelly 2001), so birds seek out even low plants. Second, although birds open the majority of flowers, bees can also be effective pollinators (Robertson *et al.* 2005) and bee foraging preferences are poorly known (Burgess *et al.* 2006). The result is no height effect in pollination service in our system. Hence, the significant pollen limitation previously reported in low-growing plants is confirmed for mistletoes at all strata.

The three-dimensional nature of forests makes investigations of ecological processes occurring above the understory prone to

sampling bias because of the difficulty of sampling the height dimension (Barker & Pinard 2001, Burgess *et al.* 2006). Although we failed to find a height effect, it is important that bias due to variation in pollination with height be considered in other systems, particularly where there is strong vertical stratification (Pearson 1971, Roubik 1993, Bernard 2001, Lim & Engstrom 2001, Schulze *et al.* 2001), and where trees present flowers at a wide range of heights.

Three of four previous studies have found significant height effects. Roubik *et al.* (1982) found no height effects for the bee-pollinated tree *Cochlospermum vitifolium* in Panama, while there were height effects found with bees visiting *Cassia biflora* in Costa Rica (Frankie & Coville 1979) and birds visiting *Metrosideros collina* in Hawaii (Carpenter 1976). Interestingly, Hingston and Potts (2005) showed that birds prefer higher flowers in *Eucalyptus globulus* in Tasmania, resulting in a higher outcrossing rate in seeds from those flowers. Hence, seed quantity and quality may both vary as a result of spatial variation in pollinator behavior.

Including ours, only two of five studies have found no height effect on pollination, but publication bias may under-represent non-significant effects. Paradoxically, on this topic negative results are good news, as they indicate that easily reached low flowers are representative of the entire population. Also, negative results help test hypotheses about where height effects on pollination are most likely. Obviously, if the foraging preferences of the pollinator are known, this may allow some predictions, but in the case of bellbirds on *P. tetrapetala*, the fruit set data refuted predictions based on general (rather than plant-specific) bird foraging data. Based on our results, we propose two hypotheses for further testing: that height effects on pollination are less likely (1) when the flowers are highly rewarding relative to contemporaneous alternative foods; and (2) when there are several alternative pollinators with different height responses. Finally, we note that the same issues arise with fruit dispersal, yet we know of no studies that have tested for a height effect on dispersal. We predict that the same principles outlined here should also apply to height effects on fruit removal rates.

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