SHORT COMMUNICATION

Weather and demographics affect *Dactylanthus* flower visitation by New Zealand lesser short-tailed bats

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Abstract: *Dactylanthus taylorii* is thought to be the only ground-flowering plant to be pollinated by a bat; the New Zealand lesser short-tailed bat (*Mystacina tuberculata*). This unique mutualistic relationship is poorly understood despite both species being threatened. We placed dataloggers around distinct clumps of *D. taylorii* inflorescences to monitor bats with implanted passive integrated transponders (PIT-tags) and quantify visitation rates and demography during the late-summer flowering season. Adult males and juveniles visited flowers more frequently than adult females. Adult males may have visited flowers to feed on nectar and offset the energy demands of advertising, lek defence and mating, and/or reduced foraging time during summer. Juvenile bats may be under increased energetic burdens due to naïve foraging behaviour and use nectar to augment low energy reserves. The frequency of visits correlated positively with mean night ambient temperature, likely because of increased prey, and therefore, bat activity. Our study is the first to examine the demographics of *M. tuberculata* visiting *D. taylorii* and serves as a baseline for future studies considering these unique and vulnerable New Zealand species.

Keywords: *Dactylanthus taylorii*; *Mystacina tuberculata*; nectarivory; parasitic flowering plant; pollination

Introduction

Nectarivory in mammals is limited to one marsupial and three families of bat species (*Pteropodidae*, *Phyllostomidae*, *Mystacindiae*; Richardson et al. 1986; Fleming et al. 2009). The New Zealand lesser short-tailed bat (*Mystacina tuberculata* henceforth *Mystacina*) is a facultative nectarivore, although it is primarily insectivorous (Arkins et al. 1999). *Mystacina* feeds on fruit, nectar and pollen from several plants (Peterson et al. 2006; McCartney et al. 2007; Cummings et al. 2014), and is the primary pollinator of *Dactylanthus taylorii* (henceforth *Dactylanthus*; Ecroyd 1996a).

Dactylanthus is New Zealand's only fully parasitic flowering plant and the only ground-flowering plant in the world to be pollinated by a bat (Ecroyd 1996b). *Dactylanthus* inflorescences emerge from under the soil surface during the flowering period (late February to early May; Ecroyd et al. 1995). Inflorescences are white to pale brown in colour and secretes copious amounts of sweet-smelling, glucose rich nectar (Ecroyd et al. 1995; Haisley Bossard 2015). *Dactylanthus* produce chemicals in the nectar typical of bat-pollinated plants, and is one of the few New Zealand plant species with traits to promote bat visitation (Ecroyd et al. 1995; Ecroyd 1996b).

The flowering period of *Dactylanthus* coincides with *Mystacina*'s singing season. During late summer males spend a third of the night singing to attract females, which likely impacts foraging time and places males under greater energetic pressure than females (Toth 2016). Additionally, juveniles, born earlier in the year, become volant and, due to naïve foraging behaviour, may be under greater energy constraints compared to adults (Rolseth et al. 1994; Adams 1996, 1997; Hamilton & Barclay 1998).

Flying insect activity correlates positively with mild temperatures (i.e. above 10°C) and both species of New Zealand bat increase activity, and likely feeding, with increased mean night ambient temperature, likely due to the interaction between increased temperature and increased abundance of flying insects (O'Donnell 2000; Christie & Simpson 2006). Nectar secretion and concentration can be influenced by factors including temperature and time of day, with greater nectar secretion associated with the active phase of the plant's pollinator (Zimmerman 1988; Corbert 1990). Although there are no data on the effects of weather on *Dactylanthus* nectar secretion, they do secrete the same amount of nectar regardless of the time of day (Ecroyd 1996a) suggesting that they may be less influenced by external factors than other flowers.

Although *Mystacina* are nationally endangered (O'Donnell et al. 2013) and *Dactylanthus* are nationally vulnerable (de Lange et al. 2013), little data are available regarding their mutualistic relationship. Predation by mammals on *Mystacina*, the absence of *Mystacina* in areas where they once occurred, and the decline of other potential pollinators threatens the viability of *Dactylanthus* on mainland New Zealand (Ecroyd 1996b; Wood et al. 2012). Insects may pollinate a small fraction of flowers, however *Mystacina* is thought to be the most important pollinator of *Dactylanthus* (Ecroyd 1996b). Given the importance of *Mystacina* to *Dactylanthus*, it is critical to increase our understanding of this unique relationship. Therefore, the goal of this study was to determine the demography (i.e. male or female, adult or juvenile) of bats visiting flowers during the flowering season. We tested the hypothesis that differences in energetic requirements among different age and sex classes of short-tailed bats would lead to differences in flower visitation. We predicted that adult males

would be the most likely to visit flowers and feed on nectar as the flowering period coincides with the male singing period, and males are likely to be under greater energetic stress than adult females or juveniles. We also tested the hypothesis that flower visitation and presumably nectar feeding, like other types of feeding, will be impacted by weather. We predicted that, like activity in other parts of their range, flower visitation by *Mystacina* would correlate positively with overnight ambient temperature and negatively with amount of rainfall.

Methods

Our study was conducted in the Pikiariki Ecological Area of Pureora Forest Park (38°26'S, 175°39'E), central North Island, New Zealand, during the peak *Dactylanthus* flowering period (February–March 2016). Pikiariki consists of 450 ha of native, mature podocarp-hardwood forests. As part of ongoing monitoring of *Mystacina* populations, it is estimated that over half of the Pikiariki bat population has been marked individually with passive integrated transponder tags (PIT-tags), with 606 tagged bats recorded at roost monitoring sites between October and November 2016. During an annual tagging event in February 2016, untagged adult and juvenile bats were PIT-tagged before the flowering season. Juvenile bats were distinguished from adults by the lack of ossification of the metacarpal-phalangeal joint on the third digit (Davis & Hitchcock 1965).

From 15 February–20 March 2016, we outfitted 15 of the most accessible clumps of *Dactylanthus* inflorescences, which bats were known to visit in the past, with PIT-tag ring antennae (Biomark HPR Plus automatic PIT-tag reader, Biomark, Idaho, USA). Antennae completely encircled inflorescences to ensure detection of visiting bats, and data loggers stored a bat's unique PIT-tag and the time of visit. Antennae took discrete readings every 60 seconds. If we recorded a bat at 00:00:00 and again at 00:01:00 we assumed this was a single visit; however, a reading at 00:00:00 and then at 00:02:00 was recorded as two separate visits. We recorded ambient temperature and rainfall in Pikiariki using a central weather station approximately 2 km from roosts and feeding areas (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Bourne, Massachussets, USA).

To determine whether there was a difference in the frequencies of total bat visitations by different age and sex classes to flowers we used a Pearson's X^2 test, followed by a post-hoc Fisher's exact test on each of the six possible pairwise comparisons (adult female vs. adult male, adult female vs. juvenile female, adult female vs. juvenile male, juvenile female vs. adult male, juvenile female vs. juvenile male) and applied the Bonferroni correction for multiple tests.

To assess the effect of weather on nightly flower visitation while controlling for pseudoreplication (due to multiple recordings from individuals), we used linear mixed effect models and logistic regression using generalised linear mixed-effect models (packages 'nlme' version 3.1-127, 'lme4' version 1.1-10 and 'MuMIn' version 1.15.6 in R version 3.1.0; R Development Core Team 2009). We used a linear mixed-effect model with frequency of visits per night as the response variable, night ambient temperature and rainfall as explanatory variables, and individual as a random effect. We used a generalised mixed-effect model to determine whether the dichotomous response variable (i.e. whether bats did or did not visit flowers) was influenced by ambient temperature and rain.

All analyses were conducted in R 3.1.0 (R Development Core Team 2009) and values are reported as the mean ± SD with *n* representing the number of nights and *N* the number of events. We assessed significance at the $P<0.05$ level.

Results

We recorded 24 tagged bats at flowers (four adult females, six juvenile females, six adult males and eight juvenile males).

We found a significant difference in flower visitation rates among different age and sex classes (X^2=31.94, d.f.=3, $P<0.01$). After post-hoc comparison, we found fewer visits by adult females (4) than expected when compared to adult males (197; $P<0.01$), juvenile females (149; $P<0.01$), and juvenile males (64; P=0.03). We found no difference between juvenile males and juvenile females (P=0.19), adult males and juvenile males (P=0.06), and adult males and juvenile females (P=0.77).

We found that mean night ambient temperature (Z=3.3, P=0.04) and rainfall (Z=-2.0, P=0.047) predicted whether bats did, or did not visit flowers. Further, the frequency of visits correlated positively with mean ambient temperature (T=3.4, d.f.=837, $P<0.01$, R^2=0.22; Fig 1).

Discussion

Our study is the first to examine the age and sex of *Mystacina* visiting *Dactylanthus*. We found mixed results for our first hypothesis. Although adult males visited flowers more frequently than adult females, they did not visit flowers more frequently than either juvenile females or males. Both juvenile females and males visited flowers more frequently than adult females. Frequency of visitation is correlated positively with mean night ambient temperature. Higher night temperatures and lack of rainfall best predict if bats visited flowers.

Mystacina exhibit sexual disparity in the timing of their sexual investment, where males engage in singing, active defence of their singing cavities, and mating during the summer when *Dactylanthus* is flowering. In comparison, female energy expenditure is likely highest when they give birth and lactate during the following spring (Carter & Riskin 2006; Toth 2016). In Pureora during winter, females defend lower body temperature and expend less energy, making them
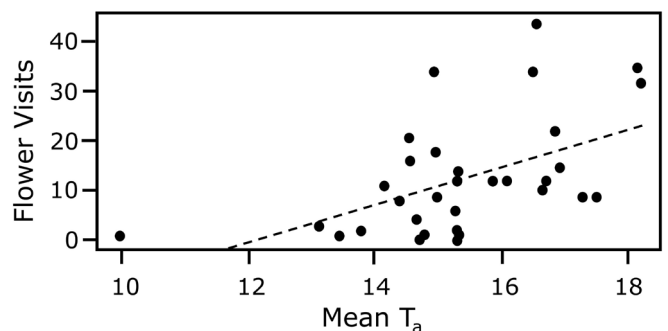
Figure 1. Total nightly visits to *Dactylanthus* by *Mystacina* as a function of mean ambient temperature °C (T_a) in Pureora Forest Park during summer 2016. Visitation increased with increasing mean T_a (T=3.4, d.f.=837, $P<0.01$, R^2=0.22; n=30, N=414).

more likely to enter their reproductive season in good body condition (Czenze et al. 2017a). During the singing season, males are likely under greater energetic pressure than females as they can sing for over a third of the night and actively defend singing trees from other males (Toth 2016). Therefore, singing males will have less foraging time than females and may be at an energetic disadvantage.

Energetic deficiencies affect food preferences (Clutton-Brock et al. 1982; Randolph et al. 1991; Welch et al. 1994; Dearing 1996) and males may be taking advantage of *Dactylanthus* nectar to recoup the energetic costs associated with singing. The large volumes of nectar in *Dactylanthus* inflorescences (0.5 ml per inflorescence per night; Ecroyd 1996a) may provide sufficient carbohydrates to quickly augment energy stores. Moreover, *Dactylanthus* nectar has a high sucrose concentration (200 mg ml^{-1}) (Haisley Bossard 2015) and *Mystacina,* unlike other nectarivorous bats, do not need to engage in expensive hovering flight to feed because they are adapted to feed on the ground (Carter & Riskin 2006). *Dactylanthus,* when flowering, may represent a stable energy source and males may optimise their energy intake by reducing foraging time. Indeed, at a different flowering site, three of four bats tracked visited a single *Dactylanthus* patch (Ecroyd 1996b). It would be useful for future studies to investigate the energetic costs of singing and how they may be offset by nectarivory.

Juvenile bats, regardless of sex, also visited flowers more often than adult females. Juvenile *Mystacina* become volant in late January–early February, and other aerially hawking bat species are inefficient at capturing flying insects in their first year compared to their adult counterparts (e.g. McGuire et al. 2009). Further, juvenile little brown bats (*Myotis lucifugus*) have more difficulty than adults accumulating fat stores during their first year, and may have higher energetic costs than adults (McGuire et al. 2009). For bats, metabolic rate while flying can be 10–15 times higher than resting (Speakman & Thomas 2003) and, despite identical fuelling indices, juvenile *M. lucifugus* lost mass while adults gained mass (McGuire et al. 2009). This difference suggests that juveniles gather the same amount of nutrients as adults but greater foraging effort is required due to their lower foraging efficiency (Davis & Hitchcock 1965; McGuire et al. 2009). Average forearm length of juvenile bats captured in Pureora during the flowering period was no different than adults; however, juveniles had lower mass and poorer body condition than adults (ZC unpubl. data). Therefore, juveniles may be finished growing during the flowering season, but may not be as efficient at capturing insects and obtaining their nightly nutrients. It may be that juveniles are under greater energetic pressure than adults and *Dactylanthus* nectar represents a high-energy/low foraging cost food source that bats can exploit to either fuel extended foraging times or augment low energy reserves.

Energetically stressed individuals appear to prefer high quality/energy foods to maximize energy or nutritional intake (Clutton-Brock et al. 1982; Randolph et al. 1991; Dearing 1996; Orr et al. 2016). We contend that during the flowering season adult females are under comparatively lower energetic pressure than adult males or juveniles. However, even if *Dactylanthus* represents such an attractive food source for adult male and juvenile bats, why did we record so few adult females at *Dactylanthus*? In part, nutrient composition may be driving diet choice. When given the choice between fruit and insects, dark capped bulbuls (*Pycnonotus barbatus*), an avian frugivore that opportunistically takes insect prey,

preferentially chose insects over fruit (Downs 2008). However, due to foraging costs, silvereyes (*Zosterops lateralis*) are unable to meet energy demands with a solely insectivorous diet and use a mixed diet of fruit and insects to achieve their energy and nutrient balance (Catterall 1985). It may be that insects, although more energetically costly to obtain, provide adult females with the macronutrients required to maintain long-term energy balance. Future studies should investigate the nutritional content of *Mystacina's* insect prey to elucidate what drives dietary preference in an omnivorous bat. Until further work is conducted on this system, the explanation for low female visitation rates remains a mystery.

Lack of rainfall and mean night ambient temperature were the best predictors of visitation and night temperature was correlated positively with nightly visitation. In other parts of New Zealand, bat activity was correlated positively with ambient temperature that likely reflects increased prey availability (Daniel 1979; O'Donnell 2000; Christie 2006; Christie & Simpson 2006). In the South Island, *Mystacina* activity was correlated negatively with rainfall (Christie & Simpson 2006). If bats were using nectar to offset an energetic deficiency caused by low insect abundance, we might expect to have recorded higher visitation rates during unfavourable nights (i.e. lower ambient temperature or higher rainfall) when foraging success was predictably low. However, the positive correlation between visitation and temperature that we observed suggests that bats may be avoiding foraging in cold wet weather (i.e. using torpor; Czenze et al. 2017b) and using nectar as a high energy, easily digestible food source to fuel foraging. It would be useful for future studies to investigate the timing of flower visitations in relation to singing and foraging to understand how/if nectarivory impacts *Mystacina*'s nightly active period.

The relationship between nectar secretion and weather is complex and dependent, among other things, on the pollination system of the plant; however, generally nectar secretion is decreased at colder temperatures (Corbet 1978). This may be a strategy by the plant to lower its energetic costs during periods of unfavourable weather and fewer pollinators (Castellanos et al. 2002). Future studies should aim to measure the volume of nectar under different ambient conditions to determine if *Dactylanthus* reduces nectar secretion during periods of low bat activity and whether this further reduces bat visitation during inclement weather.

Our study sheds light on the unique mutualistic relationship between *Dactylanthus* and *Mystacina* and provides the first evidence of sex and age biased visitation rates during the flowering period. More work is required to elucidate the energetic benefits and potential costs of nectarivory during the singing season.

Acknowledgements

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