Inter- and intraspecific priority effects mediate fruit set and fruit quality in two mass-flowering berry crops

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**Abstract**

**Introduction**

Priority effects are defined by the influence that the order in which species arrive within a community, and variation among first migrants among sites, as well as how these species interact, lead to divergent succession among sites influence species co-existence. This phenomenon has been observed in microbial bacteria communities (REFS), and can be mediated by environmental variability (Tucketr & Fukami 2014) as well as disturbance (Jiang & Patel 2008). Although these factors may facilitate and counteract the influence of priority effects, overall, priority effects are ecologically meaningful in explaining the formation of ecological communities, species turnover between communities and functional differences within meta-communities.

The presence of priority effects in floral generations of nectar microbial metacommunities (Toju et al. 2018), demonstrates that priority effects may influence pollination and plant fitness at the microbial level. However, at present, no research has centred on the influence of priority effects at the macro-ecological scale, such as in order of floral visitation among species and their interactions, leading to differential pollination success.

Animal-mediated pollination is primarily a process governed by floral visitor/pollinator communities, and its species composition, interacting sequentially upon a given plant species, which can impact pollination outcomes. As such, the directionality and order of these plant-pollinator interactions may have important impacts upon measures of pollen limitation and pollination (e.g. fruit/seed set and/or fruit weight). Further, any influence of these processes are likely also a function of floral morphology (e.g., the presence and location of floral resources and rewards) interacting with species functional traits (REF).

By potentially mediating pollination outcomes, priority effects likely manifest at both intraspecific and interspecific level. We hypothesise that differences between foraging types of eusocial bees (i.e. pollen or nectar foragers) could facilitate pollination outcomes. For example, the displacement of pollen by pollen-foragers may lead to greater overall pollination by subsequent floral visitors, independently of their forager-type. Second, interspecific priority effects could manifest through the influence of differential species behaviour during floral visits. In both cases, these effects are likely a result of not only pollinator behaviour or foraging type, both also floral structures, which may benefit or negate the influence of the first visitors.

In this study, we examine, firstly, whether priority effects manifest themselves in intra- and interspecific floral visitor `communities`, as a response of behavioural differences among pollinators interacting with two crop species, blueberry (*Vaccinium* sp.) and raspberry (*Rubus* sp.). Second, we hypothesise that priority effects within floral visit communities are diluted as floral visitation rates increase, suggesting that priority effects are strongest under higher pollen limitation. Lastly, by comparing these two plant species, which are highly morphologically dissimilar in, we test if priority effects in floral visitation are mediated by floral visitor identity, behaviour as well as floral structure and we assess how floral structure can mediate or negate facilitation by the first floral visitor.

**Materials and Methods**

**Study site**

This study was undertaken on the North Coast, New South Wales, Australia on a large-scale commercial berry farm located at Dirty Creek (29.990232°S, 153.143171°E) across two flowering seasons in 2017 and 2018.

**Plant species and floral structure**

Blueberry flowers are urceolate in shape, small (corolla length = 11mm and aperture = 3.5mm (Lyrene 1994)) and hermaphroditic. In open flowers, the anthers remain enclosed within the corolla whereas a single stigma protrudes slightly above the corolla aperture. In contrast, raspberry flowers are hermaphroditic but more open in floral display, with X~X stigmas encircled by X-X anthers. We focussed our study on two blueberry species: ‘Southern Highbush’ (SH) (*Vaccinium corymbosum,* cultivar Snowchaser) and ‘Rabbiteye’ (RE) (*V. ashei*, cultivar: Powder Blue), and one raspberry species: *Rubus idaeus,* cultivar: Driscoll Cardinal.

A figure of each would be nice here I think.

**Floral visitation**

We placed organza bags over developing shoots and tagged developing flowers in both plant species. We un-bagged flowers upon opening and observed the two dominant pollinator species present: the European honeybee, *Apis mellifera,* and the native stingless bee, *Tetragonula carbonaria,* visit designated flowers between one to 15 times in blueberry and one to 29 times in raspberry. For every visit, we noted foraging behaviour (i.e. pollen or nectar foraging on the basis of the presence of corbiculae). Once completed, these flowers were then re-bagged for duration of flowering. Fruit set was estimated one month after flowering (in blueberry only due to parthenocarpic fruit production in raspberry), and fruit weight was measured in grams in both species on an analytical balance upon ripening.

**Data analysis**

All analyses were conducted in *R* v3.5.1 (R Core Team, 2018). We specified generalised linear mixed models (GLMM) using *glmmTMB* (v.0.2.3, Brooks et al., 2017).

*Interspecific priority effects*

We estimated fruit set or fruit weight as a function of the first floral visitor in a three-way interaction with the proportion of honey bee visits and the total number of floral visits along with two crossed random terms in blueberry: *block* and *year*, and one random term: *block* in raspberry.

*Intraspecific priority effects*

We first subsetted our data to single-species compositional visits (i.e. only honeybees or stingless bees). We then estimated fruit or fruit weight as a function of the first floral visitor’s behaviour (i.e. pollen or nectar foraging) in interaction with proportion of pollen forager visits and total number of floral visits. Again, in blueberry, we included two crossed random terms: *block* and *year,* and in raspberry, one random term: *block*.

For both intra- and interspecific analyses, we modelled fruit set with a binomial distribution and fruit weight with a Gaussian distribution and assessed model residuals using *DHARMa* (v.0.2.2, Hartig 2019).

**Author contributions**

LK and RR devised and designed the study. LK and MM collected data. LK, JS and VG undertook all data analysis. LK and JS wrote the manuscript and all authors contributed substantially to the final version.