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 as the impact that the order in which species arrive within a community, and how these species interact, lead to divergent successional trajectories among sites and influence species co-existence. Priority effects can be mediated by environmental variability (Tucker & Fukami, 2014) and disturbance (Jiang & Patel, 2008) although many causes are still unknown. One way in that environmental viability influence priority effects through differences and changes in species growth rates among species; species with higher growth rates modify habitats that either hinder or facilitate the establishment of late arriving species through the pre-emption or modification of habitat resources (de Freitas & Frederickson 1978; Tilman 1980; Facelli & Facelli 1993; Loeuille & Leibold, 2008). Importantly, such responses are mediated by each species’ sensitivity to environmental variability (Chesson 2000).

Much of the research into priority effects has focussed on microbial bacteria communitiesspecifically in floral nectar microbial communities (e.g., Peay, Belisle and Fukami 2012; Tucker & Fukami 2014; Toju et al. 2018), however their importance has been observed in larval competition among frog species (Alford & Wilbur 1985), as well as coral reef fish communities (Shulman et al. 1983; Almany 2003). Interestingly, studies detailing priority effects in floral bacteria communities have provided evidence floral bacterium influence pollination through the reduction of pollinator foraging and nectar consumption, leading to reduced seed set (Vanette et al. 2013; Good et al. 2014).

Priority effects may influence other pollination-related ecological processes. Animal-mediated pollination is primarily a process governed by floral visitor/pollinator communities, and its species composition, interacting sequentially upon a given plant species (i.e. a floral visitation sequence). In this case, a priority effect may be the result of the behaviour or identity of the first visitor to a flower and this may have important impacts upon measures of pollination success (e.g. fruit set and fruit weight). Further, this effect may also, hypothetically, be mediated by floral morphology (e.g., the presence and location of floral resources and rewards) interacting with pollinator identity and behaviour. As such, these effects likely manifest at both intraspecific and interspecific levels. Previous studies (e.g., Vanette & Fukami 2014) have suggested that priority effects may be stronger at the intraspecific level, as a result of increased niche overlap and resource limitation being greater between individuals of the same species than conspecific species (Tilman 1982), however neither intraspecific or interspecific priority effects has not been tested in floral visitation sequences.

We hypothesise that the division of labour (i.e. pollen or nectar foragers) in eusocial bees could facilitate or negate pollination outcomes, due to their distinct behavioural interactions with flowers. For example, the displacement of pollen by pollen-foragers may lead to greater overall pollination by subsequent floral visitors, independently of their forager-type. Alternatively, different forager types may deplete floral resources, leading to reduced effectiveness of subsequent visitation events. Second, interspecific priority effects could manifest through the influence of each species’ distinct 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 and mating systems, for example where species differ in their pollen display (e.g., plant families such as Ericaceae which possess poricidal anthers which require vibration or buzz-pollination to release pollen as opposed other taxa, such as Rosaceae, which possess anthers laden on the outer surface) which may benefit or negate the influence of the first visitors as well as pollen incompatibility systems (i.e. the requirement autogamous (same flower), geitonogamous (clonal pollination) or allogamous (cross) pollination for plant reproductive success).Lastly, priority effects in floral visitation sequences may also be stronger under higher degrees of pollen limitation (i.e. shorter floral visitation sequences), declining in longer visitation sequences due to increases in pollen deposition.

In this study, we examine, firstly, whether priority effects manifest themselves in intra- and interspecific floral visitation sequences, as a response of behavioural differences within and between pollinators of two crop species, blueberry (*Vaccinium* sp.) and raspberry (*Rubus idaeus*). Second, we hypothesise that priority effects within floral visitation sequences 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 floral structure, we test if priority effects in floral visitation sequences are further mediated by floral structure differences interacting with pollinator behaviour.

**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**

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. Both blueberry and raspberry flowers are hermaphroditic but differ considerably in shape and displaye. Blueberry flowers are urceolate, and 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 dish-shaped, but open in floral display, with ~100 stigmas encircled by ~100 anthers (Driscoll Cardinal Patent).

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 the raspberry cultivar being highly self-fruitful (Driscoll Cardinal Patent), 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 set or fruit weight as a function of the first floral visitor’s behaviour (i.e. pollen or nectar foraging) in interaction with the 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 conceived the study and designed the experiments. LK and MM collected data. LK, JS and VG analysed these data. LK and JS wrote the manuscript and all authors contributed substantially to the final version.

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