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

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**Abstract**

**Introduction**

The compositional trajectory of ecology communities is often strongly influenced by the order of species arrival to those communities, a phenomenon termed “priority effects” (REF). Priority effects often determine how species interact and compete with each other within communities, and can drive divergent successional trajectories among sites. Priority effects can be mediated by environmental variability (Tucker & Fukami, 2014) and disturbance (Jiang & Patel, 2008), although many causes are still unknown. Environmental viability can 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).

The majority of studies on priority effects have focussed on microbial communities, specifically bacterial and yeast communities that occur in floral nectar (e.g., Peay, Belisle and Fukami 2012; Tucker & Fukami 2014; Toju et al. 2018). However, priority effects are also an important driver of competition among frog species (Alford & Wilbur 1985) and in coral reef fish communities (Shulman et al. 1983; Almany 2003). Interestingly, studies detailing priority effects in floral bacteria communities have shown that floral bacterium can reduce pollination success by reducing pollinator foraging and nectar consumption (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) suggest that priority effects may be stronger at the intraspecific level because niche overlap and resource limitation is often greater among individuals of the same species than among species (Tilman 1982).

We hypothesise that the division of labour (i.e., pollen or nectar foraging) within eusocial bee species could facilitate or negate pollination success, due to the distinct behavioural interactions of each forager type with flowers. For example, the displacement of pollen by pollen foragers may lead to more effective pollination by subsequent floral visitors, regardless of their foraging behaviour. 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, priority effects are not only likely to be driven by pollinator foraging behaviour, but also differences in floral structure and mating systems among plant species. For example, species in the plant family Ericaceae have poricidal anthers that require vibration or buzz-pollination to release pollen, whereas other plant taxa have freely available pollen and do not require buzz-pollination (REF), 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, floral visitation sequence priority effects may be stronger for flowers that are highly pollen limited (i.e. less abundant pollinator communities resulting in shorter floral visitation sequences), declining in longer visitation sequences due to increases in pollen deposition.

Here, we examine whether there are priority effects in sequences of floral visitation, both within and among insect species, for two crop species (blueberry: *Vaccinium* *corymbosum* and raspberry: *Rubus idaeus*) that have different floral morphologies. Then, test whether floral visitation sequence priority effects are diluted as floral visitation rate increase. Finally, we test if floral visitation sequence priority effects are mediated to some degree by differences in floral structure among plant species.

**Materials and Methods**

**Study site**

We conducted this study 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; May 2017 and 2018 for blueberry and February-March 2017 for raspberry.

**Plant species and floral structure**

We focussed on one blueberry species: ‘Southern Highbush’ (SH) (*Vaccinium corymbosum,* cultivar Snowchaser) and one raspberry species: *Rubus idaeus,* cultivar: Driscoll Cardinal. Both blueberry and raspberry flowers are hermaphroditic but differ considerably in shape and display. 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 blueberry and raspberry. We un-bagged flowers upon opening and observed the two dominant pollinator species (European honeybees, *Apis mellifera,* and native stingless bees, *Tetragonula carbonaria*) visit designated flowers. This resulted in sequential visits ranging from one to 15 visits in blueberry and one to 29 visits in raspberry. For each visit, we recorded foraging behaviour (i.e., pollen or nectar foraging) based on the presence of corbiculae. Once visited, each flower was re-bagged for duration of flowering to prevent further visitation. Fruit set was counted one month after flowering as the presence of absence of a developing fruit (in blueberry only due to the raspberry cultivar being highly self-fruitful (Driscoll Cardinal Patent)), and fruit weight was measured in grams (accuracy: 0.00g) 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 and 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 (categorical; honeybees or stingless bees). We then estimated fruit set or fruit weight as a function of the first floral visitor’s behaviour (categorical; pollen or nectar foraging) in interaction with the proportion of pollen forager visits (continuous) and total number of floral visits (continuous). Again, in blueberry, we included one random terms: *year,* as visitation data were collected in the block across two years,and in raspberry, one random term: *block,* as data were collected across multiple blocks.

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).

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**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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