**Title:** 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 (29.990232°S, 153.143171°E) across two flowering seasons; 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 have an open floral display, with ~100 stigmas encircled by ~100 anthers (Driscoll Cardinal Patent).

A figure of each would be nice here I think.

**Floral visitation**

For both blueberry and raspberry, we placed organza bags over developing shoots and tagged developing flowers before opening. We un-bagged flowers upon opening and observed visits from the two dominant pollinator species (European honeybees, *Apis mellifera* and native stingless bees, *Tetragonula carbonaria*). This resulted in sequential visits from honeybees (number of visits range: x-x), stingless bees (number of visits range: x-x) or a mixture of honeybees and stingless bees (number of visits range: x-x). For pollinator each visit, we recorded the foraging behaviour of the individual (i.e., either pollen or nectar foraging) based on the presence or absence of corbiculae (pollen basket on the hind leg as a result of pollen collection). Once visited for the target number of visits, each flower was re-bagged for the duration of flowering to prevent further visitation from animal pollinators. We measured fruit set as the presence or absence of a developing fruit, one month after flowering finished. This was only possible in blueberry because the raspberry cultivar was highly self-fruitful (Driscoll Cardinal Patent). We also measured fruit weight (grams) in both species using an analytical balance (accuracy: 0.00 g) upon fruit ripening.

**Data analysis**

**Do interspecific pollinator priority effects mediate fruit weight in different plant species with distinct floral structures?**

We subsetted our dataframe to only include fruit produced as a result of mixed pollinator visits (visits to flowers from both honeybees and stingless bees). We then categorised each fruit as a being produced from a flower that was initially visited either by a honeybee or a stingless bee. Using this data structure, used a generalised linear mixed effect model (GLMM) to estimate fruit weight as a function of the initial floral visitor (honeybee or stingless bee; categorical) in a three-way interaction with the total number of floral visits (continuous) and ratio of honeybee to stingless bee visits (continuous). We included two crossed random effect terms in the blueberry model (block and year; categorical) because visitation data were collected in same cropping block across two years, and one random effect term in the raspberry model (block; categorical) as data were collected across multiple cropping blocks. We then conducted model selection on this model using the “MuMIn” package (REF) and found that, in both blueberry and raspberry, the best model for predicting fruit weight included the initial floral visitor and number of floral visits terms and their interaction. Accordingly, we present model-estimates and slope contrasts for this reduced model. To calculate slope contrasts (i.e., whether there is a significant difference in model-estimated slopes for fruit weight between flowers according to the identity of their initial visitor) we used the “emmeans” package (REF). Thus, evidence of a pollinator priority effect is indicated by a significant difference in the model-estimated slope for fruit weight as a function of the number of pollinator visits between the two initial- visitor categories (i.e., honeybee or stingless bee).

**Does a mixture of floral visits from different pollinator species result in heavier fruits than visits from a single species?**

*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.

We specified generalised linear mixed effects models (GLMM) using the *glmmTMB* package (v.0.2.3, Brooks et al., 2017). For both intra- and interspecific priority effects analyses, we modelled fruit set with a binomial distribution and fruit weight with a Gaussian distribution. We assessed model residuals using the *DHARMa* package (v.0.2.2, Hartig 2019). All analyses were conducted in *R* v3.5.1 (R Core Team, 2018).

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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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