



Pollen dispersal in a population of *Passiflora caerulea*: spatial components and ecological implications

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Abstract Sexual reproduction in Angiosperms mostly depends on pollen transfer mediated by an agent. In plants pollinated by biotic agents, reproductive success and population structure are mainly determined by pollinator behavior. To further comprehend this aspect of the reproductive process, data regarding pollen transport and deposition patterns need to be gathered and analyzed. The aim of this study was to examine pollen flow dynamics in a population of the *Xylocopa* bee-pollinated *Passiflora caerulea* using fluorescent powdered dyes as pollen mimics and to analyze the relationship between inter-plant distance and fruit production. Plant arrangement,

number of flowers per plant, pollen flow and fruit set exhibited leptokurtic distributions as a function of the distance between plants. Minimum and maximum dispersal distances were 4 m and 257 m, respectively. Geitonogamous transfers were more frequent than xenogamous ones. Pollinator movements, evidenced by pollen analogues transferences, responded to plant arrangement. *Centris* sp. was recorded for the first time as a pollinator of *Passiflora caerulea*. Fruit set decayed as distance between individuals increased. The tools employed allowed to better understand the dependence between plant population structure, pollinator movement, pollen flow and fruit production, as well as to generate recommendations for crop spatial design of this promising and self-incompatible species, which will counteract the effects of harvesting from the wild.

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Introduction

Pollen vectors can carry pollen across considerable distances. However, the resultant pollen dispersal pattern is not necessarily the one that maximizes the quantity and quality of mating between plants (Harder and Barret 1996). Reproductive success in plants also

depends on being able to overcome self-incompatibility barriers, to develop fruits with viable seeds, to germinate seeds, and to establish seedlings in a suitable location. Nevertheless, pollen flow is often the major contributor to genetic exchange and therefore, a key parameter for conservation and population biology of plants (Van Rossum et al. 2011).

Pollen flow between conspecific flowers relies on several factors. Among them: microclimate, individual plant physiological and pathology state, population size, density, shape, spatial arrangement (Handel 1983), phenology, floral morphology, quantity and availability of rewards (Linsley 1978; Goulson 1999). In addition, it depends on pollinators' foraging habits and energy requirements, which in turn, respond to certain plant characteristics (Mayer et al. 2012). Pollinators are also influenced by other components of communities and ecosystems. Pollinator abundance, and consequently, the rate at which pollinators visit flowers, are determined by the availability of sympatric species floral resources (with the exception of monoleptic and monotropic pollinators, which rely upon only a single species for pollen or nectar, respectively); available nesting sites, habitat fragmentation or use of insecticides are influential as well (Goulson 1999). Pollinator behavior is affected by their activity period (diurnal and seasonal), pollinator preference for certain foraging sites, and pollinator activity at the flowers visited (search for reward, mating) (Linsley 1978). Both visitor frequency and foraging behavior at flowers can be influenced by interactions with other organisms (other pollinators, nectar and pollen thieves or robbers, insect predators) (Sazima and Sazima 1989; Goulson 1999). Finally, seasonal and inter-annual fluctuations on weather conditions affect flowering, anthesis and pollinator activity. In response, all the aforementioned factors can change over time and space (Goulson 1999), particularly on highly heterogeneous areas. This may result on mating or, by contrast, on spatio-temporal isolation of certain plants, either within or among populations.

Pollinator flight patterns are highly sensitive to several ecological factors and behavioral differences among pollinators (Handel 1983). However, studies with pollen markers or pollen analogues using different pollinator taxa and different environments have shown that mean pollen dispersal distances are relatively short (Waser and Price 1983; Girón

Vander-Huck 1984; Nilsson et al. 1992; Christensen and Olsen 1998).

Accordingly, pollen dispersal (measured as either amount of receptor flowers or deposited pollen), predicted by dispersal models (Mayer et al. 2012) or estimated using pollen analogues or even paternity analysis (Van Rossum et al. 2011) decays with distance. This results in leptokurtic curves (Waser and Price 1983; Nilsson et al. 1992; Van Rossum 2009; Van Rossum et al. 2011) with most of the pollen deposits occurring at short distances with only a few events at longer ones (Waser and Price 1983; Van Rossum et al. 2011).

Movements of pollen grains from specific sources are difficult to trace because pollen deposited on a target may have come from any direction (Handel 1983). To overcome this issue, different methods have been used: fluorescent powders (Price and Waser 1979; Waser and Price 1982, Girón Vander-Huck 1984, Thomson et al. 1986; Waser 1988; Christensen and Olsen 1998; Van Rossum and Triest 2010; Van Rossum et al. 2011), histochemical dyes that stain pollen grains (Peakall and Beattie 1991) and micro-labels in pollinia (Nilsson et al. 1992). However, fluorescent powder is usually used. In this procedure, it is assumed that the powder mimics the pollen and is carried in the same way by pollinators (Kearns and Inouye 1993). This method is a good estimator of real pollen dispersal distances because it is reliable, fast, and economical and it can be applied in natural or experimental populations of plants with biotic pollination (Handel 1983; Van Rossum et al. 2011). Moreover, pollen flow dynamics have been used as a measure of alteration and fragmentation of urbanized ecosystems. For instance, pollen flow reflects the connectivity between patches and may be applied to identify key components for managing and establishing corridors between populations or population fragments (Van Rossum and Triest 2010).

When a biotically pollinated plant is cultivated to harvest fruits and/or seeds, many other factors should be considered as well. Besides knowing the plant breeding system, identity of their pollinators' and pollinator behavior (not only among flowers of the same plant, but also of different ones), it is necessary to know the extent of pollen dispersal, whether the interest is to keep lines isolated or to maximize genetic exchange (Handel 1983), or even to maximize productivity.

Passiflora caerulea L., probably the best-known species of the genus and bred for its ornamental interest in U.S.A. and Europe (Ulmer and MacDougal 2004), is native from Brazil to central Argentina. In the latter country, it is the most widely distributed *Passiflora* species and the only one that grows at the southern extreme of the distribution range of the genus (Deginani 2001). It develops successfully in xerophytic areas as well as in wet forests (especially at forest edges), growing on modified sandy, clayey or rocky soils, from sea level up to 1400 m. This climber spreads over other plants and over wire fences at path edges and railways, both in rural and urban areas (Mendiondo and Amela García 2006). It possesses medicinal properties (Alonso and Desmarchelier 2006) and edible fruits (Ragonese and Martínez Crovetto 1947). In Argentina, it is not commercially grown, rather harvested from the wild without reposition (Deginani 2001). This, together with an increasing urbanization rate, make wild populations declining and losing their genetic variability (Aquino 2014; Amela García, unpubl. data). Moreover, this has a negative impact on the specific fauna associated with this species, as with most of the passion vines (Amela García 1999).

Considering the above information, understanding pollinator flight patterns and potential pollen pathways in populations of *P. caerulea* may be useful to estimate optimal distances for sowing and harvesting this valuable self-incompatible species without compromising population connectivity. In this context, the objectives of this paper were to describe the spatial arrangement of plants in a population of *Passiflora caerulea* through point pattern analysis, to analyze pollen dispersal within the population using fluorescent dye particles as pollen analogues and to assess whether fruit set depends on distance of a plant to its conspecifics. The following hypothesis lead the analysis: (a) pollen transfer distances of *P. caerulea* follow a leptokurtic distribution, (b) fruit production of *P. caerulea* decays as distance between conspecifics increases.

Materials and methods

Study species

In Argentina, *Passiflora caerulea* blooms between August and late May (Amela García 1999). It is self-

incompatible (Amela García and Hoc 1997) and needs specific pollinators to produce fruits with seeds: bees of the genus *Xylocopa* (Hymenoptera, Apidae): *X. augusti*, *X. frontalis*, and *X. nigrocincta*, which are polytrophic and polylectic, i.e., they collect nectar and pollen from phylogenetically non-related sources (Amela García and Hoc 1997). When an individual visits a *Passiflora* flower, pollen is passively deposited on the back of its thorax, but some of those grains are actively transferred to the collector apparatus in the hind legs (Amela García 1999). On the other hand, little is known of the home range of these carpenter bees (Roig, pers. com.). *Xylocopa augusti*, the main pollinator, hibernates between April and August in southern Brazil (Sakagami and Laroca 1971); a similar behavior might occur in Argentina.

Anthesis of *P. caerulea* flowers consists of three stages, mainly determined by the movement of the floral pieces (Amela García and Hoc 1997), the most important being that of the stigmata whereas the anthers are available throughout the whole anthesis. During phase 1, stigmata are erect, away from pollinator access, and flowers act only as pollen donors. In phase 2, stigmata bend down to the level of the anthers so pollinators can mediate both pollen deposition and removal. Entering phase 3, stigmata rise distant from pollinators' access, so the flowers become donors of the remnant pollen for the rest of anthesis. Succession of these stages occurs during a single day: floral buds open around 10 a.m. and close by 6 p.m. The pollen is viable for more than one day (Amela García 1999; Amela García and Hoc 1997).

Study population

The population of *P. caerulea* at Ciudad Universitaria campus (C.U.) of the Facultad de Ciencias Exactas y Naturales (FCEyN), Universidad de Buenos Aires (UBA), Ciudad Autónoma de Buenos Aires, Argentina (34°38'S, 58°28'E) was studied between March 2013 and March 2014. *P. caerulea* individuals grew over perimeter fences (as do in rural areas) or, rarely, over other kind of supports such as trees, fallen trunks or posts. Utilization of a semi-urban population allowed revealing patterns more difficult to ascertain in a natural one. Fences provided an easier way to identify plant individuals and measuring distances between them. Apifauna visits exhibited a high frequency probably because of undisturbed patches

Table 1 Date and meteorological conditions of each essay

Assay	Date	Temperature (°C)	Humidity (%)	Irradiance/precipitation	Wind speed (km h ⁻¹)	Wind direction
1	12/02/2013	33.5	81	Cloudy—late heavy rain	11.3	N
2	12/10/2013	23.6	63	Sunny	24.1	SE
3	12/16/2013	31.9	61	Sunny	11.3	E
4	03/20/2014	22.8	68	Partially cloudy	8	NNE
5	03/25/2014	23.6	62	Partially cloudy	8	SE

Data provided by “Grupo de pronóstico del Departamento de Ciencias de la Atmósfera y los Océanos”, FCEyN, UBA

inside the Campus and because of its geographical location: edge of Buenos Aires city, near the river coast, adjacent to a natural reserve (Reserva Ecológica Ciudad Universitaria-Costanera Norte). At the beginning of the flowering season, we inspected all fences in C.U. campus (2497.27 m total length) to locate and identify all individuals of *P. caerulea*. We registered the location of each blooming individual with a GPS (Garmin, eTrex Legend®). Thereafter, we assigned an identification number and spatial coordinates to each plant, which served to map their spatial arrangement.

Distances between conspecific plants

Distances between each pair of spatial points were estimated, which represented a blooming individual. In addition, the nearest neighbor for each point was calculated. We tested for spatial randomness in the population using the Hopkins and Skellam Test (Hopkins and Skellam 1954). This test compares nearest-neighbor distances with randomly generated point-point distances. The proportion parameter H indicates randomness ($= 1$), spatial clustering (< 1) or regularity (> 1). Point pattern analysis was performed on RStudio: the *spDists* function (*sp* package) was used to compute the distances between each pair of points and *hopskel.test* (*spatstat* package) to perform the Hopkins–Skellam Test.

Throughout the study period, the amount and identity of flowering individuals shifted. Therefore, we computed all distances between flowering plants separately for each census in which pollen dispersal and fruit production experiments were performed (December 2013 and March 2014).

Pollen dispersal assays

Each pollen dispersal assay was performed on two consecutive days. Assays were scheduled for days with promising meteorological conditions for pollinator activity. Weather condition reports (corresponding to 2 p.m., when deposition of the pollen analogues was finished) were obtained from the Meteorological Station of the FCEyN (Table 1).

Day 1. Census and marking

At the beginning of anthesis, all blooming individuals on that day were identified, and all flowers on each individual (potential recipients) were numbered with a hard paper tag around their pedicels. Pollen donors were selected considering that donor location included a wide range of potential dispersal distances between donor and recipients of pollen analogues. The dehiscent face of the five anthers of each donor flower was carefully and completely powdered with fluorescent powder dye using a wooden flat stick. Previous exploratory analyses showed a minimal loss of fluorescent powder as it adhered quite well to the exposed pollen until saturation of the anther. Different colors (2–4) were used for different donor plants.

Passiflora deposits pollen on the thorax dorsum of *Xylopa* where these insects cannot remove it. When they groom the pollen off their body and transfer it to their scopas, they reach the edges of the thorax dorsum, but not the center. It is quite common to see *Xylocopa* specimens arriving to or departing from plants with a large yellow spot on its thorax dorsum (at least on the center of the thorax). It was possible to observe that the dyes were deposited in an equivalent manner as the pollen would have been deposited on the bee thorax dorsum, leaving a spot of different color that the bees did not remove at least before abandoning the plant.

Besides, the fact that dyes were encountered on recipient plants (that were not marked), evidenced that bees at least kept enough dye to deliver between plants.

Day 2. Collection, observation, and recording of pollen analogue deposition

The day after anthesis, all flowers identified the day before as potential recipients were collected and placed in paper bags. In the laboratory, each flower was inspected under a UV light with a magnifying glass for the presence of fluorescent powder dyes. According to the color of the pollen analogue on each of the collected flowers, the donor plant was identified. No dye fell off the stigmata, as they were protected by the incurved perianth of the closed flowers.

Floral visitors' movements and identities

The following data for each pollinator that visited a flower was recorded during 15 min on each plant after powdering the anthers with pollen analogues (between 11 a.m. and 2 p.m.): taxonomic identity, direction of arrival, deposition of powder dyes on its back, number of visited flowers on the same plant, and direction of departure.

collected, and the quantity of fruits produced counted. Many neighboring plants were closer than 5 m away from each other. Because of their climber habit, some of their foliage overlapped. Therefore, a 5 m minimum distance was established to consider plants apart. Data were grouped in 5 m intervals, determined by the plant they belonged to and the distance to the nearest conspecific neighbor in blossom. Then, a fructification percentage was calculated for each interval. Afterward, linear dependency between fructification percentage and the distance to the nearest conspecific blooming plant was tested. Lastly, differences between fructification percentages among populations were analyzed through Fisher's Exact Test.

Geitonogamy and Xenogamy

Geitonogamy (G) and xenogamy (X) were estimated for each plant and assay, respectively, applying the formulas below. Afterward, a mean was calculated for the whole population.

Formula 1. Geitonogamy:

$$G = \frac{\text{Number of effective recipient flowers whose dye colour matches the plant it belongs to}}{\text{Total number of potential recipient flowers in the donor plant}}$$

Formula 2. Xenogamy:

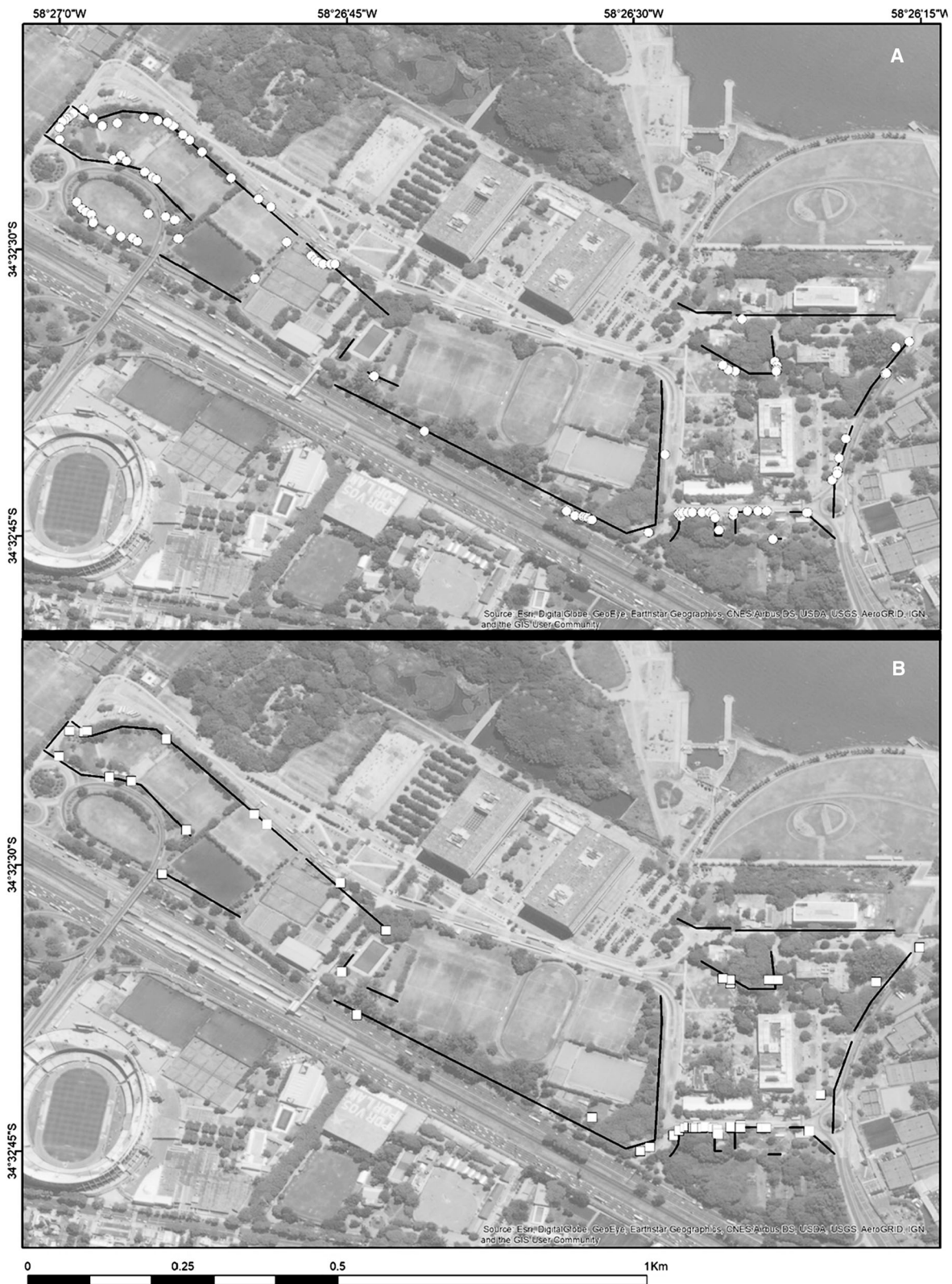
$$X = \frac{\text{Number of effective recipient flowers in the population whose dye colour does not match the plant it belongs to}}{\text{Total number of potential recipient flowers in the population}}$$

Fruit set in relation to the distance between blooming neighboring plants

Flowers in anthesis exposed to pollinators' visits were marked with hanging hard paper tags around their pedicels. Once anthesis was over, tagged flowers were isolated in mesh nylon bags in order to preserve developing fruits from frugivores. By the time fruits were fully developed (20–25 days), the bags were

Statistical analysis

Fisher's Exact Test was performed with Infostat (Di Rienzo et al. 2011). Regression and correlation tests, as well as Kruskal–Wallis test and Box-Plots, were performed with R Studio (R Development Core Team 2016). The following R-packages were used for data-processing and distance-based analysis: *readr* (Wickham et al. 2017), *raster* (Hijmans 2017), *rgdal* (Bivand



◀ **Fig. 1** Spatial distribution of individuals in blossom of *P. caerulea* in Ciudad Universitaria, Buenos Aires, Argentina. **a** Dec-2013 (white circles, $n = 98$); **b** Mar-2014 (white squares, $n = 47$). Black lines mark the extent of fences on which the plants climbed

et al. 2018), *sp* (Pebesma and Bivand 2005), *SDMTools* (VanDerWal et al. 2014), *spatstat* (Baddeley et al. 2015).

Results

In both censuses, individuals of *P. caerulea* exhibited a clustered distribution ($A < 1$; $p < 0.05$) according to the Hopkins–Skellam Test for Complete Randomness.

Distribution of minimum distances between blooming individuals of *P. caerulea* was similar in both opportunities (Kruskal–Wallis = 0.64; $p = 0.42$), in which a considerable proportion of plants were relatively close to each other rather than set apart (Fig. 1). On December 2013, 75% of the individuals were closer than 20 m from their nearest neighbors, but on Mar-2014, 75% of the individuals were less than 60 m apart from their nearest neighbors (Fig. 2) and a higher proportion of plants were 60–120 m away from their nearest neighbor. In spite of the few observed differences between December and March, blooming individuals maintained the clustered distribution in both phenological periods.

Most of the flowers (70–90%) bloomed on plants whose distances to the nearest neighbor were less than 15 m on December 2013 and less than 10 m on March 2014 (Fig. 3).

The following pollinators were recorded visiting flowers during the assays: *Xylocopa augusti*, *Xylocopa artifex*, *Xylocopa frontalis*, and *Centris* sp. In addition, domestic bees collected pollen and lepidopterans tried to suck nectar. *X. augusti* was the most frequent visitor; females performed most of the visits, though some males were observed on flowers on March 2014.

The results of the first assay were excluded from the pollen dispersal analysis because of extremely low transfer events; a sudden non-forecasted storm occurred in the study area (Table 1) which made visitors' activity decrease. In the rest of the assays (Fig. 4), fluorescent powder was deposited on 37–69%

of blooming individuals (Table 2). Pollen analogues dispersal distances ranged from 2.15 to 262.71 m (Fig. 5), but most of the events occurred at relatively short distances, e.g., less than 60 m (Fig. 4, Table 2). Dispersal distances did not differ significantly between experiments ($H = 2.13$, $p = 0.7104$). The proportion of flowers with pollen analogues deposited decayed as distance to source increased in all cases (Fig. 5). Regression analysis showed a significant and negative relationship between these variables in all four assays (Fig. 5). Pollen analogue dispersal distance increased with the minimum distance between blooming plants (Fig. 6). However, the regression linear model was non-significant, therefore, suggesting a moderate and positive trend (Pearson's correlation coefficient = 0.7046). Lastly, the index of geitonogamous transfers was above 0.83 in all assays (Table 2), which implied a high rate of pollen analogue transfers between flowers in the same plant whereas the proportion of xenogamous transfers ranged from 0.04 to 0.70 (Table 2), indicating a moderate rate of pollen transfer between flowers of different individuals.

Most pollinators displayed fixed flight patterns: parallel to the fences in which *P. caerulea* individuals grew. In a few cases in which a pollinator arrived to a plant from a flight route transversally orientated to the fence, the departure was parallel to the fence when the pollinator abandoned the plant. Field observations revealed that most of the recorded arrivals occurred along the East–West axis, in either one sense or the other, on plants whose flowers faced primarily north.

The mean production of fruits during December 2013 was significantly higher than that of March 2014: 63% ($n = 63$) vs. 26% ($n = 54$) (Fisher's Exact Test $p = 0.375661$, $p = 0.000079$). Percentage of fructification decreased as minimum distance between conspecific plants increased for December 2013 (Fig. 7, circles). Correlation between fructification percentage and distance between nearest plants was negative ($r = -0.828$) and significant ($p = 0.0214$) (Fig. 7). The model explains 63% of the observed variability. A different behavior was observed on the data obtained on March 2014 (Fig. 7, squares): the highest fruit set was not found on plants whose nearest neighbors were closer (< 5 m) but rather on plants whose nearest neighbors were 20–35 m apart.

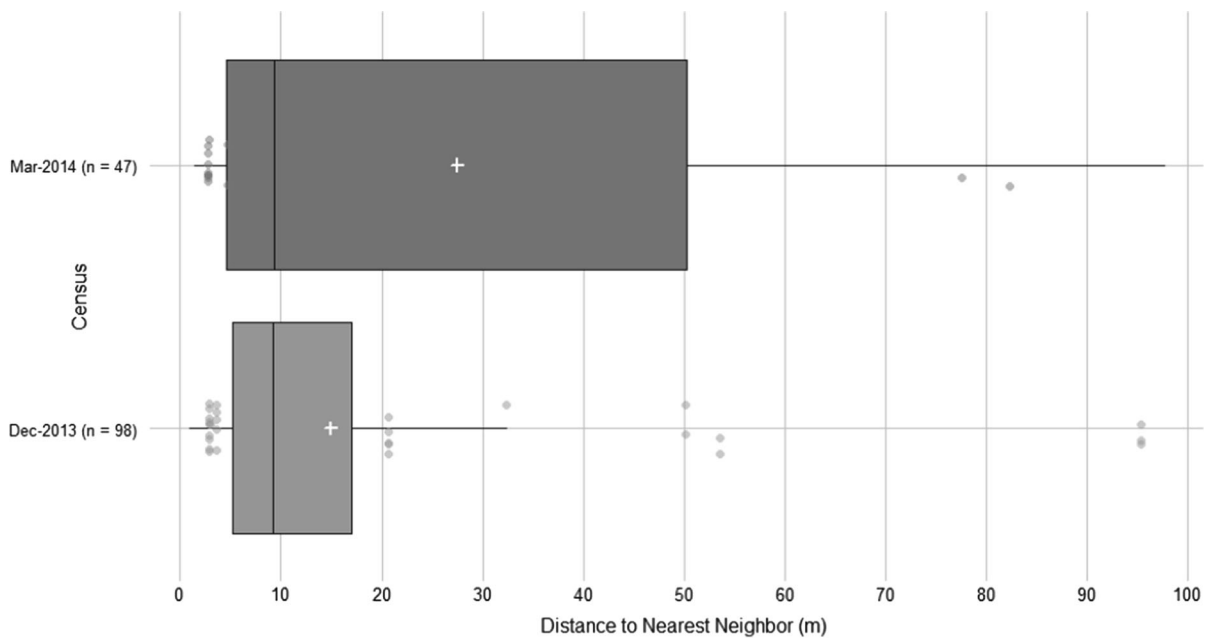


Fig. 2 Box plot representing distribution of distances to nearest neighbor in a population of *P. caerulea* on Dec-2013 and Mar-2014. The white cross marks the mean distance between con-specific neighbors on each census

Discussion

Spatial arrangement of blooming individuals

In both censuses, presence of blooming individuals of *P. caerulea* in C.U. was consistent with previously registered blooming periods by Amela García and Hoc (1997), being the flowering peak on December 2013.

Unevenness on the amount and identity of flowering individuals between both censuses was due to population dynamics and individual differences among plants such as phenological stage, phytopathology condition, and mortality. Nevertheless, flowering individuals maintained a gregarious pattern over time. Despite the fact censuses only accounted for blooming plants, it may be assumed that the remaining individuals repeated the same distribution pattern, already acknowledged by previous observers in C.U. (Bergonzi, pers. com.), who considered all plants in the population. This pattern was independent of the number of individuals and probably originated from synzoochorous and endozoochorous seed dispersal performed by Passerines (Mendiondo and Amela García 2006). In consequence, most of the individuals were relatively close to their neighbors and therefore, the

proportion of “far away” or isolated individuals was low.

The higher standard deviation in distances between nearest blooming neighbor in March 2014 might be due to the disappearance of flowering individuals in crowded areas, and their prevalence in less crowded ones, in the context of decreasing flowering individuals toward the end of the flowering period.

Differences between censuses and pollen dispersal assays within the population suggest the existence of two “neighborhoods” because no pollen analogue exchange was detected between them. This means crossing is not panmictic between them (Wright 1943 in Kerns and Inouye 1993). We named them *western* neighborhood and *eastern* neighborhood. The *western* one was richer in insects, either specific herbivores of *Passiflora* (that were not in the *eastern*) and other insects, probably because of the proximity to less urbanized areas and less exposure to pedestrian and vehicular disturbance. Between them, there was an area with minor plant density: it was more shaded, subjected to exhaustive pruning and to permanent and greater vehicular disturbance (adjacent to a highway). This could result in lower fruit set (due to lower frequency of pollinator visits) and lower seed dispersal (due to lower frequency of bird visits).

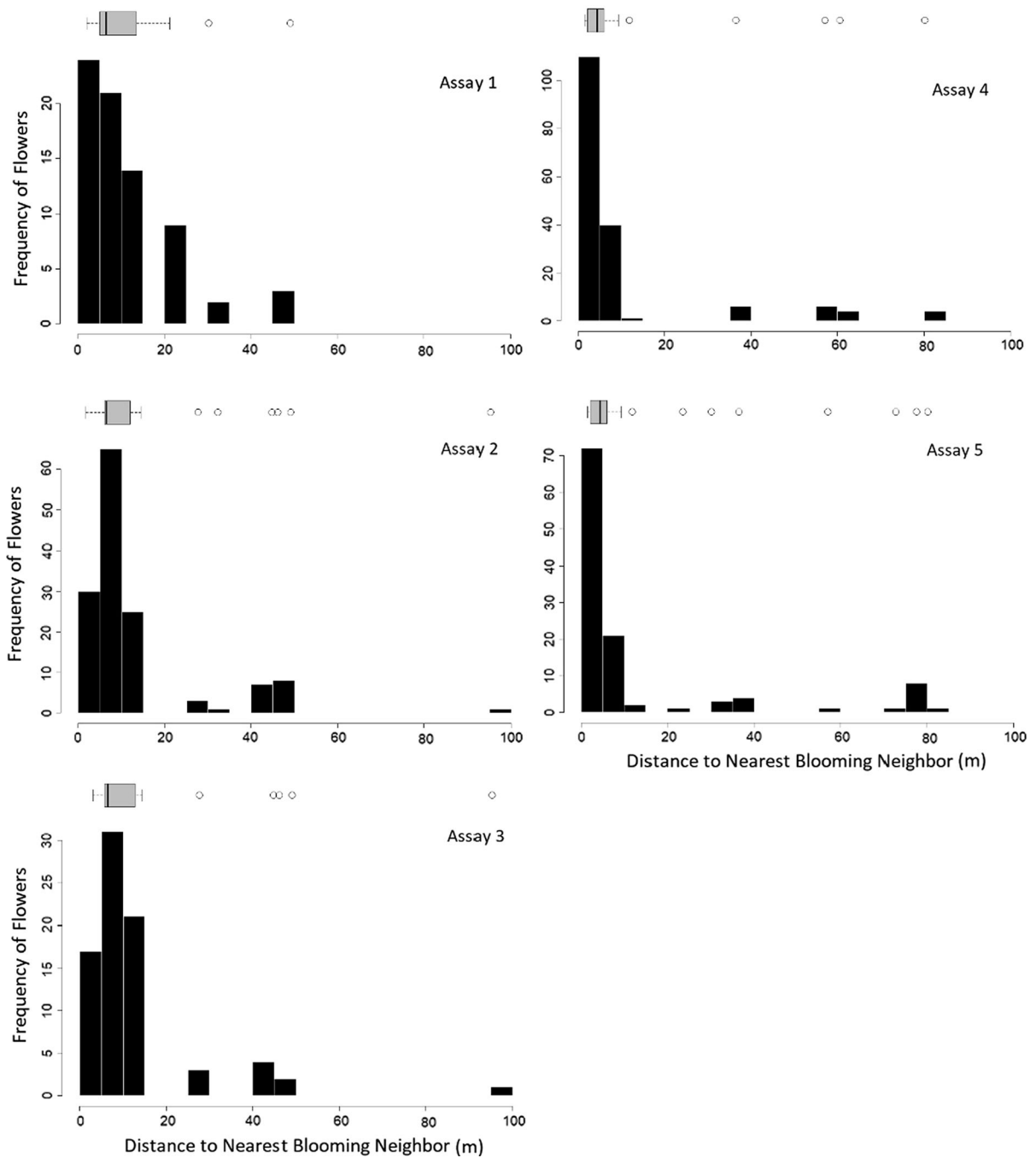


Fig. 3 Frequency distribution and Box-Plot of flowers in each assay performed in the population of *P. caerulea* on Dec-2013 and Mar-2014

Nevertheless, fruit set occurred on all blooming individuals in the population (although in different proportion among individuals), which indicates that flowers were pollinated, and consequently, there must

had been pollinators' nests nearby. In the few records that involve *P. caerulea* and nesting sites of its pollinators, nests of *X. artifex* and *X. augusti* were found less than 30 m away from *P. caerulea*

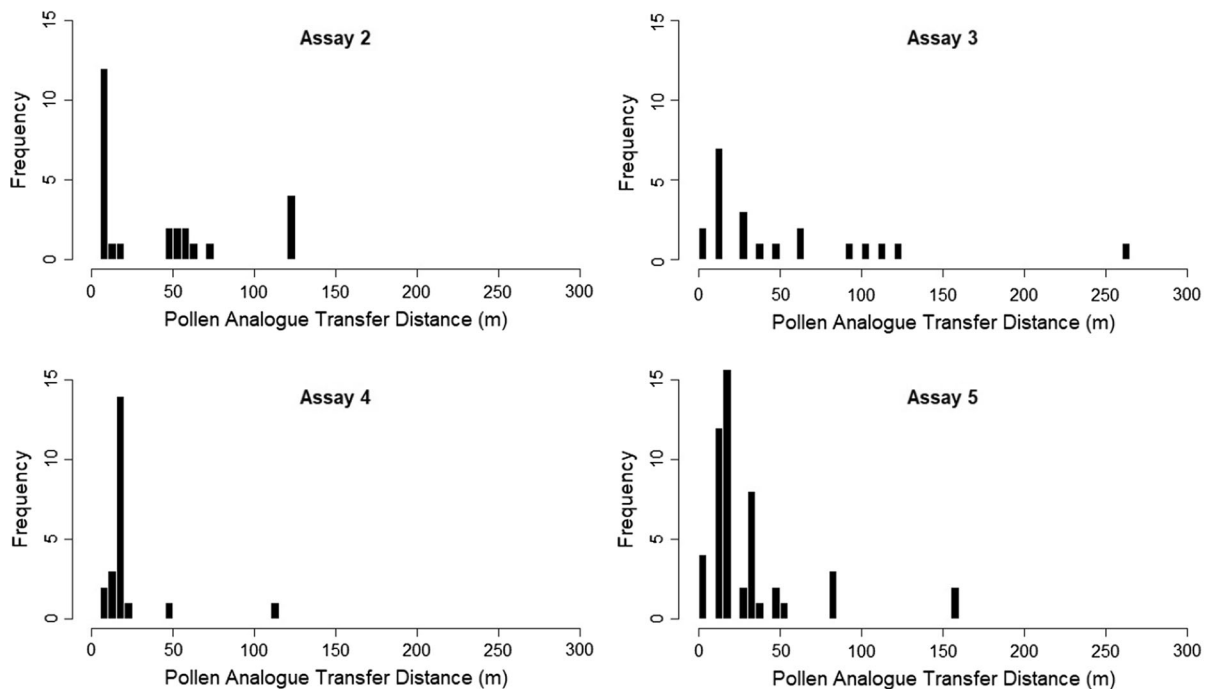


Fig. 4 Frequency distribution of fluorescent powdered dye transfer in four assays in a population of *P. caerulea*

individuals or even adjacent to them (Amela García and Hoc 1997; Amela García et al. 2013).

Pollen analogue dispersal

One of the most remarkable features regarding zoophilous pollen dispersal is its leptokurtic distribution, a shared pattern among most pollinators' movements (Handel 1983). Leptokurtosis results from interaction between either physical or behavioral characteristics (Richards 1986): plant distribution, usually contagious; occurrence of more rewarding patches that concentrate the activity of pollinators; lesser proportion of flights to distant patches; tendency to carry pollen to nearer plants; short-term foraging flights. Leptokurtosis was recurrent in the population of *P. caerulea* studied: it was observed on its plant spatial arrangement, consequently on flower distribution among plants, and for pollen analogue dispersal.

Pollen analogues' dispersal decayed as distance to source increased. Most pollen analogues were deposited at short distances, with very few exceptions. These results are consistent with those reported for other plant species and pollinators (Waser and Price 1983; Thomson et al. 1986; Thomson and Thomson

1989; Nilsson et al. 1998; Christensen and Olensen 1998; Van Rossum 2009; Van Rossum and Triest 2010; Van Rossum et al. 2011). Based on pollen analogue's dispersal patterns, pollinator's flight distances might also show a leptokurtic nature. This is consistent not only with previous findings on pollen analogues dispersal distances involving different plant and pollinator species (Tonsor 1985; Kameyama et al. 2000; Van Rossum et al. 2011; Zhang et al. 2018), but also across several biology fields such as landscape ecology, population ecology, and connectivity, which deal with different dispersal events or organisms (Fraser et al. 2001; Rodriguez 2010).

When blooming individuals are relatively close to each other, most of pollinators' flights occur on the same plant or among adjacent plants (Waser and Price 1983). Levin and Kerster (1969) found that correlation between mean plant distance and pollinator's flight distances was 0.9; they concluded that bees respond in a similar way to spacing between individual plants of various species. An equivalent trend was observed in *P. caerulea*: correlation of mean minimum distance between blooming individuals and pollen analogues' dispersal distance was 0.7, though not as high as the ones reported by other authors (Levin and Kerster

Table 2 Data of each essay carried out with fluorescent powder dyes

Assay	Distance to nearest blooming individual (m) Mean \pm SD	Number of blooming plants (<i>n</i>) and flowers per plant (Mean \pm SD)	Number of pollen analogue transfers	Mean dispersal distance (m) Mean \pm SD	Proportion of recipient individuals (%)	Index of xenogamous transfers (0–1)	Index of geitonogamous transfers (0–1)
1	11.53 \pm 10.65	20 (3.04 \pm 1.83)	3	14.69 \pm 0.00	5	0.04	1
2	15.84 \pm 16.99	16 (3.88 \pm 4.79)	26	40.08 \pm 41.62	69	0.60	1
3	20.99 \pm 24.84	19 (3.95 \pm 3.60)	22	51.46 \pm 61.29	68	0.33	1
4	17.66 \pm 24.78	24 (7.25 \pm 6.23)	22	23.12 \pm 21.65	37	0.16	0.83
5	19.93 \pm 26.16	24 (4.63 \pm 4.57)	52	28.98 \pm 31.47	67	0.7	0.9

1969; Mustajärvi et al. 2001; Schulke and Waser 2001; Pasquet et al. 2008; Jakobsson et al. 2015; Pérez-Mendez et al. 2018).

Most of the effective recipient flowers revealed by deposition of pollen analogues were relatively near the source, so most pollinators' flights must have involved moving between plants relatively close to each other. Spatial arrangement of blooming *P. caerulea* individuals determined flower distribution in the population and, therefore, may have stimulated pollinators to forage in more rewarding patches, where the required effort in terms of flight distances was lower. However, this pattern could have been obtained if pollinators had visited more or less equally distant flowers sequentially without going back to the pollen analogue source. The exact tract of the pollinator flight route cannot be assessed with this method.

Greater dispersal distances were recorded on assays in which the flowers/plant ratio was lower and a slightly negative correlation between quantity of flowers per plant and mean dispersal distance was evidenced. It is expected that if there are fewer flowers per plant, pollinators will be forced to fly longer distances in search for food.

Pollinators' movements

The majority of seedlings of *P. caerulea* are established near fences in the study area, primarily because of foraging habits of the perching bird species that feed on the fruits and scatter the seeds (Mendiondo and Amela García 2006). Thus, it is expected that pollinator movements should respond to this pattern somehow. Mapping pollen analogues transfers allowed the identification of a prevalence of East–West flight routes, in both senses, mostly in a linear patch of *P. caerulea* with a higher density of blooming individuals and flowers. Therefore, most of pollinator's foraging activity was carried out nearby.

Xylocopa and *Centris* are “trap-line” pollinators. This kind of pollinators visit the same group of plants every day and presumably in the same order (Janzen 1971). Plants pollinated by trap-liners are usually found in low densities, produce little amount of flowers each day regularly, and focus their anthesis on a specific time of the day (Janzen 1971). Most of these aspects occur in *P. caerulea*, as well as in other

Fig. 5 Relationship between deposition of fluorescent dye powder and distance to fluorescent dye powder source for each of the experiments involving more than one donor plant and more than one receptor

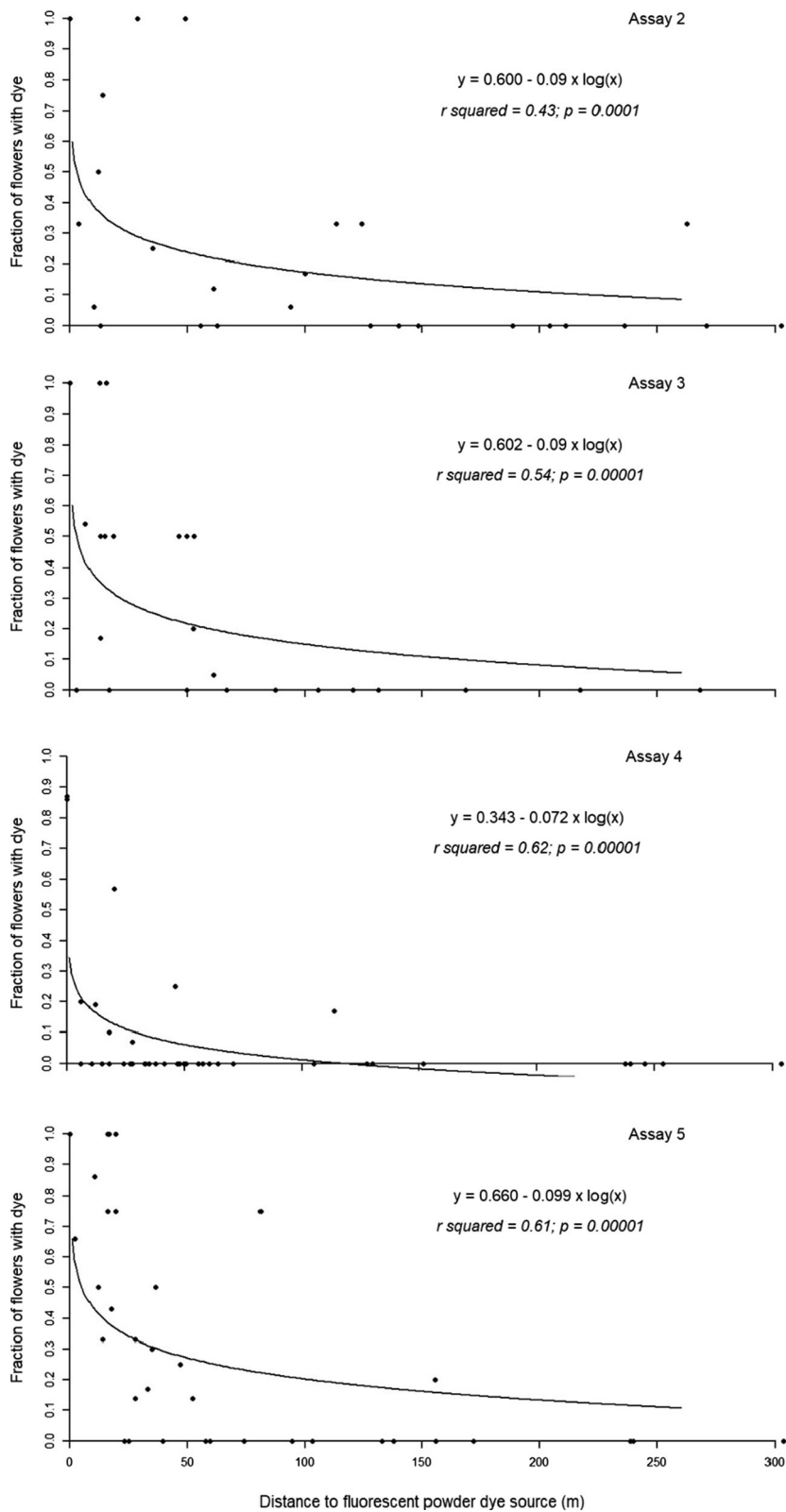


Fig. 6 Relationship between distance to nearest blooming neighbor of plants in blossom and mean dispersal distance of pollen analogues for each of the essays performed on Dec-2013 and Mar-2014

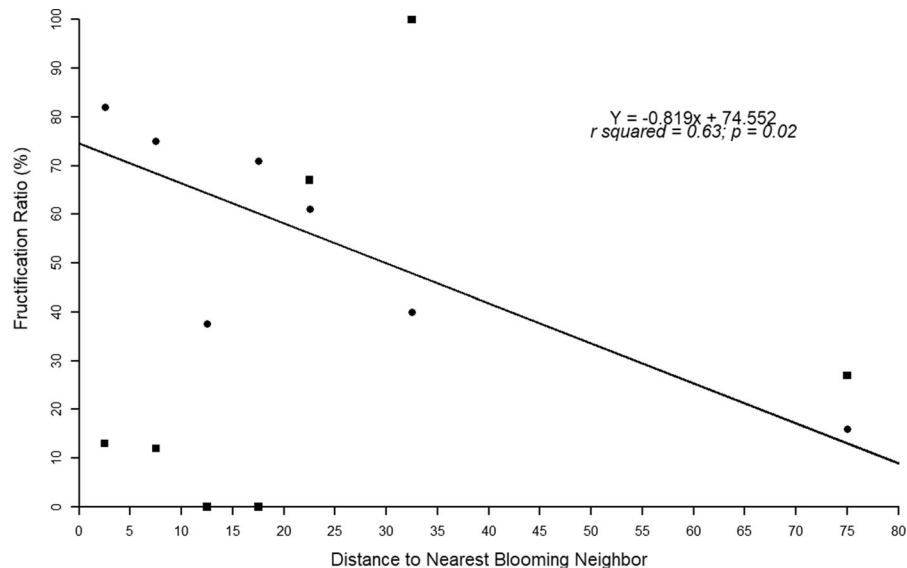
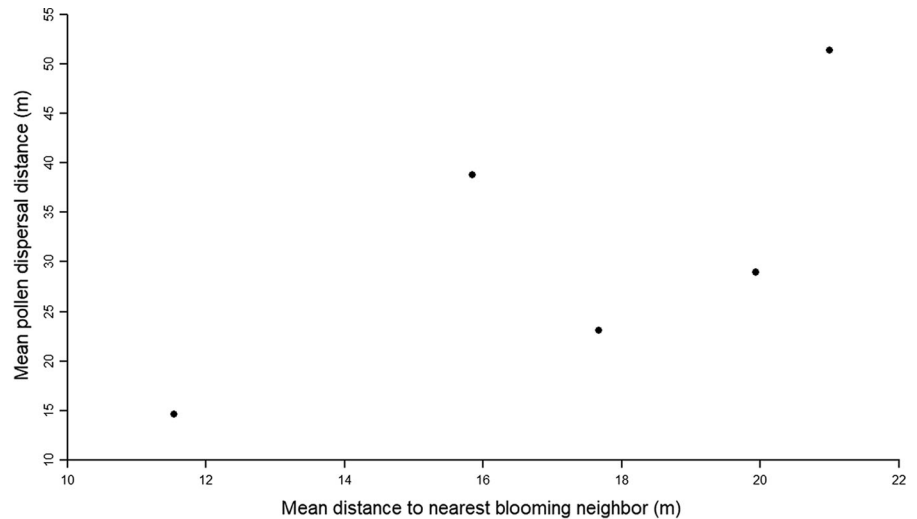


Fig. 7 Relationship between fruit set and distance to nearest blooming neighbor on Dec-2013 (circles) and Mar-2014 (squares). Linear regression and its coefficients correspond to data collected on Dec-2013. No linear regression fitted to Mar-2014 data

Passiflora species with the same pollinator taxa (*Xylocopa* and *Centris*) that were marked and registered visiting the same plants for several days (Amela García and Hoc 1998).

Loss of fluorescent particles during flights could lead to underestimation of the number of more distant flowers receiving pollen, thus exaggerating the initial decline of the leptokurtic curve. However, exploratory tests and observation on pollinators' activity confirmed neither pollen nor dye fell from pollinators and no differences of behavior between pollinators

exposed to fluorescent dyes and those foraging on non-powdered flowers were observed. Although no assumptions can be made about their behavior outside of the foraging area, arrival, foraging, and departure from flowering individuals seemed ordinary.

Fruit production

Although the number of blooming individuals and floral offer per plant was similar between each essay in December and March (Table 2), the significant decline

of fruit set at the end of the flowering season may have been due to a decrease in the number of flowering individuals (Fig. 1). Besides, although pollen analogues dispersal assays were performed with flowers that displayed all phases of anthesis on December 2013 and March 2014, physiological aspects, such as those intervening in producing flowers whose stigmata do not descend (Amela and Hoc 2012) may have taken place. This kind of behavior, which turns a proportion of the bisexual *Passiflora* flowers into solely pollen donors, was related to a decrease in resource allocation to fruit production in unfavorable times (May and Spears 1988). This scarce fraction of flowers that did not develop fruits may have been responsible for the lack of correlation between fruit percentage and distance to nearest blooming neighbor on March 2014. Therefore, if *P. caerulea* is to be cultivated for fruit yield, the proportion of fruits that could be harvested must be taken into account with respect to the phenological stage of the crop.

In *P. caerulea*, a strong negative correlation between percentage of fructification and distance between conspecific individuals was observed in the peak of the flowering season. Leptokurtosis on pollen analogue transfers' distribution would explain a lower probability of pollen deposition between distant individuals (thus becoming pollen-limited) and an increased pollen competition between nearby individuals (most of the pollen received by a greater proportion of individuals could be from one donor). In cultivated passion vines, fruit set increases as distance between plants decreases (Haddad García 1968; Gachanja and Ochieng 1988).

On the basis of the results obtained, recommendations for cultivation of *P. caerulea* related to optimal sowing distances could be given as follows. Considering minimum dispersal distance was 4.55 ± 2.00 m and that pollinators moved more frequently among plants separated by short distances provided there were available flowers (reported high index of geitonogamy; Table 2), the optimum distance for a crop of *P. caerulea* would be less than 5.00 m, both between rows and between plants of the same row. However, since each individual plant can reach almost 10 m width, it would be advisable to space individuals every 10 m to ensure optimal development of each plant, and consider leaving 5 m between rows for tillage activities. At the same time, nesting sites for pollinators should definitely be considered, as the

decrease in pollinator visits diminished fructification of other cultivated *Passiflora* species (Sazima and Sazima 1989; Miranda Lasprilla, pers. com.). In the same way, without the intervention of pollinators, *P. caerulea* does not set fruit unless flowers are manually pollinated, which is time consuming and expensive. Accordingly, it is important to optimize sowing distance between plants of different genotypes of this self-incompatible species in order to minimize transfer of geitonogamous pollen and maximize transfer of xenogamous pollen and therefore, productivity.

Conclusions

While there may be differences between dispersal patterns of real pollen and fluorescent powder dyes, this work provides further evidence that pollen analogues are a feasible way to estimate the dynamics of pollen flow. The results support the idea that the pattern of pollen dispersal in *P. caerulea* is closely related to the foraging activity of its pollinators (*Xylocopa* spp.). Consecutively, pollinators' activity responds to the arrangement of flowers on each plant and hence on the spatial arrangement of plants in the population, as well as its shape, which in this case is set along linear patches of vegetation. Using pollen analogues allowed detection of presumptive partially or completely reproductively isolated plants, as could be the case for those located more than 263 m away (maximum dispersal distance recorded) from its nearest conspecific neighbor. Consequently, a strong dependency between fruit set and distance between conspecific blooming individuals was manifest, being those closer to each other the ones that yielded more fruits per plant; however, this was detected in the peak of the flowering season but not toward the end, probably due to reduction of blooming individuals and to physiological changes. These results are consistent with the leptokurtic nature of identified pollen transfer distances of this self-incompatible species, along with previously published records for other plant/pollinator species, using different techniques to estimate pollen dispersal.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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