



# Forest connectivity boosts pollen flow among populations of the oil-producing *Nierembergia linariifolia*

Pablo Y. Huais · Gabriel Grilli ·  
Leonardo Galetto

Received: 8 September 2021 / Accepted: 21 June 2022  
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

## Abstract

**Context** The process of forest fragmentation determines landscapes with isolated forest patches immersed in a distinct matrix. This process may hinder pollinator movement throughout the landscape, which may negatively impact on pollen flow among native plant populations.

**Objectives** We evaluated the effect of the loss of forest connectivity on pollen dispersal by specialized native bees in the oil-producing and self-incompatible *Nierembergia linariifolia*.

**Methods** We estimated pollen flow between plants of *N. linariifolia* at an agroecosystem with remnant forest of central Argentina. Six plant populations (source populations) were treated with fluorescent dyes as pollen analogues, and stigmata of recipient plants were collected to seek for dye particles. Dye

deposition rate was assessed for plants that were connected through remnant forest to a source population or unconnected by a crop matrix, and at increasing distances to a source population.

**Results** Deposition rate per plant was higher in connected than in unconnected plants, and decreased with increasing distances to a source population in an exponential fashion. Most of the dispersal events between connected plants occurred at the vicinity of a source population. Long dispersal events (up to 1259 m) were recorded between plants located at neighbouring forest patches separated by an agricultural matrix.

**Conclusions** Landscape connectivity through forest remnants is key to enhance pollen flow between self-incompatible plants such as *N. linariifolia*. Besides, the evidence of pollen dispersal through the agricultural matrix pinpoints the essential role of native pollinators in maintaining pollen flow among unconnected plant populations in fragmented landscapes.

**Keywords** Forest loss · Habitat fragmentation · Landscape connectivity · Habitat stepping stones · Pollen dispersal · Fluorescent dyes

---

P. Y. Huais (✉) · G. Grilli · L. Galetto  
Instituto Multidisciplinario de Biología Vegetal, FCEyN  
(CONICET-Universidad Nacional de Córdoba), Vélez  
Sarsfield 1611, CC 495, Córdoba, Argentina  
e-mail: pablo.huais@unc.edu.ar

P. Y. Huais  
Departamento de Matemática, Facultad de Ciencias  
Exactas, Físicas y Naturales, Universidad Nacional de  
Córdoba, Córdoba, Argentina

L. Galetto  
Departamento de Diversidad Biológica y Ecología,  
Facultad de Ciencias Exactas, Físicas y Naturales,  
Universidad Nacional de Córdoba, Córdoba, Argentina

## Introduction

During the past decades, natural habitats around the globe have greatly been converted into croplands mainly driven by agricultural intensification,

determining large extensions of a few crop types (Foley et al. 2005; Green et al. 2005). In general, the conversion of forests into farmlands has negative consequences for biodiversity and ecosystem functioning (Fahrig 2003; Foley et al. 2005). Land use changes have been particularly intense in the “Chaco Forest” and in the “Chaco Serrano Forest” at central Argentina, which have experienced a massive contraction due to its conversion mostly to soybean and maize crops (Zak et al. 2008; Hoyos et al. 2012; Hansen et al. 2013; Cáceres 2015; Vallejos et al. 2015). This process has left a mosaic of forest patches of diverse size and shape with deep implications on ecosystem functions and services (Defries et al. 2004; Díaz et al. 2015). Particularly, an unprecedented plant species loss over time related to habitat loss and fragmentation (a decrease of 24% of species richness, mainly driven mainly by non-woody, short-lived species) has been reported for the highly fragmented “Chaco Serrano Forest” (Cagnolo et al. 2006; Aguilar et al. 2018).

The processes of habitat loss and fragmentation negatively affect pollination and plant fitness due to the erosion of plant-pollinator relationships in many ways (e.g. Cunningham 2000; Aizen et al. 2002; Aguilar et al. 2006). Habitat loss reduces pollinator richness and density, and habitat fragmentation affects their behaviour and mobility throughout the landscape (Hadley and Betts 2012; Volpe et al. 2016). Particularly, pollinator mobility between unconnected habitat patches can vary according to the nature of the matrix (Jules and Shahani 2003), which may be more or less permeable for pollinators. This disruption in plant-pollinator relationships generally translates into decreases of visitation rates and pollen deposition on stigmata, which may determine a downfall in plant fitness (Cunningham 2000; Aguilar and Galetto 2004; Aguilar et al. 2006; Garibaldi et al. 2011). Moreover, isolated populations are susceptible to suffering demographic, environmental and genetic changes such as inbreeding, genetic drift and disruption of the gene flow pattern (Lienert 2004; Leimu et al. 2010; Wanderley et al. 2020).

The study of pollen flow between unconnected plant populations is important to develop conservation and management strategies (Wanderley et al. 2020). In general, the evidence shows that pollen dispersal of many insect-pollinated plant species mainly occurs at the vicinity of a source population, and

rapidly decreases at short distances in an exponential fashion (e.g. Townsend and Levey 2010; Van Rossum 2010; Van Rossum and Triest 2010, 2012; Van Rossum et al. 2011, 2012, 2015; Mayer et al. 2012; Van Geert et al. 2014). This pattern of rapid decrease in pollen dispersal has been observed at fragmented landscapes with natural remnant habitats surrounded by urban (Van Rossum 2010; Van Rossum and Triest 2012) and/or agricultural matrices (Van Geert et al. 2014). In addition, previous works on fragmented landscapes showed a lower (or almost null) pollen dispersal between unconnected than between connected plant populations (e.g. Townsend and Levey 2010; Van Rossum and Triest 2012; Van Geert et al. 2014). In general, studies on pollen flow are scarce due to the difficulty of estimating pollen dispersal in natural populations (e.g. Austerlitz et al. 2004; Adler and Irwin 2006; Townsend and Levey 2010; Van Geert et al. 2010; Ottewell et al. 2012; Wanderley et al. 2020). Usually, pollen dispersal is estimated by using fluorescent dyes: flowers are treated with dye of a particular colour, whose presence is then determined on other non-treated flowers with specialized lamps or fluorescence microscopes. This inexpensive method has been proven to be efficient for pollen dispersal estimation both in experimental and natural plant populations (Young 2002; Townsend and Levey, 2010; Van Geert et al. 2010).

Here, we investigated the effects of the “Chaco Serrano Forest” fragmentation on the process of pollen flow between populations of *Nierembergia linariifolia* (Solanaceae). *N. linariifolia* is self-incompatible, as it requires cross-pollination for fertilization and seed production (Cocucci 1984, 1991). The experiments of pollen limitation carried out by Cosacov et al. (2008) at different landscapes showed that this species is pollen-limited for fruit and seed production. The flowers of this species offer oils as a resource for pollinators, which is collected by specialized native bees. Given that these oil-collecting bees are the main pollinators of *N. linariifolia* (Cosacov et al. 2008), from a functional point of view this plant species can be considered as highly specialized (Fenster et al. 2004). Populations of this species occur in open fields, road-sides and disturbed areas where soil have been recently exposed, and plants are displayed in patches of several flowering individuals. Therefore, in agroecosystems of central Argentina with forest remnants surrounded by a matrix of crops,

populations of *N. linariifolia* are more frequently found at the edge of forest patches than inside them, although populations of this species may also thrive at canopy gaps within forest fragments. All these features make this species an interesting and adequate model for the study of pollen flow at fragmented landscapes.

The questions we address are: (i) Does the loss of connectivity due to forest fragmentation affect pollen flow mediated by bees between populations of *N. linariifolia*? (ii) What is the pattern of pollen dispersal in relation to the distance between plants of *N. linariifolia* connected through remnant forest and between plants unconnected by a matrix of crops? The main hypothesis of this work poses that the presence of forest remnants immersed in a matrix of crops influences the foraging behaviour of pollinators, affecting the process of pollen flow between plants of *N. linariifolia*. Hence, the expected results are: (a) higher pollen flow between plants connected by forest remnants than between plants unconnected by a matrix of crops; (b) a decrease of pollen dispersal with increasing distances to the source plants in an exponential fashion, both for connected and unconnected plants. Finally, we discuss the implications of the results for biodiversity conservation and management strategies of pollinators and plant populations at fragmented landscapes.

## Materials and methods

### The species

*N. linariifolia* (Solanaceae; commonly named “chuscho”) is a perennial sub-shrub and self-incompatible species from South America, and it is found in the Chaco Region of Argentina, Uruguay, Paraguay and Brazil (Cocucci and Hunziker 1995). Its oil-producing flowers consist of a fertile column emerging from the centre of the corolla, which is usually flat and white, with lilac tones at the centre. The column is composed of a stigma in its apical zone, surrounded by five stamens (Cocucci 1991). The stigma is receptive and the pollen is viable throughout the flower life. This species blooms between October and March, and individual plants bloom for 3–4 weeks with each individual flower lasting 2–3 days (Cosacov et al. 2008). This plant species, like others of the same genus,

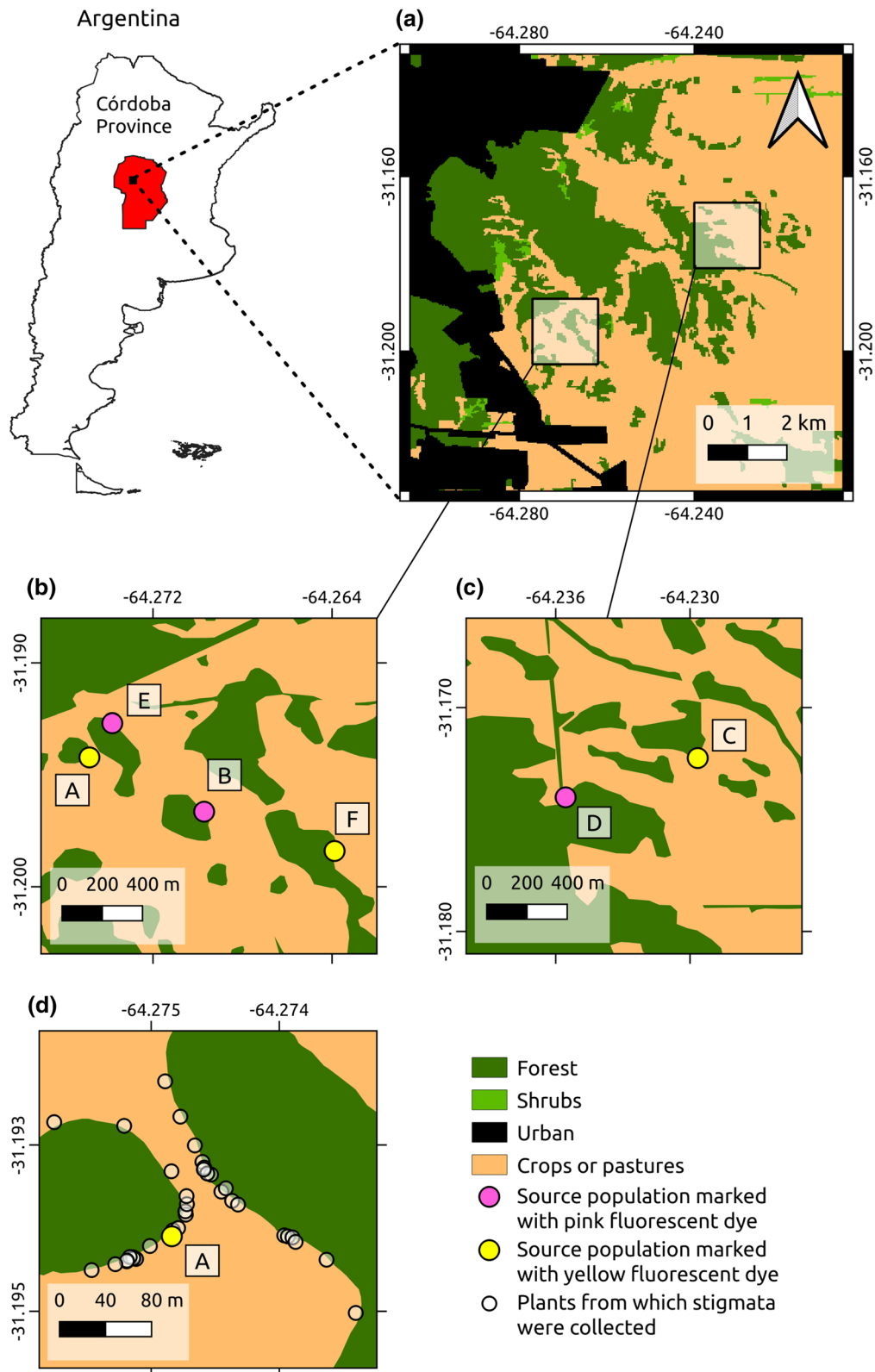
contain toxic substances in the leaves which make it a poisonous species for livestock and other animals (Buschi and Pomillo 1987; Odini et al. 1995; Torino et al. 2017).

Several works have reported the solitary bees *Chalepogenus parvus*, *Ch. nigripes*, *Ch. cocucci*, *Ch. neffi*, *Ch. nigriventris*, *Tapinotaspis chalybaea*, *Tetrapedia* sp. and *Centris tricolor* as the main pollinators of this species (Cocucci 1984, 1991; Cosacov et al. 2008; Maubecin et al. 2021). These are bees specialized in collecting energy-rich floral oils, which can be used for larval development, nest construction and possibly for adult nutrition (Buchmann 1987; Rasmussen and Olesen 2000). Also, previous studies have recorded occasional visits of bees from Halictidae, as well as rare visits by the exotic *Apis mellifera* (Cocucci 1984, 1991; Cosacov et al. 2008), which are not oil-collecting bees and probably visit the flowers in search of pollen grains. Species of the solitary and specialized oil-collecting bees, which are the most frequent pollinators of *N. linariifolia*, also visit other flowers of the community at the studied region (species of Amaranthaceae, Asteraceae, Convolvulaceae, Fabaceae, Passifloraceae, Solanaceae, etc.) to collect pollen and nectar (e.g. Aguiar 2003; Gonçalves et al. 2012; Musicante 2013).

The foraging ranges of the mentioned oil-collecting bee species have not been studied in detail, but some related previous works may offer some approximations. For instance, the observations made by Janzen (1971) suggest large foraging ranges for the bees of the genus *Centris*, a trait that may be extended to *C. tricolor*. Moreover, previous studies have shown a positive relationship between bee body size and their foraging ranges (Gathmann and Tschardt 2002; Greenleaf et al. 2007; Hofmann et al. 2020). This well-established pattern may suggest short foraging ranges for those small-sized species such as *Ch. parvus*, *Ch. nigripes*, *Ch. cocucci* and *Ch. neffi* (Maubecin et al. 2021), as well as for the small-sized Halictidae. Conversely, large foraging ranges have been evidenced for the exotic *A. mellifera* (Beekman and Ratnieks 2000).

### Study area

The study was carried out in an agroecosystem with remnant forest, at the vicinity of Córdoba city (Córdoba Province, Argentina) (Fig. 1), which is



**Fig. 1 a** Study area. This was located in Córdoba province, Argentina, in an agroecosystem at the vicinity of Córdoba city. **b, c** Locations of source populations with plants treated with pink or yellow fluorescent dyes. Although only two colours of fluorescent dye were used, populations were treated by pairs (one population treated with pink and the other with yellow fluorescent dye) at different time-periods, in order to avoid ambiguity on the origin of the fluorescent dye (see Table 1 for more details). **d** An example of the sampling context showing one source population of *N. linariifolia* (population A) and the location of the nearest plants from which stigmata were collected: some plants (translucent white circles) located at the same forest patch of the source population (connected plants) and other plants located at a neighbouring patch with a matrix of maize crop between them (unconnected plants)

characterized by the presence of numerous forest patches of diverse size and shape, usually immersed in a matrix of crops. Farmers of the region regularly apply pesticides and herbicides to control pests and weeds of different crops and these applications have been increasing during the last years, which impose a threat to pollinators and native plant populations (Ferreira et al. 2017; Galetto et al. 2022). The forest patches belong to the “Chaco Serrano Forest”, where the vegetation is a semiarid shrub-forest, with native and exotic plant species (Cabido et al. 2018). During the sampling period (summer season 2019–2020), the matrix in the surroundings of the selected forest patches was composed by maize crops, before its flowering.

#### Estimation of pollen flow with fluorescent dyes

Pollen dispersal by pollinators was estimated with fluorescent dyes. Before the experiment, we walked along the edge of several forest patches looking after and georeferencing flowered populations of *N. linariifolia*. Also, the use of fluorescent dyes was preliminary tested for this species, by treating some flowers and observing pollinator visits to treated flowers. Several flower visits by native bees (Fig. 2) were recorded, including bees from genera *Chalepogenus* and *Tapinotaspis*, and the large-sized *C. tricolor*.

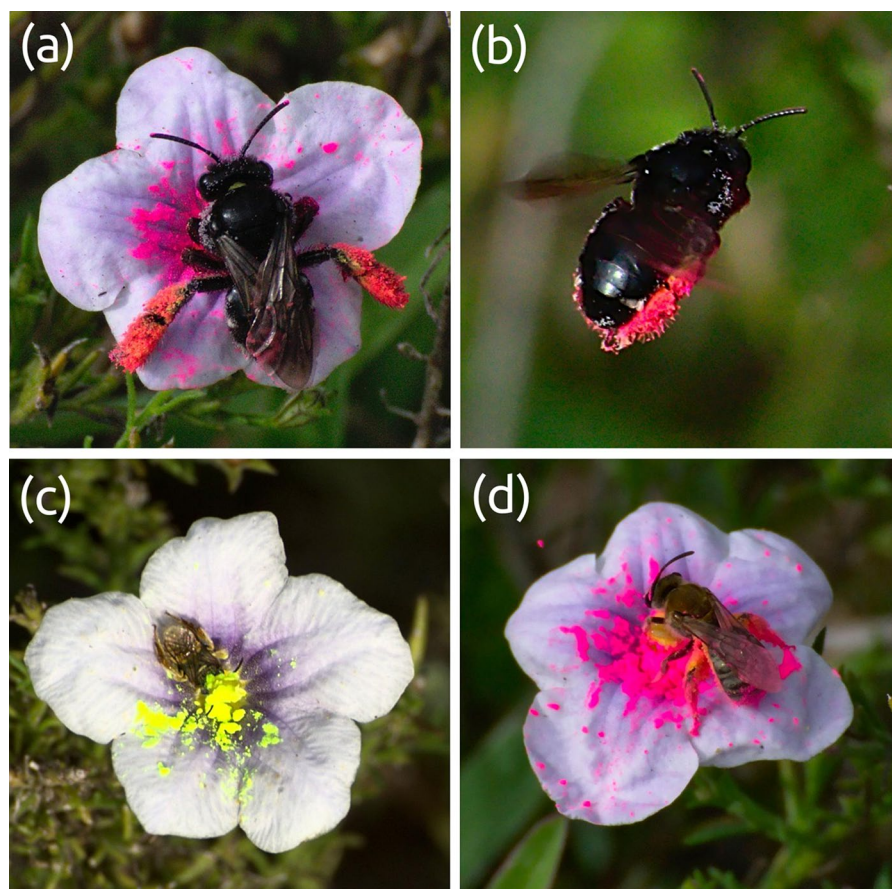
During each morning of the experiment (between 7 and 8 am), we selected two flowered populations of the species located at distinct forest patches (hereafter, source populations) to apply fluorescent dyes of different colours (pink and yellow) on all recently open flowers of several plants in each population (Fig. 1b–c; see Table 1 for more information).

The geographical coordinates of the central point of each source population were recorded with the use of a GPS. With the help of a thin wooden stick, a bit of fluorescent dye was carefully deposited over the flower column (stigma and stamens) (Fig. 3a) of each targeted flower. After 48 hrs, during the morning of the third day since the beginning of the experiment (i.e. the day when fluorescent dyes were added to the flowers), we proceed with the collection of stigmata. With the help of tweezers, stigmata were extracted from some flowers of plants to which no fluorescent dye was previously applied located at each source population, and of plants located at the same forest patch and at neighbouring patches, at increasing distances from each source population (see Fig. 1d for an example). All stigmata were extracted from plants located at the edge of forest patches. Each collected stigma was mounted on semi-permanent slides in polyvinyl-lactic acid-glycerol for ulterior analysis in the laboratory; and the geographical coordinates of the location of each plant from which stigmata were collected was recorded with a GPS.

The described procedure of flower marking with fluorescent dyes of a pair of plant populations and the later collection of stigmata in the landscape was repeated three times (over a month and half during spring/summer season), totalling data collection from six source populations. Samplings were done during sunny days and with favourable weather conditions for pollinator activity (25–32 °C during the day and no rainfalls), which were relatively constant during each sampling event (i.e. between the initial flower marking and the final collection of stigmata). Between 10 and 20 flowered plants were treated with fluorescent dyes per population, with a mean of 160 treated flowers per population (Table 1). A total of 1660 stigmata from 159 plants were collected to analyse pollen flow among plants of *N. linariifolia* ( $10.44 \pm 4.11$  flowers per plant; range 2–23 flowers per plant).

At the laboratory, each collected stigma was observed under a fluorescence microscope (Zeiss Axiophot) with the use of different filters and magnifications in order to seek for the presence of yellow and pink fluorescent dyes particles (Fig. 3b). Each stigma was categorized with the presence/absence of fluorescent dye particles of each colour.

**Fig. 2** Native bees visiting the flowers of *N. linariifolia* treated with fluorescent dyes, with particles adhered to their bodies. **a** *T. chalybaea* visiting a flower treated with pink fluorescent dye. **b** *T. chalybaea* in flight, departing from the treated flower with particles of pink dye. **c** *Ch. parvus* visiting a flower treated with yellow fluorescent dye. **d** Bee from Halictidae visiting a flower treated with pink fluorescent dye



**Table 1** Information regarding plant populations treated with fluorescent dyes (i.e. source populations)

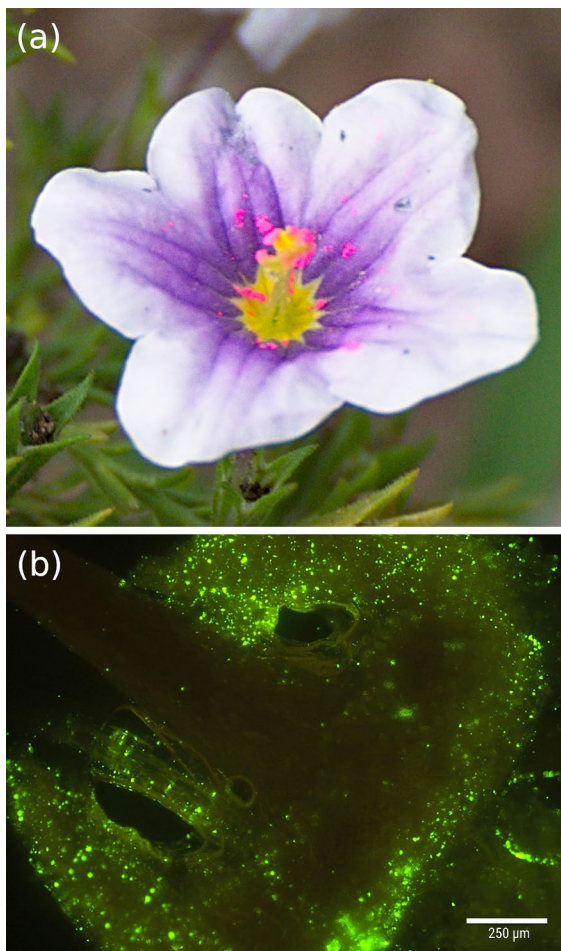
Population	Day	Latitude	Longitude	Colour of fluorescent dye	# of flowered plants	# of treated plants	# of treated flowers
A	09/12/19	-31.1942140	-64.2748388	Yellow	19	10	130
B	09/12/19	-31.1966405	-64.2697172	Pink	16	10	89
C	17/01/20	-31.1722425	-64.2296589	Yellow	22	10	87
D	17/01/20	-31.1739987	-64.2355577	Pink	18	15	208
E	22/01/20	-31.1926885	-64.2738182	Pink	22	16	225
F	22/01/20	-31.1983980	-64.2638915	Yellow	21	20	210
				Total	118	81	949

Note that, although only two colours of fluorescent dye were used, we did not mark two populations with fluorescent dye of the same colour at the same time-period

### Data analysis

We calculated the proportion of stigmata with deposition of pink or yellow fluorescent dye for each plant, which was the main response variable (hereafter,

deposition rate per plant). Given that each plant had the possibility of receiving fluorescent dye particles from two different source populations at the same time (treated with yellow or pink fluorescent dye; see Fig. 1), two records were calculated per plant, one for



**Fig. 3** **a** Flower of *N. linariifolia* treated with pink fluorescent dye. This was deposited with the help of a thin wooden stick on the fertile column of the flowers (stigma and stamens). **b** Photo of a stigma with a great number of deposited particles of yellow fluorescent dye (that appears green under the fluorescent microscope with adequate filters; Zeiss Axiophot, magnification 5×1×)

each colour of fluorescent dye. This procedure led to have a number of records depicting the deposition rates of yellow or pink fluorescent dye equal to the double of the number of plants (159 plants \* 2 source populations = 318 records; one record equals to one row in the final dataset). Each plant was associated with the deposition rate of fluorescent dye of one colour, a “distance” to each source population (continuous factor, in meters), and a “class”, a discrete factor with two levels: plants connected to a source population through remnant forest (i.e. located at the same forest patch of the source population); or unconnected

plants, those located at a neighbouring forest patch separated from the source population by matrix of maize crop. The observed distances for connected plants ranged from 1 to 470 m, while for unconnected plants ranged from 54 to 1440 m. The maximum distance for unconnected plants (i.e. 1440 m, the absolute maximum distance at which we sought for fluorescent dye deposition) was higher than the maximum distance for connected plants due to the inclusion of plants of the distant neighbouring source population that was tested at the same time (with fluorescent dye of different colour; see Fig. 1). Data from all six populations were combined and analysed in a unique dataset.

Firstly, we compared the deposition rate between classes of plant (connected or unconnected), and frequency histograms were made for the deposition rate of each class of plant. Secondly, we evaluated the relationship between the deposition rate and the distance to the source population, combining all data and separately for plants connected and unconnected to a source population. Lastly, we calculated the proportion of successful dispersal events (i.e. stigmata with deposition of fluorescent dye particles) with the distance to a source population, for connected and unconnected plants. Each dataset with records from connected and unconnected plants was separately sorted by the variable “distance” (distance of each plant from which each stigma was collected, from the lowest to the highest observed distance), and an accumulated number of stigmata with deposition for each increasing distance was calculated. Then, the accumulated number of stigmata with deposition at each observed distance was divided by the total number of stigmata with deposition (total number of successful events for connected or unconnected plants) in order to get an accumulated proportion of stigmata with dye deposition. This analysis allowed us to determine the distance at which particular proportions of stigmata with dye deposition (i.e. proportion of successful dispersal events) were found for each class of plant (for example, to determine the distance at which 50% of successful dispersal events were observed for connected or unconnected plants). In order to get fair comparisons, for the models and plots in which we compared between classes of plant (connected vs. unconnected to a source population) we used a partial dataset, which included records from the unconnected plants located at a distance equal or lower to

the maximum observed distance for connected plants (471 m); and records from the connected plants located at a distance equal or higher to the minimum observed distance for unconnected plants (54 m). The use of this partial dataset did not apply for the analysis of the proportions of successful dispersal events with the distance to a source population, where the complete dataset was used (records from plants located at any distance to a source population) in order to get a clear picture of the accumulation pattern for connected plants at short distances from a source population.

We analysed the deposition rate per plant in relation to the class of plant (connected/unconnected) and to the distance to a source population with generalized linear mixed models with a binomial family as a link function and the number of stigmata as weights of the model (Zuur et al. 2009). For a better model fitting, the distance to a source population was transformed with natural logarithm. This variable was later retransformed to the original scale (in meters), as well as the predicted values, for plotting. Also, overdispersion was corrected with the incorporation of an individual level effect (Harrison 2014). All models and plots were made with R (R Core Team 2020). Particularly, we used the function *glmer* from the *lme4* package (Bates et al. 2015), and the package

*DHARMA* (Hartig 2020) for residuals analysis. Plots were made with the *ggplot2* package (Wickham 2016), and the prediction curves were calculated with the *ggeffects* package (Lüdtke 2018).

## Results

Overall, 46% of total recorded plants exhibited at least one stigma with fluorescent dye deposition of any colour. The total recorded plants included those plants susceptible of receiving fluorescent dyes from two source populations (i.e. fluorescent dyes of two different colours), at any distance to a source population, either connected or unconnected (see M&M for more details on the construction of the dataset). Particularly, when analysing data with comparable distances (partial dataset; see M&M for more details), 80% of the records from connected plants had at least one stigma with fluorescent dye deposition compared to 52% of the records from unconnected plants. The observed distances for successful dye dispersal events considering all sampled plants ranged from 1 to 1259 m (see Table 2 for more details on total, connected and unconnected plants for the complete and partial dataset).

**Table 2** Statistical information relative to the deposition of fluorescent dye particles on stigmata of total, connected and unconnected plants, for the complete dataset (records from plants located at any distance to a source population) and par-

tial dataset (records from plants located at comparable distances from a source population between plant classes (from 54 to 471 m); see M&M for details)

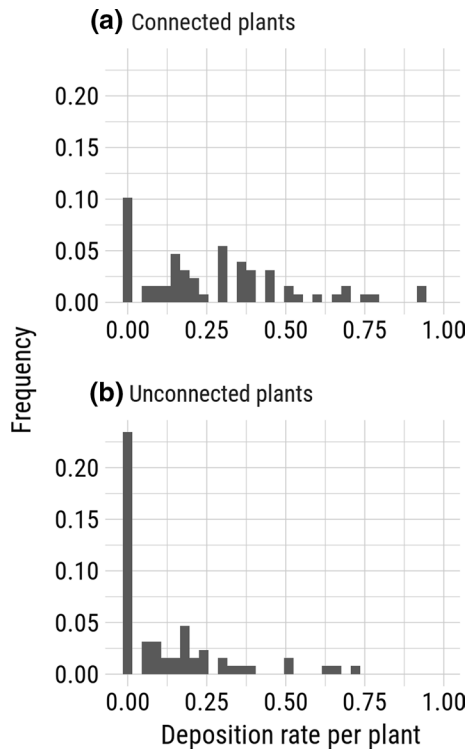
	Complete dataset (all distances)			Partial dataset (distances between 54 and 471 m)		
	Total plants	Connected	Unconnected	Total plants	Connected	Unconnected
Proportion of stigmata with deposition	0.46	0.87	0.25	0.66	0.80	0.52
Deposition rate per plant						
Range	0–1	0–1	0–0.73	0–1	0–1	0–0.73
Median	0	0.38	0	0.14	0.25	0.07
Mean $\pm$ Std. Err	0.18 $\pm$ 0.02	0.45 $\pm$ 0.03	0.05 $\pm$ 0.01	0.21 $\pm$ 0.02	0.29 $\pm$ 0.03	0.13 $\pm$ 0.02
Distance of successful dispersal events						
Range	1–1259	1–471	54–1259	56–471	56–471	56–464
Median	115.79	65.77	150.07	150.07	140.82	133.82
Mean $\pm$ Std. Err	169.66 $\pm$ 19.71	111.92 $\pm$ 13.31	314.85 $\pm$ 54.3	181.50 $\pm$ 14.52	189.42 $\pm$ 17.80	166.29 $\pm$ 25.84

In order to get fair comparisons between connected and unconnected plants, the columns related to the partial dataset should be analysed. *Std. Err.* standard error

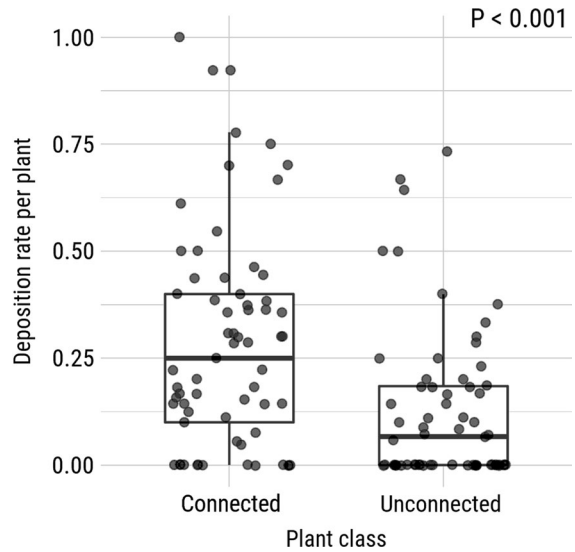


Plants unconnected to a source population showed a higher number of null depositions on their flowers compared with connected plants, when analysing data with comparable distances (Fig. 4). None of the unconnected plants exhibited the total number of collected stigmata with deposition of fluorescent dye (i.e. deposition rate = 1). In this sense, the deposition rate was higher in plants connected to a source population (i.e. located at the same forest patch;  $0.29 \pm 0.03$ ) than in unconnected plants ( $0.13 \pm 0.02$ ) ( $Z = -4.38$ ;  $P < 0.001$ ) (Table 2; Fig. 5).

In general, the deposition rate of a given plant decreased with increasing distances to a source population (i.e. without differentiating between connected and unconnected plants;  $Z = -15.03$ ;  $P < 0.001$ ; Fig. 6a). In the same way, the deposition rate decreased with increasing distances to a source



**Fig. 4** Frequency histograms of deposition rates per plant (# stigmata with dye/# of collected stigmata), for plants connected (a;  $n=65$ ) and unconnected (b;  $n=63$ ) from a source population. Note the higher proportion of unconnected plants with null deposition compared to the connected plants. The records included in this graphical analysis correspond to plants located at comparable distances between plant classes (partial dataset; see M&M for details)



**Fig. 5** Deposition rate (# stigmata with dye / # of collected stigmata) of connected ( $n=65$ ) and unconnected ( $n=63$ ) plants. The records included in this analysis correspond to plants located at comparable distances between plant classes (partial dataset; see M&M for details). The boxplot indicates the median (horizontal line), the 25th and 75th percentiles (lower and upper hinges) and each percentile  $\pm 1.5 * IQR / \sqrt{n}$  ( $IQR$  interquartile range,  $\sqrt{n}$  squared root,  $n$  number of samples) (whiskers)

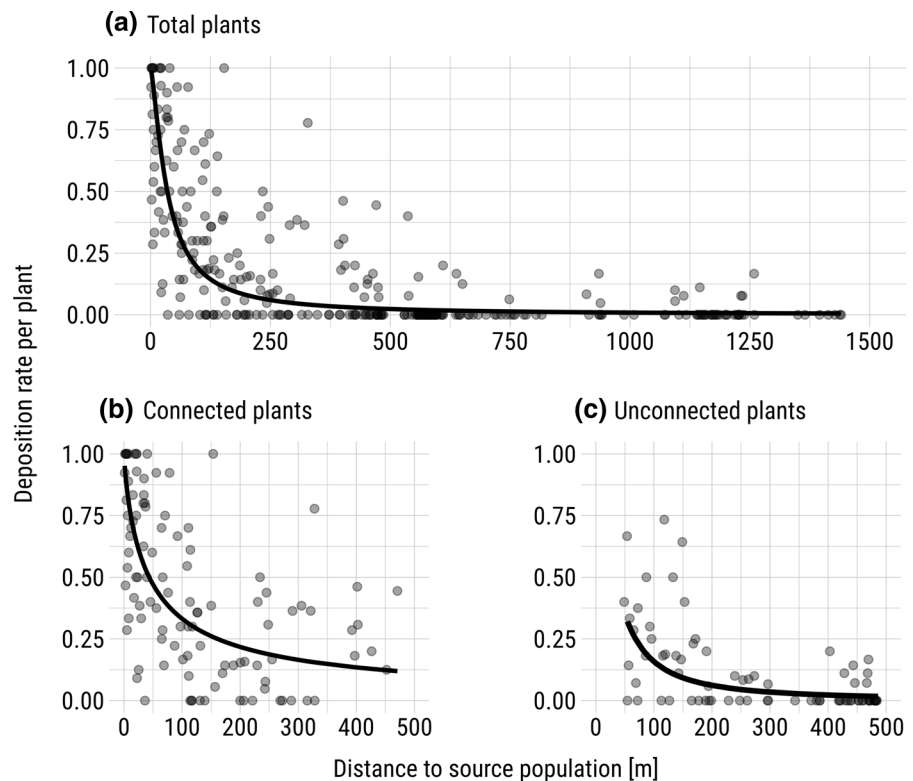
population for connected ( $Z = -7.51$ ;  $P < 0.001$ ; Fig. 6b) and unconnected plants ( $Z = -8.22$ ;  $P < 0.001$ ; Fig. 6c).

Between connected plants, the 50% of successful dispersal events (i.e. stigmata with deposition of fluorescent dye particles) occurred at approximately 36 m or less from a source population, compared to a distance of approximately 111 m or less for unconnected plants (Fig. 7). Indeed, most of the successful dispersal events occurred at the vicinity of source populations (Fig. 7), especially between connected plants, given the observed distances at which 75% of successful dispersal events occurred between connected (138 m) and unconnected plants (446 m).

### Discussion

The main result of our work shows that the loss of connectivity due to forest fragmentation negatively affects pollen flow in the oil-producing and self-incompatible *N. linariifolia*. Plants connected to a

**Fig. 6** Deposition rate (# stigmata with dye/# of collected stigmata) in relation to the distance to a source population for **a** total plants ( $n = 318$ ), **b** connected plants ( $n = 107$ ) and **c** unconnected plants ( $n = 211$ ). The maximum distance for unconnected plants was truncated to 500 m for a better comparison with connected plants. Note in **a** the presence of some deposition events at distances further from 500 m, which correspond with plants that were unconnected to a source population. The curve indicates the predicted values according to the final statistical model. This analysis included records from connected and unconnected plants from all observed distances (complete dataset; see M&M for details)

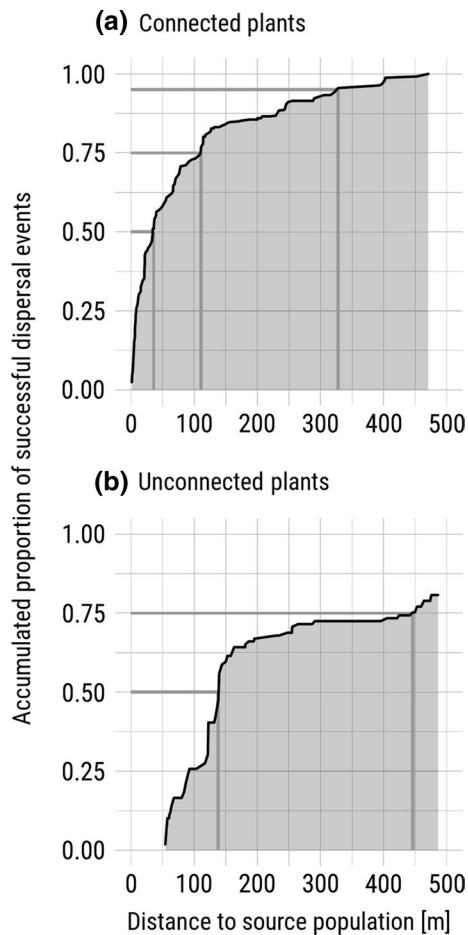


source population showed higher deposition rates of fluorescent dye particles compared to plants unconnected by a crop matrix of non-flowered maize. In this sense, connected populations of *N. liniariifolia* within a forest patch significantly increased the deposition of fluorescent dye, and most dispersal events occurred at short distances. Nevertheless, bees were also capable of dispersing fluorescent dye particles between unconnected plants of *N. liniariifolia*, evidencing the potentiality of pollen dispersal even at considerable distances within the studied agroecosystem.

Given the entomophilic nature of the flowers of *N. liniariifolia*, results indicate that specialized bees are acting as successful pollen dispersers across the agroecosystem with fragmented forest. The presence of remnant forest greatly improved pollen flow between plants, as evidenced by the higher deposition rates on plants connected to a source population (more than twice of the mean deposition rate per plant) and by the lower proportion of plants with null pollen deposition compared with unconnected plants. These results are consistent with several previous works in which a significant boost in pollen dispersal was found between plant populations that

were connected through habitat corridors (also called “linear landscape elements”) (Townsend and Levey 2010; Van Geert et al. 2010; Van Rossum and Triest 2012). Nevertheless, both Van Geert et al. (2010) and Van Rossum and Triest (2012) found almost no dispersal events between unconnected plant populations of *Primula* species, whose flowers are mostly visited by bees. The few dispersal events between unconnected plant populations may be explained because of the high levels of landscape fragmentation (Van Geert et al. 2010), where remnant habitats were represented by thin linear landscape elements composed by ditches; or because of a harsh urban composition of the matrix (Van Rossum and Triest 2012), which may hinder pollinator mobility throughout the landscape. Conversely, in our work we found a considerable number of dispersal events between unconnected plants, a striking result that may be explained because the studied landscape is composed by several forest patches of considerable size and relatively close to each other, favouring pollinator movements throughout the landscape and between neighbouring patches.

Van Geert et al. (2014) showed higher rates of fluorescent dye particles through habitat lineal corridors



**Fig. 7** Accumulated proportion of successful dispersal events (i.e. stigmata with deposition of fluorescent dye particles) in relation to the distance to a source population, for connected (a) and unconnected (b) plants. Horizontal lines indicate the accumulated proportions of 0.5, 0.75 and 0.95; and their intersection with the accumulated curve determine the vertical lines, which indicate the distances at which the mentioned accumulated proportions were reached. The maximum distance for unconnected plants was graphically truncated to 500 m for a better comparison with connected plants. The accumulated proportion of 0.95 for unconnected plants was reached at a distance of 1094 m. This analysis included records from connected and unconnected plants from all observed distances (complete dataset; see M&M for details)

when the matrix was composed by pastures compared to matrices composed by arable fields or a combination of these and pastures. In this sense, matrices composed by crops with intensive agricultural practices such as monocultures of soybean or maize (as in our work) may differentially affect pollinator movement patterns compared with matrices composed

by diverse crops, or by a combination of crops and pastures (Jules and Shahani 2003). Moreover, the effect of mass-flowering crops on diluting pollinators throughout the landscape has been evidenced in previous works (e.g. Holzschuh et al. 2011, 2016; Stanley and Stout 2014), which may affect pollination of plants within remnant patches as well as pollen flow between unconnected plant populations. For instance, previous works show that flowered maize crops are usually foraged by *A. mellifera* and even other native bees, which collect pollen grains (Danner et al. 2014; Malerbo-Souza et al. 2018), indicating that the presence of mass-flowering maize crops can affect pollinator foraging activity. More studies are needed to evaluate the influence of different matrix compositions, such as mass-flowering maize crops, on the foraging behaviour of pollinators throughout the landscape and on the process of pollen dispersal on native plants.

The deposition rate of fluorescent dye on recipient plants decreased in an exponential fashion with the distance to source populations, in agreement with previous works on pollen dispersal for plant species mainly pollinated by bees (Townsend and Levey 2010; Van Geert et al. 2010; Van Rossum and Triest 2010, 2012; Aquino and Amela García 2019) or butterflies (Townsend and Levey 2010). Consistently with the mentioned studies, in our work the great majority of dispersal events were observed at the vicinity of source populations (75% of dispersal events at distances less than 100 and 400 m between connected plants and unconnected plants, respectively). This result, especially for plants connected through remnant forest, may be explained by the potentially short (but yet unknown) foraging ranges of the native solitary bee species that commonly visit the flowers of *N. liniarifolia*. Previous studies have shown a positive relationship between bee body size and their foraging ranges (Gathmann and Tscharntke 2002; Greenleaf et al. 2007). For instance, the small size of bee species from the genus *Chalepogenus* (Maubecin et al. 2021) and of the observed Halictidae bees that commonly visit the flowers of *N. liniarifolia* may support the reported pattern of short dispersal events. Related to this, some previous works have reported the presence of high habitat patch fidelity for several solitary bee species (e.g. Kapyla 1978; Franzén et al. 2009). Also, the dispersal events at short distances from the source population could be

also explained by the activity of the large-sized bee *C. tricolor*, whose genus (*Centris*) has been described as a “trap-line” pollinator (i.e. a pollinator that visits the same group of plants every day and presumably in the same order) (Aquino and Amela García 2019). If the described behaviour patterns take place for the bee species visiting *N. linariifolia*, these may influence the maximum foraging distances by solitary bees, even between plants connected through remnant forest, and would partially explain the short dispersal events of fluorescent dye.

Interestingly, several dispersal events between plants unconnected by a matrix of maize crops happened at far distances from a source population (up to 1259 m), differing from previous studies on pollen dispersal (also by using fluorescent dye particles as pollen analogues), where almost no dispersal events were found between unconnected plant populations (Van Geert et al. 2010; Van Rossum and Triest 2012; Van Rossum et al. 2015; Aquino and Amela García 2019). Nevertheless, Van Rossum (2010) recorded long dispersal events (up to 2.6 km) among unconnected populations of *Centaurea jacea* at a landscape with an urban area matrix, and postulates bumblebee species as the responsible for these long dispersal events. On one hand, the long dispersal events found in our work cannot be explained by direct dispersal of potentially short foraging pollinators such as the small bees from the genus *Chalepogenus* or bees from Halictidae. On the other hand, the large-sized native bee *C. tricolor* may be capable of large foraging flights (see the observation made by Janzen (1971) for the genus *Centris*), which may explain these long dispersal events. The ability of performing very long flights by *A. mellifera* would explain part of the reported long dispersal events (Beekman and Ratnieks 2000), but visits of this exotic bee species to *N. linariifolia* are very rare (Cosacov et al. 2008). Although *C. tricolor* is the best candidate as the responsible for the long pollen dispersal events between *N. linariifolia* plants, more experiments on the foraging behaviour of the different native pollinator species should be carried out to better understand pollen dispersal throughout the landscape. Another possible explanation for the observed long dispersal events is the occurrence of secondary pollen transference (or in this case, transference of fluorescent dye particles), a process that occurs when pollinators remobilize pollen grains that had already been

deposited to the stigma of a flower by a previous pollinator (i.e. “remobilization” of pollen grains) (Thomson and Eisenhart 2003), although the occurrence of this process should be properly tested for *N. linariifolia*. Ultimately, the observed long distance dispersal events emphasize the important role of pollinators in improving pollen flow between unconnected plant populations in fragmented landscapes.

Although the use of fluorescent dye as a pollen analogue has been proven to be an acceptable proxy of pollen dispersal at different contexts and for diverse plant species (e.g. Austerlitz et al. 2004; Adler and Irwin 2006; Townsend and Levey 2010; Van Geert et al. 2010; Ottewell et al. 2012; Van Rossum and Triest 2012; Wanderley et al. 2020), this method may not completely correspond with the actual process of pollen dispersal happening at the field. Fluorescent dye particles are smaller than pollen grains and may move farther, but they are also less sticky, so they may underestimate effective pollen dispersal (Adler and Irwin 2006). The use of quantum dots appears as a novel method to track the fate of individual pollen grains (Minnaar and Anderson 2019). These are semiconductor nanocrystals that act as pollen labels, and emit bright light when exposed to UV light. Although it needs further verification at the field, this promising technique appears to be a better, more precise and relatively inexpensive alternative to fluorescent dyes to estimate pollen dispersal.

#### Implications for conservation and management

The results of this work have direct implications for the conservation and management of remnant forest in agroecosystems. Particularly, results emphasize the role of remnant forest in connecting plant populations, which improves pollen flow throughout the landscape, and highlight the important role of native bees in enhancing pollen dispersal from a source population to unconnected recipient plants (i.e. separated by a distinct matrix such as crops), even probably at long distances. It is interesting to stress out the contrasting effects that forest loss and fragmentation may have to the persistence of the populations of plant species such as *N. linariifolia*. Given that populations of *N. linariifolia* mainly thrive at degraded and unshaded habitats, such as the edges of forest patches and canopy gaps, this species would be benefited by processes that promote the creation of these types of

open habitats, such as the processes of forest loss and fragmentation. However, these processes also have profound negative effects on plant biodiversity and plant-pollinator interactions over space and time (e.g. Cagnolo et al. 2006; Aguilar et al. 2006, 2008; Galetto et al. 2022). The loss of plant richness may have cascading effects on pollinator abundance and diversity (Hadley et al. 2012; Ferreira et al. 2013), and consequently affect the pollination of plants highly dependent on specialized pollinators such as *N. linariifolia*. Indeed, Cosacov et al. (2008) showed that this species is pollen-limited for fruit and seed production through quantity (pollinator visitation rates and number of pollen grains deposited on stigmata) and quality (compatibility of the deposited pollen grains) components. Pollen limitation in *N. linariifolia* could be partially explained by restrictions on pollen dispersal by pollinators inhabiting highly degraded and fragmented landscapes, as exhibited in the present study.

Our results support the necessity of developing forest management and restoration strategies to preserve and enhance native bee richness and abundances, which could also improve pollen flow throughout the landscape (Menz et al. 2011) and maintain the pollination service for native plants and crops (Galetto et al. 2022). In this sense, the creation of linear forest corridors, hedgerows and the conservation of field margins with native plant species (i.e. linear landscape elements) may improve pollen dispersal, as it would facilitate native bees in reaching isolated plants (Van Geert et al. 2010; Townsend and Levey 2010; Cranmer et al. 2012; Kormann et al. 2016). Also, the preservation of even small forest patches, or the generation of new ones with native flora, may act as stepping stones for native bees (for instance, patches of habitat providing floral resources and nesting sites) which would also improve pollen flow towards isolated plant populations (Lander et al. 2010; Menz et al. 2011; Van Rossum and Triest 2012). Particularly in highly degraded landscapes, restoration of native flora should take into account a thorough selection of native plant species to be planted, in order to offer diverse resources for different pollinator species throughout the year (Menz et al. 2011). Finally, the adoption of ecological intensification practices will enhance abundance and richness of pollinators (IPBES 2016). Among others, these practices involve the preservation of high-quality

natural or semi-natural habitats inside farms and in the surroundings, an increased crop diversity (which implies a more complex matrix for pollinators), and the reduction of agrochemicals applications, all practices that may counterbalance the negative impacts of intensive monoculture agriculture (Kennedy et al. 2013; Galetto et al. 2022). Overall, the mentioned strategies would improve biodiversity conservation, increase gene flow between unconnected plant populations, enhance genetic variability and prevent inbreeding, which may redound in an increase of plant fitness (Lienert 2004; Leimu et al. 2010; Wanderley et al. 2020).

## Conclusions

Our work contributes to the body of empirical research studying the effects of habitat fragmentation on pollen flow between unconnected plant populations, by focusing on a self-incompatible species with specialized oil-producing flowers such as *N. linariifolia*. By using fluorescent dyes as pollen analogues, the results show an increase of pollen flow between plants inhabiting the same forest patch, and a decrease of pollen dispersal with increasing distances to a source population. Deposition rates per plant were higher in connected than in unconnected plants, and long dispersal events (> 1000 m) were recorded between plants located at neighbouring forest patches separated by an agricultural matrix. Overall, our results highlight the importance of forest connectivity throughout the landscape to enhance pollen flow in *N. linariifolia* and pinpoint the essential role of native bees in connecting plants unconnected by a matrix of crops, supporting the urgent need to develop strategies of forest management and restoration in order to conserve native bees populations and pollination services at fragmented landscapes.

**Acknowledgements** We thank anonymous reviewers for useful suggestions and comments, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba (UNC) and FONCYT for financial support. We thank the landowners for the access to the fields.

**Author contributions** PYH conceived the initial idea. PYH, GG and LG planned the experiment. PYH and GG performed the experiment. All authors discussed the results and contributed to the final manuscript.

**Funding** Foncyt, PICT-2015-0538. “Efectos de la biodiversidad sobre el funcionamiento de agro-ecosistemas: evaluación experimental a distintas escalas espaciales”. Proyecto Consolidar SECYT (UNC), Res. 411/18. 2018-2021. “Fragmentación de bosque y pérdida de biodiversidad por el cambio en el uso del suelo: evaluación de umbrales genéticos y ecológicos en agroecosistemas”.

**Data availability** The data that supports the findings of this study is available at the Dryad Digital Repository (<https://doi.org/10.5061/dryad.08kpr55c>).

**Code availability** Not applicable.

## Declarations

**Conflict of interest** Not applicable.

**Ethical approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

## References

- Adler LS, Irwin RE (2006) Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Ann Bot-Lond* 97(1):141–150
- Aguiar CML (2003) Flower visits of Centris bees (Hymenoptera: Apidae) in an area of caatinga (Bahia, Brazil). *Stud Neotrop Fauna Environ* 38(1):41–45
- Aguilar R, Galetto L (2004) Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui*(Solanaceae). *Oecologia* 138(4):513–520.
- Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecol Lett* 9(8):968–980
- Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches. *Mol Ecol* 17(24):5177–5188
- Aguilar R, Calviño A, Ashworth L, Aguirre-Acosta N, Carbone LM, Albrieu-Llinás G, Nolasco M, Ghilardi A, Cagnolo L (2018) Unprecedented plant species loss after a decade in fragmented subtropical Chaco Serrano forests. *Plos One* 13(11): e0206738
- Aizen MA, Ashworth L, Galetto L (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? *J Veg Sci* 13(6):885–892
- Aquino DS, Amela García MT (2019) Pollen dispersal in a population of *Passiflora caerulea*: spatial components and ecological implications. *Plant Ecol* 220(9):845–860
- Austerlitz F, Dick CW, Dutech C, Klein EK, Oddou-Muratario S, Smouse PE, Sork VL (2004) Using genetic markers to estimate the pollen dispersal curve. *Mol Ecol* 13(4):937–954
- Bates D, Maechle M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67(1):1–48
- Beekman M, Ratnieks FLW (2000) Long-range foraging by the honey-bee *Apis mellifera* L. *Funct Ecol* 14(4):490–496
- Buchmann SL (1987) The ecology of oil flowers and their bees. *Annu Rev Ecol Syst* 18:343–369
- Buschi CA, Pomilio AB (1987) Pyrrole-3-carbamidine: a lethal principle from *Nierembergia hippomanica*. *Phytochemistry* 26(3):863–865
- Cabido M, Zeballos SR, Zak M, Carranza ML, Giorgis MA, Cantero JJ, Acosta ATR (2018) Native woody vegetation in central Argentina: classification of Chaco and Espinal forests. *Appl Veg Sci* 21(2):298–311
- Cáceres DM (2015) Accumulation by dispossession and socio-environmental conflicts caused by the expansion of agribusiness in Argentina. *J Agrar Change* 15(1):116–147
- Cocucci AA (1984) Polinización en *Nierembergia hippomanica* (Solanaceae). *Kurtziana* 17:31–47
- Cocucci AA (1991) Pollination biology of *Nierembergia* (Solanaceae). *Plant Syst Evol* 174(1):17–35
- Cocucci AA, Hunziker AT (1995) Estudios sobre Solanaceae. XLI. *Nierembergia linariaefolia* y *N. pulchella*: sus sinónimos y variedades. *Darwiniana* 33(1):33–42
- Cagnolo L, Cabido M, Valladares G (2006) Plant species richness in the Chaco Serrano Woodland from central Argentina: Ecological traits and habitat fragmentation effects. *Biol Conserv* 132(4):510–519
- Cosacov A, Nattero J, Cocucci AA (2008) Variation of pollinator assemblages and pollen limitation in a locally specialized system: the oil-producing *Nierembergia linariifolia* (Solanaceae). *Ann Bot-Lond* 102(5):723–734
- Cranmer L, McCollin D, Ollerton J (2012) Landscape structure influences pollinator movements and directly affects plant reproductive success. *Oikos* 121(4):562–568
- Cunningham SA (2000) Depressed pollination in habitat fragments causes low fruit set. *P R Soc B-Biol Sci* 267(1448):1149–1152
- Danner N, Härtel S, Steffan-Dewenter I (2014) Maize pollen foraging by honey bees in relation to crop area and landscape context. *Basic Appl Ecol* 15(8):677–684
- Defries RS, Foley JA, Asner GP (2004) Land-use choice : balancing human needs and ecosystem function. *Front Ecol Environ* 2(5):249–257
- Díaz S, Demissew S, Carabias J, Joly C, Lonsdale M, Ash N et al (2015) The IPBES conceptual framework: connecting nature and people. *Curr Opin Environ Sustain* 14:1–16
- Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annu Rev Ecol Syst* 34(1):487–515
- Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD (2004) Pollination syndromes and floral specialization. *Annu Rev Ecol Syst* 35:375–403
- Ferreira PA, Boscolo D, Viana BF (2013) What do we know about the effects of landscape changes on plant-pollinator interaction networks? *Ecol Indic* 31:35–40
- Ferreira F, Torres C, Bracamonte E, Galetto L (2017) Effects of herbicide glyphosate on non-target plant native species form Chaco forests from Argentina. *Ecotox Environ Saf* 144:360–368

- Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR et al (2005) Global consequences of land use. *Science* 309(5734):570–574
- Franzén M, Larsson M, Nilsson SG (2009) Small local population sizes and high habitat patch fidelity in a specialised solitary bee. *J Insect Conserv* 13(1):89–95
- Galetto L, Aizen MA, Arizmendi MC, Freitas BM, Garibaldi LA, Giannini TC et al (2022) Risks and opportunities associated with pollinators' conservation and management of pollination services in Latin America. *Ecol Austral* 32:055–076
- Garibaldi LA, Steffan-Dewenter I, Kremen C, Morales JM, Bommarco R, Cunningham SA et al (2011) Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecol Lett* 14(10):1062–1072
- Gathmann A, Tschardt T (2002) Foraging ranges of solitary bees. *J Anim Ecol* 71(5):757–764
- Gonçalves L, Silva CI, Buschini MLT (2012) Collection of pollen grains by *Centris* (Hemisiella) *tarsata* Smith (Apidae: Centridini): is *C. tarsata* an oligolectic or polylectic species? *Zool Stud* 51(2):195–203
- Green RE, Cornell SJ, Scharlemann JPW, Balmford A (2005) Farming and the fate of wild nature. *Science* 307(5709):550–555
- Greenleaf SS, Williams NM, Winfree R, Kremen C (2007) Bee foraging ranges and their relationship to body size. *Oecologia* 153(3):589–596
- Hadley AS, Betts MG (2012) The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. *Biol Rev* 87(3):526–544
- Hansen MC, Potapov PV, Moore R, Hancher M, Turubanova SA, Tyukavina A et al (2013) High-resolution global maps of 21st-century forest cover change. *Science* 342(6160):850–853
- Harrison XA (2014) Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ* 2:e616
- Hartig F (2020) DHARMA: residual diagnostics for hierarchical (Multi-Level/Mixed) regression models. R package version 0.3.3.0. <https://CRAN.R-project.org/package=DHARMA>
- Hofmann MM, Fleischmann A, Renner SS (2020) Foraging distances in six species of solitary bees with body lengths of 6 to 15 mm, inferred from individual tagging, suggest 150 m-rule-of-thumb for flower strip distances. *J Hymenopt Res* 77:105
- Holzschuh A, Dormann CF, Tschardt T, Steffan-Dewenter I (2011) Expansion of mass-flowering crops leads to transient pollinator dilution and reduced wild plant pollination. *Proc R Soc B-Biol Sci* 278(1723):3444–3451
- Holzschuh A, Dainese M, González-Varo JP, Mudri-Stojnić S, Riedinger V, Rundlöf M et al (2016) Mass-flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecol Lett* 19(10):1228–1236
- Hoyos LE, Cingolani AM, Zak MR, Vaieretti MV, Gorla DE, Cabido MR (2012) Deforestation and precipitation patterns in the arid Chaco forests of central Argentina. *Appl Veg Sci* 16(2):260–271
- IPBES (2016) The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany
- Janzen DH (1971) Euglossine bees as long-distance pollinators of tropical plants. *Science* 171(3967):203–205
- Jules ES, Shahani P (2003) A broader ecological context to habitat fragmentation: why matrix habitat is more important than we thought. *J Veg Sci* 14(3):459–464
- Kapyla M (1978) Foraging distance of a small solitary bee, *Chelostoma maxillosum* (Hymenoptera, Megachilidae). *Ann Entomol Fenn* 35:63–64
- Kennedy CM, Lonsdorf E, Neel MC, Williams NM, Ricketts TH, Winfree R et al (2013) A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecol Lett* 16(5):584–599
- Kormann U, Scherber C, Tschardt T, Klein N, Larbig M, Valente JJ, Hadley AS, Betts MG (2016) Corridors restore animal-mediated pollination in fragmented tropical forest landscapes. *P R Soc B-Biol Sci* 283(1823):20152347
- Lander TA, BDH, Harris SA (2010) Fragmented but not isolated: contribution of single trees, small patches and long-distance pollen flow to genetic connectivity for *Gomortega keule*, an endangered Chilean tree. *Biol Conserv* 143(11):2583–2590
- Leimu R, Vergeer P, Angeloni F, Ouborg NJ (2010) Habitat fragmentation, climate change, and inbreeding in plants. *Ann NY Acad Sci* 1195:84–98
- Lienert J (2004) Habitat fragmentation effects of fitness of plant populations: a review. *J Nat Conserv* 12(1):53–72
- Lüdtke D (2018) ggEffects: tidy data frames of marginal effects from regression models. *J Open Sour Softw* 3(26):772
- Malerbo-Souza DT, da Silva TG, de Andrade MO, de Farias LR, Medeiros NMG (2018) Factors affecting the foraging behavior of bees in different maize hybrids. *Revista Brasileira De Ciências Agrárias* 13(3):1–8
- Maubecin CC, Rocamundi N, Palombo N, Aguirre LA, Cocucci AA, Sérsic AN (2021) Teasing out the functional groups of oil-collecting bees in the light of the pollination of *Nierembergia* flowers. *Arthropod-Plant Inte* 15(5):809–819
- Mayer C, Van Rossum F, Jacquemart AL (2012) Evaluating pollen flow indicators for an insect-pollinated plant species. *Basic Appl Ecol* 13(8):690–697
- Menz MHM, Phillips RD, Winfree R, Kremen C, Aizen MA, Johnson SD, Dixon KW (2011) Reconnecting plants and pollinators: challenges in the restoration of pollination mutualisms. *Trends Plant Sci* 16(1):4–12
- Minnaar C, Anderson B (2019) Using quantum dots as pollen labels to track the fates of individual pollen grains. *Methods Ecol Evol* 10(5):604–614
- Muscante M (2013) Fragmentación del hábitat y su efecto sobre comunidades de polinizadores y antófilos (Hymenoptera: Aculeata) en el Bosque Chaqueño Serrano. PhD Thesis. Fac. Cs. Ex., F. y Naturales, UNC. Córdoba, Argentina
- Odini A, Rivero R, Correa FR, Mendez C (1995) Intoxicación por *Nierembergia hippomanica* en bovinos y ovinos. *Veterinaria (montevideo)* 31(127):3–8

- Ottewell K, Grey E, Castillo F, Karubian J (2012) The pollen dispersal kernel and mating system of an insect-pollinated tropical palm *Oenocarpus Bataua*. *Heredity* 109(6):332–339
- R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rasmussen C, Olesen JM (2000) Oil flowers and oil-collecting bees. *Scand Assoc Pollinat Ecol Honours Knut Faegri* 39:23–31
- Stanley DA, Stout JC (2014) Pollinator sharing between mass-flowering oilseed rape and co-flowering wild plants: implications for wild plant pollination. *Plant Ecol* 215(3):315–325
- Thomson JD, Eisenhart KS (2003) Rescue of stranded pollen grains by secondary transfer. *Plant Spec Biol* 18(2–3):67–74
- Torino F, Colque Caro LA, Martinez OG, Micheloud JF (2017) Intoxicación experimental con *Nierembergia linariifolia* var. *linariifolia* en cabras. *FAVE-Sección Ciencias Veterinarias* 16(2):66–69
- Townsend PA, Levey DJ (2010) An experimental test of whether habitat corridors affect pollen transfer. *Ecology* 86(2):466–475
- Vallejos M, Volante JN, Mosciaro MJ, Vale LM, Bustamante ML, Paruelo JM (2015) Transformation dynamics of the natural cover in the Dry Chaco ecoregion: a plot level geodatabase from 1976 to 2012. *J Arid Environ* 123:3–11
- Van Rossum F (2010) Reproductive success and pollen dispersal in urban populations of an insect-pollinated hay-meadow herb. *Perspect Plant Ecol* 12(1):21–29
- Van Rossum F, Triest L (2010) Pollen dispersal in an insect-pollinated wet meadow herb along an urban river. *Landsc Urban Plan* 95(4):201–208
- Van Rossum F, Triest L (2012) Stepping-stone populations in linear landscape elements increase pollen dispersal between urban forest fragments. *Plant Ecol Evol* 145(3):332–340
- Van Geert A, Van Rossum F, Triest L (2010) Do linear landscape elements in farmland act as biological corridors for pollen dispersal? *J Ecol* 98(1):178–187
- Van Geert A, Triest L, Van Rossum F (2014) Does the surrounding matrix influence corridor effectiveness for pollen dispersal in farmland? *Perspect Plant Ecol* 16(4):180–189
- Van Rossum F, Stiers I, Van Geert A, Triest L, Hardy OJ (2011) Fluorescent dye particles as pollen analogues for measuring pollen dispersal in an insect-pollinated forest herb. *Oecologia* 165(3):663–674
- Van Rossum F, Vereecken NJ, Brédard E, Michez D (2012) Pollen dispersal and fruit production in *Vaccinium oxycoccos* and comparison with its sympatric congener *V. uliginosum*. *Plant Biol* 15(2):344–352
- Van Rossum F, Leprince N, Mayer C, Raabová J, Hans G, Jacquemart AL (2015) What influences pollen dispersal in the clonal distylous *Menyanthes trifoliata* (Menyanthaceae)? *Plant Ecol Evol* 148(2):199–212
- Volpe NL, Robinson WD, Frey SJK, Hadley AS, Betts MG (2016) Tropical forest fragmentation limits movements, but not occurrence of a generalist pollinator species. *PLoS ONE* 11(12):1–13
- Wanderley AM, dos Santos EKR, Galetto L, Benko-Iseppon AM, Machado ICS (2020) Pollen flow within and among isolated populations of two rare, self-compatible plant species from inselbergs of Northeast Brazil. *Plant Ecol* 221(4):229–240
- Wickham H (2016) *ggplot2: elegant graphics for data analysis*. Springer-Verlag, Cham
- Young HJ (2002) Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). *Am J Bot* 89(3):433–440
- Zak MR, Cabido M, Cáceres D, Díaz S (2008) What drives accelerated land cover change in central Argentina? Synergistic consequences of climatic, socioeconomic, and technological factors. *Environ Manag* 42(2):181–189
- Zuur A, Ieno E, Walker N, Saveliev A, Smith G (2009) *Mixed effect models and extensions in ecology with R*. Springer, New York

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.