BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research



Cite this article: González-Varo JP, Díaz-García S, Arroyo JM, Jordano P. 2019 Seed dispersal by dispersing juvenile animals: a source of functional connectivity in fragmented landscapes. *Biol. Lett.* **15**: 20190264. http://dx.doi.org/10.1098/rsbl.2019.0264

Received: 8 April 2019 Accepted: 5 June 2019

Subject Areas:

behaviour, ecology

Keywords:

cross-habitat spillover, DNA barcoding, intraspecific variability, natal dispersal, mobile links

Author for correspondence:

Juan P. González-Varo e-mail: jpgvaro@outlook.com

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9. figshare.c.4551821.



Community ecology

Seed dispersal by dispersing juvenile animals: a source of functional connectivity in fragmented landscapes

Juan P. González-Varo^{1,2,3}, Sarah Díaz-García¹, Juan M. Arroyo¹ and Pedro Jordano¹

¹Integrative Ecology Group, Estación Biológica de Doñana (EBD–CSIC), Sevilla, Spain ²Terrestrial Ecology Group, IMEDEA (UIB–CSIC), Esporles, Spain ³Research Unit of Biodiversity (CSIC-UO-PA), Universidad de Oviedo, Mieres, Spain

🔟 JPG-V, 0000-0003-1439-6475

Juvenile animals generally disperse from their birthplace to their future breeding territories. In fragmented landscapes, habitat-specialist species must disperse through the anthropogenic matrix where remnant habitats are embedded. Here, we test the hypothesis that dispersing juvenile frugivores leave a footprint in the form of seed deposition through the matrix of fragmented landscapes. We focused on the Sardinian warbler (Sylvia melanocephala), a resident frugivorous passerine. We used data from field sampling of bird-dispersed seeds in the forest and matrix of a fragmented landscape, subsequent disperser identification through DNA-barcoding analysis, and data from a national bird-ringing programme. Seed dispersal by Sardinian warblers was confined to the forest most of the year, but warblers contributed a peak of seed-dispersal events in the matrix between July and October, mainly attributable to dispersing juveniles. Our study uniquely connects animal and plant dispersal, demonstrating that juveniles of habitatspecialist frugivores can provide mobile-link functions transiently, but in a seasonally predictable way.

1. Introduction

Highly mobile animals that actively move in fragmented landscapes and connect habitats through ecological processes—i.e. by transporting propagules, nutrients or diseases—are termed 'mobile links' (*sensu* [1]; e.g. [2,3]). Frugivores play a major role as mobile links by dispersing seeds in the increasingly fragmented and deforested landscapes of the planet [4,5], fostering regeneration of disturbed habitats and functional connectivity among patches of remnant vegetation [3,6–8]. Such mobile-link services can be unevenly distributed among species within local frugivore assemblages [3] because distinct frugivore species may vary in their response to landscape alteration [9]. Yet, we know virtually nothing about within-species differences in mobile-link services provided by consistent groups of individuals (e.g. different genders or age groups), despite the recently recognized relevance of intraspecific variability in other functional components of frugivore-mediated seed dispersal [10,11].

Every year, millions of juvenile animals perform a dispersal event that occurs only once in their lives, from their birthplace to their future breeding territories, known as natal dispersal [12,13]. In fragmented landscapes, natal dispersal means that many juveniles of habitat-specialist species must leave natural habitats to travel across the low-quality habitats of the matrix [14,15]. Juvenile frugivores could therefore mediate distinctive seed-dispersal services through this 'cross-habitat spillover' [16], operating as mobile links transiently but in a seasonally predictable way.

2



Figure 1. (*a*) Breeding phenology of the Sardinian warbler in the Iberian Peninsula (based on [14]); drawing: Daniel García. (*b*) Study design: we placed seed traps (red rectangles) beneath trees and shrubs in a Mediterranean forest (n = 28) and beneath isolated trees in an adjacent agricultural matrix (n = 28). (*c*) Boxplot (median, quartiles and 10th/90th percentiles; circles: mean values) showing the bimonthly number of seeds dispersed by Sardinian warblers (identified through DNA barcoding) per seed trap in forest and matrix, during two sampling years. Different letters denote significant differences among bimonths in the matrix (Tukey post hoc tests: adjusted *p*-values < 0.05). (Online version in colour.)

Here, we test the hypothesis that dispersing juvenile frugivores leave a landscape-scale footprint in the form of seed deposition through the matrix of fragmented landscapes. We focused on a resident avian frugivore-the Sardinian warbler (Sylvia melanocephala, Sylviidae)-as a study species because it is a resident species that breeds locally, produces dispersing juveniles and occurs in the landscape throughout the year, allowing comparisons between seasons. We used an exceptional dataset resulting from (i) a 2-year field sampling of bird-dispersed seeds in forest and matrix of a fragmented landscape, and (ii) subsequent DNA-barcoding analysis that unveiled the contribution of the Sardinian warbler to seed deposition patterns. Finally, we combined this information with a national bird-ringing dataset in order to link seasonal patterns of seed dispersal in forest and matrix with seasonal captures of adult and juvenile warblers in analogous habitats.

2. Material and methods

(a) The frugivore species

The Sardinian warbler is an approximately 11-g passerine that is resident in the southern Iberian Peninsula [17] and shows a marked territorial behaviour and site-fidelity [18]. This warbler plays an important role throughout the Mediterranean Basin as a seed disperser of many fleshy-fruited species [19]. Although it can occur in a wide variety of habitats, it typically prefers open forests with abundant understory [17]. Juvenile dispersal in our study landscape is expected to begin between late June and early July (i.e. after emancipation), according to the laying, incubation, nesting and fledgling phenology in warm Iberian regions (figure 1*a*) [17]. Sexual maturity is reached during the first year of life [17].

(b) Study landscape

The study was conducted in a lowland landscape (40-60 m a.s.l.) located in southern Spain (Cádiz province; 36° 39' N, 5° 57' W). The study plot has a size of *ca* 900 × 700 m and includes a Mediterranean forest remnant surrounded by an agricultural matrix (approx. 65% of landscape cover) (electronic supplementary material, figure S1). A detailed description is provided in [3]. Briefly, the forest has a tree layer composed of large Mediterranean

oaks (*Quercus* spp.) and an understory of treelets and shrubs dominated by fleshy-fruited species. The matrix is mostly devoted to cereal crops but preserves some isolated trees, mainly oaks (approx. 80%) and wild olive trees (*Olea europaea* var. *sylvestris*, approx. 20%); hence, the wild olive is virtually the only fruit species in the matrix [3]. The avian frugivore assemblage in this landscape is dominated by migratory species [20], the Sardinian warbler being the main resident species inhabiting the forest.

(c) Seed-dispersal data

We sampled community-wide seed dispersal mediated by birds in the forest and matrix of the study landscape (figure 1*b*). We conducted periodic sampling surveys (fortnightly) for 2 years, from November 2013 to October 2015; thus, including the entire fruiting periods of local fleshy-fruited species. We placed seed traps beneath tree and shrub canopies to quantify the magnitude of bird-mediated seed rain in each habitat type. Seed traps were 0.22 m² plastic trays covered with wire mesh to prevent post-dispersal seed predation. We monitored a total of 56 seed traps, 28 in the forest, beneath the canopy of different oak trees and shrubs, and 28 in the matrix, beneath distinct isolated oaks at distances ranging from 5 to 190 m from the forest edge (electronic supplementary material, figure S1).

In each sampling survey, we recorded the number of birddispersed seeds per seed trap and sampled individual seeds or droppings with seeds for DNA-barcoding analysis. We did so by putting each sample with a minimum of handling into a 1.5- or 2.0-ml sterile tube. Tubes were labelled and stored in a freezer at -20° C until DNA extraction. We additionally sampled bird-dispersed seeds visually detected, particularly beneath isolated oaks in the matrix, where we expected a very low contribution by Sardinian warblers. With this, we aimed at increasing sample sizes because DNA-barcoding identification generally fails (PCR failure) in 5–10% of samples [3,20,21]. Conversely, we generally collected just a subsample of the seeds in the forest when seed traps received many seeds of certain plant species [20]. A detailed description of this section is available in the electronic supplementary material.

(d) Seed disperser identification through DNA barcoding

DNA of animal origin can be extracted from the surface of defecated or regurgitated seeds, allowing the identification of the frugivore species responsible for dispersal events [3,20,21]. This approach allowed us to know the contribution (%) of Sardinian warblers to the magnitude of seed deposition, for different seed species and periods of the year, quantified in the seed traps we placed in the forest and matrix of our study landscape. Detailed laboratory protocols for DNA extraction, PCR and sequencing can be found in [3,21]. Resulting sequences (length: median = 414 bp; range = 80-417 bp) were aligned and edited using SEQUENCHER 4.9, and then identified at the species level using the 'BARCODE OF LIFE DATA' identification system (BOLD: http://www.boldsystems.org; [22]). We successfully identified the disperser species in 1669 bird droppings (90.2% of 1851 analysed) containing a total of 1907 seeds. We classified species-level identifications as dispersal events mediated by either 'Sardinian warblers' (447 samples with 472 seeds) or 'other species' (1222 samples with 1435 seeds); the identity of the 'other species' can be found in [3,20].

(e) Bird-ringing data

We used a ringing database provided by SEO/Birdlife of Sardinian warblers captured ($n = 126\,891$) in Spain between 1975 and 2018 [23] in order to link seasonal patterns of seed deposition with seasonal captures of juvenile individuals in analogous habitats. We filtered the original database by classifying bird age categories (EURING classification) as 'juveniles' or 'adults' and excluding birds with unknown age. We clustered the original habitat classification of each ringing capture into 'woodland' and 'open habitats', analogous to the forest and matrix in our study landscape. Finally, we selected a subset of the warmer Spanish provinces, dominated by the thermo- and meso-Mediterranean bioclimate, aiming to use ringing data from localities with the climatic conditions as close as possible with those of our study landscape (thermo-Mediterranean). The final subset included 33 457 ringing captures, including 22 536 juveniles and 10921 adults. A detailed description of data filtering is available in the electronic supplementary material.

(f) Data analysis

All data analyses were performed using R 3.5.2 (R Development Core Team 2018). We used bimonths, a six-level factor (January-February, March-April, etc.), as the temporal scale of data aggregation per seed trap. Bimonths provided a proper temporal resolution while preventing a dominance of zeros in our dataset. We used DNA-barcoding identifications in different bimonths of each sampling year, habitats, microhabitats and 50-m distance classes from the forest edge in the matrix, to calculate the relative contribution of Sardinian warblers to seed rain of different plant species in those spatio-temporal combinations ($f_{warbler} =$ $n_{\rm warbler}/n_{\rm total}$). We then estimated the number of seeds dispersed by Sardinian warblers in each seed trap as $SR_{warbler} = SR_{total} \times$ f_{warbler} ; where SR_{total} is the total number of seeds of a plant species found in each seed trap in different bimonths of each year (see a similar procedure in [20]). These estimates were rounded and converted into integers. Finally, we aggregated the accumulated number of seeds dispersed by Sardinian warblers per seed trap and bimonth (seed species pooled), which resulted in 672 data points; i.e. 28 seed traps $\times 2$ habitats $\times 6$ bimonths $\times 2$ years. We then fitted a negative binomial generalized linear mixed-model using the package lme4 (v. 1.1-19) [24] to analyse, through a two-way ANOVA design (Habitat × Bimonth), bimonthly differences in the magnitude of seed rain (i.e. number of seeds per trap) dispersed by Sardinian warblers in forest and matrix. We included the seed-trap identity as a random factor to account for the repeated measures per trap. The significance of fixed effects (*p*-values of Wald χ^2 tests) was computed using the 'Anova' function of the package car (v. 2.1-6) [25].

Finally, we used non-parametric Spearman's rank correlation tests to test for a positive relationship (i.e. one-tailed test) between the average bimonthly seed rain dispersed by Sardinian warblers in the forest and matrix of our study landscape and the accumulated number of ringing captures of juvenile individuals in analogous habitats (n = 6 bimonths). We did not expect this relationship for adults. A detailed description of data analysis is available in the electronic supplementary material.

3. Results

A total of 1651 bird-dispersed seeds of 10 fleshy-fruited species fell in the seed traps during the study. Sardinian warblers were identified as the disperser of 396 seeds (24%) belonging to four plant species: *Rhamnus alaternus* (209), *Pistacia lentiscus* (166), *Rhamnus lycioides* (20) and *Rubus ulmifolius* (1). Spatially, warblers dispersed 349 seeds (88%) in the forest and 47 (12%) in the matrix. Seasonally, there was a marked seed-dispersal peak in both habitats between July–August (highest peak) and September–October (figure 1*c*). Apart from this peak, seed dispersal in the matrix was negligible the rest of the year (figure 1*c*). According to these patterns, Habitat (Wald $\chi_1^2 = 50.0$, $p < 10^{-15}$) and Bimonth (Wald $\chi_5^2 = 51.6$, p = 0.344), had highly significant effects on warbler-mediated seed rain.

Notably, the bimonthly mean seed rain dispersed by Sardinian warblers in the forest and matrix of our study landscape was strongly correlated with the accumulated number of juvenile warblers captured bimonthly in similar habitats within warm Spanish regions, while unrelated to the number of adults (figure 2). This correlation held with the percentage of juvenile individuals per bimonth, which is independent of any seasonal difference in the mist-netting effort (electronic supplementary material, figure S2).

4. Discussion

Our results strongly support our hypothesis that dispersing juvenile frugivores leave a landscape-scale footprint in the form of seed deposition through the matrix of fragmented landscapes. We found a marked peak in the seed-dispersal function mediated by Sardinian warblers in our study landscape between July and October (figure 1). This period matches the expected period of natal dispersal, which may occur several weeks after emancipation [17]. Importantly, this period neither coincides with the seed-dispersal peaks mediated by other frugivore species (electronic supplementary material, figure S3) nor with the peak in fruit availability at the study site (October–February; J. P. González-Varo 2018, unpublished data).

The seed-dispersal peak in the forest clearly reflected the increase in the population density of Sardinian warblers after the recruitment of new juveniles, whereas the peak in the matrix reflected the spillover of dispersing juvenile warblers from the forest. Both ideas are supported by the strong correlation between the bimonthly seed-rain magnitude in the forest and matrix of our study landscape and the number of juvenile warblers captured bimonthly by bird ringers in analogous habitats (figure 2). The spillover from the forest was also supported by a clear distance-decay pattern in warbler-mediated seed rain in the matrix (from 0 to 200 m from the forest edge; electronic supplementary material, figure S4).

Our study uniquely connects animal and plant dispersal, showing that habitat-specialist frugivores can operate as mobile links in a seasonally predictable way, during their juvenile



Figure 2. Bimonthly mean seed rain (n_{seeds} per seed trap) recorded in (*a*) the forest and (*b*) the matrix of our study landscape in relation to the accumulated number of adult (grey dots) and juvenile (black dots) Sardinian warblers captured bimonthly between 1975 and 2018 in similar habitats, in a subset of warm Spanish provinces. Spearman's (ρ) rank correlation tests are shown above the panels for juveniles and adults (ns: p > 0.5; **p = 0.007; ***p = 0.001). (Online version in colour.)

dispersal through the matrix of fragmented landscapes. Importantly, such transient and distinctive seed-dispersal services can foster regeneration in disturbed habitats and plant population connectivity [3]. As natal dispersal is a common ecological process within vertebrates [12,26], the main frugivores and seed dispersers of fleshy-fruited plants [27], our findings might be broadly generalizable across biomes and plant–frugivore assemblages.

Ethics. 'Servicio de Cría Caballar de las Fuerzas Armadas' provided permission to work at the study site.

Data accessibility. Data are available from the Dryad Digital Repository at: https://doi.org/10.5061/dryad.97q0n06 [28].

Authors' contributions. J.P.G.-V. conceived the study. J.P.G.-V. and P.J. planned the sampling design and collected the field data. J.M.A.

performed laboratory work. J.P.G.-V. and S.D.-G. analysed the data and drafted the article with inputs from P.J. All authors revised and approved the manuscript. All authors agree to be held accountable for the content of this paper.

Competing interests. The authors have no competing interests.

Funding. Spanish MINECO (CGL2017-82847-P) and Junta de Andalucía Excellence Projects (RNM–5731) awarded to P.J., Severo Ochoa Award for Centres of Excellence in R + D + I (SEV-2012-0262) and a postdoctoral fellowship from the Severo Ochoa Program awarded to J.P.G.-V. (SEV-2012-0262). While writing this paper, J.P.G.-V. was funded by a Spanish 'Ramon y Cajal' fellowship (RYC-2017-22095). Acknowledgements. The 'Molecular Ecology Laboratory' (Estación Biológica de Doñana, LEM–EBD–CSIC; ISO9001:2015 and ISO14001:2015 certifications) provided logistical support. SEO/BirdLife (Oficina de Anillamiento) provided the ringing data. Benno Simmons kindly checked the English grammar and style.

References

- Lundberg J, Moberg F. 2003 Mobile link organisms and ecosystem functioning: implications for ecosystem resilience and management. *Ecosystems* 6, 87–98. (doi:10.1007/s10021-002-0150-4)
- Kremen C *et al.* 2007 Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecol. Lett.* **10**, 299–314. (doi:10.1111/j. 1461-0248.2007.01018.x)
- González-Varo JP, Carvalho CS, Arroyo JM, Jordano P. 2017 Unravelling seed dispersal through fragmented landscapes: frugivore species operate unevenly as mobile links. *Mol. Ecol.* 26, 4309–4321. (doi:10.1111/mec.14181)
- 4. Ellis EC, Klein Goldewijk K, Siebert S, Lightman D, Ramankutty N. 2010 Anthropogenic transformation

of the biomes, 1700 to 2000. *Glob. Ecol. Biogeogr.* **19**, 589–606. (doi:10.1111/j.1466-8238.2010. 00540.x)

- Haddad NM *et al.* 2015 Habitat fragmentation and its lasting impact on Earth's ecosystems. *Sci. Adv.* 1, e1500052. (doi:10.1126/sciadv.1500052)
- Timóteo S, Correia M, Rodríguez-Echeverría S, Freitas H, Heleno R. 2018 Multilayer networks reveal the spatial structure of seed-dispersal interactions across the Great Rift landscapes. *Nat. Commun.* 9, 140. (doi:10.1038/s41467-017-02658-y)
- González-Castro A, Yang S, Carlo TA. 2019 How does avian seed dispersal shape the structure of early successional tropical forests? *Funct. Ecol.* 33, 229–238. (doi:10.1111/1365-2435.13250)
- 8. Auffret AG *et al.* 2017 Plant functional connectivity—integrating landscape structure

and effective dispersal. *J. Ecol.* **105**, 1648–1656. (doi:10.1111/1365-2745.12742)

- Schleuning M, Fründ J, García D. 2015 Predicting ecosystem functions from biodiversity and mutualistic networks: an extension of trait-based concepts to plant – animal interactions. *Ecography* 38, 380–392. (doi:10.1111/ecog.00983)
- Zwolak R. 2018 How intraspecific variation in seeddispersing animals matters for plants. *Biol. Rev.* 93, 897–913. (doi:10.1111/brv.12377)
- Snell RS *et al.* 2019 Consequences of intraspecific variation in seed dispersal for plant demography, communities, evolution, and global change. *AoB Plants* plz016 (doi:10.1093/ aobpla/plz016)
- 12. Greenwood P, Harvey P. 1982 The natal and breeding dispersal of birds. *Annu. Rev. Ecol. Syst.*

13, 1–21. (doi:10.1146/annurev.es.13.110182. 000245)

- Paradis E, Baillie SR, Sutherland WJ, Gregory RD. 1998 Patterns of natal and breeding dispersal in birds. J. Anim. Ecol. 67, 518-536. (doi:10.1046/j. 1365-2656.1998.00215.x)
- Anders A, Faaborg J, Thompson III F. 1998 Postfledging dispersal, habitat use, and home-range size of juvenile wood thrushes. *The Auk* **115**, 349–358. (doi:10.2307/4089193)
- Matthysen E, Adriaensen F, Dhondt AA. 1995 Dispersal distances of nuthatches, *Sitta europaea*, in a highly fragmented forest habitat. *Oikos* 72, 375–381. (doi:10.2307/3546123)
- Blitzer EJ, Dormann CF, Holzschuh A, Klein A-M, Rand TA, Tscharntke T. 2012 Spillover of functionally important organisms between managed and natural habitats. *Agric. Ecosyst. Environ.* **146**, 34–43. (doi:10.1016/j.aqee.2011.09.005).
- Aparicio R. 2016 Curruca cabecinegra Sylvia melanocephala (Gmelin, 1789). In Enciclopedia virtual de los vertebrados españoles (eds A Salvador,

M Morales). Madrid, Spain: Museo Nacional de Ciencias Naturales.

- Bas JM, Pons P, Gómez C. 2005 Home range and territory of the Sardinian warbler *Sylvia melanocephala* in Mediterranean shrubland. *Bird Study* 52, 137–144. (doi:10.1080/00063650509461383)
- Herrera CM. 1984 A study of avian frugivores, birddispersed plants, and their interaction in Mediterranean scrublands. *Ecol. Monogr.* 54, 2–23. (doi:10.2307/1942454)
- González-Varo JP, Arroyo JM, Jordano P. 2019 The timing of frugivore-mediated seed dispersal effectiveness. *Mol. Ecol.* 28, 219–231. (doi:10.1111/ mec.14850)
- González-Varo JP, Arroyo JM, Jordano P. 2014 Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. *Methods Ecol. Evol.* 5, 806-814. (doi:10.1111/2041-210X.12212)
- Ratnasingham S, Hebert PDN. 2007 BOLD: The Barcode of Life Data System (http://www. barcodinglife.org). *Mol. Ecol. Notes* 7, 355–364. (doi:10.1111/j.1471-8286.2007.01678.x)

- Anónimo. 2018 Banco de datos de anillamiento del remite ICONA – Ministerio de Medio Ambiente. Datos de anillamiento y recuperaciones en España. (Madrid, Ministerio de Agricultura, Alimentación y Medio Ambiente, SEO/BirdLife, ICO, EBD-CSIC y GOB.
- 24. Bates D, Maechler M, Bolker B. 2013 *Ime4*: linear mixed-effects models using S4 classes.
- Fox J, Weisberg S. 2011 An R companion to applied regression, 2nd edn. Beverley Hills, CA: SAGE Publishing.
- Clobert J, Baguette M, Benton TG, Bullock JM. 2012 Dispersal ecology and evolution. Oxford, UK: Oxford University Press.
- Herrera CM. 2002 Seed dispersal by vertebrates. In Plant – animal interactions: an evolutionary approach (eds CM Herrera, O Pellmyr), pp. 185–208. Oxford, UK: Blackwell Science.
- González-Varo JP, Díaz-García S, Arroyo JM, Jordano P. 2019 Data from: Seed dispersal by dispersing juvenile animals: a source of functional connectivity in fragmented landscapes. Dryad Digital Repository. (https://doi.org/10.5061/dryad.97q0n06.2)

Seed dispersal by dispersing juvenile animals: a source of functional connectivity in fragmented landscapes

Juan P. González-Varo^{1,2,3}, Sarah Díaz-García¹, Juan M. Arroyo¹ & Pedro Jordano¹

¹Integrative Ecology Group. Estación Biológica de Doñana (EBD–CSIC). Sevilla. Spain
²Terrestrial Ecology Group. IMEDEA (UIB–CSIC). Esporles. Spain
³Research Unit of Biodiversity (CSIC-UO-PA), Universidad de Oviedo. Mieres. Spain

Author for correspondence:

J.P. González-Varo

e-mail: jpgvaro@outlook.com

Appendix S1. Detailed methodology.

Figure S1. Aerial photograph of the study landscape.

Figure S2. Correlations between the bimonthly mean seed-rain recorded in the forest and the matrix of our study landscape in relation to the percentage of captured Sardinian warblers that were juveniles in each bimonth in similar habitats.

Figure S3. Bimonthly seed rain in the forest and matrix of our study landscape mediated by Sardinian warblers and other frugivore species

Figure S4. Distance-decay pattern in the number of seeds per seed trap dispersed by Sardinian warblers with increasing distances to the forest edge in the matrix.

Appendix S1. Detailed methodology

Letters match the corresponding subsections in the main text.

(c) Seed dispersal data

We sampled community-wide seed dispersal mediated by birds in the forest and the matrix of the study landscape (figure S1). We conducted periodic sampling surveys (fortnightly) during two whole years, from November 2013 to October 2015; thus, including the entire fruiting periods of local fleshy-fruited species. We placed seed traps beneath trees and shrubs canopies to quantify the magnitude of bird-mediated seed rain in each habitat type; trees and shrubs concentrate most seed deposition by Sardinian warblers, which use these vegetation elements as perches, whereas seed deposition in open interspaces is virtually zero [1]. Seed traps consisted of plastic trays (40 cm \times 55 cm, 8 cm weight) with small holes (1 mm diameter) to allow the drainage of rainwater, and covered with wire mesh $(1 \text{ cm} \times 1 \text{ cm})$ to prevent post-dispersal seed predation. We monitored a total of 56 seed traps, 28 in the forest and 28 in the matrix. In the forest, seed traps were placed beneath the canopy of different oak trees (n = 12) and shrubs without fleshy fruits (n = 12) or with large fleshy fruits (n = 4) not consumed locally by Sardinian warblers (i.e. hawthorns *Crataegus* monogyna; see [1] and Results). This way, we ensured that seed deposition accounted for actual dispersal events, both in forest and matrix, involving horizontal movements away from the canopy of the source plants. The distances among seed traps varied from 5 m to 530 m. In the matrix, all seed traps were placed beneath distinct isolated oaks (n = 28). The distances between target oaks ranged from 10 to 610 m, and distances to the forest edge ranged from 5 to 190 m (figure S1).

In each sampling survey, we recorded the number of bird-dispersed seeds per seed trap and sampled individual seeds or droppings with seeds for DNA-barcoding analysis. We did so by putting each sample with a minimum of handling into a 1.5- or 2.0-ml sterile tube. Tubes were labelled and stored in a freezer at -20° C until DNA extraction. We additionally sampled bird-dispersed seeds through direct searches, particularly beneath isolated oaks in the matrix, where we expected a very low contribution by a woodland-dependent species like the Sardinian warbler. With this, we aimed at increasing sample sizes for DNA-barcoding identifications because PCR generally fails in 5–10% of samples [1-3], and this leads to a fraction of seed-dispersal events assessed in our seed traps to have an 'unknown' disperser. This loss is more problematic where seed deposition in the seed traps is lowest, i.e. in the matrix. Conversely, we generally collected just a subsample of the seeds collected

from seed traps placed in the forest when they received many seeds of a given species (see [3]). Note that we only used DNA barcoding identifications to calculate the relative contribution of Sardinian warblers to seed rain ($f_{warbler}$) – see details in section (f) Data analysis. The magnitude of seed rain was only measured in the study seed traps.

(e) Bird ringing data

We used a ringing database provided by SEO/Birdlife of Sardinian warblers captured (n = 126,891) in Spain between 30/10/1975 and 09/02/2018 [4] in order to link bimonthly patterns of seed-deposition with bimonthly captures of juvenile and adults individuals in analogous habitats. We filtered the original database by classifying bird age codes (Table S1; EURING classification: <u>https://euring.org/data-and-codes/euring-codes</u>) as juveniles or adults. Birds ringed as chicks (code 1) or with an inaccurate or unknown age (codes 0 or 2 respectively) were excluded. Birds assigned to code 3 were classified as juveniles, as this category corresponds to birds hatched during the current calendar year. The other categories (codes from 4 to 9) were classified as adults, except those individuals belonging to code 5 that were ringed between January and February (i.e. prior to the year's breeding period), which we classified as juveniles. We did so because the calendar year is used throughout this coding system. Thus, a bird identifiable at the end of December as 'First-year' (code 3) immediately turns into 'Second-year' at the beginning of January (code 5). Rather than the calendar year, our classification considers the species calendar in relation to the timing of the first breeding season.

We clustered the original habitat classification (60 habitat categories) of each ringing capture into 'woodland' and 'open habitats' (15 and 18 categories, respectively), analogous to the 'forest-shrubland' and 'matrix' in our study landscape. We excluded captures assigned to 27 habitat categories that were not comparable to our forest-matrix design (e.g. coastal habitats, wetlands, urban habitats; [5]).

Finally, we selected a subset of the warmer Spanish provinces*, dominated by thermo- and meso-Mediterranean bioclimate [6], aiming to use ringing data from localities with the climatic conditions as close as possible with those of our study landscape (thermo-Mediterranean).

The final subset included 33,457 ringing captures, including 22,536 juveniles and 10,921 adults.

Age code	Definition
0	Age unknown, i.e. not recorded
1	Pullus: nestling or chick, unable to fly freely, still able to be caught by hand
2	Full-grown: able to fly freely but age otherwise unknown
3	First-year: full-grown bird hatched in the breeding season of this calendar year
4	After first-year: full-grown bird hatched before this calendar year; year of birth otherwise unknown
5	2nd year: a bird hatched last calendar year and now in its second calendar year
6	After 2nd year: full-grown bird hatched before last calendar year; year of birth otherwise unknown
7	3rd year: a bird hatched two calendar years before, and now in its third calendar year
8	After 3rd year: a full-grown bird hatched more than three calendar years ago (including present year as one); year of bird otherwise unknown
9	4th year: a bird hatched three calendar years before, and now in its fourth calendar year

Table S1. EURING age codes used to classify ringed birds as 'juveniles' or 'adults'.

* Selected geographical provinces from the dataset (not all of them are administrative provinces):

HUELVA, CADIZ (including GIBRALTAR), SEVILLA, CORDOBA, MALAGA, GRANADA, JAEN, ALMERIA, BADAJOZ, CACERES, TOLEDO, CIUDAD REAL, ALBACETE, MURCIA, ALICANTE, VALENCIA, CASTELLON, TARRAGONA, BARCELONA, GIRONA, MALLORCA, MENORCA, IBIZA Y FORMENTERA

(f) Data analysis

All data analyses were performed using R 3.5.2 (R Development Core Team 2018). We used bimonths, a six-level factor (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sept-Oct, and Nov-Dec), as the temporal scale of data aggregation per seed trap. Bimonths provided a proper temporal resolution while preventing a dominance of zeros in our dataset. We used DNA-barcoding identifications in different bimonths of each sampling year, habitats, microhabitats and 50-m distance classes from the forest edge in the matrix, to calculate the relative contribution of Sardinian warblers to seed rain of different plant species in those spatiotemporal

combinations ($f_{\text{warbler}} = n_{\text{warbler}} / n_{\text{total}}$). We then estimated the number of seeds dispersed by Sardinian warblers in each seed trap as $SR_{warbler} = SR_{total} \times f_{warbler}$; where SR_{total} is the total number of seeds of a plant species found in each seed trap in different bimonths of each year (see a similar procedure in [3]). These estimates were rounded and converted into integers. Finally, we aggregated the accumulated number of seeds dispersed by Sardinian warblers per seed trap and bimonth, which resulted in 672 data points; i.e. 28 seed traps \times 2 habitats \times 6 bimonths \times 2 years. We then fitted a negative binomial generalised linear mixed-model (nb-GLMM) using the R package *lme4* (v. 1.1-19) [7] to analyse, through a two-way ANOVA design (Habitat \times Bimonth), bimonthly differences in the magnitude of seed rain (i.e. number of seeds per trap) dispersed by Sardinian warblers in forest and matrix. Results from a previously fitted Poisson GLMM (AIC = 1135.8) were the same but this showed a worse fit ($\Delta AIC > 50$) to the data than the nb-GLMM (AIC = 1084.0). The nb-GLMM was also better fitted to the data than a zero-inflated Poisson GLMM (AIC = 1087.0) ran in the R package glmmTMB (v. 0.2.3) [8], whose results were also consistent with those from the nb-GLMM. We included the seed-trap identity as a random factor to account for the repeated measures per trap. The significance of fixed effects (*p*-values of Wald χ^2 tests) was computed using the "Anova" function of the package car (v. 2.1-6) [9].

Finally, we used nonparametric Spearman's tests to test for a positive relationship (i.e. one-tailed test) between the average bimonthly seed rain dispersed by Sardinian warblers in the forest and matrix of our study landscape and the number of ringing captures of juvenile individuals in analogous habitats (n = 6 bimonths). We did not expect this relationship for adults. We used one-tailed tests because there is evidence that the abundance of frugivorous birds can predict the local abundance of bird-dispersed seeds (see [10]). Moreover, there is no reason to expect a negative relationship between seed deposition and the abundance of either juvenile or adult birds, but simply a lack of correlation with the age

group that has had a minor/negligible contribution to seasonal differences in seed-deposition

patterns.

References

1. González-Varo J.P., Arroyo J.M., Jordano P. 2014 Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. *Methods Ecol Evol* **5**(8), 806-814. (doi:10.1111/2041-210X.12212).

2. González-Varo J.P., Carvalho C.S., Arroyo J.M., Jordano P. 2017 Unravelling seed dispersal through fragmented landscapes: Frugivore species operate unevenly as mobile links. *Mol Ecol* **26**(16), 4309-4321.

3. González-Varo J.P., Arroyo J.M., Jordano P. 2019 The timing of frugivore-mediated seed dispersal effectiveness. *Mol Ecol* **28**(2), 219–231. (doi:doi:10.1111/mec.14850).

4. Anónimo. 2018 Banco de datos de anillamiento del remite ICONA - Ministerio de Medio Ambiente. Datos de anillamiento y recuperaciones en España. (Madrid, Ministerio de Agricultura, Alimentación y Medio Ambiente, SEO/BirdLife, ICO, EBD-CSIC y GOB.

5. González-Varo J., Díaz-García S., Arroyo J., Jordano P. 2019 Data from: Seed dispersal by dispersing juvenile animals: a source of functional connectivity in fragmented landscapes. (Dryad Digital Repository.

6. Rivas-Martínez S., Penas A., Díaz T. 2004 Bioclimatic map of Europe, thermoclimatic belts. *Cartographic Service University of León, Spain.*

7. Bates D., Maechler M., Bolker B. 2013 Bates, D., Maechler, M. & Bolker, B. *lme4*: linear mixed-effects models using S4 classes. (

8. Brooks M.E., Kristensen K., van Benthem K.J., Magnusson A., Berg C.W., Nielsen A., Skaug H.J., Machler M., Bolker B.M. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal* **9**(2), 378-400.

9. Fox J., Weisberg S. 2011 *An R Companion to Applied Regression (2nd ed)*. California, USA, SAGE Publishing.

10. García D., Zamora R., Amico G.C. 2010 Birds as suppliers of seed dispersal in temperate ecosystems: Conservation guidelines from real-world landscapes. *Conserv Biol* **24**(4), 1070-1079. (doi:10.1111/j.1523-1739.2009.01440.x).

Figure S1. Aerial photograph of the study landscape in South Spain (900 m in longitude \times 700 m in latitude). The cells of the grid are 100 \times 100 m in size. Circles denote the oak trees where the seed traps were placed in the matrix.



Figure S2. Bimonthly mean seed rain (seeds per seed trap) recorded in (*a*) the forest and (*b*) the matrix of our study landscape (2013–2015) in relation to the percentage of captured Sardinian warblers that were juveniles in each bimonth in similar habitats (i.e. $\%_{juveniles} = 100 \times n_{juveniles} / [n_{adults} + n_{juveniles}]$); ringing data from a subset of warm Spanish provinces between 1975 and 2018. Spearman's (ρ) tests are shown above the panels.



Figure S3. Boxplot (median, quartiles, and 10th/90th percentiles; circles: mean values) showing the number of seeds per seed trap and bimonth in forest and matrix, during the two years of this study, dispersed by (*a*) Sardinian warblers and (*b*) by other frugivore species. Panel (*a*) is the same one showed in Figure 1*c* in the article, shown here for comparative purposes with (*b*). Note the square-root scale in the *y*-axis in (*b*).



Figure S4. Boxplot (median, quartiles, and 10th/90th percentiles; circles: mean values) showing the number of seeds dispersed by Sardinian warblers during the two years of this study in seed traps placed in the forest (n = 28) and at different distance classes from the forest edge in the matrix (n = 8, 7, 5 and 8). Note the square-root scale in the *y*-axis.

