nature portfolio

https://doi.org/10.1038/s41586-021-03665-2

Supplementary information

Limited potential for bird migration to disperse plants to cooler latitudes

In the format provided by the authors and unedited

Supplementary Information for

Limited potential for bird migration to disperse plants to cooler latitudes

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Supplementary Methods

Details on species identification for the 'MOBILELINKS' networks

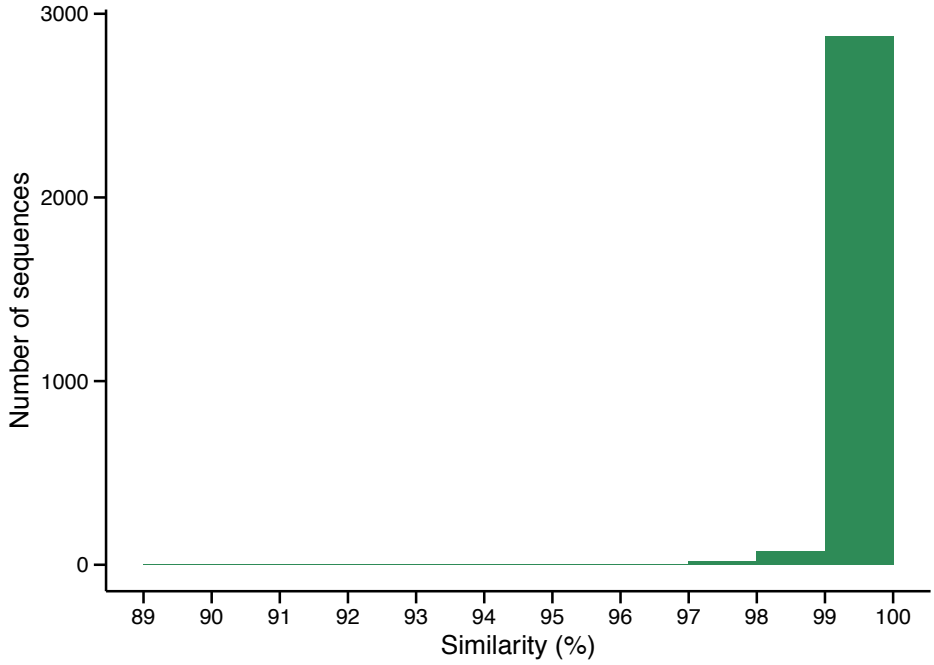
Seven out of the 13 study networks were sampled within the EU project 'MOBILELINKS' (H2020-MSCA-IF-2014-656572) at seven study sites in Spain, United Kingdom, Germany, Italy and Poland (Extended Data Table 1). For these networks, community-wide seed dispersal by frugivorous birds was sampled in seed traps and transects as detailed in the Methods section. Bird-dispersed seeds (individual seeds or droppings with seeds) were sampled into 1.5- or 2.0-ml sterile tubes that were labelled and stored in a freezer at −20°C until the extraction of avian DNA from the seed surface. We then conducted DNA-barcoding analysis to identify the bird species responsible for seed dispersal events (see below). Seed species were identified visually after DNA extraction (see details below).

Bird species identification through DNA barcoding

We used DNA barcoding to identify the bird species that dispersed the seeds. DNA of animal origin can be extracted from the surface of defecated or regurgitated seeds (see Fig. 1 in González-Varo et al.[1]), allowing the identification of the bird species responsible of each dispersal event[2]. Note that this is a type of environmental DNA (eDNA), as trace DNA is sampled from the environment, in this case, bird faeces or regurgitations collected from seed traps and transects[3]. Disperser species identification was based on a 464-bp mitochondrial DNA region (COI: cytochrome *c* oxidase subunit I). For DNA extraction, we used a GuSCN/silica protocol, incubating each seed directly in extraction buffer (added to the tube where the seed was sampled in the field), as described in González-Varo et al.[2]. Briefly, a volume of 450 or 500 µL of extraction buffer[4] (0.1 M Tris-HCl pH 8.0, 0.1 M EDTA pH 8.0, 0.01 M NaCl, 0.5% SDS and 0.25 mg/mL Proteinase K) plus 22 µL of 10% PVP-360 (polyvinylpyrrolidone) and 2 µL of β-mercaptoethanol, as adjuvants or supplements against PCR inhibitors, were added to the 1.5 or 2.0 mL tubes containing seeds, which were incubated in rotation at 50 ºC for 1 h 40 minutes. Supernatant (~ 425–475 µL) was transferred to a new 2.0 mL tube; then, 0.5 volume (~ 215–240 µL) of binding buffer (5 M GuSCN, 0.1 M Tris-HCl pH 6.4, 0.02 M EDTA pH 8.0 and 1.3% Triton X-100) and 120 µL of silica suspension were added and the mix was incubated in rotation at room temperature for 1 h 40 minutes in the dark. This step allows the binding of DNA to silica particles in the presence of high salt concentration. After centrifugation (2 min at 4000 rpm), the supernatant was discarded, and the silica pellet was resuspended in 400 µL of binding buffer and transferred to columns (MoBiTec, Germany, product # M1002S) with a glass microfiber filter (Whatman Grade GF/B 1.0 µm) on the top of the 10 µm column filter. After centrifugation (1 min at 13,000 rpm), silica particles retained in the column were washed at least twice using 450 µL of washing buffer (50% Ethanol, 10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0 and 125 mM NaCl). Columns were placed in new tubes and DNA was eluted twice, first with 60 µL of ultrapure water and then with 50 µL of diluted TE buffer (5 mM Tris-HCl pH 8 and 0.1 mM EDTA pH 8.0).

For PCR amplification, we used the primers COI-fsdF (5′–GCATGAGCCGGAATAGTRGG–3′) and COI-fsdR (5′–TGTGAKAGGGCAGGTGGTTT–3′) following the PCR protocol described in González-Varo et al.[2] to amplify the whole 464-bp COI DNA region. We also combined the primers COI-fsd-degF (5′–GGAGCCCCAGACATAGCAT–3′) and COI-fsdR to amplify a smaller fragment of 272-bp that covers part of the 464-bp COI DNA region, following the nested PCR protocol described in González-Varo et al.[1] (nested-PCR reactions using the COI-fsd-degF and COI-fsdR primer set on the AWCintF2-AWCintR4 amplicon as template, following Alcaide et al.[5]). The reason for targeting these shorter fragments is that we realized –after our first studies using DNA barcoding to identify seed dispersers[1,2]– that ~200 bp fragments are long enough to discriminate bird species, and PCRs were more successful when sampling seeds under rainy scenarios[1], where avian DNA is more likely to be degraded into smaller fragments. PCR amplifications were performed by increasing the concentration of primers and Taq to overcome the expected low avian DNA amount in samples, and by increasing the concentration of bovine serum albumin (BSA) to overcome the possible PCR inhibitors. The final 30 µL volume of the PCR cocktail contained 3.0 µL (1×) buffer (67 mM Tris-HCL pH 8.8, 16 mM $(NH_4)_2SO_4$, 10 mM KCl, 0.01% stabilizer), 1.2 µL (2.0 mM) $MgCl_2$, 1.05 µL (0.5 mg/mL) BSA (Roche Diagnostics, Barcelona, Spain), 0.3 µL (0.25 mM) dNTP, 1.8 µL (0.60 µM) × 2 primers (COI-fsdF and COI-fsdR; see above), 0.2 µL (1.0 U) Taq DNA polymerase (Bioline, London, UK), 12.65 µL ultrapure water, and 8 µL of the DNA extract (mean ± SD: 8.4 ± 5.9 ng/µL of total DNA, n = 39 samples; quantified with NanoDrop® ND-1000 Spectrophotometer, NanoDrop Technologies Inc., Wilmington, DE, USA). Reactions were undertaken in a Bio-Rad DNA Engine® Peltier Thermal Cycler with an initial 4 min of denaturation at 94 °C; 42 cycles at 94 °C for 45 s, annealing at 54 °C for 45 s and extension at 72 °C for 45 s; and final extension of 6 min at 72 °C. After verifying successful amplification by agarose gel electrophoresis, excess primers and dNTPs were removed using enzymatic reaction of Antarctic phosphatase buffer, Antarctic phosphatase and *Escherichia coli* exonuclease I (all New England Biolabs, UK). We only sequenced one strand (forward primer) of the amplified COI fragments because the electrophoretic patterns were clear (trimming initial 5′ region for low quality) and resulting sequences (length: mean = 336 bp, median = 393 bp, quartiles$_{1–3}$ = 226–411 bp; average quality > 90% in SEQUENCHER) allowed successful discrimination between species. Sequencing reaction was carried out using the BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions and labelled fragments were cleaned on SephadexTM G-50 (GE Healthcare, UK) plates before electrophoresis in an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Amplified DNA fragments were aligned and edited using SEQUENCHER 4.9, BioEdit v. 7.0.9 and Chromas v. 2.5.1. The sequences obtained were identified using the 'BARCODE OF LIFE DATA' identification system (BOLD5: http://www.boldsystems.org[6]). BOLD accepts sequences from the 5′ region of the COI gene and returns species-level identification, assigning a percentage of similarity to matched sequences. Species identification in our sequences was generally based on a 98–100% similarity with matching sequences (Supplementary Fig. 1), whereas second ranked species typically had a similarity <95% with the scored sequences[2]. The exceptions were the species pairs 'common starling (*Sturnus vulgaris*) – spotless starling (*S. unicolor*)', and

'European green woodpecker (*Picus viridis*) – Iberian green woodpecker (*Picus sharpei*)', for which the COI gene is unable to discern between these sister species owing to the low degree of genetic differentiation (<2%)[7]. In these cases, species discrimination after DNA barcoding was based on the geographical location of the study sites (green woodpecker and starlings) and field observations (starlings). In ~2% of our sequences, BOLD was unable to match them to any record due to low sequence quality as a result of non-specific amplification. In these cases, we used BLAST (http://www.blast.ncbi.nlm.nih.gov)[8] to match our sequences to sequences from GenBank, using the link '*Blast sequence on GenBank*' from the BOLD report. We successfully identified the disperser species of 2991 samples (i.e. 2991 sequences; 123–1753 per network) including 3014 interaction events between a bird-plant species pair, and containing 4812 seeds (144–2193 per network); overall 3234 samples containing 5181 seeds were analyzed, with an identification success of 92.5%. Identification failure was due to PCR failure (non-specific or very low quality DNA amplification). DNA-barcoding analysis was conducted at the Doñana Biological Station ('Molecular Ecology Laboratory', LEM–EBD–CSIC) and at the University of Oviedo (Research Unit of Biodiversity's molecular laboratory). All barcoding sequences obtained in the present study are publicly available in the data file 'MOBILELINKS_DNA_barcoding_data.csv' deposited at the DRYAD repository (https://doi.org/10.5061/dryad.15dv41nx3).



Supplementary Fig. 1 | Histogram showing the distribution of similarity values (*n* = 2991 sequences) between the sequences obtained by means of DNA-barcoding analysis for bird-species identification and the best matching sequence in BOLD or BLAST. In the vast majority of sequences (98.63%, *n* = 2950) similarity values were above 98%, whereas in a small fraction (1.37%, *n* = 41) of sequences similarity values ranged between 89.9% and 98%. The lower values were attributable to DNA of low quality as a result of non-specific amplification, which was expected to some extent considering the nature of our samples (i.e. environmental DNA). However, we were confident about the correct identification of the bird species for this small fraction because we obtained a high number of hits belonging to the same species on each sequence evaluated and similarity values were clearly lower for second ranked species.

Seed species identification

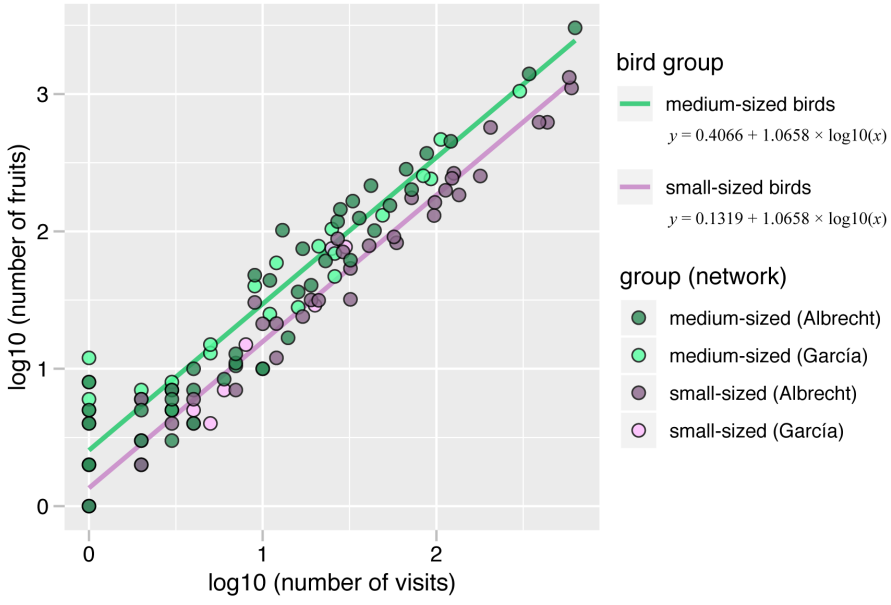
After bird DNA was extracted from the surface of the seeds, we visually identified seed species according to their morphology. To do so, we compared the seeds against a personal reference collection (owned by JPGV) and pictures from a guide of seeds of European fleshy-fruited species that includes plants from the Mediterranean and temperate biomes[9]. The exception were 11 samples whose initial identification was not possible and for which we conducted DNA-barcoding analysis using chloroplast MaturaseK gene (MatK)[10,11] following protocols for plant-species identification used in ref.[12]. Briefly, prior to DNA extraction, seeds were washed up, dried and grounded to a fine powder by breaking them into several fragments using liquid nitrogen, pestle and mortar, and then by getting the final fine powder using a homogenizer device. We transferred around 50 mg of this powder to a 1.5 mL tube. Seed DNA was extracted using DNeasy® Plant Mini kit following manufacturer's instructions. We used MatK-390F (5′–CGATCTATTCATTCAATATTTC–3′) and MatK-1326R (5′–TCTAGCACACGAAAGTCGAAGT–3′) primers, which amplify a middle fragment (~800-bp) of chloroplast MatK gene[11]. PCR reactions were set up in a final volume of 10 µL including 5 µL of GoTaq® Green Master Mix 2× (Promega Corporation, Madison, WI, USA), 0.4 µL (10 mM) of primers MatK-390F and MatK-1326R and 1 µL (~50 ng/µL) of DNA. All PCRs were performed using both negative (distilled water) and positive samples (i.e. samples with MatK sequences previously obtained). Reactions were performed on a G-Storm GS2 thermal cycler (Somerton Biotechnology Centre, Somerset, UK) under the following conditions: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30 s with an annealing temperature of 45°C for 30 s and extension at 68°C for 1 min, and final elongation at 72°C for 5 min. PCR products were checked by electrophoresis in 1.5% agarose gels stained with GelRedTM nucleic acid gel stain (Biotium, Inc., Hayward, CA, USA). Negative and positive samples worked out well and we did not find any signal of contamination. Positive amplifications were purified using the E.Z.N.A. Gel Extraction Kit OMEGA. Sequencing reactions were performed using the Perkin Elmer BigDye v. 3.1 (Applied Biosystems, Carlsbad, CA, USA) terminator reaction mix in a volume of 10 µL using 1 µL of PCR product and the primers MatK-390F and MatK-1326R. PCR conditions were: initial denaturation at 94 °C for 2 min followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 60 °C for 2 min, followed by a final extension at 60 °C for 1 min. The final product was purified and sequenced on an ABI PRISM® 3130xl Genetic Analyzer. This analysis was conducted at the University of Oviedo (Research Unit of Biodiversity's molecular laboratory).

Seed species from these 11 samples were identified through the following three steps: (1) we obtained a short list of species from the best sequence matches in BLAST[8]; (2) we used such short list to identify candidate fleshy-fruited plant species that were present around the study sites; and (3) we used the final reduced list of candidate plant species to identify seed species visually according to seed morphology, as explained above. Thus, this DNA-barcoding analysis served us to short list and guide visual identification. For example, we identified *Sorbus aucuparia* seeds from Bauerbach (Germany) by: (1) obtaining a short list of best sequence matches that included several *Sorbus* spp.; (2) checking that from such short list *S. aucuparia* was the only species present in the landscape; and (3) visually confirming

seed identity. All seed samples are stored by JPGV at the laboratory of Botany in the University of Cádiz (Spain), and plant sequences are publicly available in the data file 'MOBILELINKS_DNA_barcoding_data.csv' deposited at the DRYAD repository (https://doi.org/10.5061/dryad.15dv41nx3).

Homogenization of network interaction weights

We expressed the interaction weights (w_{ij}) of all networks as the number of seeds of each plant species *i* (or the seed-rain density as seeds per m^2) dispersed by each bird species *j*. Yet, in networks based on feeding observations, interaction weights were originally expressed as number of bird visits to focal plants[13]. We thus converted number of visits into number of seeds dispersed through the following two steps. First, we converted visits into fruits consumed using the parameters of a linear mixed model fitted to data from two European networks[14,15] for which the number of both visits and fruits consumed were recorded for each pairwise interaction (see Supplementary Fig. 2). This model included 'bird group', a two-level fixed factor differentiating 'small-sized birds' (< 20 g) and 'medium-sized birds' (> 60 g), because there is a gap in body weight between 20 g and 60 g among the study bird species. Birds body weight was obtained from EltonTraits 1.0[16]. We used this grouping factor to account for the fact that larger birds can consume more fruits per visit than smaller ones. Bird species, plant species and interaction identity (some interactions were sampled in both networks) were included as random factors to account for the repeated measures per species and interaction. A model with a single slope and two intercepts had a better fit to the data (AICc = –7.75) than a model with two slopes and two intercepts (AICc = –1.64). The fixed effects of this model explained 92% of variance in number of fruits ($R^2_{GLMM(m)} = 0.924$)[17].



Supplementary Fig. 2 | Number of fruits consumed by frugivorous birds in pairwise interactions with different plant species in relation to the number of visits to those plants and bird body size. Regression lines were obtained from a linear mixed model (after log10-transformation) fitted with the R package *lme4* (v. 1.1-19)[18]. 'García' is the network from ref.[14]. 'Albrecht' is the network network from ref.[15].

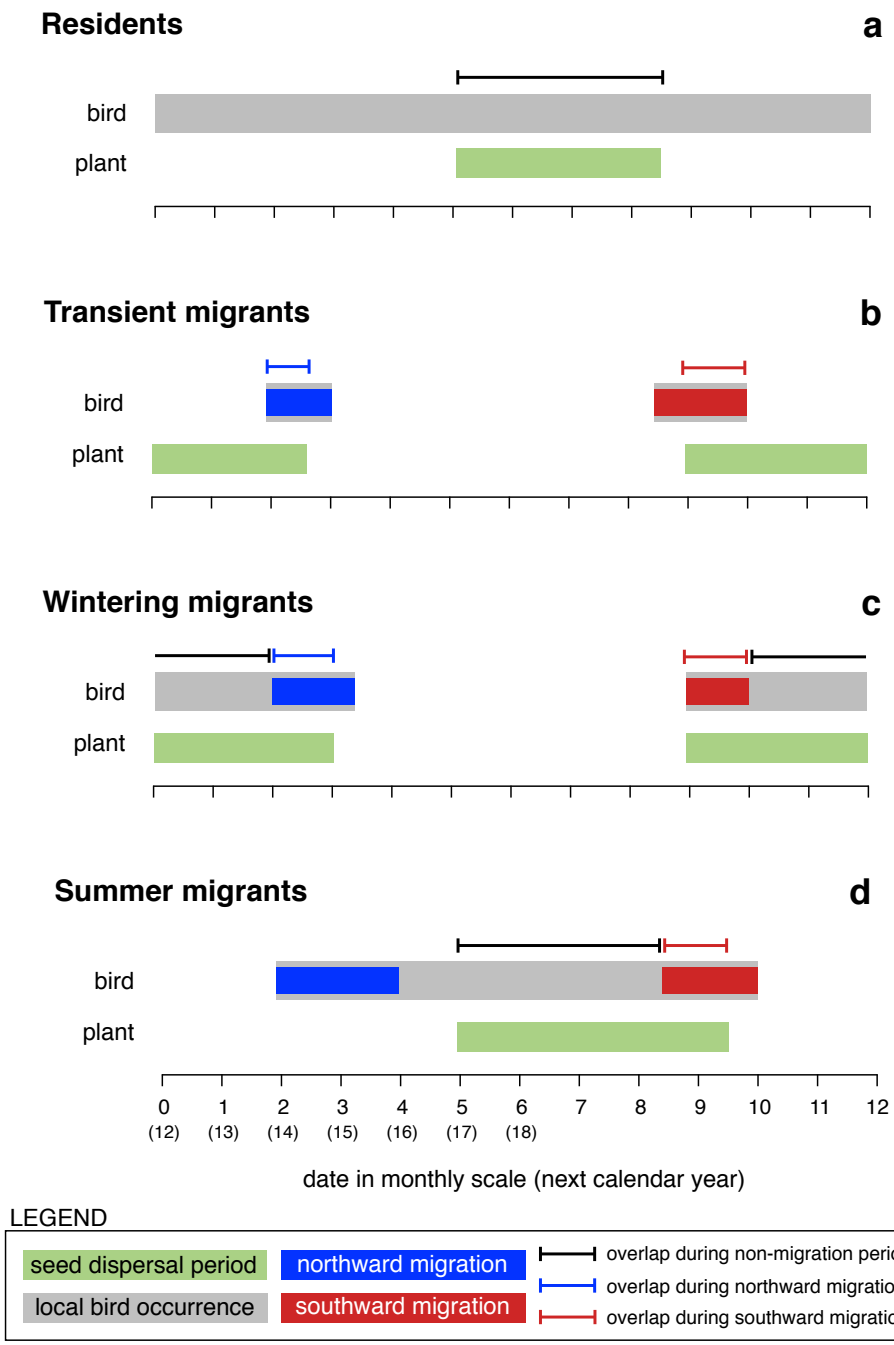
In a second step, we converted fruits consumed into seeds dispersed by multiplying the former by the average number of seeds per fruit of each plant species, which was obtained from the literature[9,19-21] and from data generated by the authors. Where the product did not result in an integer, values were rounded to the nearest integer.

Migratory strategy of birds and total phenological overlaps with fruiting plants

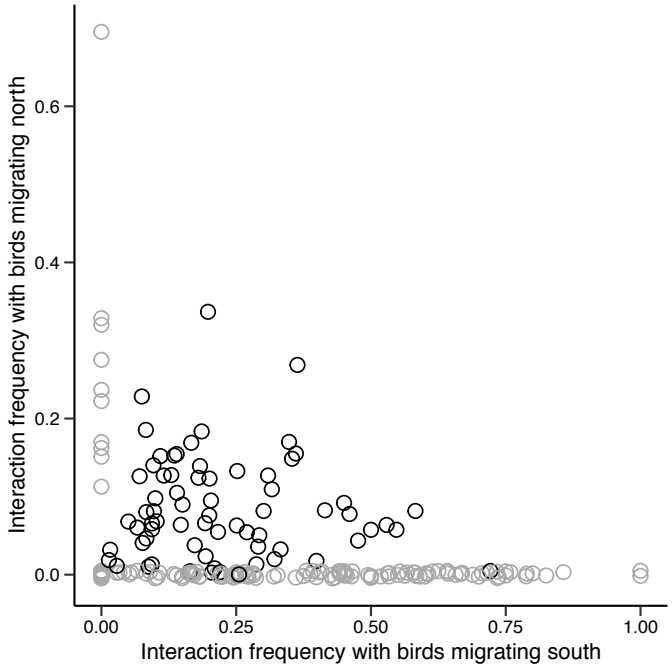
We calculated the total phenological overlap ($O_{total\text{-}ij}$) as the whole period during which a bird species coincides locally with the seed-dispersal period of each plant species in the study networks. When bird populations were fully or partially resident ($P_{migrants} < 1$), the bird species occurs locally all year round and, thus, $O_{total\text{-}ij}$ was equal to the length of the seed-dispersal period ($O_{total\text{-}ij} = D_{end\text{-}i} - D_{start\text{-}i}$). When bird populations were transient (only occur locally during migration), $O_{total\text{-}ij}$ was equal to the sum of phenological overlap during northward and southward migrations ($O_{total\text{-}ij} = O_{north\text{-}ij} + O_{south\text{-}ij}$). In the case of wintering migrants, their occurrence in the local communities spans from their arrival at the beginning of the southward migration (S_{start}) to the end of their departure at the end of the northward migration (N_{end}); thus, for wintering migrants: $O_{total\text{-}ij} = \min(D_{end\text{-}i}, N_{end\text{-}j}) - \max(D_{start\text{-}i}, S_{start\text{-}j})$. In the case of summer migrants, their presence in local communities spans from their arrival at beginning of the northward migration (N_{start}) to their complete departure at the end of the southward migration (S_{end}); thus, for summer migrants: $O_{total\text{-}ij} = \min(D_{end\text{-}i}, S_{end\text{-}j}) - \max(D_{start\text{-}i}, N_{start\text{-}j})$. Whenever $D_{end\text{-}i}$ extended to the next calendar year ($D_{end\text{-}i} > 12$), we added 12 to the migration dates in order to calculate the actual $O_{total\text{-}ij}$ (Supplementary Fig. 3).

Non-mutually exclusive interactions with south- and north-migrating birds

We checked for correlation in plant-level interactions with birds migrating southwards and northwards (Supplementary Fig. 4). The prevalence of interactions was not significantly interrelated between the southward and southward migrations (χ^2 test = 0.977, $P = 0.3230$), which means that plants dispersed by birds migrating south were not less likely to be dispersed by birds migrating north. Similarly, the frequency of realized interactions (non-zero frequencies) was not significantly interrelated between southward and northward migrations (Spearman's $\rho = -0.065$, $P = 0.5973$), which means that plants with a high interaction frequency with birds migrating south did not necessarily have a low interaction frequency with birds migrating north. This lack of interdependence between migrations in terms of prevalence is explained by the fact that the same plant species can be dispersed during both migrations (Extended Data Fig. 2). When considering all data, including zeros, the interaction frequencies during the southward migration were negatively related to those during the northward migration (Spearman's $\rho = -0.275$, $P < 0.0001$), although the predictive power of this relationship was very small. This minor interdependence between migrations in terms of interaction frequency is partly explained by the frequency of interactions with non-migrating birds ($F_{non\text{-}i,}$), the third component of the total interaction frequency (Extended Data Fig. 2).



Supplementary Fig. 3 | The phenological overlaps between the seed-dispersal period of a plant species and both the local occurrence and migration periods of interacting bird species depend on the migratory strategy. (**a**) Resident birds occur locally all year round and, thus, the total phenological overlap (O_{total}) is equal to the length of the seed-dispersal period. (**b**) Transient migrants only occur locally during migration, thus, O_{total} equals the sum of phenological overlaps during northward (O_{north}) and southward (O_{south}) migrations. (**c**) Wintering migrants occur locally from their arrival at the beginning of the southward migration to their complete departure at the end of the northward migration. The calculation of O_{total} with wintering migrants includes a non-migration overlap during the winter, between O_{south} and O_{north}. (**d**) Summer migrants occur locally from their arrival at beginning of the northward migration to their complete departure at the end of the southward migration. The calculation of O_{total} with summer migrants also includes a non-migration overlap during the summer, between O_{north} and O_{south} migrations. Note that in (**b**) and (**c**), the seed-dispersal period starts in the beginning of October (date = 9.0) and ends in the next calendar year (date + 12).



Supplementary Fig. 4 | Scatter plot of plant-level interaction frequencies with birds migrating south ($F_{south\text{-}i}$) and north ($F_{north\text{-}i}$). Grey circles denote zero frequencies in any migration, whereas black circles denote non-zero frequencies in both migrations (n = 217 'plant species–network' combinations). Colour codes help to visualize the two response variables analyzed: the prevalence (presence/absence) of interactions with birds during each migration and the interaction frequency when these interactions occurred (non-zero frequencies).

Supplementary Discussion 1

Relevance of long-distance seed dispersal by migratory birds

The mean global velocity at which organisms need to shift their distributional range under climate change to retain the same temperatures has been estimated at 4.2 km per decade, although estimates for certain regions are of tens of km per decade[22,23]. Therefore, tracking current climate change requires long-distance seed dispersal, particularly in the case of woody plant species with generation times of several years to decades[24], which may require dispersal events well over 10 km. Non-migrating frugivorous birds typically disperse seeds within 1 km of source plants[25], whereas resident frugivorous mammals, like martens and foxes, typically disperse seeds within 3 km of source plants[26].

Migratory birds are expected to assist plants to track climate change via long-distance seed dispersal[27,28], because they can transport viable seeds over tens or even hundreds of kilometers in short time periods[28-30]. Yet, measuring seed-dispersal distances mediated by migratory birds is challenging, as it requires sampling a seed at its destination site after being ejected by a migratory bird and identifying where did it come from. Although this information has been already obtained empirically within relatively small landscapes (few km^2) using DNA markers[1,31], the huge scale of bird migrations has prevented applying the same approach to broader spatial scales. This explains why the study by Viana and collaborators[29] constitutes the most important direct empirical evidence on long-distance seed dispersal by migratory birds. The authors did not identify the source of the seeds found in the gut of birds caught in migration by Eleonora's falcons (*Falco eleonorae*) in the Canary Islands (Atlantic Ocean), but they inferred that seeds came from distances longer than 170 km according to the distance to the African coast and the movement of falcons obtained through GPS tags[29]. A similar inference was made by Fridriksson[32] on a volcanic island near Iceland, where the author found seeds in the guts of snow buntings (*Plectrophenax nivalis*) arriving from the British Isles, during their northward spring migration.

Seed-dispersal kernels (i.e. probability density functions of dispersed seeds relative to the distance from maternal plants) are characterized by a decaying tail at the longest distances[33,34]. The latter means that the percentage of birds carrying seeds in their guts found by Viana and collaborators[29] (1.2% for distances longer than 170 km) is expected to be higher at shorter distances (e.g. between 20 and 50 km, or between 50 and 100 km), which still represent long-distance dispersal events. The percentage recorded by Viana and collaborators[29] becomes even more important if we consider that the bird species captured by falcons in the middle of the sea were not amongst the most important migratory seed-dispersers of European fleshy-fruited species. In other words, the percentage of birds carrying seeds in their guts at long-distances is likely to be much higher amongst the most important bird species identified in our study (see Fig. 3c).

Besides the direct empirical evidence, there are multiple lines of indirect evidence that support the relevance of migratory birds for long-distance seed dispersal. Movement data and gut retention times of ingested seeds can be integrated into mechanistic models to estimate seed dispersal distances. Viana and collaborators[30] estimated maximum dispersal distances of aquatic organisms mediated by migrating waterbirds above 1000 km. In this study, the

authors used quick ringing recoveries of waterbirds, in which only a few days elapsed between ringing and recovery dates. These mechanistic models also suggest that long-distance seed dispersal events provided by birds during migration are relatively frequent (up to 3.5% of dispersal distances above 100 km), and that small migratory birds can have a high potential to act as long distance seed dispersal vectors[35]. Quick recoveries from frugivorous birds, the birds addressed in our study, also reveal fast migratory movements, with recoveries a few days after ringing at distances farther than 500 km (https://euring.org). A remarkable example comes from a European robin (*Erithacus rubecula*; 833960) ringed on 08-04-1981 in The Belgium (Duinbergen) and recovered after four days 1,000 km away, in the Shetland Islands (Out Skerries) (data from the BTO; https://www.bto.org/our-science/projects/ringing). Moreover, the gut retention time of passerines that are similar in size to the most important birds identified in our study (Fig. 3c) can be as long as >200 minutes[36]. Gut retention times typically exhibit a large variation around central tendency measures, even in small passerines, and such variation is crucial for long-distance seed-dispersal[37]. Thus, indirect evidence from the long distances travelled in short time periods by migratory birds, their flight speed[38] and gut retention times[36] is congruent with predictions of mechanistic models[35] and with direct evidence provided by Viana and collaborators[29] in the Canary Islands. The billions of birds migrating recurrently, every year, in the same directions, make these events predictable and significant despite their very low frequency. Indeed, even a very small frequency applied to very large numbers, like thousands of millions of European migrants, and to a process (migration) recurrent every year, is expected to result into a significant phenomenon; only the European population of the blackcap (*Sylvia atricapilla*) was estimated to range between 50–100 million individuals in 2000, and that of the European robin between 80–160 millions (https://birdsoftheworld.org).

Finally, there are more sources of indirect evidence supporting the importance of migratory birds for long-distance seed dispersal, including island colonization by fleshy-fruited plants[39-41] and large-scale patterns of plant genetic structure along migratory routes[42]. The fact that most seeds of fleshy fruits sink and lose their viability in sea water suggest that endozoochorous avian dispersal is the most plausible explanation for the colonization of remote oceanic islands by fleshy-fruited species[39]. Although gulls have been reported to consume the fruits and disperse the seeds of wild fleshy-fruited species associated to coastal habitats (e.g. *Corema album* and *Empetrum nigrum*) and cultivated fleshy-fruited species such as olives (*Olea europaea* var. *europaea*)[43], it seems very unlikely that gulls have mediated the colonization of the huge diversity of fleshy-fruited plants occurring on oceanic islands (e.g. more than 80 species from more than 45 genera in the Canary Islands[44]). Migratory birds are the most likely explanation and the study by Viana and collaborators[29] endorses this idea. In addition, a recent study has found strong large-scale genetic structure among populations of a fleshy-fruited plant (*Pistacia lentiscus*) distributed throughout the Mediterranean Basin between the eastern and western migratory routes in the Mediterranean, but reduced genetic distances along the north-south axis connecting southern Europe and northern Africa within each route. These findings reveal the footprint of migratory birds on plant populations, diluting genetic structure even between intercontinental populations and are consistent with the empirical evidences discussed above.

Supplementary Discussion 2

Assessing potential biases

We assessed two sources of potential biases that might affect the results reported in Fig. 2: sampling methods of study networks and approach used to obtain seed-dispersal periods.

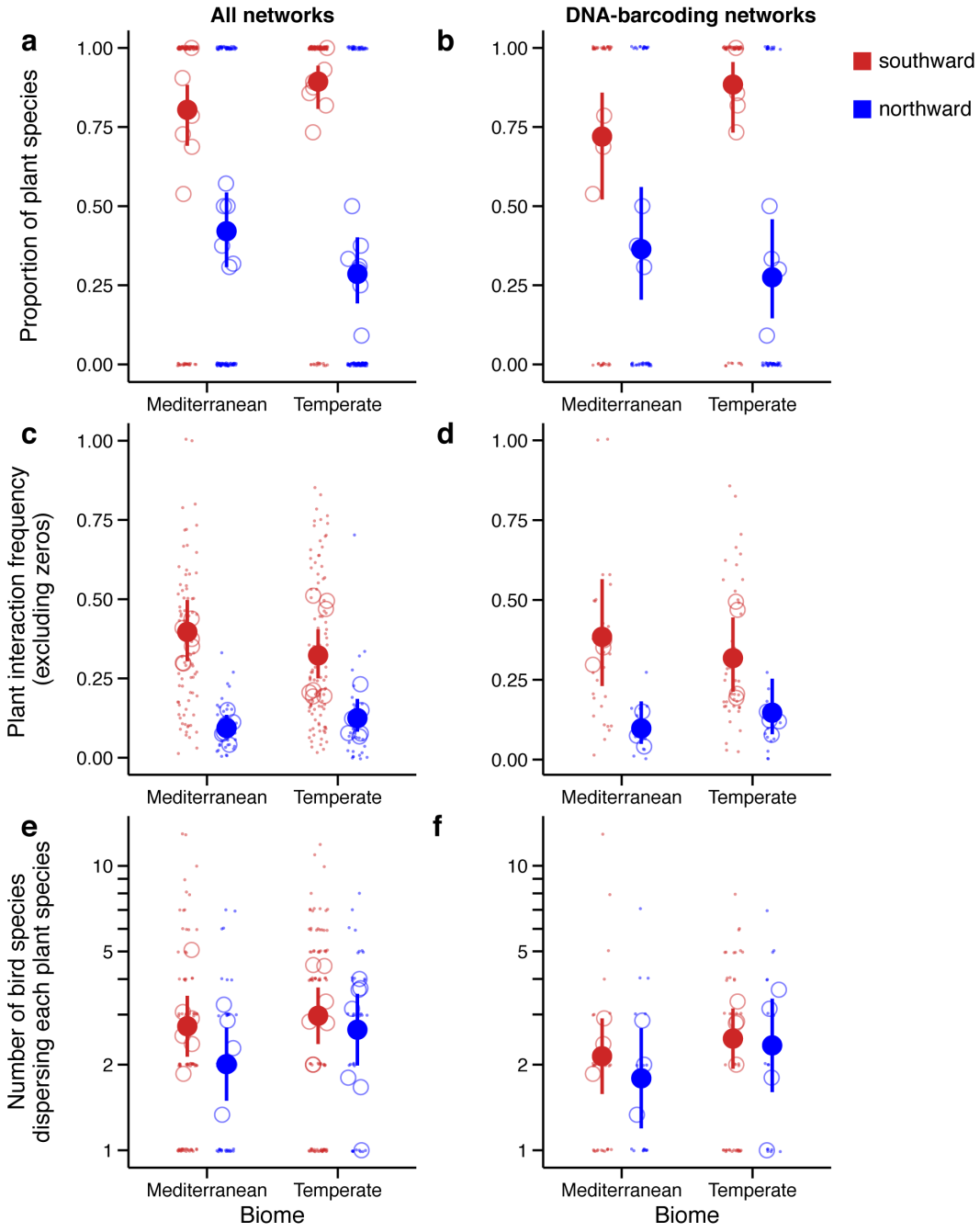
Networks obtained with different sampling methods

We assessed a potential bias of analysing networks that were obtained with different sampling methods (focal plant observations of birds feeding on fruits, dietary analysis of birds captured in mist nets, and field sampling of bird-dispersed seeds and subsequent disperser identification by means of DNA-barcoding analysis). To do so, we repeated the analyses using only a subset of networks obtained through the same sampling method: DNA barcoding. DNA barcoding was the sampling method with more network replicates (7 out of the 13) and more evenly distributed across biomes (3 Mediterranean and 4 temperate) (see Extended Data Table 1). We found virtually identical results despite the reduced statistical power (Supplementary Fig. 5; see larger error bars in right panels). This indicates that the main results reported in our study (Fig. 2) are not an artefact of analysing networks obtained with different sampling methods. The significance of the fixed-effects (at $P \leq 0.05$) was the same as in the main analyses based on all networks (shown in Extended Data Table 3) for the proportion of plant species interacting with birds during migration and the frequency of these interactions when they occurred, that is, 'Direction' and the 'Direction × Biome' interaction had significant effects. The only difference in this subset was the non-significant effects of 'Direction' on the number of bird species dispersing each plant species, but this variable showed small differences between directions in the main results: "*... plants were dispersed by more bird species migrating south than north (estimated mean = 2.9 and 2.3 species per plant, respectively; direction: P = 0.017), a small but consistent difference across biomes (Fig. 2c and Extended Data Table 3)*".

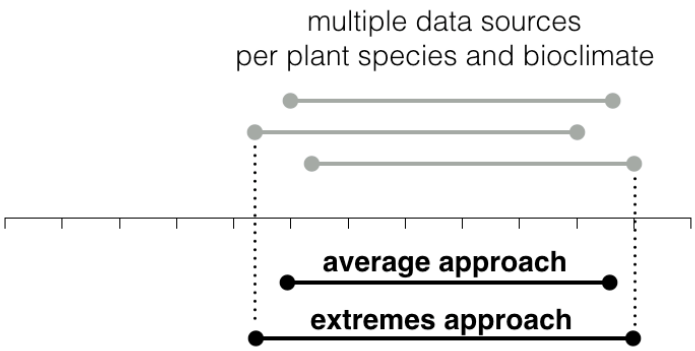
Average approach used to obtain seed-dispersal periods

We assessed a potential bias in our results for using a conservative approach to obtain seed-dispersal periods. As explained in the Methods, we obtained unique start and end dates for each 'plant species–bioclimate' combination by averaging dates across data sources, because in many cases there were several data sources for a plant species in the same bioclimate. With this procedure, we aimed at conservatively obtaining the most representative and generalizable seed-dispersal period of each plant species within each bioclimate (Supplementary Fig. 6). We repeated the analyses using the minimum start date and the maximum end per 'plant species–bioclimate' combination, that is, the longest fruiting of a plant species in a bioclimate, which represents a less conservative approach (Supplementary Fig. 6). Again, we found virtually identical results (Supplementary Fig. 7) indicating that the main results reported in our study (Fig. 2) are not an artefact of the conservative approach used to estimate seed-dispersal periods. The significance of the fixed-effects (at $P \leq 0.05$) was the same as in the main analyses (shown in Extended Data Table 3) for the proportion of plant species interacting with birds during migration and the frequency of these interactions when they occurred, that is, 'Direction' and the 'Direction × Biome' had significant effects. The

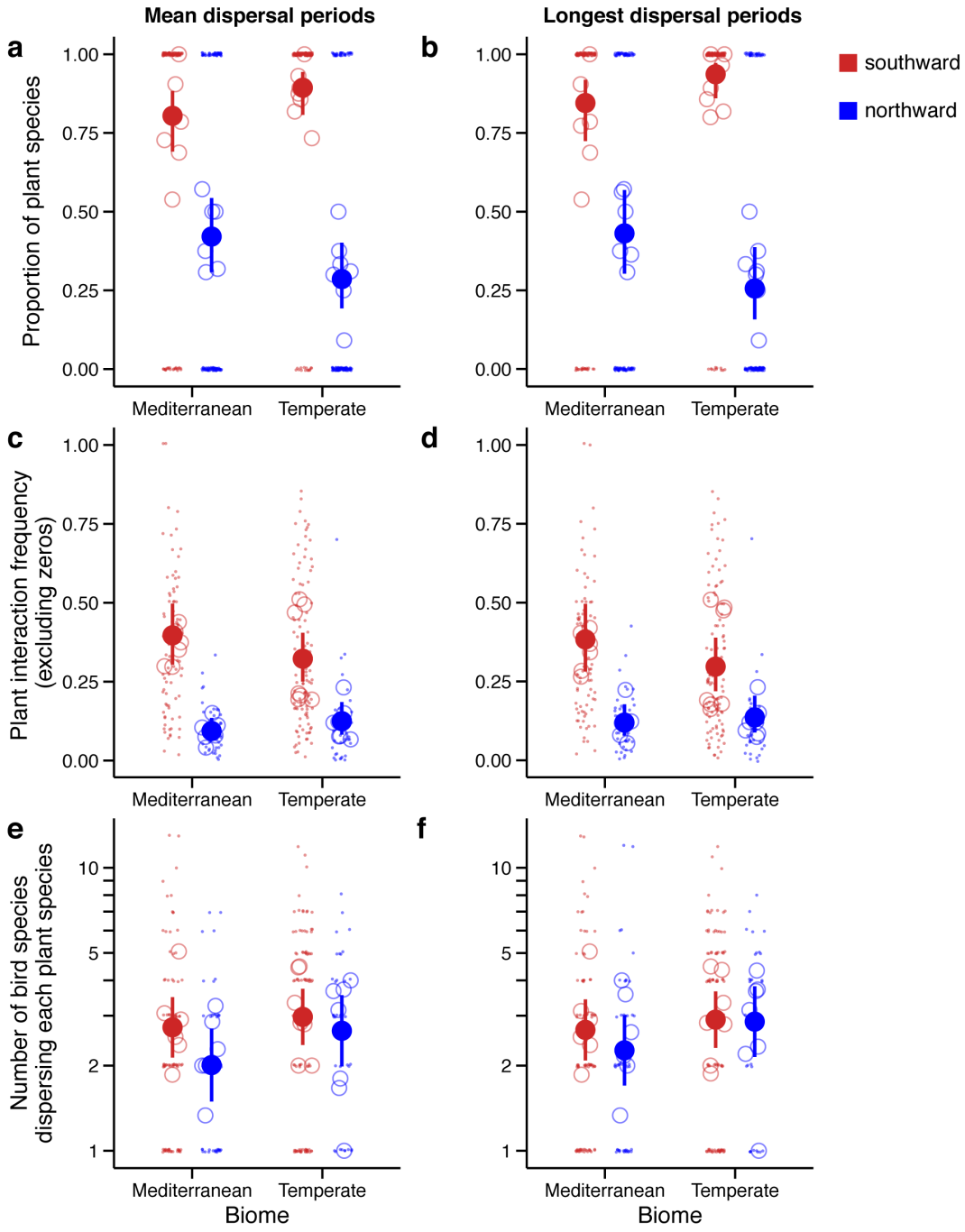
only difference was the non-significant effect of 'Direction' on the number of bird species dispersing each plant species but, as stated above, this variable showed small differences between directions in the main results.



Supplementary Fig. 5 | Seed-dispersal interactions of plants with migratory birds in relation to migration direction and biome in all study networks (a, c, e) and in networks obtained with DNA barcoding (b, d, f). Left panels including with all networks are the same panels of Fig. 2 (a, b, c). Large dots and bars denote means ± 95% confidence intervals estimated by generalized linear mixed models predicting (**a**, **b**) the proportion of plant species interacting with birds during migration (**a**: *n* = 434 observations from 13 networks across plant species and directions; **b**: *n* = 186 observations from 7 networks), (**c**, **d**) the frequency of interactions with migrating birds when these occurred (zeros excluded; **c**: *n* = 260 observations from 13 networks; d: *n* = 104 observations from 7 networks), out of the total seed-dispersal interactions, and (**e**, **f**) the number of bird species dispersing each plant species (**e**: *n* = 260 observations from 13 networks; **f**: *n* = 104 observations from 7 networks). Circles denote mean values for each seed-dispersal network, whereas tiny dots denote plant-level data.



Supplementary Fig. 6 | Approaches used to obtain seed-dispersal periods from multiple data sources for each 'plant species–bioclimate combination'. We used the *average approach* in the main analyses, a conservative approach aiming to obtain the most representative and generalizable seed-dispersal period of each plant species within each bioclimate. We repeated the analyses using the *extremes approach*, a less conservative approach aiming to obtain the longest seed-dispersal period of each plant species within each bioclimate.



Supplementary Fig. 7 | Results of seed-dispersal interactions of plants with migratory birds in relation to migration direction and biome obtained through the *average approach* (a, c, e) and through the *extremes approach* (b, d, f) (see Supplementary Fig. 6). Left panels including with all networks are the same panels of Fig. 2 (a, b, c). Large dots and bars denote means ± 95% confidence intervals estimated by generalized linear mixed models predicting (**a**, **b**) the proportion of plant species interacting with birds during migration (n = 434 observations from 13 networks across plant species and directions in both panels), (**c**, **d**) the frequency of interactions with migrating birds when these occurred (zeros excluded; n = 260 observations in **c** and 266 in **d**, from 13 networks in both panels), out of the total seed-dispersal interactions, and (**e**, **f**) the number of bird species dispersing each plant species (n = 260 observations in **e** and 266 in **f**, from 13 networks in both panels). Circles denote mean values for each seed-dispersal network, whereas tiny dots denote plant-level data.

Supplementary References

1 González-Varo, J. P., Carvalho, C. S., Arroyo, J. M. & Jordano, P. Unravelling seed dispersal through fragmented landscapes: Frugivore species operate unevenly as mobile links. *Mol. Ecol.* **26**, 4309-4321, doi:10.1111/mec.14181 (2017).

2 González-Varo, J. P., Arroyo, J. M. & Jordano, P. 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 (2014).

3 Bohmann, K. *et al.* Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* **29**, 358-367, doi:https://doi.org/10.1016/j.tree.2014.04.003 (2014).

4 Longmire, J. L., Maltbie, M. & Baker, R. J. *Use of" lysis buffer" in DNA isolation and its implication for museum collections*. Vol. 163 (Museum of Texas Tech University, 1997).

5 Alcaide, M. *et al.* Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. *PLoS ONE* **4**, e7092, doi:10.1371/journal.pone.0007092 (2009).

6 Ratnasingham, S. & Hebert, P. D. N. 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 (2007).

7 Lovette, I. J., McCleery, B. V., Talaba, A. L. & Rubenstein, D. R. A complete species-level molecular phylogeny for the "Eurasian" starlings (Sturnidae: *Sturnus*, *Acridotheres*, and allies): Recent diversification in a highly social and dispersive avian group. *Mol. Phylogen. Evol.* **47**, 251-260, doi:http://dx.doi.org/10.1016/j.ympev.2008.01.020 (2008).

8 Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389-3402, doi:10.1093/nar/25.17.3389 (1997).

9 Torroba Balmori, P., Zaldívar García, P. & Hernández Lázaro, Á. *Semillas de frutos carnosos del norte ibérico: Guía de identificación*. (Ediciones Universidad de Valladolid, 2013).

10 CBOL, P. W. G. *et al.* A DNA barcode for land plants. *Proc. Natl Acad. Sci. USA* **106**, 12794-12797, doi:10.1073/pnas.0905845106 (2009).

11 Cuénoud, P. *et al.* Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *Am. J. Bot.* **89**, 132-144, doi:10.3732/ajb.89.1.132 (2002).

12 Laiolo, P., Illera, J. C., Meléndez, L., Segura, A. & Obeso, J. R. Abiotic, biotic, and evolutionary control of the distribution of C and N isotopes in food webs. *Am. Nat.* **185**, 169-182, doi:10.1086/679348 (2015).

13 Simmons, B. I. *et al.* Moving from frugivory to seed dispersal: Incorporating the functional outcomes of interactions in plant–frugivore networks. *J. Anim. Ecol.* **87**, 995-1007, doi:doi:10.1111/1365-2656.12831 (2018).

14 García, D. Birds in ecological networks: insights from bird-plant mutualistic interactions. *Ardeola* **63**, 151-180, doi:10.13157/arla.63.1.2016.rp7 (2016).

15 Farwig, N., Schabo, D. G. & Albrecht, J. Trait-associated loss of frugivores in fragmented forest does not affect seed removal rates. *J. Ecol.* **105**, 20-28, doi:10.1111/1365-2745.12669 (2017).

16 Wilman, H. *et al.* EltonTraits 1.0: Species-level foraging attributes of the world's birds and mammals. *Ecology* **95**, 2027-2027 (2014).

17 Nakagawa, S., Schielzeth, H. & O'Hara, R. B. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133-142, doi:10.1111/j.2041-210x.2012.00261.x (2013).

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

19 Snow, B. & Snow, D. *Birds and berries*. (T and A D Poyser, 1988).

20 Stiebel, H. *Frugivorie bei mitteleuropäischen Vögeln (PhD thesis)*, Universität Oldenburg, (2003).

21 Jordano, P. Data from: Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant-animal interactions. doi:doi:10.5061/dryad.9tb73 (1995).

22 Loarie, S. R. *et al.* The velocity of climate change. *Nature* **462**, 1052-1055,|doi:10.1038/nature08649 (2009).

23 Diffenbaugh, N. S. & Field, C. B. Changes in ecologically critical terrestrial climate conditions. *Science* **341**, 486-492, doi:10.1126/science.1237123 (2013).

24 Nathan, R. *et al.* Spread of North American wind-dispersed trees in future environments. *Ecol. Lett.* **14**, 211-219, doi:10.1111/j.1461-0248.2010.01573.x (2011).

25 Breitbach, N., Böhning-Gaese, K., Laube, I. & Schleuning, M. Short seed-dispersal distances and low seedling recruitment in farmland populations of bird-dispersed cherry trees. *J. Ecol.* **100**, 1349-1358, doi:10.1111/1365-2745.12001 (2012).

26 González-Varo, J. P., López-Bao, J. V. & Guitián, J. Functional diversity among seed dispersal kernels generated by carnivorous mammals. *J. Anim. Ecol.* **82**, 562-571, doi:10.1111/1365-2656.12024 (2013).

27 Bauer, S. & Hoye, B. J. Migratory animals couple biodiversity and ecosystem functioning worldwide. *Science* **344**, doi:10.1126/science.1242552 (2014).

28 Viana, D. S., Santamaría, L. & Figuerola, J. Migratory birds as global dispersal vectors. *Trends Ecol. Evol.* **31**, 763-775, doi:10.1016/j.tree.2016.07.005 (2016).

29 Viana, D. S., Gangoso, L., Bouten, W. & Figuerola, J. Overseas seed dispersal by migratory birds. *Proc. R. Soc. B* **283**, doi:10.1098/rspb.2015.2406 (2016).

30 Viana, D. S., Santamaría, L., Michot, T. C. & Figuerola, J. Migratory strategies of waterbirds shape the continental-scale dispersal of aquatic organisms. *Ecography* **36**, 430-438, doi:10.1111/j.1600-0587.2012.07588.x (2013).

31 Jordano, P., García, C., Godoy, J. A. & García-Castaño, J. L. Differential contribution of frugivores to complex seed dispersal patterns. *Proc. Natl Acad. Sci. USA* **104**, 3278-3282, doi:10.1073/pnas.0606793104 (2007).

32 Fridriksson, S. *Evolution of Life on a Volcanic Island*. 198 (Butterworth & Co (Publishers) Ltd., 1975).

33 Nathan, R. *et al.* Mechanisms of long-distance seed dispersal. *Trends Ecol. Evol.* **23**, 638-647, doi:http://dx.doi.org/10.1016/j.tree.2008.08.003 (2008).

34 Nathan, R., Klein, E., Robledo-Arnuncio, J. J. & Revilla, E. in *Dispersal Ecology and Evolution* (eds Jean Jean Clobert, Michel Baguette, Tim G. Benton, & James M. Bullock) 187-210 (Oxford University Press, 2012).

35 Viana, D. S., Santamaría, L., Michot, T. C. & Figuerola, J. Allometric scaling of long-distance seed dispersal by migratory birds. *Am. Nat.* **181**, 649-662, doi:10.1086/670025 (2013).

36 Rehm, E., Fricke, E., Bender, J., Savidge, J. & Rogers, H. Animal movement drives variation in seed dispersal distance in a plant-animal network. *Proc. R. Soc. B* **286**, 20182007, doi:doi:10.1098/rspb.2018.2007 (2019).

37 Guttal, V., Bartumeus, F., Hartvigsen, G. & Nevai, A. L. Retention time variability as a mechanism for animal mediated long-distance dispersal. *PLOS ONE* **6**, e28447, doi:10.1371/journal.pone.0028447 (2011).

38 Alerstam, T., Rosén, M., Bäckman, J., Ericson, P. G. P. & Hellgren, O. Flight speeds among bird species: allometric and phylogenetic effects. *PLoS Biol.* **5**, e197, doi:10.1371/journal.pbio.0050197 (2007).

39 Esteves, C. F., Costa, J. M., Vargas, P., Freitas, H. & Heleno, R. H. On the limited potential of Azorean fleshy fruits for oceanic dispersal. *PLOS ONE* **10**, e0138882, doi:10.1371/journal.pone.0138882 (2015).

40 Burns, K. C. A multi-scale test for dispersal filters in an island plant community. *Ecography* **28**, 552-560, doi:10.1111/j.0906-7590.2005.04221.x (2005).

41 Carlquist, S. The biota of long-distance dispersal. V. Plant dispersal to Pacific Islands. *Bulletin of the Torrey Botanical Club* **94**, 129-162 (1967).

42 Martínez-López, V., García, C., Zapata, V., Robledano, F. & De la Rúa, P. Intercontinental long-distance seed dispersal across the Mediterranean Basin explains population genetic structure of a bird-dispersed shrub. *Mol. Ecol.* **29**, 1408–1420, doi:10.1111/mec.15413 (2020).

43 Calviño-Cancela, M. Gulls (Laridae) as frugivores and seed dispersers. *Plant Ecol.* **212**, 1149-1157, doi:10.1007/s11258-011-9894-2 (2011).

44 Arechavaleta, M., Rodríguez, S., Zurita, N. & García, A. *Lista de especies silvestres de Canarias. Hongos, plantas y animales terrestres. 2009*. (Gobierno de Canarias, 2010).