Universidad de Sevilla

Estación Biológica Doñana CSIC

Super-generalist species in frugivory mutualisms

Ecological interactions and their ecosystem implications

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DOCTORAL THESIS

DOCTORAL THESIS 2024

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Recommended citation:

Quintero, E. (2024). Super-generalist species in frugivory mutualisms: ecological interactions and their ecosystem implications. PhD Thesis. Universidad de Sevilla, Sevilla, Spain. https://idus.us.es.

Cover illustration and front pages in general introduction and discussion are an original creation of Maria León. Illustrations in the front page of the four chapters are an original creation of Paula Martin Art.

Estación Biológica de Doñana (EBD-CSIC)
Departamento de Ecología y Evolucion

Universidad de Sevilla
Facultad de Biología
Doctorado en Biología Integrada

Super-generalist species in frugivory mutualisms:
Ecological interactions and their ecosystem implications

Memoria presentada por Elena Quintero Borrero para optar al Título de Doctora por la Universidad de Sevilla.

Fdo.: Elena Quintero Borrero

Sevilla, 2024

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CERTIFICAN:

Que la tesis doctoral titulada “*Super-generalist species in frugivory mutualisms: Ecological interactions and their ecosystem implications*” presentada por Elena Quintero Borrero ha sido realizada bajo nuestra dirección y tutoría, y reúne los méritos suficientes para optar al grado de Doctor por la Universidad de Sevilla ante el Tribunal designado a tal efecto. Y para que así conste y surta los efectos oportunos, firmamos el presente certificado en Sevilla, a 25 de enero de 2024.

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Vº Bº del tutor,

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This thesis received financial support from “la Caixa” Foundation fellowship for Doctoral studies at Spanish Universities or Research Centres (ID 100010434) with code LCF/BQ/DE18/11670007, as well as by the projects CGL2017-82847-P and PID2022-136812NB-I00 from Agencia Estatal de Investigación, Ministerio de Ciencia e Innovación and project PAIDI_2020-P20_00736 from Consejería de Economía, Conocimiento, Empresas y Universidad, Junta de Andalucía. In addition, this thesis received support by a Fulbright fellowship for pre-doctoral research abroad, co-funded by Junta de Andalucia and by project LIFEWATCH-2019-09-CSIC-13 funded by European Fund for Regional Development (FEDER-EU).

Contents

Abstract

Studying interactions among species and their multiple forms and outcomes has become a central question of ecological research. Mutualisms have changed the way ecologists understand the functioning of ecosystems. Generalised mutualistic networks, especially among free-living species, have been observed to include highly heterogeneous assemblages, in which most species establish few interactions while a minority of species establish a wide range of interactions. These highly connected species are referred to as super-generalists. They are characterised for connecting otherwise isolated groups of interacting species or modules and for providing stronger cohesion to ecological networks. Their characteristics make them fundamental for local biodiversity and the structure and stability of interactions.

In this PhD thesis we aim to better comprehend the super-generalist strategy and the mutualistic interactions at the individual level. For this purpose, we use as a model organism *Pistacia lentiscus* L. (Anacardiaceae), a woody shrub species abundant in the Mediterranean Basin, together with the coterie of frugivorous animals that consume its fruits and disperse its seeds. In Chapter 1 we review sampling methods used to record frugivory interactions, assessing their strengths, caveats, and convenience in different contexts. We discuss different approaches for combining data gathered using different methodologies. In Chapter 2, we explore the effectiveness of the frugivory/seed-dispersal mutualism between individual plants of *P. lentiscus* and its avian frugivore assemblage, assessing the level of reciprocity in reward exchange and dependence asymmetry among partners. In Chapter 3 we investigate the delayed outcomes of animals' fruit consumption in early seedling recruitment of *P. lentiscus* plants. Lastly, in Chapter 4 we analyse individual-based networks of different plant species and regions of the world, we compare their topology and structure with that of species-based networks, and explore the specialisation and interaction profile of individual plants within populations.

We highlight the significant advancements brought by emerging methods like camera traps and molecular tools, which allow recording interactions across large spatiotemporal scales and even tracking the movement of individual seeds by animals. We also show how data integration improves network completeness and representativity, and compare different data merging approaches for adjacency matrices. We document how the effectiveness of interactions established between *P. lentiscus* individual plants and their frugivores is mainly determined by fruit consumption frequency (the component with highest variation), leading to highly reciprocal exchange of services but still highly asymmetric dependence among partners. Despite being mostly determined by the number of fruits consumed, the dispersal service provided by birds is decoupled from microhabitat suitability: frugivores deposit a smaller number of seeds in the most suitable microhabitats. These results underscore the role of different frugivores in spatial recruitment of heterogeneous landscapes. Finally, we demonstrate that the structure of individual-based networks is very similar to that of species-based networks. Plant individuals presented similar interaction profiles irrespective of the species or regions their population belonged to. Within populations, plants present low to medium levels of specialisation, and few frugivore species contribute most interactions in all studied networks.

Overall, these results suggest that super-generalist species may evolve when they combine sets of traits that make them accessible and attractive to a diversified assemblage of frugivores. Numerical effects, such as abundant fruit crops, facilitate plenty of frugivory interactions, characterised in most cases by high reciprocity. In exchange for a fair nutritious reward, plants secure their recruitment thanks to a vast amount of seeds dispersed. Finally, our results indicate that the variation encountered in the way plant individuals structure their interactions is highly consistent across populations of generalised mutualisms worldwide.

Collectively, the four chapters in this thesis contribute to better understanding the origin and maintenance of super-generalist species within complex ecological networks, by focusing on the biological scale at which interactions actually occur in nature, that is, the individual scale.

Resumen

Estudiar las interacciones entre especies junto a sus múltiples formas y resultados se ha convertido en una cuestión central de la investigación en ecología. Los mutualismos han cambiado la forma en que los ecólogos entienden el funcionamiento de los ecosistemas. Se ha observado que las redes mutualistas generalizadas, especialmente entre especies de vida libre, incluyen conjuntos muy heterogéneos, en los que la mayoría de las especies establecen pocas interacciones, mientras que una minoría de especies establece una amplia variedad de interacciones. Estas especies altamente conectadas se denominan super-generalistas. Se caracterizan por conectar grupos de especies o módulos que de otro modo estarían aislados, y por proporcionar una mayor cohesión a las redes ecológicas. Sus características las convierten en especies fundamentales para la biodiversidad local así como para la estructura y estabilidad de las interacciones.

En esta tesis doctoral pretendemos comprender mejor la estrategia super-generalista y las interacciones mutualistas a nivel individual. Para ello, utilizamos como organismo modelo *Pistacia lentiscus* L. (Anacardiaceae), una especie arbustiva leñosa abundantemente distribuida por la cuenca mediterránea, junto a los animales frugívoros que consumen sus frutos y dispersan sus semillas. En el Capítulo 1 se revisan los métodos de muestreo utilizados para registrar las interacciones de frugivoría, evaluando sus ventajas, inconvenientes y conveniencia en diferentes contextos; y se discuten diferentes enfoques para combinar los datos recogidos utilizando diferentes metodologías. En el Capítulo 2, exploramos la eficacia en el mutualismo frugivoría/semilla-dispersión entre plantas individuales de *P. lentiscus* y las aves frugívoras que consumen sus frutos, evaluando el nivel de reciprocidad en el intercambio de recompensas y la asimetría de dependencia entre los socios. En el Capítulo 3, investigamos los resultados aplazados del consumo de frutos por animales en plantas de *P. lentiscus* para el reclutamiento temprano de plántulas. Por último, en el Capítulo 4 analizamos redes basadas en individuos para diferentes especies de plantas y regiones del mundo, comparando su topología y estructura con la de redes basadas en especies, y exploramos la especialización y el perfil de interacción de plantas individuales dentro de sus poblaciones.

Destacamos los importantes avances que han supuesto métodos emergentes como las cámaras trampa y técnicas moleculares, que permiten registrar interacciones a una amplia escala espacio-temporal e incluso rastrear el movimiento de semillas individuales por parte de animales. También mostramos cómo la integración de datos mejora la completitud y representatividad de las redes ecológicas, y comparamos distintos métodos de combinación de datos para matrices de adyacencia. A continuación, documentamos cómo la eficacia de las interacciones establecidas entre plantas individuales y sus frugívoros viene determinada principalmente por la frecuencia de consumo de frutos (el componente con mayor variación), lo que conduce a un intercambio de servicios altamente recíproco, pero manteniendo una dependencia altamente asimétrica entre los socios. A pesar de estar determinado principalmente por el número de frutos consumidos, el servicio de dispersión proporcionado por las aves está desvinculado de la idoneidad del microhábitat: los frugívoros depositan un menor número de semillas en los microhábitats más adecuados. Estos resultados resaltan el papel que los distintos frugívoros tienen en el reclutamiento en paisajes heterogéneos. Por último, demostramos que la estructura de las redes basadas en individuos es muy similar a la de las redes basadas en especies. Las plantas individuales presentaron perfiles de interacción similares independientemente de la especie o región a la que perteneciera su población. Dentro de las poblaciones, las plantas presentan niveles de especialización medio-bajos, y unas pocas especies de frugívoros contribuyen a la mayoría de las interacciones en todos las redes estudiadas.

Estos resultados sugieren que las especies super-generalistas pueden evolucionar cuando combinan conjuntos de rasgos que las hacen accesibles y atractivas para un conjunto diversificado de frugívoros. Efectos numéricos como la alta abundancia de frutos facilitan gran cantidad interacciones frugívoras, caracterizadas en la mayoría de los casos por una elevada reciprocidad. A cambio de la recompensa nutritiva que provee la pulpa de los frutos, las plantas aseguran su reclutamiento gracias a una gran cantidad de semillas dispersadas. Por último, nuestros resultados indican que la variación encontrada en la forma en que las plantas individuales estructuran sus interacciones es muy consistente en las poblaciones de mutualismos generalizados de todo el mundo.

En conjunto, los cuatro capítulos de esta tesis contribuyen a comprender mejor el origen y la persistencia de especies super-generalistas dentro de redes ecológicas complejas, centrándose en la escala biológica a la que se producen realmente las interacciones en la naturaleza, es decir, la escala individual.

List of publications

Quintero, E., Isla, J., & Jordano, P. (2022). Methodological overview and data-merging approaches in the study of plant–frugivore interactions. *Oikos*, e08379, 1–18. https://doi.org/10.1111/oik.08379

Quintero, E., Rodríguez-Sánchez, F., & Jordano, P. (2023). Reciprocity and interaction effectiveness in generalised mutualisms among free-living species. *Ecology Letters*, 26(1), 132–146. https://doi.org/10.1111/ele.14141

Quintero, E., Arroyo, J., Dirzo, R., Jordano, P., & Rodríguez-Sánchez, F. (2024). Lasting effects of avian-frugivore interactions on seed dispersal and seedling establishment *Journal of Ecology*, 00, 1-17. https://doi.org/10.1111/1365-2745.14260

Quintero, E., Arroyo-Correa, B., Isla, J., Rodríguez-Sánchez, F., & Jordano, P. Downscaling mutualistic networks from species to individuals reveals consistent interaction niches and roles within populations. *Submitted, January 2024.* https://doi.org/10.1101/2024.02.02.578595

GENERAL INTRODUCTION

Ecological communities are composed of a diverse set of species that rely on each other for survival. Through a myriad of interactions, species manage to obtain essential nutrients, protection and opportunities for reproduction, among many other requirements (Thompson 1982, Bronstein 2015). Documenting this intricate assembly of ecological interactions has fascinated scientists dating back to the time of Aristotle and has become fundamental for understanding ecosystem functioning (Hutchinson *et al.* 2019). Given the diverse nature and complexity of ecological interactions, ecologists have categorised them according to the result for both interacting species, whether it is positive, negative or null. Among these interaction types, competition and predation have garnered the attention of most traditional studies aiming to understand species diversity as well as the natural dynamics of ecosystems, initially overlooking the importance of other ecological interactions (Boucher 1985, Hale & Valdovinos 2021, Simha *et al.* 2022). Nevertheless, mutualistic interactions also play a vital role in ecosystems, by providing reciprocal beneficial services for any two interacting species (Bronstein 2001). Through mutualisms, our vision of ecosystem functioning has been reshaped, revealing the profound impact that cooperation can have on the resilience of ecological communities (Koffel *et al.* 2021). But even within mutualisms, nature shows a gradient in the effectiveness of the services provided between species (Rodríguez-Rodríguez *et al.* 2017, Simmons *et al.* 2018, Gómez *et al.* 2019), actually defining continua between antagonistic-mutualistic extremes (Gómez *et al.* 2023a).

Generalised plant-animal mutualisms among free-living species (*e.g.*, seed dispersal, pollination) often involve animals using food resources provided by plants (Ollerton 2006). Generalisation in this context, refers to the reduced reliance on the partner, not equivalent to the specificity reported for intimate mutualistic and symbiotic interactions (Bronstein 2009). Variance in interaction outcomes can arise from the different degrees of dependence that animal partners have on these resources, *e.g.,* partial frugivory among animal seed dispersers, or variable pollinator reliance on nectar or pollen. Opportunistic species have emerged throughout the evolution of mutualisms, taking advantage of available resources and providing in exchange services of varying effectiveness, even to the point of being null or harmful, as in the case of nectar robbers or seed predators (Ollerton 2006). This diversity in mutualistic services contributes to their rich complexity, and poses challenges for the study of their ecological importance and evolutionary process (Bueno *et al.* 2013, Genrich *et al.* 2017, García *et al.* 2018). A lasting challenge in the study of mutualisms is to understand the determinants of variable interaction outcomes, *i.e.*, the variance in the establishment of interactions, that ultimately determines fitness effects.

The generalised nature of frugivory interactions

Within mutualisms, frugivory interactions play a fundamental role in the reproduction and natural regeneration of many plants that depend on animals for the dispersal of their seeds (*i.e.*, zoochorous; Howe & Miriti 2004, García *et al.* 2010). The formal onset of scientific research on frugivory and seed dispersal can be at least traced back to Ridley's seminal publication in 1930, which explored the mechanisms of seed dispersal, or even to previous essays (Hill 1883, Beal 1898). Even before, the experiments by Linnaeus on germination of seeds consumed by animals, were also replicated by Darwin in his experiments on seed survival to soaking in salty water to assess plant dispersal potential (Darwin 1857). However, after the pioneering work by Ridley, it took nearly 30 years for further, significant advancements in the field. This progress was re-initiated by Van der Pijl's book (Van der Pijl 1969) on higher plants' seed dispersal principles and by the seminal works of Barbara and David Snow on frugivorous birds in Trinidad (Fleming & Estrada 1993). A more formal, hypothesis-driven, approach to the study of plant-frugivore mutualisms was later developed in the early seventies, with the pioneer work of Snow (1971), McKey (1975), and Howe & Estabrook (1977).

Frugivory is perhaps one of the most generalised mutualisms, where multiple species are able to exploit the reward provided by a single species and where obligate mutualisms are extremely rare. And while in frugivory we may not find abundant instances of high specialisation between species, as in pollination or ant-plant mutualisms (Blüthgen *et al.* 2007, Phillips *et al.* 2020, Guimarães *et al.* 2007), we still find convergence and trait complementarity between fruits and animals in the colour, nutrients, size and shape of fruits, and in the gape size, body mass, beaks or nutritional preferences of animals (*e.g.*, Herrera 1984a, Jordano 1995, Levey & Martínez del Rio 2001, Lomáscolo *et al.* 2010, Onstein *et al.* 2017).

The analysis of generalisation patterns in these mutualisms still lacks, however, an assessment of the ecological correlates of generalisation in the "setup" of interactions among individual partners. How do different individuals in a population of, say, a fleshy-fruited plant species, share and "use" the available coterie of animal partners? Are there super-generalized individuals, able to interact with every partner frugivore species? What are the key characteristics of these individual plants? On the other hand, what is the distribution of generalisation among animal frugivores? Are there species able to interact with most of the plants in a given population?

Super-generalist species role in ecological webs

When assembling interaction networks for multi-species mutualistic systems, the resulting distribution of interactions and their frequencies is not homogeneous. Multiple studies have contributed to the understanding of the structure and topology of mutualistic networks, showing that low intimacy networks (*sensu* Guimarães *et al.* 2007) have low and asymmetric connectance. That is, many species establish only a few interactions while a few species are highly connected (Jordano *et al.* 2003, Bascompte *et al.* 2006, Bascompte & Jordano 2007). This minority of highly connected species are the so-called super-generalists and they can have disproportionately large effects in evolution processes (Olesen *et al*. 2007). These species occupy the core of complex ecological networks having multiple interactions that involve rare and specialised species (Guimarães *et al*. 2011), and thus play an important role in sustaining biodiversity.

The super-generalist concept is tightly related to other more traditional concepts in ecology for characterising important species, such as keystone or foundation species. Keystone is a concept initially applied in trophic cascade studies to identify those species whose removal can have disproportionately large consequences on network stability (Paine 1969, Power *et al*. 1996, Cottee-Jones & Whittaker 2012). The idea of foundation species, on the other hand, refers to highly abundant species with a central role in community structuring and biodiversity sustenance (Dayton 1972). Although these concepts may be similar, they also refer to different ecological processes and can vary in certain characteristics as, for example, their natural abundances (see Ellison 2019 for a comprehensive review of different categorisations used for important species). This PhD thesis sets its focus on super-generalist species from an ecological interaction perspective, specifically examining those species that play a central role in mutualistic assemblages due to their broad diversity of interactions. Our understanding of the evolutionary origins of the super-generalist strategy and the conditions that enable these species to persist remains incomplete. In this thesis we aim to further deepen into the study of super-generalist species by studying their interaction configurations, species and individuals interdependence and the mutualistic outcomes for involved partners.

Challenges for sampling and making robust inferences of frugivory interactions

One of the earliest challenges ecologists encounter when studying frugivory systems is the task of obtaining reliable estimates for characterising species assemblages in a way that accurately reflects their natural occurrences. Robust estimates of interaction network patterns can only be derived from studies with sufficient sampling effort (Jordano 2016). Currently, a wide array of methodologies have been developed to sample frugivory interactions, along with recent innovative technologies for monitoring ecological data (Hartig *et al.* 2023). Noteworthy, in the last decade there has been an emergence of innovative and pioneering techniques such as molecular analysis (DNA-barcoding) for inferring the identity of frugivores on dispersed seeds (González-Varo *et al*. 2014) or metabarcoding to infer fruit species in animal's diet (Velarde-Garcéz *et al*. 2024). The development of advanced technology has also allowed researchers to massively record interactions remotely through the use of camera traps and AI tools for data processing (Norouzzadeh *et al*. 2018). Additionally, over the last years, there has been an increasing trend in the scientific community towards data sharing and the creation of open repositories to store and share field-collected data (Hampton *et al*. 2013). Handling these vast amounts of data poses new challenges to ecologists, such as the integration of different sources. Combining data offers the advantage of increasing information of plant-frugivore assemblages and improving the robustness of current inferences (Almeida & Mikich 2018). This emerging field provides exciting opportunities for developing effective techniques to manage and integrate data from various sources, ultimately leading to the generation of more accurate and deeper insights into complex mutualistic systems.

Quantifying benefits, reciprocity and dependence among partners in frugivory mutualisms

In order to unravel the implications of super-generalist species' interactions, it is first necessary to study how these species "configure" their interactions at the individual level and what is the outcome in terms of fitness for individuals and species involved in mutualisms. Given the diversity of partners interacting with super-generalist species, it is expected that interaction outcomes will differ for the species implied. First efforts in the field of frugivory were to develop quantitative approaches that could measure animal contributions to plant seed dispersal (Howe & Estabrook 1977, Howe *et al.* 1985, Howe 1986). In 1993, Schupp proposed a common framework for estimating partner effectiveness by splitting the mutualistic service into two components: quantity (number of seeds dispersed) and quality (the probability that a dispersed seed will produce a new reproductive adult). Numerous studies have since then implemented this framework, and its application has expanded to other mutualistic interaction types and perspectives (Schupp *et al.* 2010, 2017, Gómez *et al.* 2022).

Although mutualisms benefit both partners in terms of fitness, there has been a tendency to prioritise the view of one partner over the other: the plant's perspective, *i.e.*, phytocentric perspective. Most empirical studies primarily focus on animal's effectiveness for plant fitness, disregarding plant's effectiveness for animal fitness. Yet, to fully comprehend the ecological and evolutionary implications of mutualisms, it is crucial to examine the exchange of services and the fitness effects for both partners. In fact, few studies have explicitly explored partners' co-dependence in frugivory systems (*e.g.*, Herrera 1984b, Reid, 1990, Burns 2003, Guerra & Pizo 2014, González-Castro *et al.* 2022). These studies have highlighted the importance of considering the reciprocal benefits experienced by both partners in frugivory mutualisms to understand their persistence and stability.

With the purpose of exploring interdependence in mutualisms, in this PhD thesis we employ two concepts that can bring insights into their stability: the reciprocity in rewards and the symmetry in interaction dependence. We define reciprocity as an indicator that tells us whether the exchange of resources is "fair" or balanced among different partners in the community or whether there are interactions in which some partners obtain more reward than others. Alternatively, the concept of dependence between partners refers to the proportion by which one actor receives its resources from a specific partner, relative to the total resources it obtains. It also reflects whether partners' levels of mutual dependence are similar (symmetric dependence) or if there exists a significant difference (asymmetric dependence). Using a market theory analogy can be useful to illustrate reciprocity and dependency concepts: high reciprocity markets translate into fair prices and proportional rewards regardless of the identity of the partner involved (sellers or buyers). Low reciprocity markets on the other hand, would present varying prices for the same product quantity or quality and so being impossible to establish a common value for an average service, causing some transactions to be much more effective or beneficial than others. Dependence instead refers to the distribution of supply and demand between partners. In a symmetric system, dependence in supply and demand would be similar and balanced (*e.g.*, a local market where buyers and sellers distribute their dependencies evenly), conversely, in an asymmetric system, one partner has little dependence while the other relies heavily (*e.g.*, a "monopoly" market, where buyers depend fully on a company, but the company relies little on each specific buyer). These aspects of reciprocity and dependence have been explored with more detail in intimate symbiotic systems such as mycorrhizae (*e.g.*, Noë & Kiers 2018). Reciprocity in rewards has been little explored in generalised, low intimacy mutualisms, yet it has been repeatedly reported that dependence between species is often asymmetric (*e.g.*, Jordano 1987a, Bascompte *et al.* 2006, Schleuning *et al.* 2016, González-Castro *et al.* 2022). By examining partner co-dependence in a super-generalised mutualism, we can gain valuable insights into the structuring of plant-frugivore interactions upon which asymmetric dependencies emerge, and the mechanisms that promote their long-term persistence (Chomicki *et al.* 2020). Moreover, understanding the fitness effects for both interacting partners can shed some light into the selective pressures driving the evolution of frugivory-seed dispersal mutualisms (Cosmo *et al.* 2023).

Delayed consequences of frugivory on plant natural regeneration

Ascertaining the impact of the high diversity of interactions that super-generalist species establish on their fitness can help us to gain a deeper understanding into their evolutionary success. When the super-generalist species is an endozoochorous plant, it is relevant to understand whether all interactions held by frugivores are equally effective or whether frugivores play varying roles throughout the demographic process. Additionally, exploring how super-generalists cope with limiting factors for their reproductive success, such as low seed viability or high predation rates, can provide us with valuable information on their survival strategies and population dynamics. When combining this demographic approach with a detailed analysis of individual-based interaction networks, we can effectively bridge network patterns with demographic and fitness variation consequences.

Plant population regeneration involves various, sequential, demographic stages, including flowering, fruiting, seed dispersal, seedling emergence, establishment, and subsequent growth (Harper 1977, Wang & Smith 2002). A broad definition of seed dispersal thus encompasses much more than, and beyond, the fruit removal stage (Schupp *et al*. 1989), due to the delayed effects of dissemination. These stages are essential for recruitment and population growth, and can be limited by several factors. Ultimately, recruitment will be determined by the joint probabilities of arrival and survival in the habitat mosaic, so that limitation processes act in several stages of the sequence.

One of the earliest limitations affecting the reproductive fitness of plants after flowering is the production of viable seeds. Many plant species experience seed abortion or produce unfilled seeds (parthenocarpy; Fuentes & Schupp 1998). Furthermore, seeds are often preyed-upon by insects and vertebrates or infected by pathogens before dispersal (Isla *et al*. 2022). Primary causes for seed abortion are thought to be related to resource or pollen limitation (Verdú & García-Fayos 1998). Parthenocarpy, on the other hand, has been hypothesised to have evolved to reduce seed loss to pre-dispersal predation (Traveset 1993, Fuentes & Schupp 1998, Verdú & García-Fayos 2001). The combination of these pre-dispersal losses can significantly reduce the number of viable propagules available for subsequent demographic stages (*e.g*., Heyes *et al*. 2023) and even cause a lack of enough propagules to reach suitable sites for recruitment (*i.e*., seed limitation, Muller-Landau *et al*. 2002).

Additionally, endozoochorous plants require sufficient mobilisation by frugivores for their seeds to reach suitable places for survival. Limitation by insufficient fruit consumption at this stage may constraint later stages of regeneration. Ultimately, the location where seeds are deposited will be dependent on their dispersers' heterogeneous use of the landscape (Jordano & Schupp 2000, Wenny 2001, Russo & Augspurger 2004, Lavabre *et al*. 2014). As a result, frugivores' foraging site (microhabitat) preferences play a key role in shaping seed dispersal patterns, impacting plant recruitment by depositing seeds in various microhabitats (Venable & Brown 1993, Wenny & Levey 1998). Frugivores providing high-quality dispersal will predominantly carry seeds to more suitable locations where seeds have greater probability of escaping predation or experience better microclimatic conditions for seedling establishment and subsequent growth (Verdú & García-Fayos 1996b, Gómez-Aparicio 2008). Thus, the impact of frugivores on plant recruitment is not solely determined by the quantity of seeds they disperse, but also by the long-term consequences of their dispersal. Pre- and post-dispersal processes of plants are often studied in isolation and few studies have successfully managed to connect frugivore consumption with seedling recruitment as this requires abundant data gathering (*e.g*., Herrera *et al*. 1994, Jordano & Herrera 1995, Schupp 1995, Jordano & Schupp 2000, Rey & Alcántara 2000, Côrtes *et al*. 2009, Rother *et al*. 2013, Donoso *et al.* 2016). Treating jointly dissemination and establishment processes can enhance our understanding of the role of frugivores along demographic transitions from fruit production to seedling establishment, and can become useful to predict the consequences of environmental changes and animal fluctuations for plant regeneration. When such analysis is carried out at the individual-level scale, such demographic consequences can be linked to the role of interaction strength and diversity in plant-frugivore assemblages.

Ecological interactions configuration at individual plant level

Super-generalist species, having numerous connections to different partner species, can exhibit a wide range of variation in how individuals within the species structure their interactions. For instance, individual members may display a generalised behaviour by interacting with the entire assemblage of frugivores the species is documented to, or individuals may specialise and interact only with a subset of the species. Intraspecific variation in the structuring of frugivory and seed dispersal interactions will play a key role in determining their success of their outcome (Snell *et al*. 2019). This places the analysis of individual-based interaction networks in a position analogous to the analysis of niche variation (Van Valen 1965, Bolnick *et al*. 2007), where one explores how individuals vary in resource use. In the case of frugivore mutualisms, the variation between individual plants' interaction establishment will be motivated by frugivores (the mobile partner). Frugivores exert different preferences for plant traits and consequently forage heterogeneously in a given plant population (*e.g*., Sallabanks 1993, Poulsen *et al*. 2002). This differential foraging by frugivores translates into different assemblage and interaction frequencies for any given individual plant in their population, and sets the "interaction niche" of each individual, *i.e*., how each individual "uses" the array of available partners in the assemblage. Assessing how this variation in the way interactions ensembles within the population can improve our understanding of super-generalist species strategy at the individual level.

Network theory has emerged as a valuable tool to study complex systems (Strogatz 2001), specifically the study of species assembly in many systems ranging from food webs to mutualisms (Fontaine *et al*. 2011). Previous research has identified underlying common structural and topological properties in species-networks (McCann *et al*. 1998, Mora *et al*. 2018). Significant advancements have been made in understanding the assembly of mutualistic interactions between species (Bascompte & Jordano 2014). Mutualistic networks have been observed to be highly heterogeneous, with species engaging in interactions that can span a wide range of scales (*i.e.*, a majority of species having only a few interactions while a minority of species exhibiting high connectance with the other species) (Jordano *et al*. 2003, Fortuna & Bascompte 2008). These networks are usually nested, where specialist species interact with a small subset of the species with which other generalist species interact (Jordano *et al*. 2003, Bascompte & Jordano 2007).

In recent years, more attention has been drawn to the fact that interactions in nature occur at the individual level, despite the convenience of aggregating them into species for analytical and synthesis purposes (Guimarães 2020, Nakazawa 2020). This shift in perspective has been argued to allow a deeper understanding into the intricacies of mutualism structuring and ecosystem stability. For example, higher levels of intraspecific variation has been proven to promote mutualism feasibility (Arroyo-Correa *et al*. 2023). Researchers are only now beginning to unravel the structure and dynamics of individual-based networks. But it remains unclear how scaling down from the species level to the individual level influences the assembly of interactions and if there is an underlying pattern that transcends different ecological contexts, such as phylogeny, geographic location, or trait diversity.

The study of individual variation in resource use has traditionally focused on antagonistic interactions, such as trophic niches (Bolnick *et al*. 2003, Araújo *et al*. 2011). However, recently there has been a growing interest in applying niche theory to understand individual variation in mutualistic interactions (Dupont *et al*. 2011, Tur *et al*. 2014, Albrecht *et al*. 2018, Phillips *et al*. 2020, Koffel *et al*. 2021, Arroyo-Correa *et al*. 2023, Gómez *et al*. 2023b). By downscaling the interaction focus to individuals, we can study how variation among individuals in their partner "use" is distributed within the population and understand the levels of specialisation in frugivore assemblages. Studying individual specialisation in frugivory systems can be specially revealing, given that mutualists directly affect the reproductive outcome of individuals by influencing population dynamics and trait selection.

Node-level metrics are a powerful tool for characterising interaction profiles as they define different aspects in the way interactions are established. Several frugivore studies have used node-level network metrics to understand the role and significance of individual plants in their population (*e.g.*, Guerra *et al.* 2017, Crestani *et al.* 2019, Vissoto *et al.* 2022, Isla *et al.* 2023). However, these studies have focused on few metrics in isolation and are limited to specific plant populations. By further exploring how individuals establish their mutualistic interactions and comparing them across populations, species, and regions, we can address important structural questions. For instance, we can investigate whether certain individual interaction profiles are consistently observed across different populations or how prevalent these profiles are. Additionally, we can explore potential relationships between these interaction patterns and specific individual traits within the population.

Model organism

The Mediterranean Basin is a highly relevant biogeographical region, being home to a high diversity of plant species (Médail & Quézel 1997, Rodríguez-Sánchez *et al.* 2008). It is estimated that, on average, 56% (47-64 %) of all woody species in local sites of this region rely on vertebrates for the dispersal of their seeds (endozoochorous; Herrera 1984c, Jordano 2000). *Pistacia lentiscus* (Anacardiaceae) is an evergreen woody shrub distributed along the Mediterranean Basin and can often be found dominating the landscape at low elevations (Zohary 1952, Palacio *et al.* 2005, Martínez-López *et al.* 2020). The species lineage originated during the Oligo-Miocene period, exhibiting plant characteristics that are considered evolutionary anachronisms due to their adaptation to past tropical environments rather than the current Mediterranean climate (Palamarev 1989, Verdú & García-Fayos 2002). *Pistacia lentiscus* is dioecious, with anemophilous pollination, and its fleshy-fruits are a staple food for frugivorous birds (Jordano 1989). During the fruiting season (September-March) *P. lentiscus* produces large amounts of fruits that are consumed by a great variety of species (Fig. I.1). Not only resident species but aso migrants rely on *P. lentiscus* fruits for obtaining necessary nutrients and energy (González-Varo *et al.* 2019a). Between the months of September and October trans-Saharan migrants (*e.g.*, *Sylvia borin*, *Curruca communis, Ficedula hypoleuca* or *Phoenicurus phoenicurus*) consume big quantities of *P. lentiscus* fruits while making stop-overs before crossing the Gibraltar strait to their wintering grounds in Africa. It is also around October that bird species from the North of Europe start arriving to overwinter in the Mediterranean scrubland and need to gain enough energy to migrate back to their breeding grounds in spring (winter residents, *e.g.*, *Turdus philomelos, Sylvia atricapilla* or *Erithacus rubecula*). The reliance of all these bird species on fleshy fruits makes *P. lentiscus* a key fruiting resource in Mediterranean scrublands, behaving as a perfect candidate to study the super-generalist strategy in frugivory networks. *Pistacia lentiscus* fruits have been reported to be one the most consumed and dispersed species in respect to other fruiting resources in the Mediterranean lowland communities (Herrera 1984b, Jordano 1984, 1987b, 1988a, García 2016, Parejo-Farnés *et al*. 2020a).

Fruits of *P. lentiscus* are single-seeded drupes that transition from red colour when unripe to a vibrant black when ripe. The fruits often contain empty seeds as a result of either parthenocarpy, embryo abortion or pre-dispersal seed predation by the wasp (Grundwag 1976, Jordano 1989). The wasp *Megastigmus pistaciae* of the superfamily Chalcidoidea oviposits on the fruit, where the larvae will consume the endosperm from within, rendering the seed unviable (Traveset 1993, Verdú & García-Fayos 1998). Seed maturation and viability are associated, with black fruits generally being more viable than red ones (Jordano 1989). Viability has been reported to be dependent on water resources available, and the frequency of empty seeds varies greatly from year to year, as well as among populations (mean: ~30 %, range: 7% to 45%) (Jordano 1988b, 1989, Verdú & García-Fayos 1998, 2002, Albaladejo *et al*. 2009).

The fruits of *P. lentiscus* are consumed in three different ways by the avian community: (1) gulper birds act as legitimate dispersed shallowing the whole fruit and regurgitating or defecating the seeds intact; (2) seed-predators destroy the seeds by breaking the seed coat in half and feeding on the embryo (mostly Fringillidae family); (3) pulp-peckers peck on the pulp usually leaving the fruit attached by the peduncle to the branch (mostly Paridae family). All frugivores strongly prefer the black fruits as they have higher lipid content, and higher proportions of filled seeds, yet they also consume red fruits extensively (Jordano 1989, Trabelsi *et al.* 2012).

Figure I.1. Some of the most common species of frugivorous birds known to consume and disperse *Pistacia lentiscus* fruits. In clockwise order starting from top left and with their migrant behaviour code in brackets: *Saxicola rubicola* (R), *Curruca melanocephala* (R), *Turdus philomelos* (W), *Sylvia borin* (TS), *Sylvia atricapilla* (W), *Phoenicurus phoenicurus* (TS), *Erithacus rubecula* (W), *Turdus merula* (R) *and Chloris chloris* (R; this last species acts as seed predator). Migrant codes: R – resident, W – wintering migrant, TS – trans-Saharan migrant.

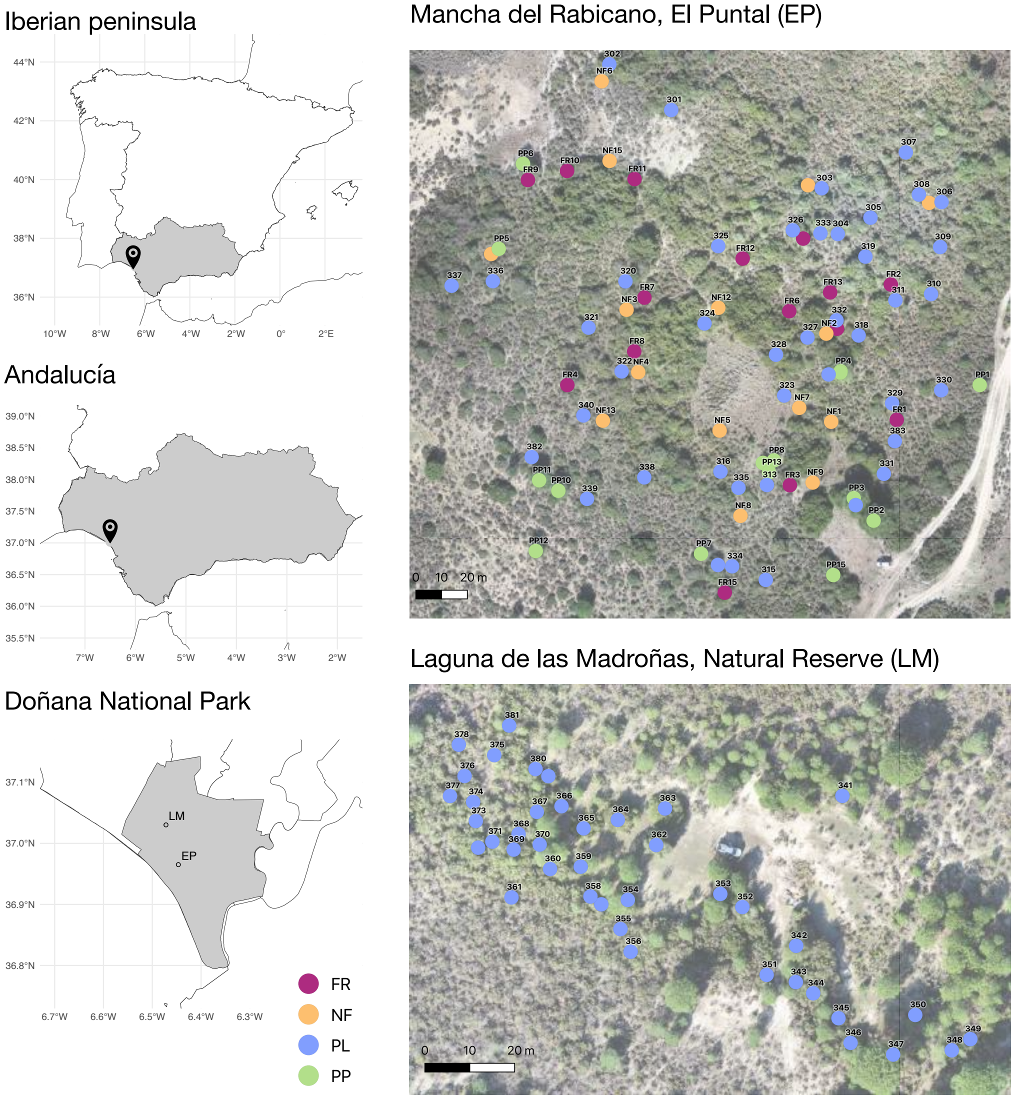


Figure I.2. Map of study sites and sampling points in Doñana National Park where the two *Pistacia lentiscus* populations were sampled. Points indicate sampling locations and colour microhabitat type: female *Pistacia lentiscus* (PL), other fleshy fruited species (FR), non-fleshy fruited species (NF), pine trees (*Pinus pinea*; PP). Note open area (OA) microhabitat is not shown in the map because it was sampled using transects.

Study system and brief overview of fieldwork

We sampled two populations in Doñana National Park (Huelva, Andalucía, Spain): La Mancha del Rabicano, in El Puntal site (EP), and Laguna de las Madroñas, within the natural reserve (LM) (Fig. I.2 and I.3). Both areas consist of Mediterranean sclerophyllous shrubland dominated by lentiscs (*Pistacia lentiscus*) coexisting with other fleshy-fruited species such as *Phillyrea angustifolia, Olea europaea var. sylvestris, Asparagus aphyllus* and *Myrtus communis* (Fig. I.3). The presence of pine trees (*Pinus pinea*) is scattered at EP, but more abundant at LM. Other abundant non-fleshy fruited species present in the area are *Erica arborea, Ulex parviflorus, Halimium halimifolium* and *Cistus salviifolius* (Allier *et al*. 1974; Rivas-Martínez *et al*. 1980). In each population we marked 40 individual female plants of *P. lentiscus*, as we focus on the individual plant perspective (Fig. I.2). This sampling included all the female fruiting plants found in the LM population. In the EP site, individual plants were haphazardly selected to encompass the range of plant size and fruit crop production in the area.

Figure I.3. Study sites at Doñana National Park. EP site, left, LM site, right.

Fieldwork was performed during two fruiting seasons: 2018-2019 and 2019-2020. The first season we monitored plants from EP site and started sampling in mid-November, missing the first months when trans-Saharan migrants are more abundant. This dataset is used in Chapter 1 to compare field-sampling methods for detection of frugivory interactions. The second season took place in both populations (EP and LM) and sampling was performed throughout the entire fruiting season. This dataset is used in Chapters 2, 3 and 4 where analyses are based on the complete frugivore assemblage.

To characterise intraspecific variation, we measured some individual plant attributes, including the initial crop in both fruiting seasons, plant dimensions, and fruit characteristics. Additionally, we assessed the fruiting neighbourhood using drone aerial images. We collected fruits from individual plants in both fruiting seasons and estimated unviability rates due to abortion, parthenocarpy, and wasp predation. Furthermore, we measured the black to red colour ratio, as well as the weights of the fruit, pulp, and seeds (Fig. I.4).

Figure I.4. *Pistacia lentiscus* photographs. Top left: individual plant with most common shrub rounded shape. Top right from up to down: male flowers, female flowers and detail of fruit predated by *Megastigmus pistaciae* wasp. Bottom left: detail of branches crowded with fruits. Bottom right: detail of mature black fruits with red unripe fruits.

Plant-animal frugivory interactions were sampled using two methodological approaches: DNA-barcoding of bird faecal and regurgitated samples and continuous-monitoring cameras. Samples for DNA-barcoding were collected using seed traps that were located beneath the crown of all focal plants. In the EP site, we also installed continuous-monitoring cameras that were set facing individual plants. Each plant was recorded once in the first season 2018-2019, and nine times distributed fortnightly along the fruiting season of 2019-2020.

To infer post-dispersal consequences, we selected five microhabitats in EP site based on the differential seed deposition and establishment success expected due to bird landscape-use patterns, post-dispersal predation pressure and microclimatic conditions (Jordano & Schupp 2000, García *et al*. 2005, Gómez-Aparicio 2008) (Fig. I.2). Microhabitats selected were: under *Pistacia lentiscus* conspecifics (PL), under other fleshy fruited species (FR), under non-fleshy fruited species (NF), under pine trees (*Pinus pinea*; PP), and open ground areas (OA). Differential seed dissemination by birds in microhabitats was inferred through DNA-barcoding analysis of dispersed seeds (Fig. I.5). In addition to seed traps located under focal plants, *i.e.*, PL microhabitat, we also placed seed traps in FR, NF and PP microhabitats. Given the low seed rain density and wide extension in the OA microhabitat, we opted to conduct walking transects to collect seeds. To measure post-dispersal outcomes, we conducted experiments in each microhabitat to estimate the probabilities of seed to survive post-dispersal predation, as well as the probabilities of seedling emergence and survival during the first and second summer (Fig. I.5).

Figure I.5. Photographs of sampling methods used to capture frugivory interaction and experiments performed in the field. Top left: Monitoring camera (GoPro Hero® 7 White) recording in front of an individual plant. Two photos in top right: seed predation experiment with rodent exclusion control with a detail of seeds predated by rodents. Two photos in middle left: seed emergence and survival station experiment with details of 1 year old seedlings of *P. lentiscus*. Bottom: trays (seed traps) used to capture samples for DNA-barcoding. Detail of regurgitated clean seed with some faeces without seeds, and detail of dispersed seed in faeces.

Objectives

The present PhD thesis aims at understanding how super-generalist species shape the network of ecological interactions, explore more in depth the development of their strategy in mutualistic systems, their evolutionary success and their implications in ecosystem functions. Using *Pistacia lentiscus* as a model organism, we investigate the complexity and diversity of interactions supported by the species. This PhD thesis encompasses some recent advances in the study of frugivory mutualisms and is structured as follows:

Chapter 1 provides an overview of the current methods employed to document plant-animal frugivory interactions and proposes different approaches for data combination from different sources to maximise information and obtain more robust inferences. Additionally, we provide two study cases to illustrate the combination approaches proposed and compare their performance. This chapter was born out of a reflection after a symposium with my colleague Jorge Isla on the need to bring together the enormous diversity of methods that exist for sampling frugivory interactions.

Chapter 2 seeks to understand how the ecological interactions within a generalised mutualistic system are structured and how the outcome of the interactions affects both partners. To do so, we focus on eighty individual plants of the super-generalist species *Pistacia lentiscus* in two populations. Using the effectiveness framework, our aim is to determine whether the exchange of resources within the mutualism occurs in a balanced manner (*i.e.*, "a fair two-way transfer"). We explore whether there is reciprocity in the exchange of rewards between individual plants and the set of frugivorous birds that consume their fruits. In addition, we examine how partner dependencies are distributed from the two perspectives (*i.e.*, plant's and animal's) and assess whether there is symmetry in the mutual reliance on resources.

Chapter 3 investigates the role of avian frugivores and seed viability in early plant recruitment. Frugivorous birds act as a key link between fruit consumption and plant recruitment in different microhabitats by considering their landscape use and heterogeneous seed deposition. We consider the reproductive cycle of *P. lentiscus* plants from fruit production to recruitment of 2-year-old seedlings. We estimate the number of propagules and the transition probabilities through the following demographic stages: (1) fruit consumption, (2) avian seed predation, (3) viable seed dispersal to different microhabitats, (4) escaping post-dispersal rodent predation, (5) seedling emergence and (6,7) seedling survival to two consecutive summers. This chapter aims to unravel the demographic consequences of frugivore interactions and the post-dispersal consequences of dispersed seeds for the reproductive success in this Mediterranean shrub species.

Chapter 4 extends the findings from the interaction patterns of individual plants in our study population to other frugivory systems worldwide. By compiling a set of individual-based network data from Asia, America, and Europe, we aim to understand how ecological interactions are structured. These networks focus on the individuals of specific plant species and their frugivore assemblage. We set three main objectives: 1) Compare the architectural and structural properties of individual-based networks with what is known about species-based networks to test for differences in their topologies that allow their differentiation and explore potential unifying principles underlying their topology. 2) Using classical niche theory we explore how variation in frugivory interactions occurs at the plant population level by quantifying individual niche-partitioning and frugivore interaction allocation. 3) Finally, we characterise different aspects of the interaction profiles of individual plants and assess their distribution within the population. Combining network and niche theories with an individual-based focus, we hope to gain insight into the mechanisms underlying the assembly and functioning of mutualistic communities.

CHAPTER 1

Methodological overview and data-merging approaches in the study of plant-frugivore interactions.

Quintero, E., Isla, J., & Jordano, P. (2022). Methodological overview and data-merging approaches in the study of plant–frugivore interactions. Oikos, e08379, 1–18.

Abstract

Recording species interactions is one of the main challenges in ecological studies. Frugivory has received much attention for decades as a model for mutualisms among free-living species, and a variety of methods have been designed and developed for sampling and monitoring plant-frugivore interactions. The diversity of techniques poses an important challenge when comparing, combining, or replicating results from different sources with different methodologies. With the emergence of modern techniques, such as molecular analysis or multimedia remote recorders, issues when combining data from different sources have become especially relevant. We provide an overview of all the techniques used for monitoring endozoochorous primary seed dispersal, focusing on a critical appraisal of the advantages and limitations, as well as the context-dependency nature, of the different methods. We propose five data merging approaches potentially useful to combine frugivory interactions data from different methodologies. Additionally, we provide two case studies where we combine empirical data from plant-animal interactions in Mediterranean shrublands using different methodologies. Data merging resulted in a net increase in the number of distinct pairwise interactions recorded and compensated biases inherent to different methods, resulting in more robust estimation of network topological descriptors. These case studies clarify the context-dependent character of the merging approaches, highlighting the value of collecting detailed information on the sampling effort in terms of reliable results and reproducibility. Finally, we discuss the trends with different methodological approaches used in the last decades and future perspectives in this field.

Keywords: frugivory, methods, plant-animal interactions, endozoochory, seed dispersal, ecological networks

Introduction

One of the greatest challenges that ecologists face is to properly determine the biodiversity present in their study systems, *i.e.*, the presence and relative abundance of species (Magurran 1988). An important facet, yet a frequently dismissed one, in biodiversity analysis is to document how species interact with one another, and what are the outcomes of these interactions (Valiente-Banuet *et al*. 2015). Scholar accounts of the myriad connections among species date back at least to al-Jāhiz in the 9th century or even earlier to Aristotle in the 4th century BCE (Egert 2007). Yet, the more formal onset of the ecology of interactions took place later, fostered by late 18th-century naturalists. Pioneer studies of ecological interactions were focused on trophic cascades within food webs (Cohen 1978, Polis & Strong 1996), and later unfolded into the analysis of complex networks of ecological interactions in the late '90s (see Bascompte & Jordano 2014). Effectively incorporating the quantification and analysis of ecological interactions is essential to recent efforts to preserve the value of Biodiversity (IPBES 2019) yet we are still far from achieving this goal, not only by assessing the actual richness and diversity of interactions in nature, but also by assessing the ecological services associated to them.

Frugivory has received much attention for decades and a variety of methods have been designed and developed to track how encounters between animal frugivores and plants result in seed dispersal events for the plants and food resource provisioning for the animals (Estrada & Fleming 1986, Fleming & Estrada 1993, Levey *et al.* 2002, Dennis *et al.* 2007). This reciprocal service is the basis of coevolved plant-frugivore mutualistic interactions and implies enormous consequences for forest regeneration and ecosystem functioning (Howe & Smallwood 1982). A crucial aspect of interaction sampling, besides recording the mere presence of an interaction, is also measuring its relative frequency and its impact, *i.e.,* the outcome of interactions in terms of fitness effects for the interacting partners.

The study and monitoring of seed dispersal events became increasingly apparent from late eighties with the publication of the first volume of FSD (Estrada & Fleming 1986), and direct observation and census at focal plants became a standard method to inventory plant-frugivore interactions with multiple objectives. Yet, new methods have emerged in the last decades allowing indirect, delayed recording of these interactions and opening new possibilities for research on frugivory and seed dispersal (see *e.g.,* Forget & Wenny 2005, Carlo *et al.* 2009, González-Varo *et al.* 2014). The diversity of techniques available to monitor species interactions pose the important challenge of comparing results obtained with different methodologies, replicating the results or incorporating interaction data from different sources. With the emergence of modern techniques, such as DNA-based molecular analysis (Valentini *et al.* 2009, Carreon-Martínez & Heath 2010, González-Varo *et al.* 2017, Mata *et al.* 2019), this has become especially relevant. Merging data from different sources allows us to maximise information and improve research potential for any kind of frugivory and seed dispersal study. Combining the distinct data types and information yielded by such a diversity of methods, can become a difficulty and even a limitation if there are no well-established guidelines.

Given the wide spectrum of seed dispersal interactions that exist, in the first part of this manuscript we provide a methodological overview where we primarily focus on endozoochorous seed dispersal. Our goal in this part is not a comprehensive review of methods, rather we aim to offer a critical appraisal of the advantages and limitations as well as the context-dependent nature of the major sampling methods, focusing on methods complementarity, reproducibility and sampling effort. In the second part of this manuscript we propose and illustrate five different merging approaches to combine datasets originated with different sampling methodologies. The merging data approaches we describe here may be also applicable to other interaction forms aside endozoochorous seed dispersal, such as synzoochory, epizoochory or secondary seed dispersal (see *e.g.,* Costa *et al.* 2014, Gómez *et al.* 2019) or even other types of interactions like pollination, host-parasite or plant-plant facilitation. To exemplify and validate the described merging methods we provide two case studies, using empirical data where we compare and combine different methodologies using an interaction network approach. Finally, we discuss the trends in the use of different approaches over the last decades and the future perspectives in this field. We hope that this overview and the combination strategies proposed here can serve as a useful reference for researchers when approaching future frugivory studies and may complement other papers dealing with plant-frugivore interactions and thorough field-sampling approaches.

Study focus, scale and resolution

Depending on the study's objective, the term 'interaction' and its measurement can vary greatly. The strength of an interaction (*i.e.*, the effect magnitude of its outcome, in addition to its frequency of occurrence) can change depending on the focus of study and how its outcome for the partners is measured. Therefore, the study question will determine when and how interactions are monitored (Niquil *et al*. 2020).

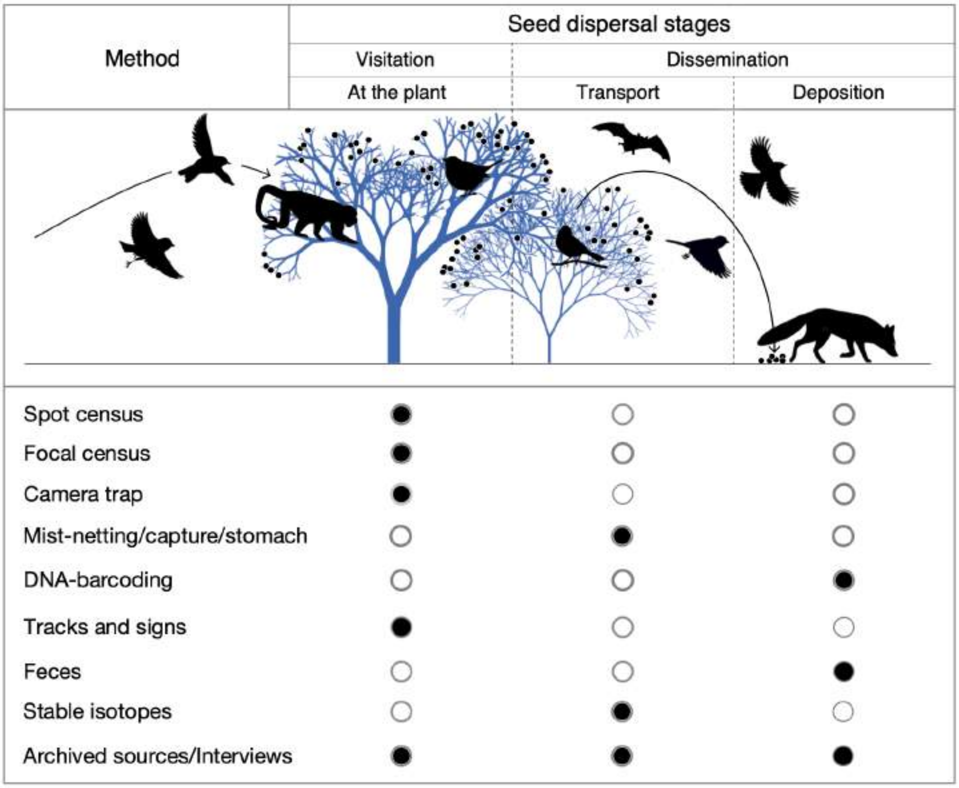
Focus may be directed towards the plant partner (*i.e.,* phytocentric), the animal partner (*i.e.,* zoocentric) or both (Jordano 2016). These approaches impose different sampling challenges and information, varying in their characteristics, accessibility, visibility, potential biases, logistic limitations, sampling effort demand, etc. In many cases the goals themselves can clearly establish the characteristics of the study focus (*e.g.,* understand the role in seed dispersal of juvenile vs. adult individual animal frugivores, Gonzalez-Varo *et al.* 2019b). However, sometimes the focus of study may be more complex to define (*e.g.,* select a phytocentric or zoocentric approach when comparing seed dispersal networks between sites).

Other important aspects include the resolution and scale of the sampling, being the intra-individual level the most refined, and scaling up to the aggregation of species in groups, eventually including higher taxonomic levels, morphological or functional groups (see *e.g.*, Moran *et al*. 2004). Clearly defining the spatial and temporal scale of the study is key. Plant-frugivore interaction patterns at different spatial scales are not necessarily consistent (Jordano 1993, García & Ortiz-Pulido 2004), furthermore it becomes extremely difficult to extend analyses of *e.g.*, dispersal kernels, beyond the local scales (García & Borda-de-Água 2016). Likewise, temporal variations driven by the phenology of the species or the availability of resources will largely determine the interactions detected (Carnicer *et al.* 2009, Costa *et al.* 2020).

When combining studies or methodologies, it is advisable to look at the study focus and at the scale at which each data source has been gathered. At the end of the combining methodologies section we propose a way of correcting the divergence that may exist between scales. Also, one of the case studies illustrates an example of data merging for two methodologies with different focus; observations of foraging animals, as plant-focused, and mist-netting, as animal-focused.

A general overview of sampling methods

In order to illustrate current methodologies, we will divide sampling techniques into three intuitive categories based on the main stages of the seed dispersal process (Schupp *et al.* 2017). Depending on when we are collecting information for animal-plant interactions, the sampling will be directed towards one of these three stages (Fig. 1.1): ‘Visitation’, ‘Transport’ or ‘Deposition’. While some methods may be directed to more than one stage, we have classified them in the most representative one. The first, or early stage (‘Visitation’), refers to the actual interaction on the plant, when the animal is manipulating, removing or ingesting the fruits on the plant. The subsequent two stages refer to the dissemination process, where the seeds are first transported (*i.e.*, moved some distance away from the source plant, ‘Transport’) and then deposited (*i.e.*, disseminated), which may involve actual burying of the seed or just dropping, *e.g.,* by spitting, regurgitation or defecation (‘Dissemination’).



Method	Seed dispersal stages		
	Visitation	Dissemination	
	At the plant	Transport	Deposition
Spot census	●	○	○
Focal census	●	○	○
Camera trap	●	○	○
Mist-netting/capture/stomach	○	●	○
DNA-barcoding	○	○	●
Tracks and signs	●	○	○
Feces	○	○	●
Stable isotopes	○	●	○
Archived sources/Interviews	●	●	●

Figure 1.1. A non-exhaustive overview of the most frequently used methods for recording plant-frugivore interactions. Sampling methods are categorised based on seed dispersal stages: “Visitation”, “Transport”and “Deposition”.

Methods targeting 'Visitation'

Methods used to monitor the initial 'Visitation' stage (Fig. 1.1, Table. 1.1) are typically directed towards seed sources (*i.e.*, maternal) plant individuals with standing fruit crops, where it is possible to observe the interaction occurring. When considering the resource-harvest type of mutualism characteristic to most plant-frugivore interactions (Janzen 1983, Ollerton 2006), this typically refers to the feeding phase, when the source plant and individual animal actually interact. The methods used in this stage can be classified into direct methods that allow us to observe the interaction directly, and indirect methods based on the detectable signals of the interaction. Traditional methods are field focal observations at fruiting plants (Howe & Vande Kerckhove 1980, Snow & Snow 1988, Jordano & Schupp 2000, Stevenson *et al.* 2015), transects (Galetti & Pizo, 1996), animal visual trackings (Gestich *et al.* 2019) and spot censuses (Howe & Vande Kerckhove 1981, Rother *et al.* 2015). The implementation of technological advances such as camera traps or other multimedia recording systems (*e.g.*, action cameras) also allows us to observe the interaction taking place (Miguel *et al.* 2018, Campos-Arceiz *et al.* 2012). These non-invasive multimedia techniques avoid the interference of the observer with the animal in the field, allow continuous sampling over day and night and extended periods of time, and enable simultaneous monitoring over large study areas, thus increasing the probability of detection of rare interactions and improving the description of interactions distribution. Other indirect methods such as footprint traps allow to identify the species of animal that visits the plant (Jácome-Flores *et al.* 2020), or offerings (Garrote *et al*. 2018) that allow to quantify frugivory rates, can also be very useful, since they do not require a continued presence nor entail a high economic cost. Bill and teeth marks are signals that can be used to infer interactions too (Alves-Costa & Lopes 2001). An alternative indirect method in this phase is the estimation of the fruit removal caused by frugivores by counting the plant crop size over consecutive periods of time. This method becomes useful for plants with one exclusive frugivore (*i.e.*, exclusive frugivory on islands, Hansen & Traveset 2012; or cases of double mutualism, Gomes *et al*. 2014) as well as to discern between guilds of daytime and night-time frugivores (Palmeirim *et al*. 1989, Korine *et al*. 2000).

Methods targeting 'Transport'

Methodologies used during the 'Transport' stage are typically those where the animal is intercepted by means of capture, before any kind of fruit or seed release or deposition has taken place (Fig. 1.1, Table 1.1). The capture methods depend on the target species, for example the mist nets are the most commonly used for medium-small size birds and bats (Herrera 1984b, Costa *et al.* 2020). Live traps are used for mammals (Genrich *et al.* 2017), and for fish there are also capture methods that allow obtaining the stomach content of the captured animals (Weiss *et al.* 2016). Other sources of information include the stomach contents of animals after death from directed hunting (Remsen *et al.* 1993), fishing (Galetti *et al.* 2008, Correa *et al.* 2015) or roadkills (Vaz *et al.* 2012). Interactions can be quantified on the basis of the number of seeds of different species found in the faeces or in their stomach contents, properly accounting for pulp and/or seed remains due to potential biases generated by differential gut treatment (Oliveira *et al.* 2002, see Appendix 1B).

Methods targeting 'Deposition'

Methods targeting the 'Deposition' stage (Fig. 1.1, Table 1.1) are used when the seed has reached its final destination by means of defecation, regurgitation, spitting, scatter-hoarding, discarding or unfortunate drop. In this case, sampling is directed towards the final seed destination (except when secondary or subsequent dispersal events are involved, *i.e.*, re-caching), typically in scats or droppings. Collection of faecal samples or regurgitated seeds can be carried out in different ways. For example, using transects or established areas to collect samples in the field has been widely used to study seed dispersal by mammals (*e.g.*, González-Varo *et al*. 2013, Perea *et al.* 2013). In the case of primates, continuous monitoring of individuals can prove to be useful (Gestich *et al*. 2019). For bird dispersal, the use of seed traps is more common, since it greatly facilitates sample detection and can limit bias effects such as post-dispersal predation or secondary seed dispersal (Jordano *et al.* 2007).

Direct identification of frugivores by the shape and size of the faeces is possible for some carnivore species (Guitián & Munilla 2017). Individual tracking or identification of other frugivore droppings can be challenging, such as for reptiles, birds or bats; fortunately, new molecular techniques such as DNA-barcoding or metabarcoding offer a great potential to solve this problem. DNA-barcoding methods, allow the identification of the frugivorous species from the genetic material (animal origin) present on the seeds after their dispersal, matching the sequences obtained with reference sequences deposited in the BarCode of Life databank (Hebert *et al.* 2004, Kress *et al.* 2005, González-Varo *et al.* 2017). More and more studies are using this method, which promotes the expansion of species with available reference sequences and the optimization and adjustment of the protocol. Conversely, if we are interested in identifying the plant species consumed by a frugivore over a period of time, DNA metabarcoding techniques would be the ideal option, however see Tercel *et al.* 2021 for a discussion on potential limitations of this method. This molecular approach allows the simultaneous identification of multiple taxa from a single frugivore scat containing a mixture of DNAs by means of high-throughput sequencing of a carefully selected parts of the genome, a technique widely used in plant-herbivore interaction studies (Evans *et al.* 2016, Kartzinel *et al.* 2019).

Lastly, stable-isotopes analyses can also be useful in frugivory studies, although with less resolution than with other techniques (Galetti *et al*. 2016). This approach is based on the premise that there is a relationship between stable isotopic compositions of consumer tissues and the stable isotopic compositions of the diet (Deniro & Epstein 1978, 1981). The stable isotope technique is only useful in situations where two isotopically distinct dietary sources are available for frugivorous species (*i.e.*, relative contributions of C3, and C4, plant-based proteins to avian diets, see Hobson & Clark 1992), although it may not be useful for describing interaction patterns across multiple partners.

Alternative Methods

There are other approaches to compile interaction data, such as bibliographic searches, image repositories, interviews or word-of-mouth (*e.g.*, Koike & Masaki 2008). These interaction records do not normally come with specific information on the moment of the seed dispersal process, therefore it is not possible to assess a specific interaction detection moment. For example, for some specific studies like seed dispersal systems in remote areas, conducting interviews with native inhabitants can be a precious source of information (Cámara-Leret *et al*. 2019), due to their close relationship with the natural environment or even their use of fleshy fruits. Another example of these compilation methods could be citizen science studies (Bath-Rosenfeld 2019) or image repositories (Gonçalves dos Santos *et al.* 2019). Likewise, thorough bibliographic review in search of interaction data can provide a useful method for more general reviews or greater scope studies (Bufalo *et al.* 2016, Bello *et al*. 2017). These data gathering strategies can be very powerful, yet they come with some limitations, such as the information obtained will come from different methodologies and the sampling effort or precise georeferencing may be difficult or impossible to establish.

Table 1.1. A summary of sampling methods used in plant-frugivore interaction studies. Including information about: seed dispersal stage, sampling effort, costs, detectability, replicability, additional information that can be obtained (apart from the interaction per se), units of measurement and reference studies illustrating the usage of each method. See Appendix 1E for the full reference list of the example studies.

Method	Stage	Sampling effort	Cost	Detectability	Replicability	Additional information	Units	Ref
Direct observations	Visitation	Requires long time in the field.	Low. High cost in sampling time.	Medium-low (human presence can affect significantly).	Limited - affected by the expertise of the observer.	Foraging and fruit handling behaviour.	- Frequency (*e.g.*, visits /h and/or feeding rate) - Total count	1, 2, 3
Spot censuses	Visitation	Requires long time in the field.	Low. High cost in sampling time.	Medium-low (human presence can affect significantly).	Limited - affected by the expertise of the observer.	Foraging behaviour.	- Frequency (*e.g.*, interactions recorded/area/h) - Total count	4, 5 ,6
Animal followings	Visitation	Requires long time in the field.	Low. High cost in sampling time.	Medium-low (human presence can affect significantly).	Limited - affected by the expertise of the observer.	Foraging and fruit handling behaviour.	- Frequency (*e.g.*, interactions/time, fruits/visit) - Total count	27
Multimedia (*e.g.*phototrapping)	Visitation	Low in the field but remarkable processing video efforts.	Expensive at first (big inversion, then low (only batteries).	High for large-bodied animals. Low for small or fast animals.	Medium-high - can be challenging for high canopy plants.	Behaviour, n of fruits, feeding time, etc.	- Frequency (e.g., visits/h). - Total count.	7, 8
Footprint traps. Bill and teeth marks	Visitation	Low.	Low in material. High cost in sampling time.	Medium, useful for interactions with well-known signals.	Medium. Highly dependent on substrate type or in species signals.	Animal body size, traits of discarded fruits.	- Frequency: visits/ time unit. -Total counts: no. of contacted fruits.	9, 10

Method	Stage	Sampling effort	Cost	Detectability	Replicability	Additional information	Units	Ref
Fruit removal in the plant	Visitation	Low-mid, problematic if huge crop sizes.	Low in material. High cost in sampling time.	High with medium-low crop sizes. Subsample could be useful.	High.	Temporal activity patterns.	- Total counts: Fruit removal metrics.	11, 12
Offerings	Visitation	Low-mid, problematic if low crop size.	Low in material. High cost in sampling time.	Low specificity.	Medium-High.	Feeding preferences.	- Frequency: visits/ time. - Total counts: fruit removal metrics.	13, 7
Stomach content (after dead)	Transport	High, needs large samples. Bio-ethical concerns.	Low in material. Medium-high cost in sampling time and processing.	High.	Low - depends on sample availability.	Animal phenotypic traits. Fruit preferences.	- Frequency: seeds/sample. -Total counts: no. of fruits/seeds consumed.	14, 15
Mist netting, traps	Transport	High, needs long time to capture sufficiently-representative sample.	Mid-high.	Low - very selective method, elusive animals are hard to capture.	Mid-low.	Animal phenotypic traits. Supplementary diet resources.	- Frequency: seeds/bird - No. of seeds per stomach content or fruits consumed.	16, 17
Fecal samples or regurgitated/spat seeds (in the field)	Deposition	High, requires field work plus sample treatment and identification	Low in material. High cost in sampling time and processing.	Medium. It is difficult to detect and identify samples of rare species.	High.	Micro-habitat deposition. Other food resources. Precise spatial reference. Fruit preferences and handling.	- Seed shadow metrics. - Freq. of seeds per sample. - Faeces/seeds per transect. - No. fruits consumed.	18, 19

Method	Stage	Sampling effort	Cost	Detectability	Replicability	Additional information	Units	Ref
DNA-based molecular techniques (e.g., barcoding)	Deposition	Mid, relatively short time in the field but long lab protocol.	High both in materials and processing time.	High-rare species Low-species not adequately sampled in seed traps.	High-based on protocol.	Micro-habitat deposition. Other food resources. Precise spatial reference. Fruit preferences.	- Interaction frequency. - Seed shadow metrics. - Freq. of seeds per sample. - No. dispersed seeds.	20, 21
Stable isotopes	Transport-Deposition	Mid, may need time to capture sufficiently-representative sample.	High.	Limited, usually not species-specific detection.	High.	Possibilities for large-scale analysis. Dispersal distances.	Interaction frequency. Presence of plant material in diet.	22, 23
Interviews	Visitation-Transport-Deposition	Low.	Low cost in terms of material. High cost in time for interviews.	Medium-low (human presence can affect significantly-difficult to identify at species level).	Limited-Low. (High area-dependent effect).	Behaviour, no. of fruits, feeding time, long-term trends.	No. of positive responses for total interviews (total count).	24
Bibliographic review	Visitation-Transport-Deposition	Low, no field. Hard review and researchers contact work.	Limited material. High cost in time.	High, M&M dependent.	High.	Behaviour, no. of fruits, feeding time, frugivore community and plant resources availability.	Specific units of each paper. No. of papers. Total interactions for all sources.	25, 26

Complementary information obtained with different methods

Different stages of the plant-frugivore interaction process (Fig. 1.1) will provide varied and valuable information. The first part of the dispersal process ('Visitation') is the only stage where we are able to observe both partners together. The source plant will be exclusively present during this phase, leaving a progeny in the form of a seed, to be present in later stages. This enables us to get data on feeding rates (*e.g.*, fruits per visit, visit length), handling damage to fruits and seeds and fruit foraging behaviour (Moermond & Denslow 1985, Levey 1987, Snow & Snow 1988, Jordano & Schupp 2000). In addition, we may collect valuable information about intrinsic and extrinsic attributes of the mother plant, (*e.g.,* crop size, fruit traits, conspecific neighbourhood densities), that would not be possible otherwise (Sallabanks 1993, Miguel *et al.* 2018).

Methods targeting the second stage ('Transport') can be very useful for zoocentric studies, since they provide valuable information on dispersing animals. During this phase individual identification and marking is possible, as well as, we can gather additional data on animal body condition, morphological traits, or even measurements of gut passage time (Herrera 1984a, Remsem *et al.* 1993). Radio trackers can also be settled in captured animals to study dispersal distances (see Uriarte *et al*. 2011). This type of complementary data related to animal vectors and their behaviour, allows us to better understand how and why the interactions we detect are taking place, as well as to be able to project and model the consequences of their dispersal (Nathan *et al.* 2012).

Sampling carried out during the last seed dispersal stage ('Deposition') can be suitable for plant demographic studies (*e.g.,* Howe 1990), or animal habitat use, occupation or home range studies (*e.g.,* Gestish *et al*. 2019). Maternal genetic correlates, such as relatedness between seeds, can be obtained through molecular techniques (García *et al.* 2009) and can help disentangling spatial genetic patterns of plant growth. Methods targeting seed deposition also provide important evidence on dispersal distance and can help identify long-distance dispersal (LDD) events (Nathan *et al.* 2012), with recent extensions based on extreme events theory allowing the exploration of very long-distance events (García & Borda-de-Água 2016).

Combining sampling techniques can often increase the complementary information available. The combination of methods with different focus of study can be useful to acquire more in-depth knowledge about interactions outcome (*i.e.,* combining phytocentric and zoocentric methods).

Sampling methods: constraints, potential limitations, and sampling effort

By definition, no sample is complete; a key aspect is to evaluate how far from completeness we are when analysing a specific system with a specific sampling method. Different methods (Fig. 1.1, Table 1.1) are subjected to different constraints (*e.g.*, logistic, temporal, accessibility, economic cost or technical difficulty) and these may differentially affect sampling completeness. Having a robust sampling design is important. When monitoring comprises several individuals, species or areas, the sampling effort should be adjusted and its adequacy explicitly evaluated (*e.g.*, with accumulation methods). Otherwise, a posteriori corrections need to be incorporated to account for unequal sampling effort (Jordano 2016, Vizentin-Bugoni *et al*. 2016). Another aspect to consider is the potential bias derived from each sampling method, mostly arising from detectability biases.

Costs can be evaluated in terms of time, necessary expert workforce, economic expenses, material or logistics. Once the samples are collected, variation exists in terms of processing costs. A trade-off between collection and processing costs emerges for different monitoring interaction techniques (Appendix 1A). Genetic or high-tech methods such as meta-barcoding or camera traps are economically costly but they can reduce the laborious time spent in the field. While these methods can save time in the field, they frequently impose longer processing times for robust identification of animal visitors or during laboratory work; however, recent advances in automatic detection may contribute to alleviate this issue (Norouzzadeh *et al.* 2018).

Given the above constraints and limitations, sampling effort eventually becomes limiting for obtaining an adequate completeness of the data. Interaction accumulation curves (IAC) (Fig. 1.2) provide an excellent tool to estimate the sampling completeness of a study and its robustness (Jordano 2016, Macgregor *et al.* 2017, Mata *et al.* 2019). This method is a simple reformulation of the species accumulation curves (SAC) method (Gotelli & Colwell 2001, Chao *et al.* 2014a) that plots the cumulative number of unique pairwise interactions recorded as a function of sampling effort (Jordano 2016). Completeness can be estimated as the percentage of interaction richness detected with our sampling, where the observed interactions are divided by the total number of estimated interactions with Chao2 and multiplied by 100 (see Chacoff *et al.* 2012).

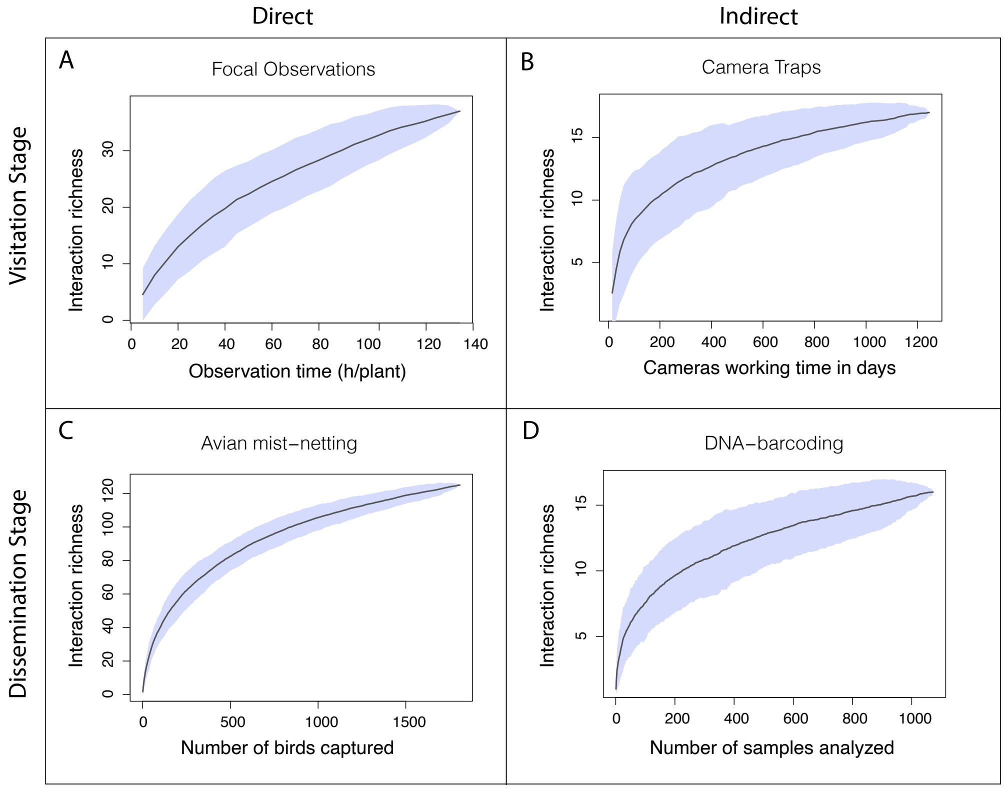


Figure 1.2. Examples of interaction accumulation curves (IAC), where the number of unique pairwise interactions (y-axis) accumulates as the sampling effort increases (x-axis). Each plot represents a different field sampling methodology with different sampling effort associated (x-axis). A) Focal Observations: frugivore visits to *Cecropia glaziovii* individual plants; where the sampling effort is represented by the number of individual trees observed. B) Camera traps: animal interactions with *Juniperus phoenicea* individual plants, where the sampling effort is represented by the number of camera-days. C) Mist-netting: plant-frugivore interactions at community level in a Mediterranean shrubland, where the sampling effort is represented by the number of samples analysed from captured birds. D) DNA-barcoding: frugivore interactions with *Pistacia lentiscus* individual plants, where the sampling effort is represented by the number of faecal samples analysed.

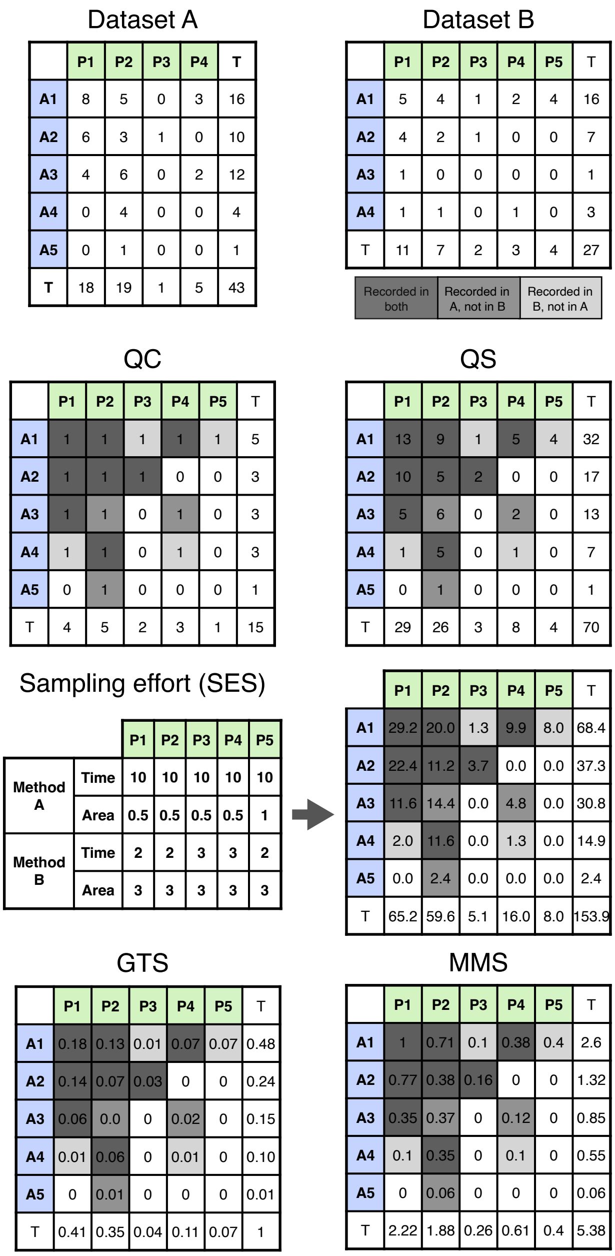
Sampling effort can be measured from different perspectives: it may represent the time spent recording or identifying interactions (*e.g.,* Fig. 1.2A and 1.2B), as well as number of samples collected (*e.g.*, Fig 1.2C and 1.2D) or the number of sites sampled. This approach provides an estimation of how many distinct pairwise interactions, among the possible ones that can be recorded in the study area, are actually recorded. Different sampling methods will saturate their accumulation curve faster than others, approaching asymptotic sample completeness to variable degrees. Most recent studies of plant-frugivore interactions report sampling completeness in some way (*e.g.*, Olesen *et al*. 2011, Bello *et al.* 2017, Acosta-Rojas *et al.* 2019, Costa *et al.* 2020).

Combining data obtained with different methodological approaches

Combining data allows overcoming the limitations of each method and obtaining a more accurate and complete representation of the interaction network (Bosch *et al.* 2009). The problem of data combination is central in frugivory studies, for example in analyses of complex networks aiming to get the maximum information from diverse sources to obtain a robust estimation of the interactions present. In this section we describe five different approaches to merge interaction data coming from different methodologies. To illustrate the data merging options we will consider, as an example, two matrices of pairwise interactions between a set of frugivore species and their food plants, assumed to result from different sampling approaches (see Fig. 1.3). Interactions are tallied and summarised as adjacency matrices, with rows representing animal species and columns indicating plant species, so that matrix elements a_{ij} can represent estimates of the presence/absence (*i.e.*, qualitative) interaction strength (*i.e.*, quantitative) between animal species i and plant species j (Bascompte & Jordano 2014).

Qualitative Combination (QC)

For all those cases where the characteristics of the datasets are hardly comparable, or if they just refer to presence/absence of the interaction (0-1), a Qualitative Combination of matrices (QC, Fig. 1.3) may be the most conservative option. This straightforward approach maximises the number of pairwise interactions recorded, taking advantage of the full detectability potential of both sampling methods. Although qualitative matrices can be useful when describing frugivory assemblages (Bascompte & Jordano 2014, Almeida & Mikich 2018), quantitative information better describes the complexity of the structure of natural systems (Banasek-Richter *et al.* 2004, Dormann *et al.* 2009).



Dataset A

	P1	P2	P3	P4	T
A1	8	5	0	3	16
A2	6	3	1	0	10
A3	4	6	0	2	12
A4	0	4	0	0	4
A5	0	1	0	0	1
T	18	19	1	5	43

Dataset B

	P1	P2	P3	P4	P5	T
A1	5	4	1	2	4	16
A2	4	2	1	0	0	7
A3	1	0	0	0	0	1
A4	1	1	0	1	0	3
T	11	7	2	3	4	27

QC

	P1	P2	P3	P4	P5	T
A1	1	1	1	1	1	5
A2	1	1	1	0	0	3
A3	1	1	0	1	0	3
A4	1	1	0	1	0	3
A5	0	1	0	0	0	1
T	4	5	2	3	1	15

QS

	P1	P2	P3	P4	P5	T
A1	13	9	1	5	4	32
A2	10	5	2	0	0	17
A3	5	6	0	2	0	13
A4	1	5	0	1	0	7
A5	0	1	0	0	0	1
T	29	26	3	8	4	70

Sampling effort (SES)

		P1	P2	P3	P4	P5
Method A	Time	10	10	10	10	10
	Area	0.5	0.5	0.5	0.5	1
Method B	Time	2	2	3	3	2
	Area	3	3	3	3	3

	P1	P2	P3	P4	P5	T
A1	29.2	20.0	1.3	9.9	8.0	68.4
A2	22.4	11.2	3.7	0.0	0.0	37.3
A3	11.6	14.4	0.0	4.8	0.0	30.8
A4	2.0	11.6	0.0	1.3	0.0	14.9
A5	0.0	2.4	0.0	0.0	0.0	2.4
T	65.2	59.6	5.1	16.0	8.0	153.9

GTS

	P1	P2	P3	P4	P5	T
A1	0.18	0.13	0.01	0.07	0.07	0.48
A2	0.14	0.07	0.03	0	0	0.24
A3	0.06	0.0	0	0.02	0	0.15
A4	0.01	0.06	0	0.01	0	0.10
A5	0	0.01	0	0	0	0.01
T	0.41	0.35	0.04	0.11	0.07	1

MMS

	P1	P2	P3	P4	P5	T
A1	1	0.71	0.1	0.38	0.4	2.6
A2	0.77	0.38	0.16	0	0	1.32
A3	0.35	0.37	0	0.12	0	0.85
A4	0.1	0.35	0	0.1	0	0.55
A5	0	0.06	0	0	0	0.06
T	2.22	1.88	0.26	0.61	0.4	5.38

Figure 1.3. Illustrative example showing five merging methods for interaction data matrices considered in this study: Qualitative Combination (QC), Quantitative Sum (QS), Sampling Effort Standardization (SES), Grand Total Standardization (GTS) and Min-Max Scaling (MMS). Matrices show the result of merging the simulated datasets A and B. For the SES approach we include sampling effort information for a simulated phytocentric study: area of the plant sampled and observation time on each plant.

Quantitative Sum (QS)

The simplest way to merge two weighted matrices without losing information, is to make a direct sum (QS, Fig. 1.3) of both datasets (see Timóteo *et al.* 2018). This merging approach can be useful to combine data with equivalent sampling efforts whenever an absolute sum of records can be achieved without sacrificing biological interpretation. Despite incorporating more detailed information than the previous approach, it also has important limitations. Merging datasets that differ greatly in their measurement units, associated sampling efforts, or spatio-temporal scales may yield unreliable results (Miranda *et al.* 2019).

Sampling Effort Standardization (SES)

Having detailed knowledge of the sampling effort associated with a given interaction survey (*e.g.*, time, area, number of individuals sampled) allows using a more realistic and reliable standardisation method, a Sampling Effort Standardization (SES, Fig. 1.3). In order to conduct the data combination, both datasets need to be referred to the same 'currency' of interaction or unit, controlling for the sampling effort (*e.g.*, visitation frequency in phytocentric studies, see Simmons *et al.* 2018, or ingestion rates in zoocentric studies). Once both matrices are standardised to a common ground, one could merge values by using the mean (option shown in Fig. 1.3) or the highest value recorded for each pairwise interaction. Averaging values can be problematic if the detectability of specific interactions differs significantly among techniques, as it may downplay or overestimate the weight of some interactions. On the other hand, selecting the maximum value for each interaction tries to harness the highest number of interactions observed but may also produce upward-biassed values with some methods. This approach is appropriate for methodologies that share the same focus of study (*i.e.,* only zoocentric or only phytocentric), as it can be challenging to find a common reference unit between a plant-focused study and an animal-focused one.

Grand Total Standardization (GTS)

When sampling techniques are very different and sampling effort correction cannot be applied, either because it is unreliable or not available, an option to collapse information is standardising by the total number of interactions recorded. This approach, which we refer to as Grand Total Standardization (GTS, Fig. 1.3), is solely based on information from the adjacency matrix, and is recommended when sampling efforts are unknown or difficult to compare. Using a GTS approach, all the values in the adjacency matrix are weighted by the total number of interactions recorded under each specific method (*i.e.,* the sum of all the matrix element values):

$$a'_{ij} = \frac{a_{ij}}{\sum_{i=1}^{A} \sum_{j=1}^{P} a_{ij}}$$

where a_{ij} is the interaction value for animal species i and plant species j, divided by the total sum of interactions in the adjacency matrix across all the A animal and P plant species.

Once both matrices are weighted by their respective total interactions, the final combined matrix can be calculated with a mean. This type of standardisation has an immediate biological interpretation: the final matrix element value for a specific pairwise interaction, a'_{ij}, indicates the probability that a randomly-chosen interaction in that community corresponds to that specific pair of partners. Merging two matrices with very different grand totals can also yield biassed results, because of the strong influence of the matrix with the lowest sampling effort. Once we divide by a grand total and calculate the relative frequency of each interaction related to that grand total, we lose any information about the sample size/effort (*i.e.,* 1/10 will weigh equal to 100/1000). Thus, even small deviations in the least sampled matrix can bias the final matrix.

Min-Max Scaling (MMS)

Min-Max Scaling is a mathematical alternative to GTS if we want to scale interactions instead of using frequentist measures (MMS, Fig. 1.3). This method normalises all unique pairwise interactions into a range of values from 0 to 1. Unique pairwise interactions are scaled by subtracting the minimum value and dividing the result by the difference between the maximum and minimum values for all pairwise interactions (a_{ij}) present in the adjacency matrix (A_{ij}):

$$a'_{ij} = \frac{a_{ij} - min(A)_{ij}}{max(A)_{ij} - min(A)_{ij}}$$

By rescaling both matrices we give a weight for each interaction on a scale of 0 to 1, and so allow comparison of the datasets, and their combination through a mean.

This mathematical approach maintains the relative distance between the interaction weights, and the results should be interpreted in terms of interaction scoring, not probability of pairwise interaction.

Other normalisation alternatives, such as those based on z-score or mean-value normalisation can be problematic for two reasons. First, the biological interpretation of the resulting merged matrix can be challenging; for example, given that the distribution of interaction frequencies is highly skewed, a z-score deviation from a "interaction frequency" mean can be misleading. Second, they produce negative values, which may preclude some types of network statistical analysis.

Preliminary considerations

We must emphasise that all the quantitative merging methods implicitly assume two comparable datasets of animal-plant interactions without major biases between them. But, it is worthy to draw attention to the eventual data differences that may hinder a successful quantitative merging, such as study scale and sampling completeness.

Often datasets differ in the temporal, spatial or taxonomic scale of resolution. Several studies may refer to incomplete phenological periods, different spatial scales or to a grouping of interactions taking place (*i.e.*, referring to a higher taxonomic level instead of single species). Substantial differences in completeness between datasets can introduce sizable biases because a subset of the records may become overrepresented in the merged dataset (*e.g.*, common species with more frequent interactions). If we are able to calculate the relative weight that a certain group of species, area, or phenologic period has in the study datasets, we will be able to refer all interactions weights to a common ground. For example, by considering the differences in length of the study, the weight of those interactions belonging to the less complete dataset can be corrected. Standardising our data based on the spatial, temporal or taxonomic scale would allow reliable comparisons between datasets.

A further issue we may encounter is a substantial difference in sampling completeness. A possibility to overcome this issue is weighting each matrix by its degree of completeness (determined from its IAC analysis) so as to have each method valued by their sampling effort coverage. Another possibility is calculating standard errors for each observed interaction probability (p) in the matrix to estimate an "uncertainty" associated with their occurrence:

$$SE(p) = \sqrt{\frac{p(1-p)}{N}}$$

where p is the probability of the pairwise interaction occurring and N is the total number of interactions recorded.

Case-dependent weighting or adjustment of the databases prior to generating an analysis matrix is recommended to generate truthful and interpretable information.

Case studies

To illustrate the advantages and shortcomings of merging data collected through different sampling methods we use two empirical case studies, with two different organisation levels. Both case studies are focused on plant-frugivore interactions taking place in the Mediterranean shrubland of Doñana National Park, Huelva, Spain. In each case study two sampling methods were used to maximise animal-plant interactions detected. The first case is an individual-based study on the avian frugivore assemblage of *Pistacia lentiscus* (Anacardiaceae) in El Puntal area, where monitoring cameras and DNA-barcoding were used to record interactions (present thesis). The second case is a community-based study aiming to document species-specific plant-frugivore interactions in Hato Ratón, where analysis of faecal samples obtained with mist-netting and focal observations were used to detect interactions (Jordano 1984, 1987c, 1989, Olesen *et al.* 2011). All detailed information on sampling methods and protocols for each study can be found in Appendix 1B.

We used the data merging approaches described above to combine sampling methodologies within each case study: Qualitative Combination (QC), Quantitative Sum (QS), Sampling Effort Standardization (SES), Grand Total Standardization (GTS) and Min-Max Scaling (MMS), but the SES method was only applied in El Puntal case study. Note that for the Hato Ratón dataset, the fact that data come from a phytocentric approach (spot-censuses at plants along transects), on one hand, and from a zoocentric approach (mist-netting avian frugivores and faecal analysis), on the other, precludes the standardisation to comparable units needed for a SES approach.

To standardise interaction data according to sampling effort (SES merge) for El Puntal, all interactions were referred to the number of visits per hour received by each individual plant (visits h^{-1} $plant^{-1}$). In order to do this conversion, we referred all DNA-barcoding data to the time in hours that seed traps were settled under individual plants, as well as to the plant cover area sampled by the seed traps. The same transformation for time and space was conducted with the monitoring cameras data. Bird visitation detected with the cameras was referred to hours and corrected by the percentage of canopy area observed in the videos.

We built bipartite interaction networks for each study, following the different merging methods for both initial adjacency matrices and the merged ones. We evaluated the resulting networks' structure with basic metrics representing complementary aspects of the structure of mutualistic networks (see Table 1.2; Appendix 1C).

Results for case studies

A. Interactions and species gain

The different sampling methods yielded different numbers of species, links and unique pairwise interactions in both case studies (Table 1.2, Fig. 1.4). This was expected, since some methods have unavoidable biases in sampling, *e.g.*, mist-netting failing to capture canopy-dwelling, large frugivorous birds, limited sampling time of GoPro cameras, etc.

DNA-barcoding was the most productive method for the El Puntal case study, identifying up to 16 frugivorous bird species, compared to only seven avian species detected by the monitoring cameras. DNA-barcoding also rendered most unique pairwise interactions between individual plants and bird species (166), compared to 91 from the monitoring cameras. Yet, cameras detected 19 new distinct pairwise interactions, so combining both methods improved the completeness of the final interaction matrices.

For the Hato Ratón case study both sampling methods provided a similar number of detected species. Mist-netting aimed and was more effective in detecting plant species consumed, while the focal observations aimed to detect foraging birds, and so was more effective in detecting animals. Mist netting noticeably recorded more unique pairwise interactions than visual censuses, although focal observations yielded an increase of 30 unique pairwise interactions when combining both methods (mostly corresponding to avian frugivore species rarely or never captured in mist nets). Regarding the total number of interactions, mist netting yielded more interactions than censuses. The remarkable number of bird species detected by exclusively either one of the methods (n = 20) in the Hato Ratón case study, and the exclusive number of pairwise links (n = 90), highlights the great potential of these methods combination and data merging approaches.

B. Consistency and complementarity of merging methods

Pearson's product-moment and Kendall's rank correlations were used to explore how the merging methods resembled each other and how consistent they were to the initial adjacency matrices in terms of both quantitative and rank correspondence (see Appendix 1D). Rather than focusing on the significance of these correlations we were interested in showing how variable these correlations are and whether they tend to be high or low for specific combinations of methods. All the final merged matrices showed high and significant Kendall's and Pearson's correlation between them, revealing consistent proportional weights and concordant rankings for all the unique pairwise interactions (Appendix 1D). However the two initial adjacency matrices in both case studies showed lower correlation between them when compared to the correlations between either the initial and merged matrices or between merged matrices resulting from different methods of data combination (Appendix 1D). This is expected from the substantial differences in species detectability intrinsic to each sampling method and the resulting different weights assigned to specific interactions.

For the El Puntal case study, the Kendall's correlations between initial matrices and merged ones were higher for DNA-barcoding method, indicating that ranking was better preserved for this specific methodology than for the cameras (probably since barcoding rendered much more interactions than the cameras, *i.e.*, 1162 vs 397 records, respectively). Yet when regarding Pearson's correlation, the matrices resulting from Grand Total Standardization (GTS) and Sampling Effort Standardization (SES) merging methods were more correlated to the cameras than to the barcoding, indicating higher quantitative consistency with the camera interactions records. The SES merged matrix differed the most from the other merged matrices in terms of Pearson's correlation, being most similar to GTS, but still significantly correlated to all.

Regarding Hato Ratón datasets, the merged matrices were all highly correlated both value- and rank-wise. Yet, the ranking (*i.e.*, Kendall's correlation) of the mist-netting methodology was better preserved than the ranking of focal observations.

In the case of specific interaction weights (*i.e.*, Pearson's correlation), those of mist-netting were better preserved for Quantitative Sum (QS) and Grand Total Standardization (GTS), while focal observations had a higher influence for Min-Max Scaling (MMS) merging.

C. Network properties

Regarding the network properties, the largest differences in assemblage patterns and resulting indexes were found between initial adjacency matrices. This result indicates that network metrics differ more between sampling methods than between merging approaches (Table 1.2).

Raw connectance for El Puntal increased when obtaining the merged adjacency matrix (Merged = 0.289, DNA-Barcoding = 0.259, Cameras = 0.142), due to matrix filling with new interactions (see Bosch *et al.* 2008). In contrast, the Hato Ratón merged matrix connectance slightly decreased relative to the mist-netting dataset due to an increase in matrix size when considering the species recorded in mist-netting and direct observations together (Merged = 0.257, Mist-netting = 0.316, Focal observations = 0.195). When considering weighted connectance for GTS and SES matrices, El Puntal showed lower values since both merging methods gave more weight to the cameras dataset (see Pearson's correlation Appendix 1D), thus more closely resembling camara weighted connectance. The same happened for the Hato Ratón dataset; the weighted connectance of the merged matrices was more similar to the specific sampling methods with which they have higher Pearson correlation (*i.e.*, QS and GTS to Mist-netting and MMS to Focal Observations).

Table 1.2. Summary of species, interaction richness and network statistics recorded with different sampling methods in two study areas, El Puntal (DNA barcoding of collected samples, and monitoring cameras on P. lentiscus individual plants) and Hato Ratón (faecal sample analysis from mist-netting bird captures and direct focal observations during censuses), within the general area of Doñana National Park (SW Spain). The table indicates the number of species (bird species in El Puntal; bird and plant species in Hato Ratón), number of distinct pairwise links, and total number of interactions recorded in the samplings. Numbers in parentheses indicate the number of shared species, links, or interactions; for Modularity, number of distinct modules. Number of interactions for Hato Ratón are rounded to the nearest integer, as faecal sample analysis yields fractional fruit consumption data. Network metrics were calculated for the two initial matrices in each case study, and for the resulting

matrices from the different merging approaches used: QC, QS, GTS and MMS for both case studies and additionally SES for El Puntal case study. Observed values of Weighted Connectance (wC), Weighted Nestedness (wNODF) and Modularity are reported, bracketed values indicate a bootstrap-estimated confidence interval.

* Note that qualitative merged matrices (QC merging method) report unweighted Nestedness (NODF) and their modularity was calculated using Beckett´s algorithm. See Appendix 1C for analysis details.

		Species	Pairwise Links	Interactions	Weighted Connectance	wNODF	Modularity
El Puntal	DNA-Barcoding	16 Birds 40 Plants	166	1162	0.308 [0.305-0.312]	34.87 [34.08-35.66]	0.171 (4) [0.168-0.173]
	Monitoring cameras	7 Birds 40 Plants	91	397	0.241 [0.239-0.245]	40.75 [39.48-42.03]	0.226 (4) [0.219-0.232]
	Qualitative Combination (QC)	16 Birds (7) 40 Plants (40)	185 (72)	1559 (634)	0.287 [0.284-0.290]	72.72* [72.17-73.27]	0.321 (8) [0.316-0.327]
	Quantitative Sum (QS)				0.308 [0.306-0.311]	39.69 [39.08-40.30]	0.148 (4) [0.145-0.152]
	Grand Total Standardization (GTS)				0.288 [0.285-0.291]	43.36 [42.63-44.08]	0.157 (4) [0.153-0.160]
	Min-max Scaling (MMS)				0.305 [0.303-0.308]	42.58 [41.77-43.39]	0.148 (4) [0.145-0.151]
	Sampling Effort Standardization (SES)				0.240 [0.237-0.243]	47.39 [46.65-48.15]	0.192 (4) [0.187-0.197]
Hato Ratón	Mist-netting	24 Birds 15 Plants	114	3541	0.095 [0.091-0.099]	65.77 [64.62-66.93]	0.120 (2) [0.111-0.129]
	Focal observations	30 Birds 14 Plants	82	2031	0.134 [0.131-0.138]	44.14 [42.86-45.42]	0.201 (4) [0.134-0.209]
	Qualitative Combination (QC)	37 Birds (17) 15 Plants (14)	143 (53)	5572 (2042)	0.217 [0.213-0.220]	63.94* [62.60-65.27]	0.348 (5) [0.340-0.356]
	Quantitative Sum (QS)				0.096 [0.092-0.100]	49.11 [47.92-50.30]	0.151 (4) [0.142-0.161]
	Grand Total Standardization (GTS)				0.103 [0.099 -0.107]	46.92 [45.69-48.16]	0.162 (4) [0.151-0.172]
	Min-max Scaling (MMS)				0.111 [0.107-0.114]	44.93 [43.50-46.36]	0.174 (4) [0.164-0.184]

The merged networks in El Puntal showed higher weighted nestedness (wNODF) values than the individual source matrices separately (Table 1.2), except for QS methods which were similar to the camera-derived network. Note that QC matrices are qualitative (*i.e.*, 0-1 values), consequently unweighted NODF was computed, making its comparison with the other wNODF values unreliable Weighted nestedness values for Hato Ratón merged matrices were intermediate between both methods. wNODF for the mist-netting derived adjacency matrix was considerably higher than the value of the focal observations censuses matrix. This is likely attributable to the limited detectability of the mist-net captures, which selectively sample a subset of all the birds present in the area.

Modularity was similar for all matrices, being highest for the qualitative merging (QC) in both case studies. It is unrealistic to compare modularity results produced by QC method with the rest of merging approaches since different algorithms are used for qualitative (Beckett´s algorithm) and weighted (Newman´s algorithm) adjacency matrices (Dormann *et al.* 2009). El Puntal network derived from monitoring cameras showed higher modularity compared to theDNA-barcoding network (Table 1.2), probably corresponding to an increase in DNA-barcoding species detectability. In Hato Ratón the modularities of weighted merged matrices were intermediate between those of the source datasets.

In general, when both sampling methods were efficient and complementary, as in Hato Ratón study, the resulting merged matrices had intermediate values for the different network descriptors. However, in El Puntal case, where sampling methods were more redundant, network descriptors for the merged matrices resembled more to either one of the initial matrices, depending on the sampling methodology with which they had a higher Pearson's correlation (GTS and SES resembling more to cameras and QS and MMS to DNA-barcoding; Appendix 1D).

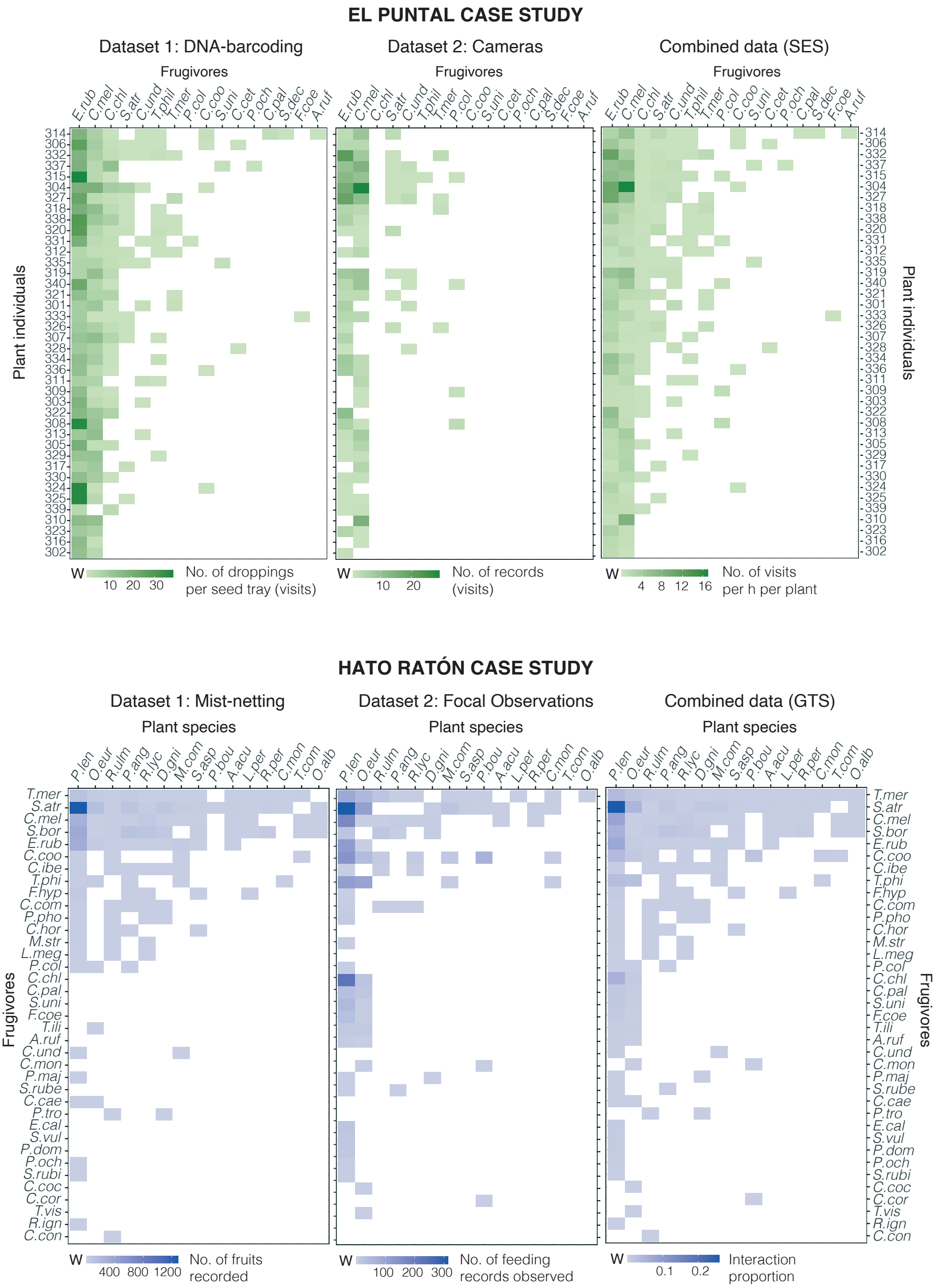


Figure 1.4. Empirical adjacency matrices for the two case studies, El Puntal (DNA barcoding of dispersed seeds and camera-trap monitoring of individual P. lentiscus plants, upper panels) and Hato Ratón (faecal sample analysis from mist-netting bird captures and direct focal

observations during censuses, lower panels). The matrices on the right correspond to merged datasets, using the SES and GTM methods, respectively. Note that for El Puntal case study rows indicate plant individuals and columns indicate frugivore species (phytocentric study), while for Hato Ratón rows indicate frugivore species and columns indicate plant species (zoocentric study). Colour shade intensities indicate relative values of interaction strength (W). Animal species codes in alphabetical order: *A.ruf = Alectoris rufa, C.cae = Cyanistes caeruleus, C.cet = Cettia cetti, C.chl = Chloris chloris, C.coc = Coccothraustes coccothraustes, C.com = Curruca communis, C.con = Curruca conspicillata, C.coo= Cyanopica cooki, C.cor = Corvus corax, C.hor = Curruca hortensis, C.ibe = Curruca iberiae, C.mel = Curruca melanocephala, C.mon = Corvus monedula,C.pal = Columba palumbus, C.und = Curruca undata, E.cal = Emberiza calandra, E.rub = Erithacus rubecula,F.coe = Fringilla coelebs, F.hyp = Ficedula hypoleuca, L.meg = Luscinia megarhynchos, M.str = Muscicapa striata, P.col= Phylloscopus collybita, P.dom = Passer domesticus, P.maj = Parus major, P.och = Phoenicurus ochruros, P.pho = Phoenicurus phoenicurus, P.tro = Phylloscopus trochilus, R.ign = Regulus ignicapilla, S.atr = Sylvia atricapilla, S.bor = Sylvia borin, S.dec = Streptopelia decaocto, S.rube = Saxicola rubetra, S.rubi = Saxicola rubicola, S.uni = Sturnus unicolor, S.vul = Sturnus vulgaris, T.ili = Turdus iliacus, T.mer = Turdus merula, T.phil = Turdus philomelos, T.vis = Turdus viscivorus. Plant species codes in alphabetical order: A.acu = Asparagus acutifolius, C.mon = Crataegus monogyna, D.gni = Daphne gnidium, L.per = Lonicera periclymenum, M.com = Myrtus communis, O.eur = Olea europaea var. sylvestris, O.alb = Osyris alba, P.ang = Phillyrea angustifolia, P.len = Pistacia lentiscus, P.bou = Pyrus bourgaeana, R.lyc = Rhamnus lycioides,R.per = Rubia peregrina, R.ulm = Rubus ulmifolius, S.asp = Smilax aspera, T.com = Tamus communis.*

Discussion

Most plant-frugivore interaction studies involve some type of sampling to gain insight into the interaction partners: their diversity, numbers, spatial and temporal trends, etc. Our results provided an overview of different alternatives for data-merging, linked to the specific stage of the animal-mediated seed dispersal process being studied. The methodological approaches used with animal frugivores and fleshy-fruited plants have diversified enormously since the pioneer, observation-based methods (*e.g.*, Howe & Vande Kerckhove 1980, Snow & Snow 1988), now including a plethora of active, passive, automated, direct, indirect, and big-data oriented methods. Rather than aiming at an exhaustive review, or even a complete comparative analysis, we focused on analysing the potential to combine multiple data sources in a biologically-insightful way.

Methodological advances in frugivory studies

With the arrival of new molecular and multimedia methods, the field of plant-frugivore interactions has expanded a great deal its exploration potential. Passive sampling methods (*i.e.*, not requiring the active presence of the researcher during the interaction) have allowed us longer sampling extensions, leading to less work time in the field but higher post-processing efforts. Both the scale of sampling (ability to record interactions over broader spatial scales) and its precision (ability to detect rare interactions) have increased immensely. Confronted with such a variety of methods an under-researched aspect has been the development of merging strategies capable of combining data coming from a variety of sources and approaches.

Some obvious biases seem, however, unavoidable; for example, geographic and habitat-type generated biases. Focal and camera-trap observations are probably better suited for tropical areas, where the spatial scale of samplings necessarily has to be more extensive than in temperate areas, just to be able to sample rare species and interactions. On the other hand, indirect methods like those based on DNA-barcoding analyses may become more limited in tropical areas because of sample processing, collection, and preservation. Furthermore, the lack of DNA sequence data for many species, some not even known, limits the use of these molecular techniques in megadiverse areas. Studies in insular habitats may require a broader combination of methodological approaches, given that their frugivore assemblages tend to include a more diverse array of frugivore higher taxa.

Combining approaches

Our analysis reveals that any combination of methods yielded better results in terms, among other things, of completeness and representability, than resorting to a single sampling method and simply ignoring potential biases inherent to it.

The high and significant correlations between different merging approaches in the two case studies analysed shows that they all produce consistent results. Provided that the sampling has been robust and sufficient, merging simply yields a more complete and thorough dataset and may compensate for sampling biases inherent to the initial methods. Accordingly, the selection of the merging method should depend mainly on the characteristics of the available data and the interpretable output needed (*e.g.*, in terms of probability, ranking, frequencies, etc.). Note that SES approach appears more limiting when facing the merging of data obtained with different study focus, such as when combining samplings of animal faeces and observations at focal plants. When the sampling methods to be combined have both the same approach, either phyto- or zoo-centric, the SES combination appears more straightforward, given that it involves similar currencies to quantify interaction strengths. The Hato Ratón case study (involving both phyto- and zoo-centric methods) suggests that merging approaches such as GTS or MMS can be a suitable tool to increase data availability in a reliable way, allowing the merging of datasets sampled with rather different approaches. Specific consideration should then be given to the biological interpretation of the merged results, *e.g.*, probabilistic estimates of interspecific interaction or pairwise interaction scoring.

While both methods in El Puntal were indirect (*i.e.*, with no disturbance because of human presence), the DNA-barcoding allows recording interactions for longer time (a passive method, sampling the seed rain), yet for a smaller plant area (*i.e.*, a limited percentage of the plant canopy surveyed). In contrast, while the monitoring cameras worked for substantially less time, they provided coverage for monitoring frugivore activity and visitation over most of the plant. This resulted in a trade-off between area and time. It is worthy to draw attention to the difference in area and time scales between methods. While the area correction scale ranged from 0 to 100% of the plant cover sampled, the time correction scale was much ample (from hours to months). This resulted in a significant detriment for the DNA-Barcoding method (the longest sampled method in time), whose interactions lost weight when equated to camera data. It is therefore important to consider the imbalance that may emerge between methods, whenever these scales are very different (Jordano 2016). Techniques allowing a correction by sampling effort will help in those cases (*e.g.*, those based on cumulative sampling effort).

Our results highlight the relevance of achieving adequate standardisation of data, ability to evaluate data completeness, ensure reproducibility, and provide details of the data merging approaches used. The qualitative combination may be applicable to rapid interactions surveys (analogous to a biodiversity survey) for large areas or regions, where only qualitative records of the interactions being present is available.

Future perspectives

We advocate for further research within mainstream ecological studies to explore data-merging strategies, an undeveloped study line in comparison to other knowledge areas with analogous problems related to data merging from diverse experimental sources (*e.g.*, Huttenhower *et al.* 2006, Steele & Tucker 2008, Lagani *et al.* 2016). This is timely, now that data gathering in plant-frugivore interactions is greatly increasing and that we have resources to provide open data access or data papers (*e.g.*, Bello *et al.* 2017).

More and more researchers are starting to share their databases in public and open repositories (see *e.g.*, Bello *et al.* 2017). The composition and the structure in which these databases are provided is a key aspect. Data is usually shared as an interaction adjacency matrix or an edge list (Bascompte & Jordano 2014), however, such a dataset contains summarised information, losing the variation sources. Sharing extended databases that contain information for the recorded individual pairwise interactions would allow answering more questions and would help data combination through more sophisticated methods. Providing high quality metadata associated with the datasets is also essential, and this can be readily accomplished using specific R or python packages implementing standard open science grammars for metadata specification (*e.g.*, Boettiger *et al*. 2021). Metadata should contain not just the basic information (author, site, dates, etc.) but also information on sampling effort and both temporal and spatial scope as much detailed as possible to ensure reproducibility (*e.g.*, number of hours of observation per individual or square metres of mist-net per time). This is fundamental for reliable dataset combination and comparison. Furthermore, providing quantitative and complementary information of the study sites and species (*e.g.*, independently-estimated species abundance or vegetation cover) can be useful to address broader questions.

Given the diversity of methods (and their combinations) developed to study plant-frugivore interactions, one of the challenges will be to select the one or those that can best help us answer our questions. Our analysis reveals that data combination approaches open new ways towards more robust sampling of plant-frugivore interactions. No specific method is probably perfect for all situations; yet when adequately combined, even disparate methods outperform single-methods in estimating interaction richness. It seems more difficult to find an interaction that cannot be sampled than to find a method to sample it.

Acknowledgements

We thank Juan Miguel Arroyo who provided essential help in the laboratory for the DNA-barcoding analysis, and Iago Ferreiro assisted with the camera-trap data preparation. We thank the logistic and facilities support form ICTS-RBD Doñana and the Doñana National Park administrators for onsite access authorizations. Discussions and comments with Francisco Rodríguez-Sánchez and Blanca Arroyo were most helpful during the preparation of the manuscript. The 7th Frugivores and Seed Dispersal Symposium, in Corbett, India, provided the excellent atmosphere to discuss and share ideas that motivated this study. Funding – Our work was funded by the project CGL2017-82847-P from the Agencia Estatal de Investigación, Spain. During their respective PhD projects, EQ is being supported by 'La Caixa' Foundation grant ID LCF/BQ/DE18/11670007, and JI by FPI grant PRE2018-085916 from the Spanish Ministry of Science and Innovation.

APPENDICES CHAPTER 1

Appendix 1A. Trade offs between collection and processing costs for different monitoring interaction techniques

Appendix 1B. Case Studies: materials and methods

Appendix 1C. Analyses of interaction network statistics and indexes for adjacency matrices estimated with different sampling methods in two study areas, Doñana National Park (SW Spain)

Appendix 1D. Analyses of consistency and complementarity between sampling methods aimed to obtain merged datasets

Appendix 1E. List of bibliographic references included in Table 1.1, as examples of study methods for plant-frugivore interactions

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Data and code for the analyses of Chapter 1 are archived in the Dryad digital repository: https://doi.org/10.5061/dryad.jm63xsjb8 and the GitHub repository: https://github.com/PJordano-Lab/MS_Oikos_FSD_Monitoring_interactions.

Appendix 1A. Trade offs between collection and processing costs for different monitoring interaction techniques

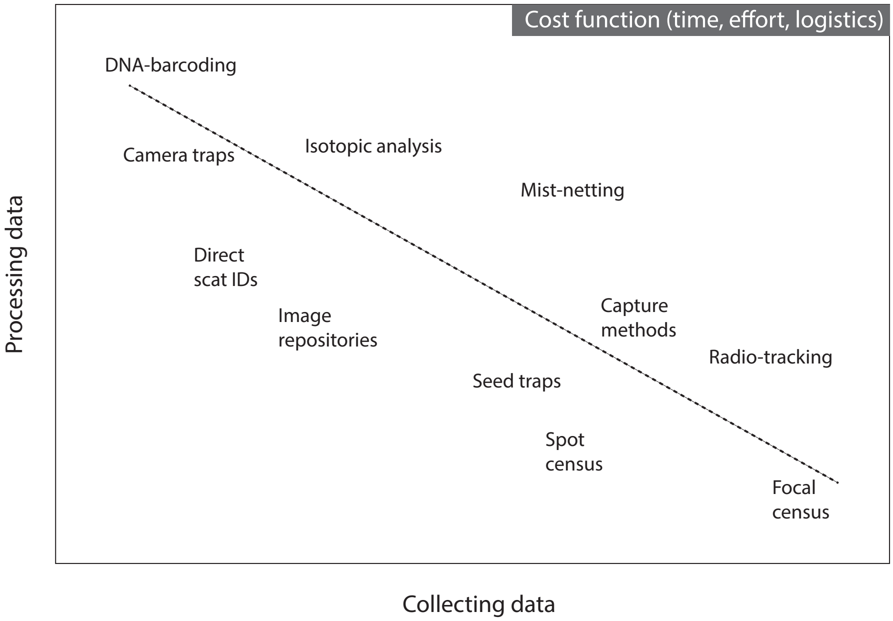


Figure A1.1. Approximate cost function trade-offs for methods used to assess plant-frugivore interactions. Different methods are placed approximately at locations that combine the costs (time, effort, logistic) of collecting the data compared to the costs (*e.g.*, laboratory work, sample analysis, video image processing) involved in data processing.

Appendix 1B. Case Studies: materials and methods

We used two empirical datasets to illustrate data merging approaches. The first study locality is a lowland Mediterranean shrubland covering ca. 15 ha in Mancha del Rabicano in the area of El Puntal, Doñana National Park, Huelva, SW Spain. A total of 40 *P. lentiscus* plants were marked and surveyed during the fruiting season of 2018-2019. Data collected for this study is partially complete, since it includes plant-frugivore interactions taking place just during the winter season, right in the middle of the fruiting peak, not depicting the complete frugivore assemblage in the area.

In order to capture all avian visitors interacting with *P. lentiscus* individual plants, we used two indirect sampling methods: one focused at the 'Visitation' stage and the other at the 'Deposition' stage. The 'Visitation' method involved placing continuous-monitoring cameras (GoPro Hero® 7 model) facing individual plants (Fig. A1.2).

Forty individual plants were filmed for approximately 2 hours in several runs in different days (total of 84.5h), and any avian visitation was recorded as an interaction, yielding a total of 397 visitation records. Cameras were operative from sunrise for ~2h recording set at maximum resolution. Data resulting from this sampling can be given as total number of records, or standardised by sampling time (no. records h^{-1}).

Figure A1.2. Interaction record using camera traps at *Pistacia lentiscus* individual plants in order to obtain estimates of individual-based plant-animal interaction networks. Camera records allow in many instances obtaining data on fruit handling, feeding rates, etc., in addition to just the visitation record. A male Sardinan warbler *Curruca melanocephala* just after picking a ripe *P. lentiscus* fruit.

The 'Deposition' method was based on DNA-barcoding identification of faecal samples collected in seed traps (plastic trays covered with 1 cm mesh wire) under the same forty individual trees (González-Varo *et al*. 2014). All samples were retrieved from seed trays located under individual plants, working for 102.7±8.9 days (mean ±SD) per plant. A total of 1371 faecal samples were analysed (mean no. per plant: 33.8±15.2). Samples were collected regardless of whether or not they had seeds, as an indicator of a visitation event. Eventually, all samples containing *P. lentiscus* seeds indicate the role of those frugivore species as legitimate dispersers. Yet, since effective dispersal is not our scope, and for the sake of comparison with the monitoring camera data, any visitation event is considered. Faecal samples were stored at -20°C and later processed following protocols described in detail by González-Varo *et al*. (2014). Avian DNA was extracted from the surface of defecated or regurgitated seeds or the surface of the scat (samples without seeds) (see Marrero *et al.* 2009), allowing the identification of the frugivore species that contributed each dispersal event or potential visit to the plant. Frugivore species identification was based on a 464-bp mitochondrial DNA region (COI: cytochrome c oxidase subunit I), employing the 'Barcode Of Life Data' identification system (BOLD: http://www. boldsystems. org; Hebert *et al.* 2004). BOLD accepts sequences from the COI gene and returns species-level identification whenever possible and assigns a percentage of similarity to matched sequences. All samples were amplified by PCR using the COI-fsdF and COI-fsdR primers (see González-Varo *et al*. 2014). This product was later sequenced and verified for its matching with COI sequences from BOLD databases. Data resulting from this sampling can be given as a total number of records with positive identification of a given frugivore species, or standardised by the sampling time with seed traps actively operating in the field (no. records/trap/day or similar).

The second case is a community-based study aiming to document species-specific plant-frugivore interactions in Hato Ratón, an area with similar landscape physiognomy to El Puntal (N Doñana Natl. Park, S Spain; Jordano 1984, 1987c, 1989, Olesen *et al.* 2011). Data collected for this study completely spans two fruiting seasons (1981-1982 and 1982-1983) and also focuses on avian frugivores.

Two sampling techniques were used: the first, focused at the 'Transport stage', using bird mist-netting to collect avian faecal samples that were subsequently examined under microscope, quantifying the presence and relative contribution of different fruit species, either by seed or exocarp remains (see Jordano 1984, 1988 for details). Estimation of dietary diversity of frugivore species by relying just on seed identification in scats invariably underestimates the actual diversity of fruits consumed (Jordano 1988a). To avoid this bias we used a microhistological technique to identify fruit species present with no seeds by examining under microscope (40X, 100X) the shape, size, and structures (trichomes, glands) of exocarp tissue cells. Similar techniques are routinely used to study the diet composition of herbivorous animals (see *e.g*., Marrero & Nogales 2005). This allowed not only the identification of fruit species when no seeds are present but also the relative volume occupied in the sample, so that an estimate of the corresponding number of fruits ingested can be derived (Jordano 1988a). For example, a given sample of Blackcap may contain seeds from just two species (*e.g*., *P. lentiscus* and *Phillyrea angustifolia*, yet remains of up to 7 different species may be present and identified under microscope (Jordano 1984, 1988). Between 6-10 mist nets were operated weekly for 1-2 days (for a total of 84 sampling days and 4080.5 mist-net hours), totalling 3541 faecal samples analysed (Jordano 1984, Olesen *et al.* 2011).

The second method focused at the 'Visitation' stage through the use of focal observations. Feeding records of frugivores visiting fruiting plants were obtained during 1.0 km-length walk censuses in the area, with 2-5 censuses carried out per month (123 sampling days), totalling 89.5 km and 2031 records. These are not focal observations spanning a given time period focusing at fruiting plants (Snow & Snow 1988), but spot censuses where interactions are recorded during short stops as the observer advances along a fixed transect. A feeding record involves a frugivore seen handling a fruit (Snow & Snow 1988); in some cases (<15 % of the records) where no handling was observed but just the visit to the plant, the number of fruits was approximated from data on feeding rate (no. fruits/visit). Data resulting from this sampling can be given as total number of records, or standardised by sampling time (no. records km^{-1} census or no. records h^{-1} or day^{-1}, or similar).

Appendix 1C. Analyses of interaction network statistics and indexes for adjacency matrices estimated with different sampling methods in two study areas, Doñana National Park (SW Spain)

Adjacency matrices were obtained for each sampling method. Connectance (C) is the proportion of observed links divided by the number of total potential links (Jordano 1987a). Since these are weighted networks, we also analysed weighted connectance (wC), which is a similar connectivity metric but based on linkage density (Bersier *et al.* 2002). To evaluate to what extent link distribution is not structured randomly, we calculated the weighted nestedness (wNODF) and the modularity (M) and number of modules (nM) of the networks. Nestedness represents the degree to which the interactions of less-connected species are a subset of those of more connected species (Ulrich *et al.* 2009). Modularity is the tendency of a network to be organised in clusters, where highly inter-connected subsets of nodes are less connected to nodes in other subsets (Olesen *et al.* 2007). Network metrics were calculated using R package 'bipartite' (version 2.15, Dorman *et al.*, 2009) in R statistical software version 4.0.3 (R Core Team 2018).

Weighted connectance, wC, measures the fraction of interactions actually occurring, out of all the potential, in which each link is weighed on the basis of its frequency. Weighted connectance was computed by the weighting the number of pairwise interactions in the network with the observed frequency of each pairwise interaction (Dormann *et al.* 2009), *i.e.*, the linkage density divided by number of species in the network (Bersier *et al.* 2002). Raw connectance was calculated for the qualitative matrices (QC merging method) that report unweighted interaction values.

Modularity (M) and number of modules (nM) were estimated using the function ComputeModules in the R package bipartite (Dormann & Strauss 2014, Beckett 2016). For unweighted networks (QC) the algorithm developed by Beckett was used while for the weighted networks the Dormann algorithm was computed. The number of distinct modules was obtained for each run of the modularity algorithm and is reported as the average number of modules found in repeated runs (N= 5). These parameters quantify the tendency of a network to be organised into distinct clusters, *i.e.,* modular networks showing distinct subsets of taxa interacting more frequently among each other than with taxa in other modules. Given that the estimation for the number of modules can vary between runs, the number of modules was calculated as the average (±SD) for 5 runs.

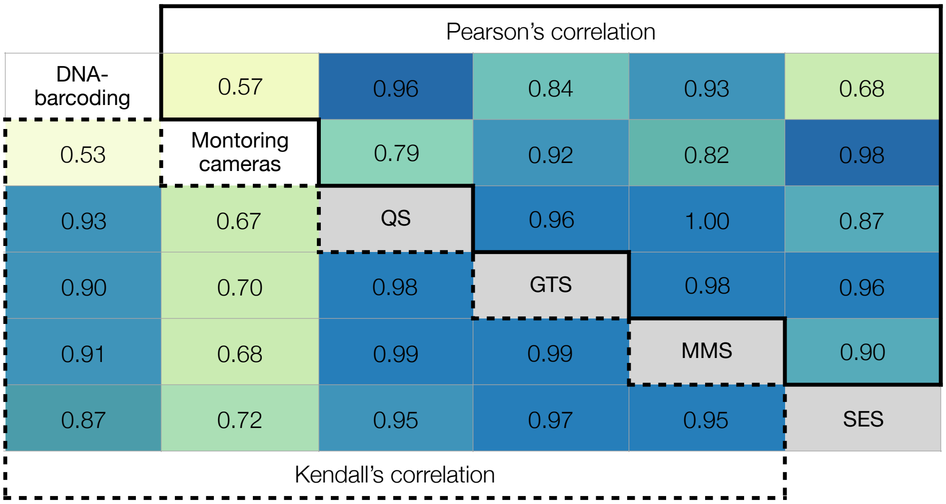
Appendix 1D. Analyses of consistency and complementarity between sampling methods aimed to obtain merged datasets

We tested the consistency in interaction value estimates by means of both quantitative (Pearson's correlation) and non-parametric, rank-based tests (Kendall's correlation). Tests were carried out to compare the adjacency matrices estimated with different sampling methods, as well as the correlation between the merged matrices and each of the original matrices being merged. Pearson's correlation is a parametric test that indicates the consistency and correlation of the interactions weight values for two methods being compared, while Kendall's correlation is a non-parametric test that indicates the correspondence in the ranking of pairwise interactions for two different methods being compared.

Pearson's and Kendall's correlation significance was tested using permutation tests (n=9999 permutations) using function perm.relation in the R package wPerm (Weiss 2015), and resulted highly significant ($p < 0.001$) for all correlations obtained.

Figure A1.3 summarises the correlation values obtained, overall suggesting a sizable degree of consistency both in the quantitative values and rank estimates for the pairwise interactions.

El Puntal

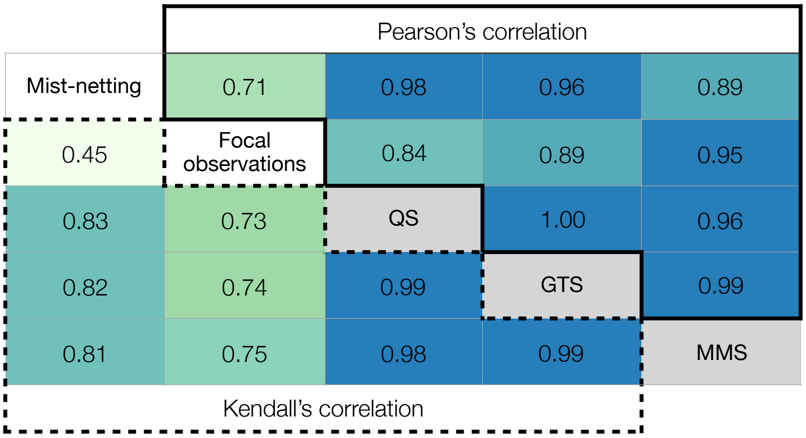


Pearson's correlation

DNA-barcoding	0.57	0.96	0.84	0.93	0.68
0.53	Montoring cameras	0.79	0.92	0.82	0.98
0.93	0.67	QS	0.96	1.00	0.87
0.90	0.70	0.98	GTS	0.98	0.96
0.91	0.68	0.99	0.99	MMS	0.90
0.87	0.72	0.95	0.97	0.95	SES

Kendall's correlation

Hato Ratón



Pearson's correlation

Mist-netting	0.71	0.98	0.96	0.89
0.45	Focal observations	0.84	0.89	0.95
0.83	0.73	QS	1.00	0.96
0.82	0.74	0.99	GTS	0.99
0.81	0.75	0.98	0.99	MMS

Kendall's correlation

Figure A1.3. Summary of Pearson's (above diagonal) and Kendall's rank correlation (below diagonal) coefficients to assess consistency between methods used to compile plant-frugivore interaction data at two study sites: El Puntal and Hato Ratón, within the general area of Doñana National Park (SW Spain). Correlations were estimated on the raw interaction data derived from the two compared methods for all the pairwise interactions. Pearson's correlation coefficients suggest high consistency of the quantitative values recorded, while Kendall rank correlations indicate high consistency in the interaction ranks according to their value. Colours indicate different interval levels for correlations from high (dark blue) to lower correlation (light green).

Appendix 1E. List of bibliographic references included in Table 1.1, as examples of study methods for plant-frugivore interactions

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CHAPTER 2

Reciprocity and interaction effectiveness in generalised mutualisms among free-living species.

Quintero, E., Rodríguez-Sánchez, F., & Jordano, P. (2023). Reciprocity and interaction effectiveness in generalised mutualisms among free-living species. Ecology Letters, 26(1), 132–146.

Abstract

Mutualistic interactions among free-living species generally involve weak links and highly asymmetric dependence among partners, yet our understanding of factors behind their emergence is still limited. Using individual-based interactions of a super-generalist fleshy-fruited plant with its frugivore assemblage, we estimated the Resource Provisioning Effectiveness (RPE) and Seed Dispersal Effectiveness (SDE) to assess the balance in exchange of resources. Plants were highly dependent on a few super-generalist frugivore species, while these interacted with most individual plants, resulting in strong asymmetries of mutual dependence. Interaction effectiveness was mainly driven by interaction frequency. Despite highly asymmetric dependences, the strong reliance on quantity of fruit determined high reciprocity in rewards between partners (*i.e.*, higher energy provided by the plant, more seedlings recruited), which was not obscured by minor variations in the quality of animal or plant service. We anticipate reciprocity will emerge in low-intimacy mutualisms where the mutualistic outcome largely relies upon interaction frequency.

Keywords: asymmetry, Bayesian, ecological networks, frugivory, Mediterranean, plant-animal interactions, reciprocity, resource provisioning effectiveness, seed dispersal, seed dispersal effectiveness

Introduction

Mutualisms are ecological interactions entailing beneficial outcomes for the interacting partners. These benefits broadly emerge from interspecific encounters where there is an exchange of resources (Kiers *et al.* 2011). Despite recent interest in interspecific exchanges, especially focusing on strict and intimate interactions (Guimarães *et al.* 2007), much of the reciprocal effect between generalised, free-living, mutualistic partners remains unexplored (Thompson 2009).

Species-level analyses of complex interaction networks have revealed highly heterogeneous structures (*i.e.*, high variance in number of interactions per species), weak levels of mutual dependence, and high asymmetry in interaction strength (Johnstone & Bshary 2008, Bascompte & Jordano 2014, Wootton & Stouffer 2016). Interaction asymmetry in complex networks of free-living species (Bascompte *et al.* 2006), as well as energy flow asymmetry in food webs (Rooney *et al.* 2006), appear as quintessential characteristics of these complex systems, closely associated with their stability (Berlow 1999). Yet our understanding of the factors behind the emergence of asymmetric interactions is very limited; for example, if generalised mutualistic interactions between free-living species entail exchanges of services, is there a "fair two-way transfer" of resources (Kiers *et al.* 2011, Chomicki *et al*. 2020) *i.e.*, is there reciprocity?

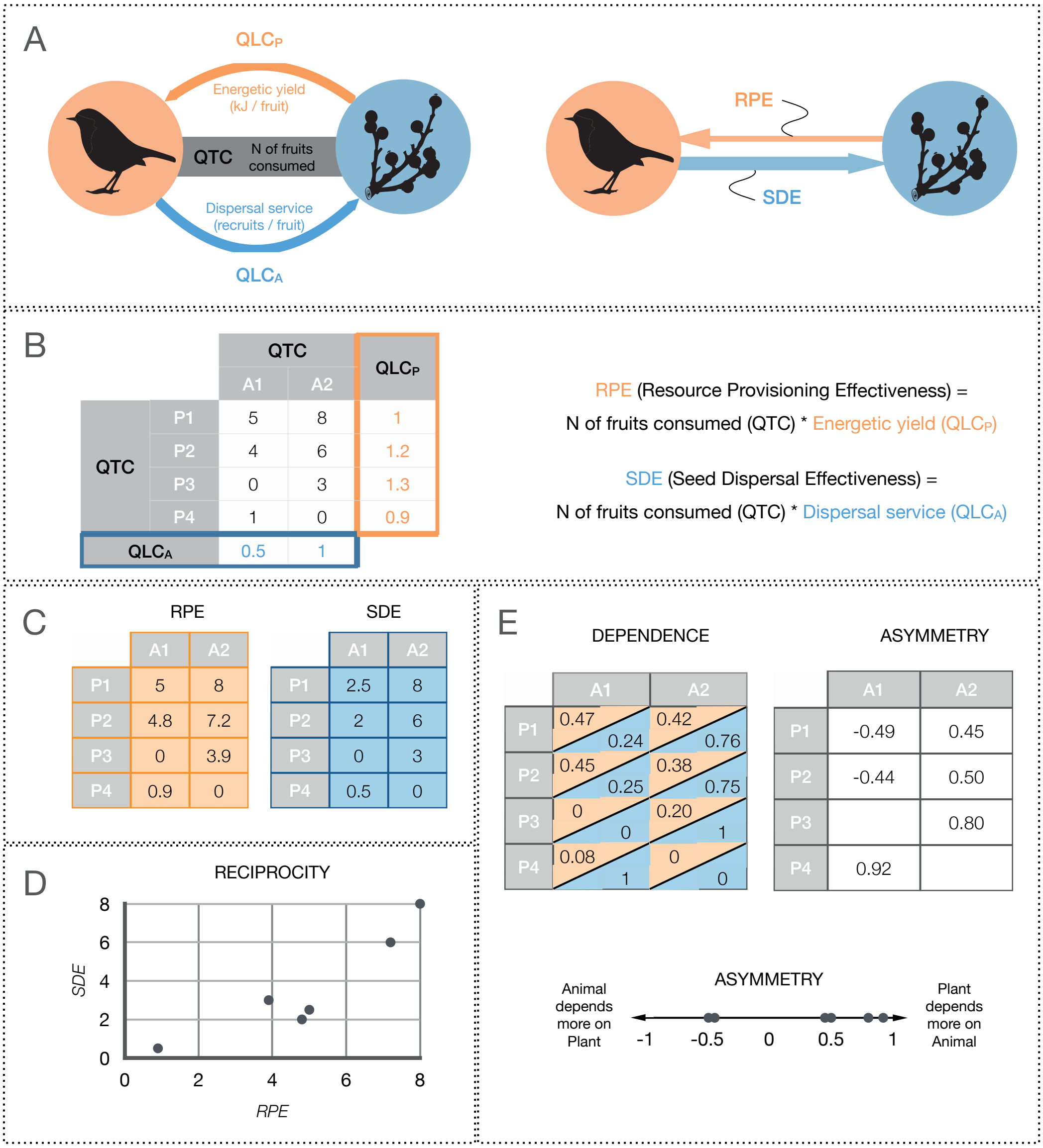
Reciprocity, as defined herein, is the existence of a positive association in the rewards provided between mutualistic partners. We consider a mutualistic system to be reciprocal if the reward provided by one organism (*e.g.*, pollen grains or fruits offered by plants) matches the reward from its mutualistic partner (*e.g.*, fertilised ovules or dispersed seeds). If an increase or decrease in reward does not return proportional changes in the reward by the other partner, so that both rewards keep balanced, those interactions would be less, or not reciprocal at all. Without an external reference, it is not possible to determine if the exchange in resources between partners is equal or fair. A population or community perspective will allow us to understand whether specific pairwise interactions are exchanging their resources at 'fair' cost, or at least at the cost set by the population or community. Aside from previous work on mycorrhizal symbioses, less intimate and 'lagged' (*i.e.*, with delayed responses beyond the interaction), the reciprocity of mutualisms has been rarely addressed. However, previous studies explore other definitions of reciprocity using different approximations that are related to the degree of partner's dependence and not to the balance in rewards exchanges (*e.g.*, Herrera 1984b, Reid 1990, Burns 2003, Guerra & Pizo 2014).

Partner dependence, *i.e.*, how much a partner relies upon another partner for its services, is a better explored aspect of mutualistic interactions. Dependence can be estimated as the proportion of service obtained from a specific partner relative to the total service obtained from all partners. Dependence differs from reciprocity in that it examines the reliance from the perspective of the partner, and not the whole population. Estimating dependence also allows calculation of interactions asymmetry by comparing the mutual dependence of both partners. Asymmetry emerges when a species/organism depends a lot on one partner but, in turn, the partner does not rely as much on that particular pairwise interaction (Jordano 1987a, Bascompte *et al.* 2006, Vázquez *et al.* 2007).

A generalised property of free-living species networks is the high frequency of weak interactions (Jordano 1987a) so that when other interactions are strong, their dependence becomes highly asymmetric. This pattern in the mode of interaction between organisms is known as disassortativity, whereby organisms that establish many interactions tend to interact with less connected organisms (Barabási 2016), and is often found in biological networks (Newman 2003). Weak links appear a characteristic feature of complex systems which are made up of highly diversified components (Granovetter 1973, Csérmely 2009). Weak links also provide support for network stability (McCann *et al.* 1998). Most previous analyses of network patterns in real-world ecosystems have considered species-level interactions. However, interaction asymmetries at the individual-level remain largely unexplored, despite likely being the most appropriate level to address interaction outcomes (Clark *et al.* 2011). Actual ecological interactions that we can observe, sample, and document, occur from interspecific encounters between individuals (Dupont *et al*. 2014, Jordano 2016). One might therefore wonder if, when looking at a more refined level (*e.g.*, from species to individuals), we could still expect asymmetry in mutual dependence.

Few studies so far have analysed interaction asymmetry beyond variation in just interaction frequency or strength, further examining differences in interaction quality (Herrera 1984b, Jordano 1987a, Guerra & Pizo 2014, González-Castro *et al.* 2022). Interaction outcomes may yield different results from those expected solely on the basis of interaction frequency (Janzen 1983, González-Castro *et al.* 2022), and so it is possible that infrequent interactions result in higher fitness than frequent interactions, affecting the reciprocity balance. A useful tool to measure the functional outcome (fitness) of mutualisms in terms of both interaction quantity and quality at the individual level is the effectiveness framework (Schupp 1993, Fig. 2.1A). Consideration of individual variation and interaction outcomes expands our understanding of the potential consequences, *e.g.*, demographic or evolutionary, of ecological interactions.

In this study we calculate the two-sided rewards for seed dispersal mutualistic interactions between plants and animal frugivores by means of the Resource Provisioning Effectiveness (RPE) and Seed Dispersal Effectiveness (SDE) frameworks (Schupp *et al.* 2017). We look at mutual reciprocity (*i.e.*, the balance in the exchange of resources) from an individual perspective in a plant population using SDE and RPE as estimates for the reward obtained in the relationship (Fig. 2.1D). We explore whether mutualistic dependencies are still asymmetrical when looking from a plant individual perspective and when incorporating both interaction frequency and quality (Fig. 2.1E). We use as study organism the plant *Pistacia lentiscus*, a species defined as super-generalist species because it interacts with a large part of the local diversity of partner species, being heavily connected to the rest of the community (Jordano *et al.* 2003, García 2016, Parejo-Farnés *et al.* 2020b). Super-generalists play a fundamental role in ecological networks because they provide great cohesion (Guimarães *et al.* 2011). A two-sided study of such mutualism at the plant individual level informs about the diversity of individual rewards, the diversity of mutualistic partners and their effects, and the consequences on resource exchange between them.



		QTC A1	QTC A2	QLCP
QTC	P1	5	8	1
	P2	4	6	1.2
	P3	0	3	1.3
	P4	1	0	0.9
QLCA		0.5	1	

RPE (Resource Provisioning Effectiveness) =
N of fruits consumed (QTC) * Energetic yield (QLCP)

SDE (Seed Dispersal Effectiveness) =
N of fruits consumed (QTC) * Dispersal service (QLCA)

RPE

	A1	A2
P1	5	8
P2	4.8	7.2
P3	0	3.9
P4	0.9	0

SDE

	A1	A2
P1	2.5	8
P2	2	6
P3	0	3
P4	0.5	0

DEPENDENCE

	A1	A2
P1	0.47 0.24	0.42 0.76
P2	0.45 0.25	0.38 0.75
P3	0 0	0.20 1
P4	0.08 1	0 0

ASYMMETRY

	A1	A2
P1	-0.49	0.45
P2	-0.44	0.50
P3		0.80
P4	0.92	

Figure 2.1. Schematic representation of this study approach to characterise plant–frugivore seed dispersal mutualisms, showing: (A) the three main subcomponents present in the mutualism between any two nodes in the network: the interaction frequency or quantity component (QTC) and the two-sided quality (QLC) of the service provided by the partners. For this example we present the animal frugivore in orange, and the plant individual in blue; plant's quality is the energetic yield per fruit (QLC_P) and bird's quality is the probability of seedling recruitment per consumed fruit (QLC_A). On the right we provide an example adjacency matrix with simulated numbers of quantity and quality data, with two animals (A1 and A2), and four plants (P1–P4). (B) The two subcomponents (quantity and quality) are combined to calculate the effectiveness of the interaction from the bird (Resource Provisioning Effectiveness, RPE) and the plant's perspective (Seed Dispersal Effectiveness, SDE). (C) Resulting calculations of

RPE and SDE using the example matrix in (A). (D) Reciprocity (*i.e.*, the balance in rewards exchange between partners) is assessed by the correlation between RPE and SDE values of all pairwise interactions. (E) Derivation of mutual dependence estimates and interaction asymmetry for plant and animal partners. Dependence values for animals (*i.e.*, how much the animal depends on each particular plant, orange upper-left cells) are calculated based on RPE values, while dependence values for plants (blue lower-right cells) are based on SDE values. The asymmetry of each pairwise interaction is calculated as the standardised difference between the two dependence values in each interaction, and ranges between −1 and +1.

Here we address three specific objectives: 1) characterise the effectiveness of the mutual beneficial service between individual plants and their frugivorous species, 2) test if the service provided between partners in terms of the amount of reward is reciprocal, and 3) explore if there exists asymmetry in the mutual dependencies when looking at a plant individual level and considering interaction outcomes; that is, accounting for interaction quality beyond interaction frequency.

Methods

Species and study site

Pistacia lentiscus (Anacardiaceae) is a dioecious, wind-pollinated, animal seed-dispersed shrub that can be considered as a 'foundation species' (Whitham *et al.* 2006) in lowland Mediterranean scrublands. Numerous resident and migrant frugivorous birds rely on *P. lentiscus* fruits as a nutritional resource (González-Varo *et al.* 2019a) and act as its seed dispersers, with infrequent consumption by mammals (Perea *et al.* 2013).

Fieldwork was conducted between the years 2019-2020 at two study sites in Doñana National Park (Huelva, SW Spain): La Mancha del Rabicano in El Puntal site (EP) and Laguna de las Madroñas (LM). Both areas consist of Mediterranean sclerophyllous scrubland dominated by *P. lentiscus* coexisting with a total of 28 fleshy-fruited species recorded in the area. We monitored 40 individual P. lentiscus plants per study site (Appendix 2A). This sampling included all the female fruiting plants found in LM population. In EP site, we chose a representative sample of female plants scattered across the site and covering the full size gradient in the population.

Interaction frequency: Quantity component (QTC)

The interaction frequency of *Pistacia lentiscus* plants with avian frugivore species was assessed through DNA-barcoding and continuous-monitoring cameras

(Quintero *et al.* 2022) during the complete fruiting season, between September 2019 and March 2020.

We placed seed traps beneath individual plants at both populations, where we collected a total of 2691 faecal and regurgitated seed samples (1913 for EP and 778 for LM). Visiting avian species were identified with DNA-barcoding analysis of collected samples. Animal-origin DNA present in the surface of the samples was extracted, amplified and then sequenced following protocols in González-Varo *et al.* (2014) with minor modifications (Appendix 2B.1). Retrieved sequences were identified using BOLD Systems database or BLAST from the NCBI. More than 90% of the collected samples were analysed (n = 2510) and the identification success was 94%.

Using monitoring cameras, we recorded animal visitation and feeding events at focal plants in one of the sites (EP). All individual plants were monitored every fortnight along the fruiting season for a total of nine times, accumulating c.19 h observation per plant. Recordings lasted c. 2.2 h and started in the early morning (Appendix 2B.2). We analysed the video recordings with the help of DeepMeerkat software (Weinstein 2018). We obtained the feeding frequency of animals (*i.e.*, fraction of visits with actual fruit consumption) and the number of fruits consumed per visit. Avian species identification was possible for 91.5% of the n = 3970 visits recorded by cameras and 24% of the interaction events included feeding records.

The total number of frugivorous bird species recorded was 27; 26 recorded with cameras and 22 with DNA-barcoding. Interaction accumulation curves (IAC) were used to determine sampling completeness (see Appendix 2B.3; Colwell & Coddington 1994, Jordano 2016). Overall, sampling completeness (*sensu* Chacoff *et al.* 2012) was 93% for both methods; 95% for cameras and 96% for DNA-barcoding.

To estimate the total number of fruits consumed by each bird species at each individual plant we multiplied four sequential steps: (1) the total number of visits at each site, (2) the probability that a given bird species visited a particular plant, (3) the probability that a visit included a feeding event, and (4) the number of fruits consumed per visit by each bird species. We estimated these quantities using Bayesian models fitted with Stan (Stan Development Team 2023) and brms package (Bürkner 2017) in R 4.1.2 (R Core Team 2021). Bayesian approach allowed us to combine information on bird visitation rates coming from DNA-barcoding and monitoring cameras, to estimate a few unobserved quantities, such as missing feeding rates for some species by sharing information across taxa, and to obtain and propagate uncertainties along multiple effectiveness components (see Appendix 2E.1 for details on each model). Since posterior distributions were often right-skewed, we report their median throughout.

Interaction outcome for the animal: Quality Component (QLC - RPE)

Plant quality was defined as the energetic reward provided per fruit consumed. Feeding behaviour was different among the avian species recorded: some birds consume the whole fruit regurgitating or defecating the seed intact (legitimate seed dispersers), others consume part of its pulp discarding the seed (pulp thieves); while other birds peel the fruit, break the seed coat and consume the embryo inside the seed (seed-predators or granivores). Because avian species consume different parts of the fruit, the energy obtained refers to the pulp for swallowers and pulp consumers, and to the seeds in the case of predators (see Table A2.1 for frugivory type categories).

We collected fruits from each plant (mean = 31 fruits, range = 17–63, Appendix 2C) and measured both pulp and seed fresh mass. Fresh mass was converted to dry mass using *P. lentiscus* % water content (Jordano 1984). To obtain the energy contained per fruit, we then multiplied the pulp and seed dry mass by their estimated energy yields: 25.25 kJ/g for pulp and 28.14 kJ/g for seed (see Appendix 2E.2).

Interaction outcome for the plant: Quality Component (QLC - SDE)

We estimated the quality of animals as seed dispersers according to the: (1) probability of seeds escaping predation by granivorous birds, (2) microhabitat use by each bird species, (3) probability of seeds escaping post-dispersal predation, and (4) probability of seedling emergence and early survival (past their first summer) in each microhabitat. We estimated these probabilities using Bayesian models as above (see Appendix 2E.3 for details). The product of these four steps determined the probability of seedling recruitment resulting from the consumption of one fruit by a specific avian consumer.

We found a few undamaged, depulped seeds (n = 36) in the seed traps beneath plants that were attributed to seed predators through DNA-barcoding. Seeds dropped during fruit handling indicated sporadic dispersal events by seed predators, whose probability was estimated using the total number of preyed-upon seeds (open seed endocarp halves) and the number of undamaged seeds found in seed traps attributed to granivores (Appendix 2D.1 and 2E.3).

The intensity of microhabitat use by the different bird species was inferred from the seed rain of *P. lentiscus* seeds collected at five microhabitats: under *Pistacia lentiscus* conspecifics (PL), under other fleshy fruited species (FR), under non-fleshy fruited species (NF), under pine trees (*Pinus pinea*; PP), and open ground areas (OA). At each microhabitat, we collected *P. lentiscus* dispersed seeds and identified the bird species through DNA-barcoding. For the PL microhabitat we used the seed traps located beneath the focal individuals plants (see above). To sample microhabitats FR, NF and PP we placed additional seed traps at 15 replicated points per microhabitat. For open areas (OA) we scanned 17, 1-m wide, transects regularly during the season. The number of dispersed seeds collected at each microhabitat allowed us to estimate the probability of dispersal to each specific microhabitat by each bird species (Appendix 2D.2 and 2E.3).

Finally, we measured post-dispersal seed predation, seedling emergence and survival at each microhabitat. To study post-dispersal predation we placed 10 seeds on a petri dish (6 replicates per microhabitat) and monitored the rate at which seeds experienced predation (mainly by rodents, see Appendix 2D.3 and 2E.3). We also installed germination stations (13 replicates per microhabitat, each containing 16 sown seeds) to estimate seedling emergence and survival (see Appendix 2D.4).

Reward estimation using the Effectiveness framework

We estimated the rewards exchanged between mutualistic partners using the Resource Provisioning Effectiveness and Seed Dispersal Effectiveness framework (RPE and SDE, Schupp *et al.* 2017, Quintero *et al.* 2020). The effectiveness of the mutualistic interaction is estimated as the product of a quantity and quality component (*i.e.*, the interaction frequency multiplied by its functional outcome; Fig. 2.1 and Fig. A2.9). The quantity component (QTC) was common for both RPE and SDE, that is, the total number of fruits consumed by a specific bird species on a given plant. Quality from the animal's perspective was the energy acquired per fruit consumed (referred to pulp or seed energy depending on bird's feeding behaviour). From plants' perspective, the quality component represents the probability that a consumed fruit becomes a seedling surviving its first summer. RPE therefore estimates the total energy provided by an individual plant to a bird species across the fruiting season, and SDE estimates the potential number of seedlings recruited coming from an individual plant by interacting with a bird species. Below we define the components for the rewards calculation:

RPE_{ij}: Total energy that bird species *j* obtained by feeding in plant *i*

$RPE_{ij} = QTC_{ij} \times QLC\_RPE_i$

SDE_{ij}: Number of plant *i* seedlings recruited through consumption by bird species *j*

$SDE_{ij} = QTC_{ij} \times QLC\_SDE_j$

QTC_{ij}: Number of fruits consumed by bird species *j* in plant *i* along the fruiting season

QTC_{ij} = Total no. of visits in population ×

Prob. that bird species *j* visits plant *i* ×

Prob. bird species *j* consumes fruit during visit ×

No. fruits bird species *j* consumes per visit

QLC_RPE_i : Energy contained per fruit pulp or seed of plant *i*

QLC_RPE_i = Fresh pulp or seed mass of plant *i* (g) ×

non-water % in pulp or seed ×

energetic yield per gram of dry pulp or seed

QLC_SDE_j: Probability that a fruit consumed by bird species *j* becomes a seedling surviving its 1st summer

QLC_SDE_j = Prob. seed escaping predation when manipulated by bird species *j* ×

$\sum_{m=1}^{5}$ [Prob. bird species *j* deposits a seed at microhabitat *m* ×

Prob. seed escaping post dispersal predation in microhabitat *m* x

Prob. seedling emerging and surviving its 1st summer in microhabitat *m*]

Reciprocity

To estimate reciprocity between partners we used Pearson correlation coefficients between the log-transformed RPE and SDE values. We aggregated the RPE and SDE values for each individual plant, *i.e.*, adding up the values for all bird species with which it interacted, resulting in the total energy provided by the plant and the number of seedlings recruited through interactions with its bird assemblage. We used 1000 samples from the posterior distribution of RPE and SDE calculated for each plant, to consider uncertainties in the estimation of reciprocity (Appendix 2F.1). A high positive correlation indicates high reciprocity, meaning that plants providing more energy (RPE) also obtain a higher number of seedlings recruited (SDE).

Dependence and asymmetry between bird species and individual plants

We calculated two mutual dependence (*d*) values for each pairwise interaction, one for the plant () and one for the animal species (); (Appendix 2F.2).

$$d_{P_i \to A_j} = \frac{SDE_{ij}}{\sum_{A=1}^{n} SDE_i}$$, for the dependence of *P. lentiscus* plant *i* on animal species *j*; and

$$d_{A_j \to P_i} = \frac{RPE_{ij}}{\sum_{P=1}^{m} RPE_j}$$, for the dependence of animal species *j* on plant *i,*

where *d* is the dependence of plant *i* on animal species *j*, or vice versa; SDE_{ij} is the estimated number of seedlings recruited coming from plant *i* via frugivore species *j*; RPE_{ij} is the amount of kilojoules plant *i* provided to frugivore species *j*; and *n* and *m* represent the total number of animal species and individual plants, respectively.

Interaction asymmetry (AS) is defined as:

$$AS_{P_i A_j} = \frac{d_{P_i \to A_j} - d_{A_j \to P_i}}{max(d)}$$

AS values can range from 1 to 1, where 0 indicates total symmetry (both partners depend on each other with the same intensity), values approaching +1 indicate that the plant is more dependent on the animal than *vice versa*, and negative values indicate that the animal is more dependent on the plant than the plant on the animal. To assess the robustness of the observed asymmetry values to variations in our sampling design we repeated the asymmetry calculations using replicated random subsets of 20, 40, and 60 plants, to examine potential effects of the number of focal plants on the distribution of asymmetry values. We also calculated the asymmetry in a randomised network of the same size (*i.e.*, 80 focal plants) following Patefield and Vázquez null models to test if the observed and randomly-expected asymmetry values differed (Appendix 2H).

Results

Plant individual-based interactions

We estimated that birds consumed a total of 2.2×10^5 fruits from the 80 marked plants at both *P. lentiscus* populations (90% credibility interval: $1.5 \times 10^5 - 6.6 \times 10^5$). This consumption represents c. 20% of the total number of fruits produced by focal plants in the 2019–2020 season (Fig. A2.21). We detected 27 bird species consuming *P. lentiscus* fruits, of which 12 are considered residents, 9 summer, or trans-Saharan, migrants and 6 winter migrants (Table A2.1). More than 85% of the consumed fruits were eaten by just three species: *Curruca melanocephala*, *Erithacus rubecula* and the seed predator *Chloris chloris*. These species behaved as super-generalists, interacting with the majority of individual plants (Fig. 2.2). The next most significant consumers were *Turdus merula* and the winter migrant *Sylvia atricapilla*.

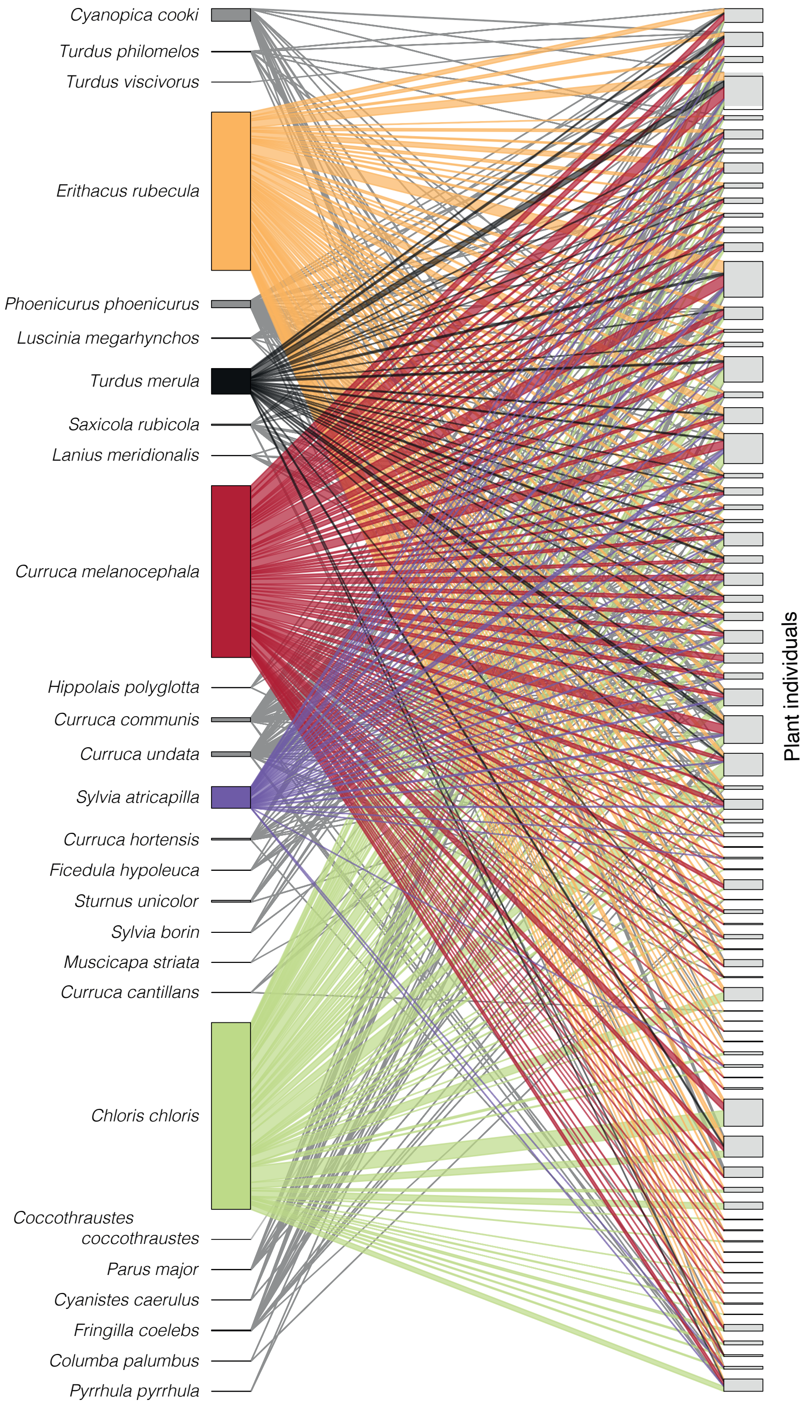


Figure 2.2. Interaction network between avian consumer species and individual *Pistacia lentiscus* plants, where the node and link width is proportional to the total number of fruits consumed on each plant. Non-legitimate dispersers (n = 7) are grouped at the end of the network.

Resource Provisioning and Seed Dispersal Effectiveness

Pistacia lentiscus plants were highly variable in Resource Provisioning Effectiveness (RPE) provided to avian species (Fig. 2.3). On a per-interaction basis a frugivore species consumed a median of 101 fruits on each plant (90% CI: 2 – 2680). We estimated that *Curruca melanocephala* and *Erithacus rubecula* ate more than 4000 fruits, and *Chloris chloris* predated more than 5500 seeds, at certain individual plants. This intensity of consumption represents, however, just a small proportion of the available crop offered: 90% of the plants had less than half their crop size removed by birds (Fig. A2.21). The quantity component accounted for almost all (93%) of the variation in RPE (Appendix 2E.5). Regarding quality, we found up to seven fold differences in the energetic content of fruits from individual plants. Birds exploited the full gradient of fruit sizes (Fig. A2.22), but in general, avian consumption was higher in plants with larger crops, canopy area, and pulp content (Table A2.5).

Seed Dispersal Effectiveness (SDE, Fig. 2.3) was also determined more by the quantity than the quality component, the latter varied little among bird species (variance partitioning: quantity = 69%, quality = 31%; Appendix 2E.5). Except for seed predators, which had negligible contributions to recruitment (because they destroyed 99.9% of the seeds consumed), the probability of recruiting a seedling per consumed fruit was similar for all bird species (median = 1.1×10^{-4}; 90% CI = 1.0×10^{-5}– 9.6×10^{-4}), with *Curruca melanocephala* emerging as the highest quality disperser, followed by other members of the Sylviidae family (Fig. 2.3; Fig. A2.18). Recruitment probabilities at the final stage were low; even the most effective pairwise interaction (involving *C. melanocephala* and plant '314'), would have resulted in SDE = 0.53 seedlings (<1 seedling) surviving the first summer.

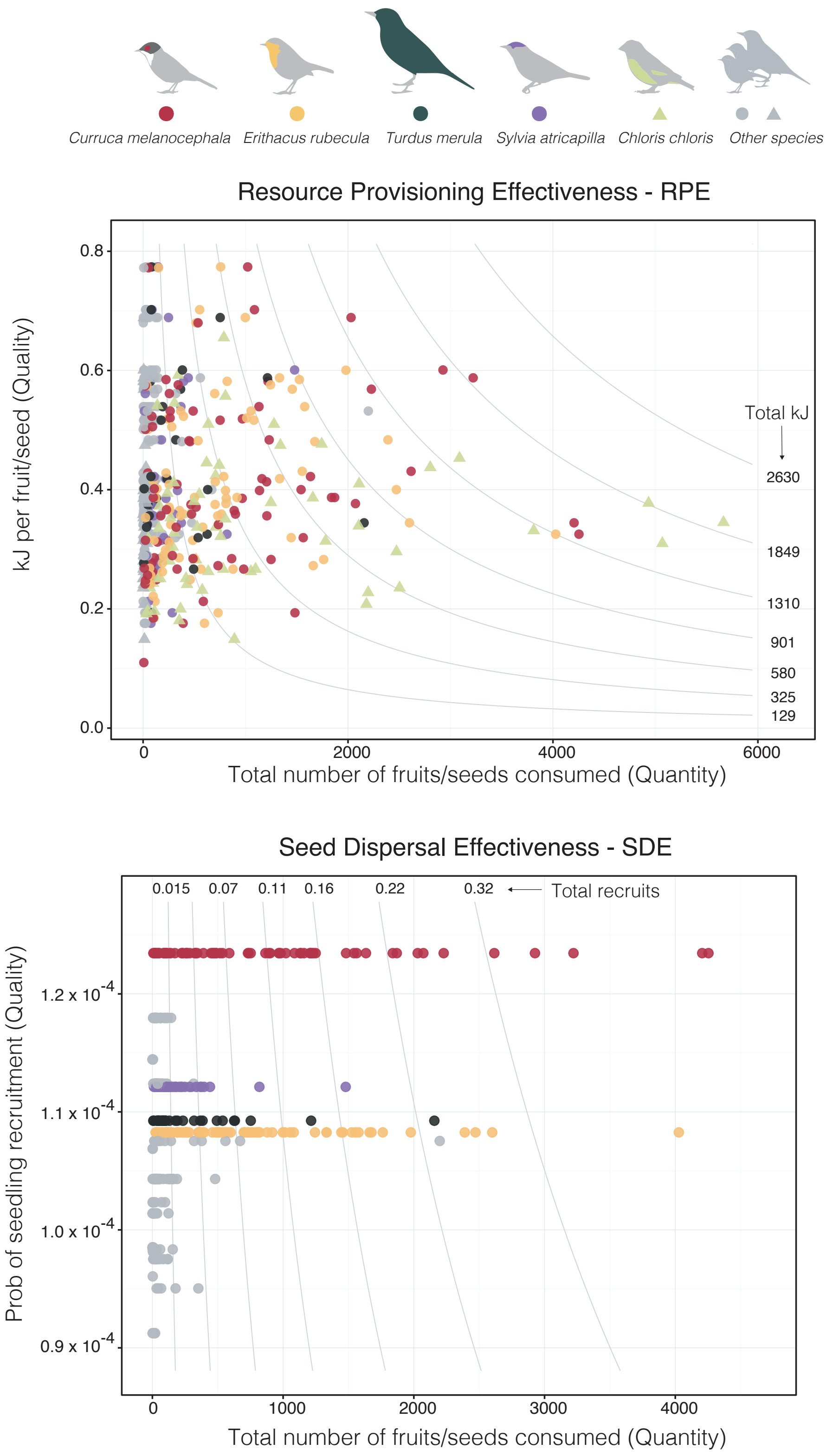

Curruca melanocephala
Erithacus rubecula
Turdus merula
Sylvia atricapilla
Chloris chloris
Other species
Resource Provisioning Effectiveness - RPE
kJ per fruit/seed (Quality)
0.0
0.2
0.4
0.6
0.8
Total number of fruits/seeds consumed (Quantity)
0
2000
4000
6000
Total kJ
2630
1849
1310
901
580
325
129
Seed Dispersal Effectiveness - SDE
Prob of seedling recruitment (Quality)
0.9 x 10^-4
1.0 x 10^-4
1.1 x 10^-4
1.2 x 10^-4
0.015
0.07
0.11
0.16
0.22
0.32
Total recruits
Total number of fruits/seeds consumed (Quantity)
0
1000
2000
3000
4000

Figure 2.3. Landscapes for Resource Provisioning Effectiveness (RPE) and Seed Dispersal Effectiveness (SDE). Each point represents an individual pairwise interaction between an individual plant and a frugivorous avian species. In both landscapes, the horizontal axis depicts the total number of fruits consumed by each bird species in each individual plant. Symbols represent feeding behaviour (circles for legitimate dispersers, and triangles for non-legitimate dispersers). In the RPE landscape, the vertical axis represents the median energy (kJ) obtained from the pulp or seed from each individual plant. In the SDE plot, the vertical axis represents the posterior median probability of recruiting a seedling from a fruit ingested by each bird species. Hence, the product of the horizontal (Quantity) and vertical (Quality) axis gives the effectiveness of each bird-plant pairwise interaction: the total energy (kJ) in the case of RPE, and total number of plant recruits for SDE. Different combinations of quantity and quality can produce equal effectiveness values, as shown by isolines. Note seed predators are not shown in the SDE landscape visualisation, because their dispersal quality is zero or close to zero and their inclusion distorts the graph (see Fig. A2.19 for complete SDE landscape).

Differences among frugivore species in dispersal quality result from their distinctive microhabitat use (Fig. A2.15) and existing trade-offs between recruitment stages in different microhabitats (Figure A2.16; A2.17). For example, seeds falling under pine trees had the highest probability of surviving rodent predation (median probability = 0.023), followed by those arriving to open areas (median probability = 0.013). Seedling emergence and survival, on the other hand, was highest in open areas and lowest beneath pines (median probability = 0.038 in OA versus 0.003 in PP). Overall, Open Area was the microhabitat with highest probability of recruitment, yet very few seeds arrived there, hence this microhabitat hardly contributed to recruitment. The high quality of *C. melanocephala* (median probability = 1.2×10^{-4}) emerged from its preferential dispersal towards the most suitable microhabitats: beneath non-fleshy fruited plants and *P. pinea*. In contrast, heavy *P. lentiscus* fruit consumers like *E. rubecula* showed medium quality (median probability = 1×10^{-4}) because it frequently deposits seeds under *P. lentiscus* plants, a microhabitat where the probability of escaping post-dispersal seed predation and seedling survival were medium to low.

Reciprocity

We found high correlation between RPE and SDE (mean Pearson r on log-log values = 0.93; Fig. A2.20). High correlation indicated high reciprocity in the interactions between individual *P. lentiscus* plants and their bird consumers: more seedlings were recruited from plants supplying more energy (Fig. 2.4). In other words, the larger the reward provided by one interaction partner, the larger the reward contributed by the other partner. This high reciprocity stems from both RPE and SDE being mainly driven by the quantity component (intensity of consumption) rather than by differences in plant and frugivores quality. As a result, more seedlings were recruited from plants which had more fruits consumed (mean slope of log SDE ~ log RPE = 0.83, SD = 0.06; Fig. 2.4), regardless of differences in the composition of their frugivore assemblages. Deviation from a slope of 1 indicates a 'diminishing return' effect, so that the number of seedlings recruited did not increase in the same proportion as the total energy provided by plants. This diminishing return was not caused by interactions with seed predators (mean slope of log SDE ~ log RPE ± SD excluding seed predators = 0.85, SD = 0.04); who damaged all plants in similar proportions. Additionally, plants presenting greater rewards had larger crop sizes and were consumed by a higher number of bird species (Fig. 2.4).

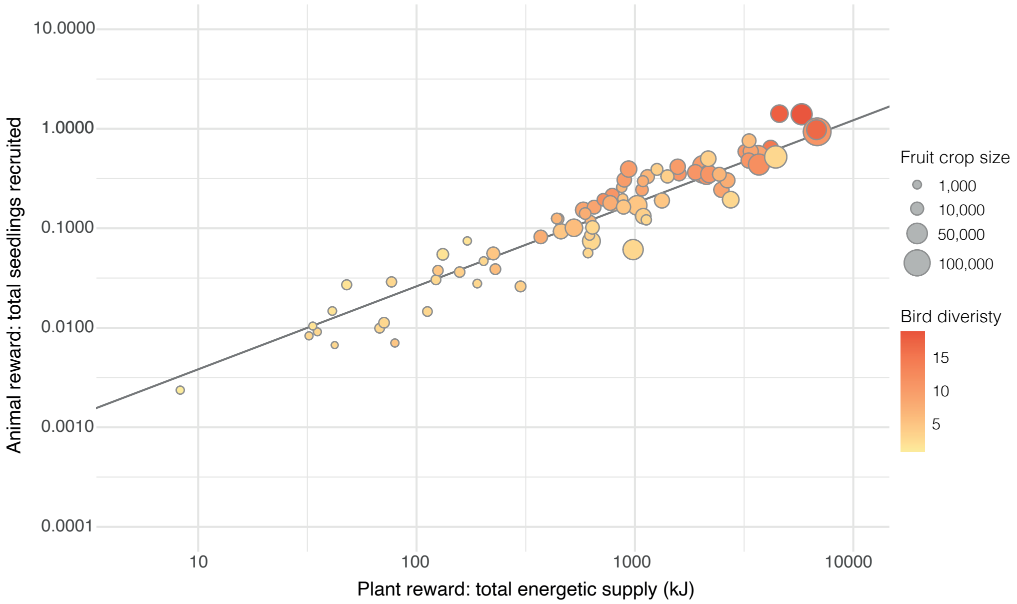


Figure 2.4. Relationship between the total energetic supply provided by individual plants (aggregating all its consumer bird species) and the number of seedlings recruited by each plant (n = 79 plants). The positive relationship indicates highly reciprocal interactions: the higher the reward offered by the plant, the higher the reward received from its bird consumers. Point size represents plants' initial fruit crop size, and colour intensity indicates the number of animal species partners, so that plants involved in larger rewards had larger crop sizes and larger number of frugivore partners. Note both axes are in logarithmic scale.

Dependence and Asymmetry

Mutual dependencies on the partner were in general low (Fig. 2.5). Most pairwise interactions (96% from birds' perspective, and 76% from plants') showed dependencies below 0.25, indicating that most interactions contributed to the partner only a small fraction of the total reward obtained (*i.e.*, energy income for birds or seedlings recruited for plants). There were, however, some strong, highly-dependent interactions, namely those involving the two main dispersers *E. rubecula* and *C. melanocephala*: plants strongly depended on both bird species for effectively dispersing their seeds and recruiting (Fig. 2.5, left). In contrast, avian species were less dependent on individual plants. Only a few rare bird species showed high dependency on specific plants (Fig. 2.5, centre).

When comparing the corresponding dependencies of each partner, we found that most bird-plant interactions were highly asymmetric (Fig. 2.5, right); 71% of interactions had absolute asymmetry values over 0.75. These asymmetry values did not deviate significantly from those obtained using null models (Appendix 2H). Two major processes caused asymmetry to emerge. First, plants depended strongly on the main avian consumers (*C. melanocephala, E. rubecula*), while these birds had low dependencies on individual plants (asymmetry values towards 1) because they were feeding and obtaining energy from many plants, hardly depending on any particular one. Second, when the animals had high dependency on a particular plant (asymmetry values towards −1), the plants in turn hardly depended on that particular bird. These interactions were dominated by seed predators (mainly *C. chloris*), pulp consumers, and locally uncommon bird species, which provided no or very limited seedling recruitment. Symmetric interactions (where both partners had similar dependency values) were scarce: only 16% of interactions had asymmetry values between −0.5 and 0.5 and were represented by strongly frugivorous and moderately abundant birds such as *T. merula*, *S. atricapilla* and *Cyanopica cooki*. In these cases of symmetric interactions, the importance of individual plants for energy provisioning was balanced with the importance of these birds as effective seed dispersers.

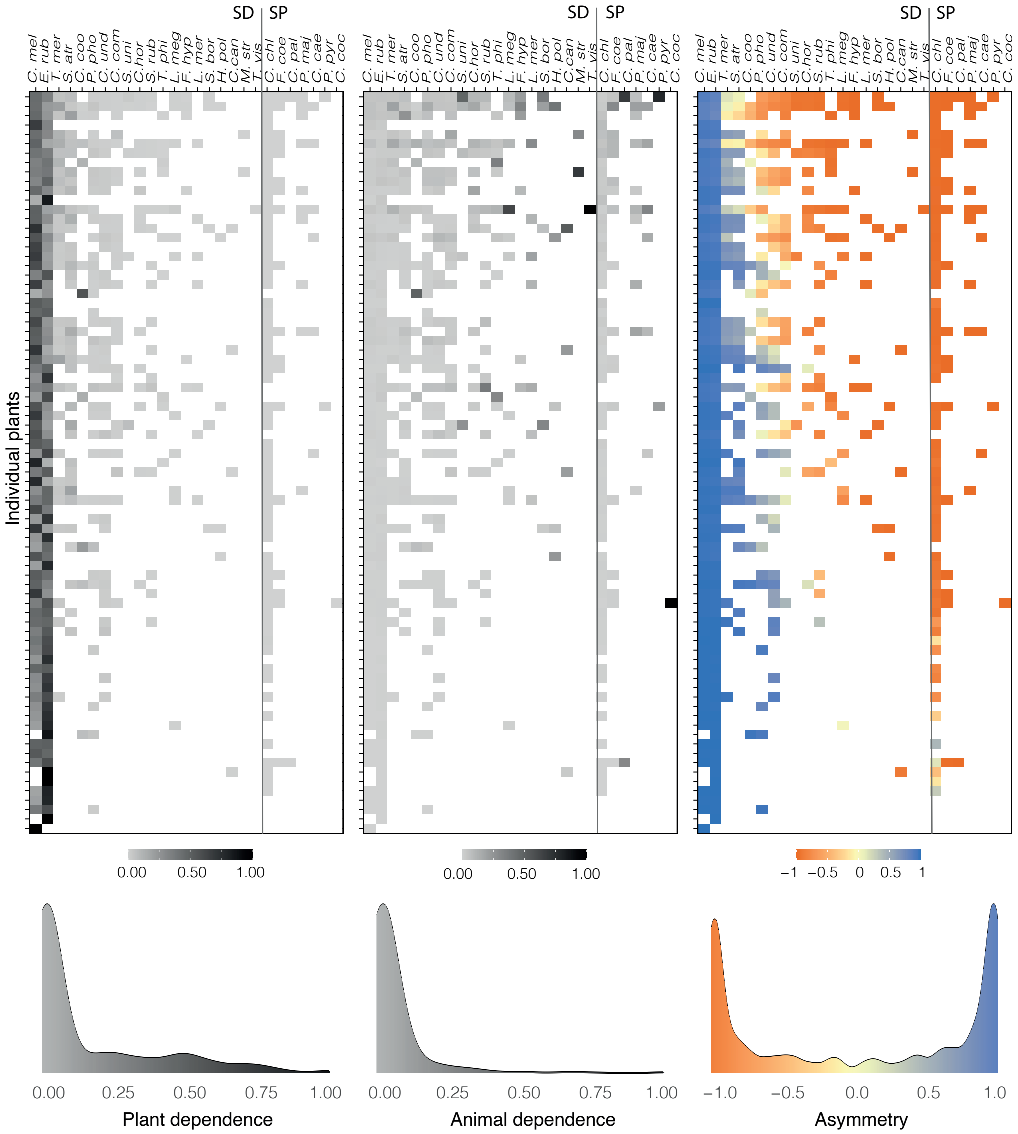


Figure 2.5. Interaction matrices between individual *Pistacia lentiscus* plants and their avian consumers. The first matrix (left) depicts how much each plant's seed dispersal effectiveness (number of seedlings recruited) depends on each bird species, whereas the second matrix (centre) shows how much the resource provisioning effectiveness (energy obtained) of each bird species depends on each particular plant. Both matrices range from 0 (no dependence at all) to 1 (total dependence on that particular partner). The third matrix (right) shows the asymmetry in dependence for each unique bird-plant pairwise interaction. Colours gradually veering toward blue (asymmetry values approaching 1) indicate interactions where the plant is more dependent on the animal than vice versa, whereas colours veering toward orange

(*i.e.*, asymmetry approaching −1) indicate interactions where the animal is more dependent on the plant. Symmetrical interactions, where the dependence of both partners is similar, are represented by yellow tones (asymmetry values close to 0). The lower graphs represent the frequency distribution of the above matrix values. Animal species codes in alphabetical order: *C.cae = Cyanistes caeruleus, C.chl = Chloris chloris, C.coc = Coccothraustes coccothraustes, C.com = Curruca communis, C.coo = Cyanopica cooki, C.hor = Curruca hortensis, C.ibe = Curruca iberiae, C.mel = Curruca melanocephala, C.pal = Columba palumbus, C.und = Curruca undata, E.rub = Erithacus rubecula, F.coe = Fringilla coelebs, F.hyp = Ficedula hypoleuca, H.pol = Hippolais polyglotta, L.meg = Luscinia megarhynchos, L.mer = Lanius meridionalis, M.str = Muscicapa striata, P.maj = Parus major, P.pho = Phoenicurus phoenicurus, P.pyr = Pyrrhula pyrrhula, S.atr = Sylvia atricapilla, S.bor = Sylvia borin, S.rub = Saxicola rubicola, S.uni = Sturnus unicolor, T.mer = Turdus merula, T.phi = Turdus philomelos, T.vis = Turdus viscivorus.*

Discussion

We report interaction patterns for a super-generalist plant species, with the aim of documenting variation in mutual dependence with animal seed dispersers at the plant individual level and degree of interaction reciprocity at the population scale. Our results allowed us to link the structure of individual-based interaction networks and the fitness consequences in local plant population recruitment.

Interaction intensity dominates partner effectiveness

Most previous studies have focused on effectiveness from a species-level, community perspective (although see Guerra *et al.* 2017, Palacio 2019, Jácome-Flores *et al.* 2020). The individual focus in *P. lentiscus* revealed ample variation in fruit consumption by animal frugivores at individual plants, while showing smaller variation in the quality of partner's reward. Both RPE and SDE variation were driven by the quantity component, rather than quality, indicating that interaction frequency *per se* is acting as a good surrogate of effectiveness, as found in previous studies (Vázquez *et al.* 2005). However, accounting for interaction quality may change interpretations of partner effectiveness in other systems (*e.g.*, rank reversals in González-Castro *et al.* 2022).

The resource provisioning effectiveness landscape (Fig. 2.3) did not reflect clear preferences of bird species for plants with energy-rich fruits. However, when aggregating the consumption data of non-granivorous birds by individual plants, we found that large plants, with larger fruit crops, producing heavier (more energetic) fruits, received a larger number of seeds dispersed (Table A2.5). Plant size and crop are well known to affect frugivory (Sallabanks 1993, Ortiz-Pulido *et al.* 2007, Schupp *et al.* 2019) and are both related to the ontogeny, growth and size hierarchies in plant populations (Weiner & Solbrig 1984). Other factors not analysed here, such as secondary compounds, fruit accessibility or fruiting neighbourhood can also be affecting consumption patterns (Moermond & Denslow 1985, Cipollini & Levey 1997, Carlo *et al.* 2007).

Legitimate seed dispersers also exhibited limited variation in the quality component of seed dispersal effectiveness (Fig. 2.3). The resulting probability of recruitment per consumed fruit was surprisingly similar between frugivore species, indicating a broad functional redundancy in their dispersal service (González-Castro *et al.* 2015). However, when considering the final seed dispersal effectiveness, two bird species (*C. melanocephala* and *E. rubecula*) emerged as the main contributors to seedling recruitment due to their high consumption. The redundancy encountered in the quality component could make the dispersal of *P. lentiscus* less susceptible to the loss of bird species or fluctuations in bird populations (Zamora 2000); however, marked changes in bird abundance, particularly of the dispersers that consume the most fruit, could compromise plant recruitment.

Reciprocity in partner rewards as a feature of mutualistic systems

Although the exchange of rewards between bird species and individual plants varied over several orders of magnitude, there was a high correlation between the rewards obtained by each partner in the interaction. High correlation between rewards points to a stable and fair two-way transfer in the exchange of mutualistic services. In the case of *P. lentiscus*, the reciprocity in the rewards stems from the strong dominance of the quantity component (intensity of consumption), common to both resource provisioning and seed dispersal effectiveness. Such high reciprocity appears characteristic of many seed dispersal systems and other generalised, resource-based mutualisms (Wheelwright & Orians 1982, Ollerton 2006). However, reciprocity in a mutualistic system could be compromised whenever there are large differences between partners quality, as occurs for example in systems with highly heterogeneous frugivore assemblages (González-Castro *et al.* 2015, García-Rodríguez *et al.* 2022). Reciprocity can also be broken when antagonists disrupt, to a variable extent, mutualistic interactions of plants with legitimate seed dispersers (Jácome-Flores *et al.* 2020);

however, mutualism breakdown scenarios have been largely examined for intimate interactions, not for free-living species (Sachs & Simms 2006, Chomicki & Renner 2017).

The deviation of reciprocity from strict proportionality (log slope = 1) could be caused by: (i) plants that produce heavier fruits have fewer seeds dispersed and get fewer seedlings recruited per amount of energy offered than small-fruited plants, (ii) highly fecund individuals (that disperse many fruits) attract both highly effective and less effective frugivores, and (iii) the fact that our analysis did not account for likely increasing seedling recruitment probabilities with increasing fruit and seed size. If bigger and more energetic fruits with larger seeds implies higher survival probability at the seedling stage (Piper 1986, Leishman *et al.* 2000), then our analysis could be underestimating the number of seedlings recruited for those plants.

Our results are consistent with previous reports showing that extremely high seed production and consumption are required to ensure recruitment, given sharp decreases in survival probability as seeds move along dissemination and establishment stages (Herrera *et al.* 1994, García-Fayos & Verdú 1998, Gómez-Aparicio 2008). Following our estimates, individual *P. lentiscus* plants would have to disperse > 8000 seeds to have just a single recruit surviving their first summer. Thus, successful plant recruitment requires huge reproductive effort from plants, even in well-functioning dispersal mutualisms with high reciprocity.

Highly asymmetric dependencies between mutualistic partners

The majority of interactions between bird species and *P. lentiscus* individual plants were highly asymmetric: when one partner depended strongly on the other, the latter depended much less on the former. The highly skewed distribution of dependence values was likely generated by the combination of varying bird abundances (Vázquez *et al.* 2007), differences in the degree of frugivory, and varying fruit production and attractiveness to frugivores from the plant individual side. Assessing individual variation in long-lived plants and the outcomes of their interactions with shorter-lived frugivores provide insights into the delayed consequences for both partners. A widely recognized constraint for coevolution between interacting species is asymmetry in generation time (and thus, evolutionary rates), violating one of the assumptions early stated by Janzen (1980) in his definition of coevolution: simultaneity. This is especially evident in interactions between short-generation, small frugivorous passerines and long-lived woody plants. Lack of simultaneity in evolutionary response has been implied, for example, to explain how megafauna-dispersed plants survived (through *e.g.*, vegetative propagation) to the Pleistocene-Holocene extinction of large mammals acting as their seed dispersers (Guimarães *et al.* 2008). Our results show that plant individuals differ in the way their mutualistic interaction assemblages are built and this results in extremely skewed contributions to population-level seedling recruitment, a delayed response to the interaction itself.

The high asymmetry between mutualistic partners' interdependence at the individual level is consistent with previous findings at the species level (Jordano 1987a, Bascompte *et al.* 2006, Guimarães *et al.* 2006, Guerra & Pizo 2014). In Herrera (1984b), most observed dependencies between frugivores and plant species were also weak or highly asymmetric. Interestingly, at the species level, *P. lentiscus* showed quite symmetric dependencies with its main seed dispersers. Our analysis revealed that, while bird species consumed *P. lentiscus* fruits heavily, they did not depend on particular plants, but rather spread their dependencies, generating highly asymmetric interactions. If individual birds could have been identified, rather than aggregated to species level, many of those plants' strong dependencies on the main consumers might in turn transform into weak links, with just a few strong interactions (*e.g.*, territorial birds strongly depending on a specific patch of *P. lentiscus*). Hence, zooming in to the individual level seems important because it may enrich our perceptions of the embedded dependencies in mutualistic networks (Tonos *et al.* 2022) and address the proper scale in order to understand emerging properties at the species-level (Clark *et al.* 2011).

The available evidence suggests that symmetric dependencies are rare in mutualistic systems (Bascompte *et al.* 2006). So far, symmetric interactions have been reported only in very specific local communities, such as honeyeater-mistletoe facultative interactions (Reid 1990) or impoverished island systems (González-Castro *et al.* 2022). The disassortativity in the way species interact seems to promote asymmetry in partners' dependence. The absence of symmetry in the dependence between species agrees with previous work arguing that reciprocal specialisations are rare (Joppa *et al.* 2009).

Concluding remarks

Interactions between the individuals of a super-generalist plant with its fruit consumers have shown to be reciprocal in terms of rewards exchange, despite partners being highly asymmetric in their mutualistic dependence. These aspects appear quite general to less intimate mutualisms among free-living species (*e.g.*, pollination, seed dispersal) that are largely dependent upon interaction frequency for the harvesting of food resources by animals. A key feature for the success of super-generalist organisms appears to be related to abundance parameters that define their interaction frequency (Fort *et al.* 2016) and, ultimately, their fitness. In contrast, highly specialised interactions most likely depend on the ability to maintain reciprocity by means of a fine-tuned quality service between interacting species, where dependencies between partners would likely be more symmetric and intimate (Guimarães *et al.* 2007, Kiers *et al.* 2011). We might expect the emergence of high-reciprocity, high-asymmetry patterns when mutualisms among free-living species rely on encounter frequencies, whose variance among species is so large as to obscure variation in the quality of outcome. Exceptions may include some mutualisms in specific environmental settings (*e.g.*, oceanic islands) or characterised by high specificity of the interaction. Further studies on the reward reciprocity of generalised mutualistic interactions will help to evidence the diversity of engagement forms between animals and plants and the mechanisms behind the perpetuation of mutually-beneficial relationships.

Acknowledgements

We are grateful to Juan Miguel Arroyo who provided essential support in the laboratory by working on the DNA-barcoding analysis. The ‘Molecular Ecology Laboratory’ (LEM–EBD–CSIC; ISO9001:2015 and ISO14001:2015 certifications) provided logistical support for molecular analyses. Camera data collection was possible thanks to the help of Lewis Barrett and especially Antonio López-Orta who assisted with long-hours of video screening. Video screening time was greatly facilitated by the use of DeepMeerkat on the EBD-CSIC data server facility, thanks to the assistance of Ben Weinstein (software developer) on parameter selection and Luis Torres on software installation. EQ is grateful to Rosario and Luis Carlos who kindly helped by measuring individual plants’ fruit attributes. Discussions with Jorge Isla, Blanca Arroyo and Luísa Genes were invaluable to help improve and elucidate the implications of this study. We thank the logistic and facilities support form ICTS-RBD Doñana and the Doñana National Park for onsite access authorizations during the fieldwork.

Funding information: Agencia Estatal de Investigacion, Spain, Grant/Award Number: CGL2017-82847-P; Universidad de Sevilla / Junta de Andalucía / FEDER EU, Grant/Award Number: US-1381388; FEDER EU, Grant/Award Number: LIFEWATCH-2019- 09- CSIC-13; Fundación la Caixa, Grant/Award Number: LCF/BQ/DE18/11670007; VI Plan Propio de Investigación (Universidad de Sevilla), Grant/Award Number: VI PPIT-US-2018-IV.2.

APPENDICES CHAPTER 2

The data and code generated for Chapter 2 are archived in the Dryad digital repository: https://doi.org/10.5061/dryad.02v6wwq6n and the GitHub repository: https://github.com/PJordano-Lab/MS_effectiveness

Appendix 2A. Plant populations and frugivore species

We sampled two study sites in Doñana National Park (Huelva, Spain): La Mancha del Rabicano in El Puntal site (EP; coords: 36.965180, -6.446582) and Laguna de las Madroñas (LM; coords: 37.030317, -6.471945). Both areas consist of Mediterranean sclerophyllous scrubland dominated by lentiscs (*Pistacia lentiscus)* coexisting with other fleshy-fruited species such as *Phillyrea angustifolia*, *Olea europaea* var. *sylvestris*, *Asparagus aphyllus* and *Myrtus communis.* The presence of pine trees (*Pinus pinea*) is scattered at EP, but more abundant at LM. The lower sclerophyllous scrubland is dominated by *Ulex parviflorus*, *Halimium halimifolium* and *Cistus salviifolius*. We used 2-4 ha plots within more extensive areas (over ca. 50 ha) of *P. lentiscus*-dominated shrubland, being surrounded by successional low shrubland dominated by *Halimium halimifolium* in drier places and *Erica arborea* in more humid locations (Allier *et al.* 1974, Rivas-Martínez *et al.* 1980).

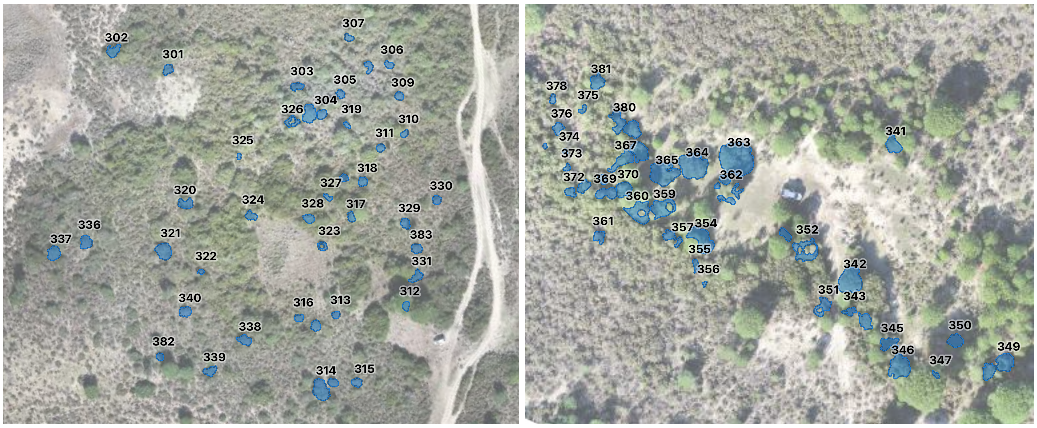


Figure A2.1. Aerial image showing individual plants of *Pistacia lentiscus* marked at El Puntal (EP) and Laguna de las Madroñas (LM) populations; 40 plants per study site. The individual plants' canopies are outlined in blue and numbered.

Pistacia lentiscus (Anacardiaceae) is a dioecious, anemophilous pollinated, animal-dispersed shrub that can be considered as a 'foundation species' (Whitham *et al.* 2006) playing a central role in the landscape physiognomy of in lowland Mediterranean scrublands. *Pistacia lentiscus* fruits are a staple food for frugivorous birds. Both the unripe (red) and ripe (black) fruits often have empty seeds as a result of either parthenocarpy, embryo abortion or pre-dispersal seed predation (Grundwag 1976, Jordano 1989). Frugivores strongly prefer the black, ripe fruits, and these typically have a higher proportion of filled, viable seeds (Jordano 1988b, 1989) yet they also consume (in lower proportion) red fruits, which frequently have empty seeds. As a result frugivores mostly disperse filled, viable seeds but together with a variable fraction of empty seeds (González-Varo *et al*. 2019a). The frequency of empty seeds varies greatly from year to year, as well as among *P. lentiscus* populations (Jordano 1988b, 1989; Verdú & García-Fayos 1998), resulting in variable amounts of empty seeds in the seed rain. In the focal study population, the mean percentage of empty seeds found in the plant canopy (estimated by floatability) was 67.5% at EP and 64.8% at LM (± 20.6% and 24.9% of SD respectively). At each site, we monitored 40 individual *P. lentiscus* plants for the complete 2019-20 fruiting season, totaling 80 focal individuals (Fig. A2.1).

Table A2.1. Frugivorous avian species considered in the study, average body mass, type of fruit consumption, and migratory status in the area. Types of frugivory acronyms: SD, seed disperser; SP, seed predator; PC, pulp consumer; PC/SD, pulp consumer with sporadic legitimate dispersal of seeds. Species are ordered by body mass (from Wilman *et al.* 2014).

Species	Body mass (g)	Type of frugivory	Migration
Columba palumbus	490.00	SD/SP	Resident
Turdus viscivorus	117.37	SD	Winter migrant
Turdus merula	102.73	SD	Resident
Cyanopica cooki	95.91	SD	Resident
Sturnus unicolor	83.66	SD	Resident
Turdus philomelos	67.74	SD	Winter migrant
Lanius meridionalis	60.43	SD	Resident
Coccothraustes coccothraustes	56.63	SP	Winter migrant
Chloris chloris	26.00	SP	Resident
Pyrrhula pyrrhula	24.26	PC/SD	Winter migrant
Fringilla coelebs	23.81	PC/SD	Resident
Curruca hortensis	21.90	SD	Summer migrant
Luscinia megarhynchos	19.60	SD	Summer migrant
Sylvia borin	18.20	SD	Summer migrant
Erithacus rubecula	17.70	SD	Winter migrant
Sylvia atricapilla	16.70	SD	Winter migrant
Parus major	16.25	PC/SD	Resident
Muscicapa striata	15.90	SD	Summer migrant
Curruca communis	15.10	SD	Summer migrant
Phoenicurus phoenicurus	14.59	SD	Summer migrant
Saxicola rubicola	14.09	SD	Resident
Ficedula hypoleuca	13.79	SD	Summer migrant
Cyanistes caeruleus	13.30	PC/SD	Resident
Curruca melanocephala	11.70	SD	Resident
Hippolais polyglotta	11.00	SD	Summer migrant
Curruca undata	10.80	SD	Resident
Curruca cantillans	9.60	SD	Summer migrant

Appendix 2B. Plant-animal interaction frequency

We used two distinct sampling methods to monitor interaction frequency of frugivores and plants: DNA-barcoding of bird faecal and regurgitated samples and continuous-monitoring cameras.

2B.1. DNA-barcoding sampling

Seed traps of 55 x 40 cm (0.22 m^2 trays) were located beneath the crown of individual plants, protected by a mesh of 1cm to prevent rodent predation. We placed one tray beneath every plant, except in four very large plants where we placed two trays. Seed traps were scanned fortnightly and all regurgitated and faecal samples in the tray were collected, regardless if they contained seeds or not. A total of 2691 samples were collected (1913 for EP and 778 for LM). On a few occasions, when the samples found in the trays were very abundant and presented identical aspect (e.g., multiple regurgitated seeds below a perch), a subset of samples were collected and the remaining count of seeds was assigned to the same species identified in the subset of samples obtained. Samples imputed this way represent 8% of the total samples obtained.

Animal-origin DNA was obtained from the surface of the samples (either scats or regurgitated seeds), was extracted and amplified using the primers COI-fsdF and COI-fsdR that target the COI region (cytochrome C oxidase subunit I; see González-Varo *et al.* 2014). Amplified DNA was then sequenced and identified using the Barcode Of Life Data (BOLD) Systems database (https://www.boldsystems.org/) or the Nucleotide Basic Local Alignment Search Tool (BLAST) from the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). DNA-barcoding analysis was carried out following the protocol described in González-Varo *et al.* (2014) with some modifications. In order to reduce time and costs, silica suspension addition step was removed, where instead DNA supernatant and binding buffer were added directly to the column with the microfiber filter. The column was then set for the second incubation period. This modification was based on the finding that DNA similarly attaches to the glass microfiber filter (Shi *et al.* 2018). Replacing silica suspension by glass microfiber filter we obtained similar identification yields and successful amplification rates. Columns brand (MoBiTec, Germany) was also replaced by another brand (ClearLine®, France, product # 007862CL ClearSpin inserted into 2 mL tube, product # 72691, Sarstedt, Germany). For the samples that failed to amplify using the COI-fsd primer pair, we amplified a smaller fragment of 272-bp from the 464-bp COI DNA región using the primers COI-fsd-degF and COI-fsdR and the nested PCR protocol described in González-Varo *et al.* (2017).

The number of samples collected under some plants was considerably high (over 200 samples in some cases). To ensure that the assemblage of avian visitors to individual plants was well characterised, we proceeded with DNA-barcoding laboratory analysis until sampling completeness was reasonably robust. A minimum of 40 samples per plant were analysed. This minimum however was subjected to sample availability, as some individuals had few samples or these were highly degraded samples not suitable for analysis. For plants with more than 40 samples, we gradually increased the number of DNA-barcoded samples until the sampling completeness curves were saturated (see Appendix 2B.3). In cases where individuals had fewer than 40 samples, we processed all the available samples for each individual that were suitable for analysis (*i.e.*, removing samples with highly degraded DNA). In total, we analysed 90% and 96% of the samples collected for EP and LM respectively. Identification success rate of the analysed samples was 94% (n = 2285). We established a quality criterion for DNA-barcoded samples, where we only considered samples over 150 bp length and over 90% of identity similarity. Most samples, however, scored over 99% similarity (mean length = 288 bp, mean similarity = 99.31%). For the minority subset of samples whose similarity was between 90%-99% (n = 228), the second species identified had to be further than 2% similarity distance, or absent in the geographical range area, as an additional quality requisite.

2B.2. Camera-trap sampling

In addition to DNA barcoding, we also used video monitoring to record animal visitation and feeding events in focal plants at EP site. Continuous-monitoring cameras (GoPro Hero® 7 White) were set facing individual plants, so that almost all of the plant could be seen from one side (Fig. A2.2). We recorded plants nine times spaced along the season, however some differences may exist between total recording times due to camera issues (see Table A2.2). Just in a few occasions, cameras turned off earlier due to battery issues or SD card was illegible. Cameras started recording between 8:00-10:00am for a period of approximately 2.2 hours. All individual plants were monitored every fortnight for a total of 9 times along the fruiting season, accumulating more than 19 hours of observation per individual plant on average (range = 18-20). Overall, cameras recorded 3790 visits by avian frugivores.

Figure A2.2. Photos captured from GoPro video recordings of *Erithacus rubecula* (top left) and *Curruca melanocephala* (bottom left) feeding on *Pistacia lentiscus* fruits. The image on the right shows the installation of the GoPro camera to record the interactions of visiting birds on individual plants.

We analysed the >700 hours of video recordings with the help of the motion detection program DeepMeerkat (Weinstein 2018). Motion detection helped to locate the specific moment of a visitation event, narrowing considerably the video screening time for analysis. DeepMeerkat was most helpful when the wind was mild, otherwise there were too many false positives caused by moving branches; in these cases the videos were fully watched to detect visitation events. We performed several trials to determine the best parameter threshold at which the DeepMeerkat algorithm was most sensitive (*i.e.,* detected most true positives), and settled on a tensorflow threshold (*i.e.*, confidence level to ignore movement detected; Weinstein 2018) of 10^{-11} and a minimum size of contour of 10^{-10}. We also carried out a parallel analysis of 22 videos to test the success rate of DeepMeerkat motion detection in comparison with detection by naked eye. Of a total of 46 interactions recorded in the test videos, four interactions were exclusively detected by DeepMeerkat and three by the naked eye, indicating very good performance of DeepMeerkat even though there is some trade-off between both approaches. The species exclusively detected with the program were mainly perching species (*P. phoenicurus* and *F. hypoleuca*) that can pass undetected to the naked eye by their stillness, whereas their fast arrival can be detected by the program. On the other hand, the three naked eye exclusive detections corresponded to *Curruca melanocephala* that tends to scurry around the plant, being easier to detect by the naked eye in a fast-paced video, but may become more cryptic for the program if the animal is moving behind vegetation.

For every visitation event we recorded the identity of the visiting species when possible, arrival and departure time, visit length, behaviour and number of fruits consumed, if any. Species identification was possible for 91% of the visits (n = 323 visits by unknown species). We extracted information on the feeding frequency of animal species (*i.e.*, fraction of visits where there was actual fruit consumption) and the number of fruits consumed per visit. We could detect feeding on fruits and/or seeds on 927 out of 3790 visits (24%), and recorded the number of consumed fruits or seeds whenever possible. A total of 37 animal species were identified visiting the individual plants. All were avian species with the exception of visiting cows (*Bos taurus*) and a rabbit (*Oryctolagus cuniculus*), none of these two mammals were feeding on the fruits of the plants. Of all the visitors recorded, 26 species were frugivorous birds (species known to feed on *P. lentiscus* fruits, even sporadically).

Table A2.2. Time in minutes spent recording individual plants along nine different periods.*B.3. Interaction accumulation curves*

ID plant	Sept.	Sept.	Oct.	Oct.	Nov.	Nov.	Dec.	Dec.	Jan.	Total time (min)
301	94	136	136	136	136	76	135	135	133	1117
302	88	135	136	136	136	136	136	135	135	1173
303	124	113	136	136	136	136	135	135	135	1186
304	96	120	136	136	136	136	135	126	135	1156
305	114	128	130	136	130	136	98	125	135	1132
306	127	135	136	136	127	136	135	121	135	1188
307	135	136	136	135	129	136	135	124	136	1202
308	35	135	136	135	128	136	135	121	135	1096
309	134	122	119	136	136	136	135	135	73	1126
310	122	124	121	136	136	136	136	135	134	1180
311	120	126	79	136	136	136	135	135	74	1077
312	131	130	130	136	109	136	136	135	135	1178
313	131	104	114	136	136	136	130	135	135	1157
314	131	136	136	134	136	136	135	135	133	1212
315	110	110	136	136	136	120	135	135	135	1153
316	115	135	136	136	136	136	135	135	135	1199
317	134	103	136	136	136	136	135	135	135	1186
318	135	129	136	135	135	136	135	135	135	1211
319	52	124	131	135	131	136	135	125	135	1104
320	112	135	133	136	135	136	135	135	135	1192
321	118	18	136	136	136	136	135	135	131	1081
322	0	135	136	136	136	136	135	135	127	1076
323	135	134	136	136	136	136	135	136	135	1219
324	135	118	136	136	136	136	135	135	135	1202
325	131	125	136	136	136	136	136	135	135	1206
326	124	105	136	135	133	136	135	127	135	1166
327	130	131	136	136	136	136	135	135	135	1210
329	100	88	123	136	136	136	135	135	47	1036
330	133	126	122	136	116	136	135	136	112	1152
331	135	128	128	136	136	136	135	135	49	1118
332	81	130	136	135	134	136	135	134	136	1157
334	50	119	136	136	100	136	135	135	135	1082
335	131	136	57	136	136	136	123	135	135	1125
336	86	119	136	136	136	136	135	135	135	1154
337	127	136	136	136	136	136	135	135	135	1212
338	118	136	136	136	136	135	136	90	132	1155
339	206	136	88	136	136	136	135	135	47	1155
340	0	136	136	136	136	135	135	135	134	1083
382	123	134	136	136	136	136	49	135	132	1117
383	87	127	125	136	136	136	135	135	75	1092

2B.3 Interaction accumulation curves

We used interaction accumulation curves (IAC, analogous to species accumulation curves) to determine both DNA-barcoding and video recording sampling completeness (Colwell & Coddington 1994, Jordano 2016). The number of samples collected in seed traps under individual lentiscs varied from 2 up to 203 for the whole fruiting season. Most plants (72 out of 80) had up to 90% of their samples analysed (see Fig. A2.3, Table A2.4). Overall sampling completeness was 93% for both methods (*sensu* Chacoff *et al.* 2012); 95% for cameras and 96% for DNA-barcoding (Table A2.3). The total number of frugivorous species recorded was 27; of which 26 were recorded with cameras and 22 with DNA-barcoding.

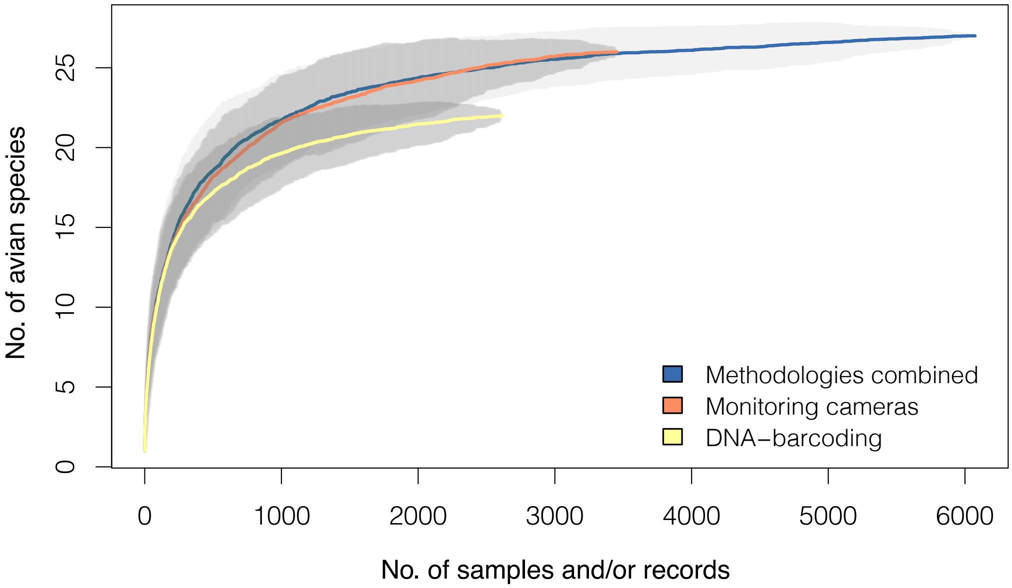


Figure A2.3. Interaction Accumulation Curves for animal interaction records with *Pistacia lentiscus* using two different methodologies, and the result of considering both together by combining the two datasets (see Quintero *et al*. 2022).

Table A2.3. Sampling completeness for interactions with *Pistacia lentiscus* using datasets resulting from different methodologies. Number of samples, number of species recorded, Chao estimator and its Standard Error, as well as completeness *(sensu* Chacoff *et al.* 2012) are provided. For the DNA barcoding samplings, results are given for EP and LM sites separately. Note that in LM site the Chao estimator is rather uncertain (SE > 10), hence the estimated completeness is rather uncertain too; in all cases, however, the empirical values fall within the ±1SE range of the estimator.

Dataset	N samples	Species	Chao	Chao SE	Completeness
Both methodologies	6073	27	29.0	3.7	0.93
Monitoring cameras	3456	26	27.5	2.3	0.95
DNA-Barcoding	2617	22	23.0	2.3	0.96
DNA-Barcoding EP	1851	21	22.0	1.9	0.95
DNA-Barcoding LM	766	16	26.0	10.2	0.62

Table A2.4. Number of species recorded per individual plant, total number of correctly identified frugivorous interactions records and its breakdown by the methodologies used. The total number of records refers to all samples/videos considered in the study (considering only successfully identified DNA-barcoding samples and only from avian frugivores). The table also shows the number of samples collected and analysed for DNA-barcoding methodology, as well as the total number of visits recorded and identified for the camera-traps. 'P' indicates the proportion of samples/videos analysed/identified with each methodology relative to the total obtained for each plant. Results are given separately for El Puntal (EP) and Las Madroñas (LM) sites, with only the former being monitored with the two methods.

EL PUNTAL SITE (EP)

Plant ID	Species	Total records	DNA-barcoding			Cameras		
			Samples collected	Samples analysed	P	Visits recorded	Visits identified	P
301	11	214	40	38	0.95	189	181	0.96
302	15	196	27	27	1.00	194	172	0.89
303	9	68	21	21	1.00	50	48	0.96
304	19	465	203	118	0.58	392	350	0.89
305	5	40	20	18	0.90	25	22	0.88
306	11	142	54	52	0.96	105	98	0.93
307	7	61	23	22	0.96	45	41	0.91

Plant ID	Species	Total records	DNA-barcoding			Cameras		
			Samples collected	Samples analysed	P	Visits recorded	Visits identified	P
308	7	134	71	71	1.00	68	63	0.93
309	9	80	35	35	1.00	45	45	1.00
310	7	87	30	29	0.97	62	58	0.94
311	8	56	23	22	0.96	36	36	1.00
312	7	88	64	60	0.94	32	29	0.91
313	9	122	44	43	0.98	83	80	0.96
314	18	307	136	102	0.75	240	205	0.85
315	10	147	51	50	0.98	119	101	0.85
316	6	46	15	15	1.00	33	31	0.94
317	5	60	28	27	0.96	38	34	0.89
318	9	213	106	92	0.87	139	125	0.90
319	11	122	43	43	1.00	84	83	0.99
320	9	208	134	113	0.84	109	102	0.94
321	11	167	65	63	0.97	120	108	0.90
322	12	110	67	64	0.96	53	49	0.92
323	11	160	61	50	0.82	116	113	0.97
324	8	71	22	22	1.00	56	49	0.88
325	8	91	41	41	1.00	54	52	0.96
326	11	120	57	57	1.00	74	66	0.89
327	10	126	79	75	0.95	66	55	0.83
329	11	124	45	43	0.96	85	82	0.96
330	9	94	33	32	0.97	66	63	0.95
331	8	91	35	35	1.00	63	59	0.94
332	11	146	52	50	0.96	109	97	0.89
334	10	139	53	52	0.98	99	91	0.92
335	8	98	41	41	1.00	60	58	0.97
336	11	147	51	51	1.00	108	99	0.92
337	17	244	65	64	0.98	205	183	0.89
338	12	206	94	84	0.89	146	125	0.86
339	7	42	16	16	1.00	28	26	0.93
340	10	120	28	28	1.00	104	94	0.90
382	8	37	19	19	1.00	20	18	0.90
383	7	118	55	55	1.00	70	65	0.93

LAGUNA DE LAS MADROÑAS SITE (LM)

Plant ID	Species	Total records	DNA-barcoding		
			Samples collected	Samples analysed	P
341	3	22	22	22	1.00
342	5	17	19	19	1.00
343	3	24	24	24	1.00
344	5	18	19	19	1.00
345	3	5	8	8	1.00
346	4	45	46	46	1.00
347	5	18	18	18	1.00
348	4	39	39	39	1.00
349	2	10	10	10	1.00
350	5	46	53	48	0.91
351	4	7	8	8	1.00
352	4	47	56	48	0.86
353	5	21	21	21	1.00
354	3	34	34	34	1.00
355	3	12	13	12	0.92
356	1	9	9	9	1.00
357	3	11	11	11	1.00
358	3	16	17	17	1.00
359	3	12	12	12	1.00
360	4	12	13	13	1.00
361	2	11	11	11	1.00
362	2	6	14	14	1.00
363	3	29	32	32	1.00
364	6	38	56	40	0.71
365	4	25	32	31	0.97
366	3	26	26	26	1.00
367	3	19	23	21	0.91
368	3	10	10	10	1.00
369	3	11	13	13	1.00
370	4	9	10	10	1.00
371	1	12	12	12	1.00
372	2	8	9	9	1.00
373	3	11	12	12	1.00
374	1	1	2	2	1.00
375	3	11	12	12	1.00
376	3	11	12	12	1.00
378	2	14	14	14	1.00
379	5	47	51	49	0.96
380	3	29	30	30	1.00

Appendix 2C. Interaction outcome for birds (quality of resource provisioning effectiveness)

To estimate differences in fruit quality provided by individual plants, we randomly collected ripe fruits (mean = 31 fruits, range = 17–63) from each individual plant at both populations and measured the whole fruit and the seed fresh mass. Pulp mass was calculated as the difference in weight between the whole fruit and the seed, *i.e.*, before and after being manually depulped (Fig. A2.4). This pulp and seed mass was later converted into energy obtained (see Appendix 2E.2), depending on the bird feeding behaviour (frugivorous or granivorous; see Table A2.1. for bird species categorization into frugivory types).

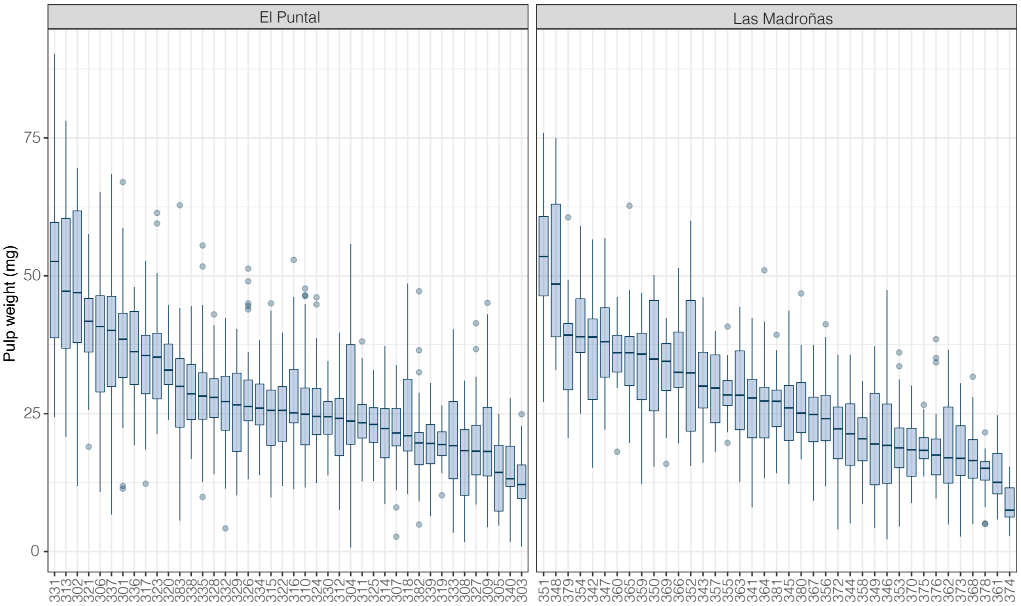


Figure A2.4. Pulp fresh mass per fruit (in mg) for individual plants in the two study populations. Each box represents the 1st–3rd interquartile range, the solid middle line represents the median, and whiskers extend to the largest or smallest value no further than 1.5 times the interquartile range; dots indicate more extreme values. Numbers at the bottom are the individual plant identification codes.

Appendix 2D. Interaction outcome for plants (quality of seed dispersal effectiveness)

In order to estimate the probabilities of seedling recruitment resulting from fruit/seed consumption by each avian consumer we considered four steps in chronological order: (1) probability of seeds to escape granivorous birds predation during handling, (2) microhabitat use patterns by each bird species, (3) probability of seeds escaping rodent post-dispersal predation in each microhabitat, and (4) probability of seedling emergence and early survival (past through their first summer) in each specific microhabitat.

2D.1. Seeds escaping avian predation

Some intact clean seeds found in the seed traps were attributed to *Chloris chloris*, *Fringilla coelebs* and *Pyrrhula pyrrhula* through DNA-barcoding (n = 36). This indicates that sporadic dispersal events by these granivores are possible if intact seeds are dropped during handling. To take this into account, we calculated the probability of seeds escaping predation by avian granivores using the total number of preyed-upon seeds (open seed endocarp halves) and the number of intact seeds attributed to granivores found in each seed tray.

2D.2. Microhabitat seed deposition

We classified the vegetation of both sites into five microhabitats for measuring seed dissemination and establishment success: (1) under *Pistacia lentiscus* conspecifics (PL), (2) under other fleshy fruited species (FR), (3) under non-fleshy fruited species (NF), (4) under pine trees (*Pinus pinea*; PP), and (5) open ground areas (OA). We expected different bird species to use these microhabitats with varying intensity, hence generating contrasting seed rain abundance and composition. Expected microhabitat variation in seed predator abundance and microclimatic conditions would also affect the fate of dispersed seeds (García *et al.* 2005, Gómez-Aparicio 2008).

In order to estimate the probability of dispersal of *Pistacia lentiscus* seeds towards each microhabitat, we collected dispersed seeds in the five microhabitats distributed along El Puntal (EP) area. For the *Pistacia lentiscus* (PL) microhabitat, we included all dispersed seeds collected in the seed trays beneath the 40 individual *P. lentiscus* plants monitored at EP site. For the other three microhabitats beneath vegetation cover (FR, NF and PP) we placed two seed-sampling trays (33 x 25.5 cm; 0.084 m^2) in 15 replicated locations per microhabitat. Lastly, for the open ground area (OA), we sampled 17 transects, 100 to 400 metres long and 1 m wide, at different times distributed along the fruiting season, and collected every faeces containing *P. lentiscus* seeds.

A total of 1664 seeds of *Pistacia lentiscus* were collected in the five microhabitats, of which 96% were analysed (n = 1594 seeds). The identity of the bird dispersing the seeds was determined through DNA-barcoding analysis, using the same protocol described above (see also Appendix 2B.1). DNA-barcoding identification success was 95%. The number of *P. lentiscus* seeds dispersed by each bird species to each microhabitat were then used to estimate their differential contributions to seed rain across microhabitats (see below).

2D.3. Seeds escaping rodent predation

To estimate post-dispersal predation rates we placed 6 experimental predation station replicates per microhabitat. Each experimental unit consisted of a petri dish open to rodents and a control plate protected with wire mesh of 1cm light to prevent rodent predation, each containing 10 seeds (Fig. A2.5). These controls allowed us to discern when the disappearance of a seed was not caused by rodents but by other animals, most likely ants. All seeds were ensured to be viable through flotation-sink experiments (Albaladejo *et al.* 2009) to avoid empty seed detection by the animals (Jordano 1989). The experimental units were checked every one or few days at the beginning of the experiment and then checks were gradually spaced over time (Fig. A2.6). Experimental units were installed in January 2019 and removed in July 2019, for a total of 131 days.

Figure A2.5. Photos of (top left) broken seeds without embryo after being preyed upon by rodents (*Mus spretus* and/or *Apodemus sylvaticus*); (top right) control (open) and experimental (protected with mesh wire) seed predation stations used in the field; (bottom left) experimental sowing station used in the field, and two-month old emerged seedlings (bottom right).

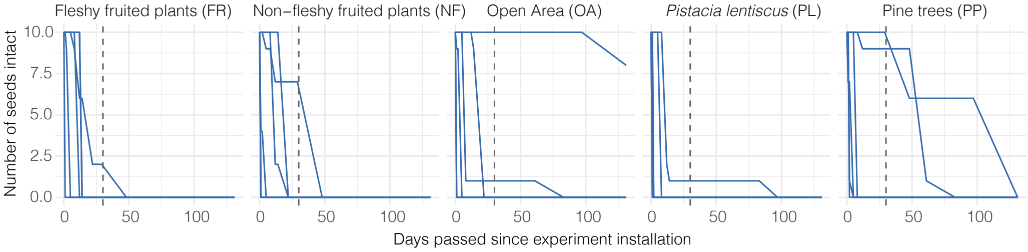


Figure A2.6. Number of seeds surviving rodent predation in the five microhabitats along time. Each line corresponds to an experimental station, each starting with 10 intact viable seeds in the beginning. The dashed vertical line represents the 30-day cutoff, which we considered as the critical period for predation as seedlings start to emerge around the fourth week since sowing.

2D.4. Seedling emergence and survival

We carried out experimental seed sowing to assess seedling emergence and early survival rates per microhabitat. These experiments were repeated for two consecutive years during the fruiting season of 2018-19 and 2019-20. We installed 6 germination stations the first season, and 7 the second season, in each microhabitat.. Germination stations consisted of 16 sown seeds spaced 1.5 cm between each other in a four by four grid, and protected by a 1cm-light wire mesh on the sides and a fibreglass mesh on top to prevent herbivory, debris and trampling (Fig. A2.5). All sowed seeds were checked to be viable through flotation-sink experiments and came from 8 and 6 different mothers for the first and second year, respectively. We ensured the mother origin of the seeds was equally distributed among all stations and microhabitats. Seeds were submerged in cold water for 24 h previous to sowing, as seedling emergence is conditioned to abundant rain events (García-Fayos & Verdú 1998, Del Campo *et al.* 2014). Germination experiments started in January of 2019 and in October of 2019. Seedling emergence and survival were monitored approximately every fortnight for the first four months after sowing and monthly thereafter until no seedlings remained alive (Fig. A2.7, Fig. A2.8).

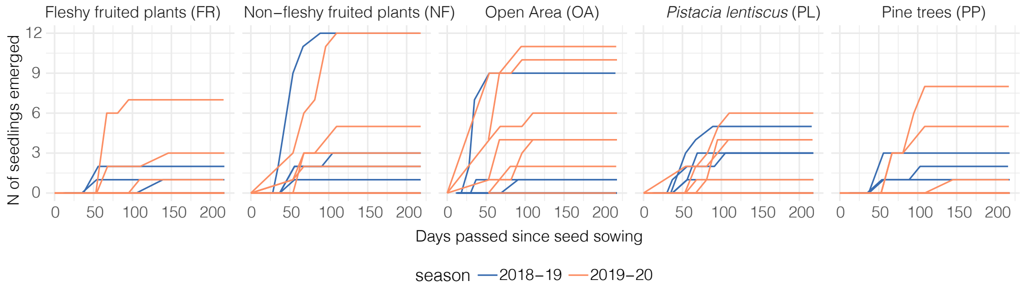


Figure A2.7. Seedling emergence dynamics in the experimental sowing units set up across the five microhabitats in two different seasons (2018-19 and 2019-20). Each line represents an experimental unit, consisting of 16 seeds. The number of experimental units per microhabitat was 6 in 2018-19 and 7 in 2019-20.

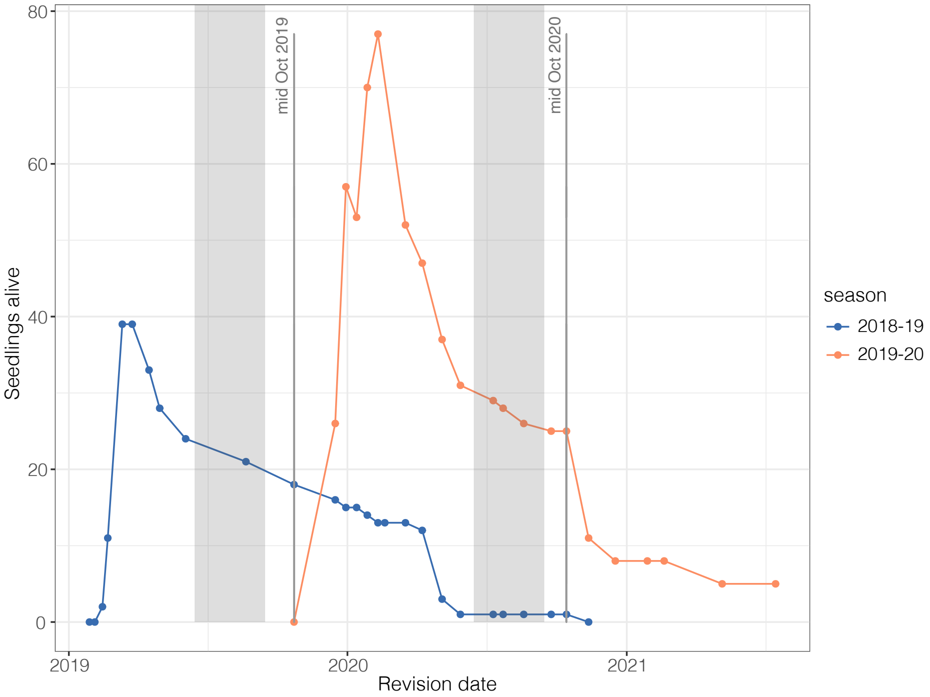


Figure A2.8. Number of seedlings recorded alive in the experimental sowing stations during two consecutive fruiting seasons, from January 2019 to July 2021. The number of seedlings recorded in a given date includes newly emerged seedlings as well as those surviving from previous dates. The shaded area in grey corresponds to the hottest months (from 15th June to 15th of September). For each season we quantified seedling survival just after their first summer (mid October, grey vertical lines).

Appendix 2E. Effectiveness calculations

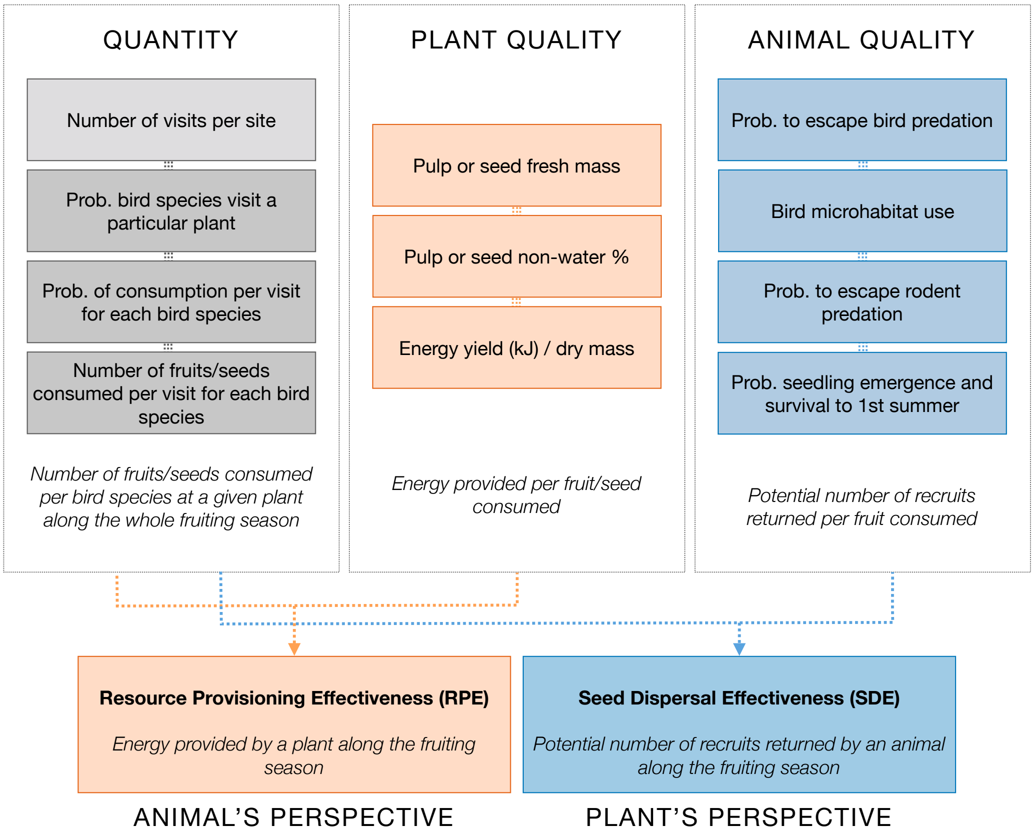


Figure A2.9. Diagram of all the elements involved in SDE and RPE calculations. The estimates for each component (*i.e.*, Quantity and Quality) are all chronological and multiplicative sequential steps (Schupp *et al.* 2017).

Calculating seed dispersal or resource provisioning effectiveness (SDE and RPE, respectively) requires large amounts of data (on bird visitation rates and fruit consumption patterns, seed rain density and post-dispersal survival, fruit weights, etc.; Fig. A2.9) which are rarely available for all the plants and bird species involved. Most effectiveness studies try to fill data gaps *ad hoc*, *e.g.*, assigning fruit consumption patterns from similar or related species, without considering uncertainties. Here we attempt a pure model-based approach to estimate all the components required to estimate SDE and RPE. In particular, we exploit hierarchical Bayesian models to share information ("borrow strength") across bird species and plant individuals, being able to obtain probabilistic estimates even for unobserved quantities. Furthermore, by using the full posterior distributions from all the estimated quantities we can propagate uncertainties and provide more realistic estimates of the uncertainty involved in such convoluted effectiveness analyses.

We explain how we estimated each component of RPE and SDE below. Once we had estimated each component, we multiplied the quantity and quality components to calculate the total effectiveness. The quantity component (*i.e.*, total number of fruits consumed by a specific bird on a given plant) was common for both the animal and plant's perspective. Quality for the animal was the energy acquired per fruit/seed consumed. Resource Provisioning Effectiveness (RPE) therefore represents the total energy acquired by a specific bird along the fruiting season from a given plant. Quality for the plant was the probability that a consumed and viable fruit becomes a seedling surviving its first summer. Seed Dispersal Effectiveness therefore indicates the potential number of seedlings recruited for a specific plant by a given bird species.

2E.1. Quantity component

We estimate the number of fruits consumed by each bird species at each individual plant combining the following quantities:

- Total number of bird visits received by plants at each site (estimated from bird droppings in seed traps beneath mother plants)
- Probability that different bird species visit a particular plant (estimated from both DNA barcoding and video cameras)
- Probability of fruit/seed consumption per visit for each bird species (estimated from video recordings)
- Number of fruits/seeds consumed per visit of each bird species (estimated from video recordings)

Total number of visits and probability of visit to each individual plant by each bird species

To estimate the probability of visit to each plant from each bird species we used data from DNA barcoding of droppings collected beneath mother plants (both sites), as well as data obtained through the analysis of video recordings (Puntal site only). Estimates from barcoding and video analysis for El Puntal site were then merged (see below).

Estimating probability of visit from barcoding data

For each of both sites, we used all bird droppings collected at seed trays beneath lentisc plants to estimate the total number of bird visits to each individual plant *i* along the fruiting season, using a hierarchical Bayesian Poisson regression:

$$Nvisit_{DNAi} \sim Poisson(\lambda_{DNAi})$$

where the log number of visits received by each individual *i* (λ_{DNAi}) was modelled as

$$log(\lambda_{DNAi}) = \mu_{visitDNA} + \alpha_{DNAi} + \text{offset}(log(\text{trap.area}_i))$$

$$\alpha_{DNAi} \sim N(0, \sigma^2_{DNAvisit})$$

In this equation, $\mu_{visitDNA}$ is the average number of bird visits across all individual plants over the season, and α_{DNAi} represents individual variation around that population average (*i.e.*, a random intercept), drawn from a Normal distribution with standard deviation $\sigma_{DNAvisit}$. We included an offset term to account for the fact that sampling effort was not constant among individuals (4 plants at El Puntal site had 2 seed trays placed beneath, while all other individuals had 1 seed tray). Hence, all parameter estimates refer to visits/m^2 of canopy area. Note this model assumes that each bird dropping corresponds to a single visit.

We used weakly informative Normal priors for all parameters, and performed prior predictive checks in all models to ensure that our priors produced reasonable estimates. $\mu_{visitDNA}$ had a Normal(4, 1) prior in log scale, corresponding to c. 50 bird visits per square metre of canopy area over the whole season. $\sigma_{DNAvisit}$ had a half-Normal prior with standard deviation = 1, *i.e.*, Normal(0, 1) truncated at 0.

Once we had estimated the number of visits to each individual plant (taking into account their total canopy area as measured from the drone image; Fig. A2.1), we could calculate the total number of visits per site (aggregating all individuals) and the relative probability of visit of each individual plant ($Pvisit_{DNAi}$) by dividing their visits by the total number of visits at the site.

Then, we estimated the probability that a given visit is from a given bird species ($Pbird_{DNAij}$). In other words, the proportion of visits from each bird species (as identified from DNA barcoding) at each plant. For that, we modelled the number of visits from each bird species *j* to each individual plant *i* following a Binomial distribution and a logit link ($log(P/(1-P))$):

$$Nvisit_{DNAij} \sim Binomial(Nvisit_{DNAi}, Pbird_{DNAij})$$

$$logit(Pbird_{DNAij}) = \mu_{birdDNA} + \alpha_{DNAi} + \alpha_{DNAj} + \alpha_{DNAij}$$

$$\alpha_{DNAi} \sim N(0, \sigma^2_{DNAplant})$$

$$\alpha_{DNAj} \sim N(0, \sigma^2_{DNAbird})$$

$$\alpha_{DNAij} \sim N(0, \sigma^2_{DNAplant-bird})$$

Hence, we used random effects for both bird species and individual plants as well as their interaction to obtain the probability of visit from each bird species to each individual plant. Standard deviation parameters had half-Normal priors with large standard deviations ($\sigma = 3$) as the variation in visitation rate among bird species is usually quite large. The prior average number of visits from a given bird species on a given plant ($\mu_{birdDNA}$) was set rather low: Normal(- 6.5, 1) on logit scale, as most bird species do not visit most plants.

Finally, we calculated the posterior probability of visit from each bird species to each individual plant ($Pvisit.bird_{DNAij}$) as the product of the probability of visit for each plant at each site ($Pvisit_{DNAi}$) and the relative probability of visit for each bird species on each plant ($Pbird_{DNAij}$ in the Binomial model above).

Estimating probability of visit from video analysis

We used similar reasoning and models to estimate the probability of visit from each bird species to each individual plant from video records. First, we estimated the number of bird visits per hour to each individual plant *i* using a Poisson distribution:

$$Nvisit_{CAMi} \sim Poisson(\lambda_{CAMi})$$

where the log number of visits received by each individual (λ_{CAMi}) was modelled as

$$log(\lambda_{CAMi}) = \mu_{CAMvisit} + \alpha_{CAMi} + \text{offset}(log(\text{recording.time}_i))$$

$$\alpha_{CAMi} \sim N(0, \sigma^2_{CAMvisit})$$

In this equation, $\mu_{CAMvisit}$ is the average number of bird visits across all individual plants, and α_{CAMi} represents individual variation around that population average (*i.e.*, a random intercept), drawn from a Normal distribution with standard deviation $\sigma_{CAMvisit}$. We included an offset term to account for different recording time among individual plants (range = c. 18 - 20 hours, Table A2.2).

We used weakly informative Normal priors for all parameters: $\mu_{CAMvisit}$ had a Normal(1.4, 1) prior (in log scale), corresponding to c. 4 bird visits per hour. $\sigma_{CAMvisit}$ had a half-Normal prior with standard deviation = 1, *i.e.*, Normal(0, 1) truncated at 0.

Once we had estimated the number of visits/h to each individual plant, we could calculate their relative probability of visit ($Pvisit_{CAMi}$) by dividing each plant's visits by the total number of bird visits to all individuals at the site.

Then, we estimated the probability that a given visit at each plant is from a given bird species ($Pbird_{CAMij}$). For that, we modelled the number of visits from each bird species *j* to each individual plant *i* following a Binomial distribution:

$$Nvisit_{CAMij} \sim Binomial(Nvisit_{CAMi}, Pbird_{CAMij})$$

$$logit(Pbird_{CAMij}) = \mu_{CAMbird} + \alpha_{CAMi} + \alpha_{CAMj} + \alpha_{CAMij}$$

$$\alpha_{CAMi} \sim N(0, \sigma^2_{CAMplant})$$

$$\alpha_{CAMj} \sim N(0, \sigma^2_{CAMbird})$$

$$\alpha_{CAMij} \sim N(0, \sigma^2_{CAMplant-bird})$$

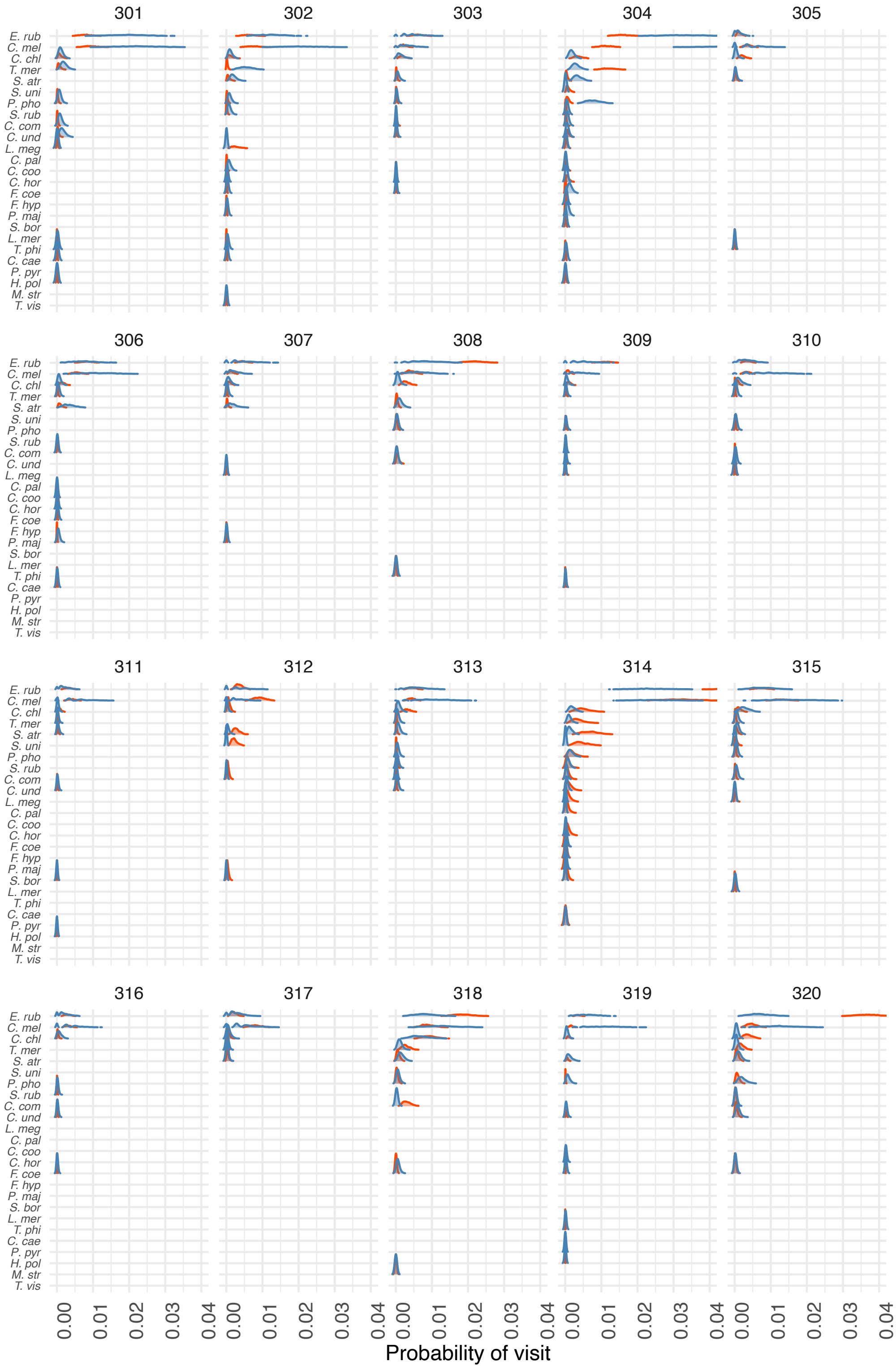
As for barcoding data, we used random effects for both bird species and individual plants as well as their interaction to obtain the probability of visit from each bird species to each individual plant. Standard deviation parameters had half-Normal priors with large standard deviations ($\sigma = 3$), and the prior average number of visits from a given bird species on a given plant ($\mu_{CAMbird}$) had Normal(-6.5, 1) prior on logit scale.

Finally, we calculated the posterior probability of visit from each bird species to each individual plant ($Pvisit.bird_{CAMij}$) as the product of the probability of bird visit for each plant at each site ($Pvisit_{CAMi}$) and the relative probability of visit for each bird species on each plant ($Pbird_{CAMij}$ in the Binomial model above).

Merging of visitation estimates from DNA barcoding and video monitoring

The parallel analyses of visitation rates from both videos and DNA barcoding data produced compatible pairwise probabilities of visit ($Pvisit.bird_{ij}$) for each plant-bird species pair at El Puntal site (Fig. A2.10). We averaged the posterior distributions of both probabilities to obtain the consensus probability of visit arising from the combination of both data sources (barcoding and videos; Fig. A2.11). This estimate could be interpreted as the consensus probability that a given bird visit at the site involves a particular bird species and individual lentisc plant. For most bird species, both methods produced quite similar probabilities of visit, and the consensus probability only reinforced those estimates. When DNA barcoding and videos suggested different probabilities of visit for some plant-bird species pair, the spread of each posterior distribution offered a natural weighting so that more uncertain estimates (from whichever method) had less influence on the final consensus probability.

At Las Madroñas site, where video recordings were not available, the pairwise probabilities of visit were estimated based on barcoding data only (Fig. A2.12).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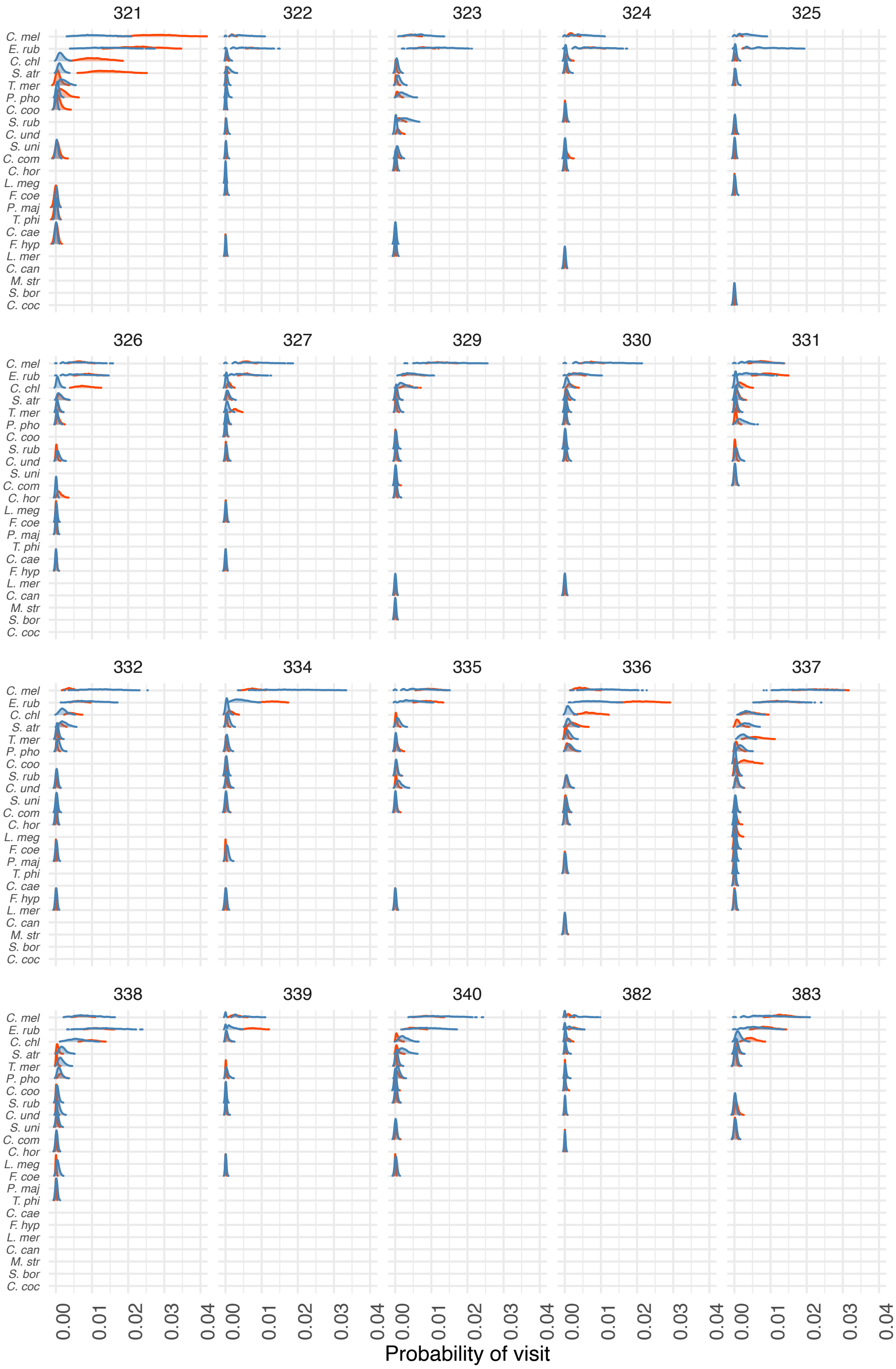

Figure A2.10. Estimated probability of visit from each bird species to each individual plant at El Puntal site. Posterior distributions in red and blue colours represent estimates arising from DNA barcoding and video cameras, respectively. Panel numbers represent different plant IDs.

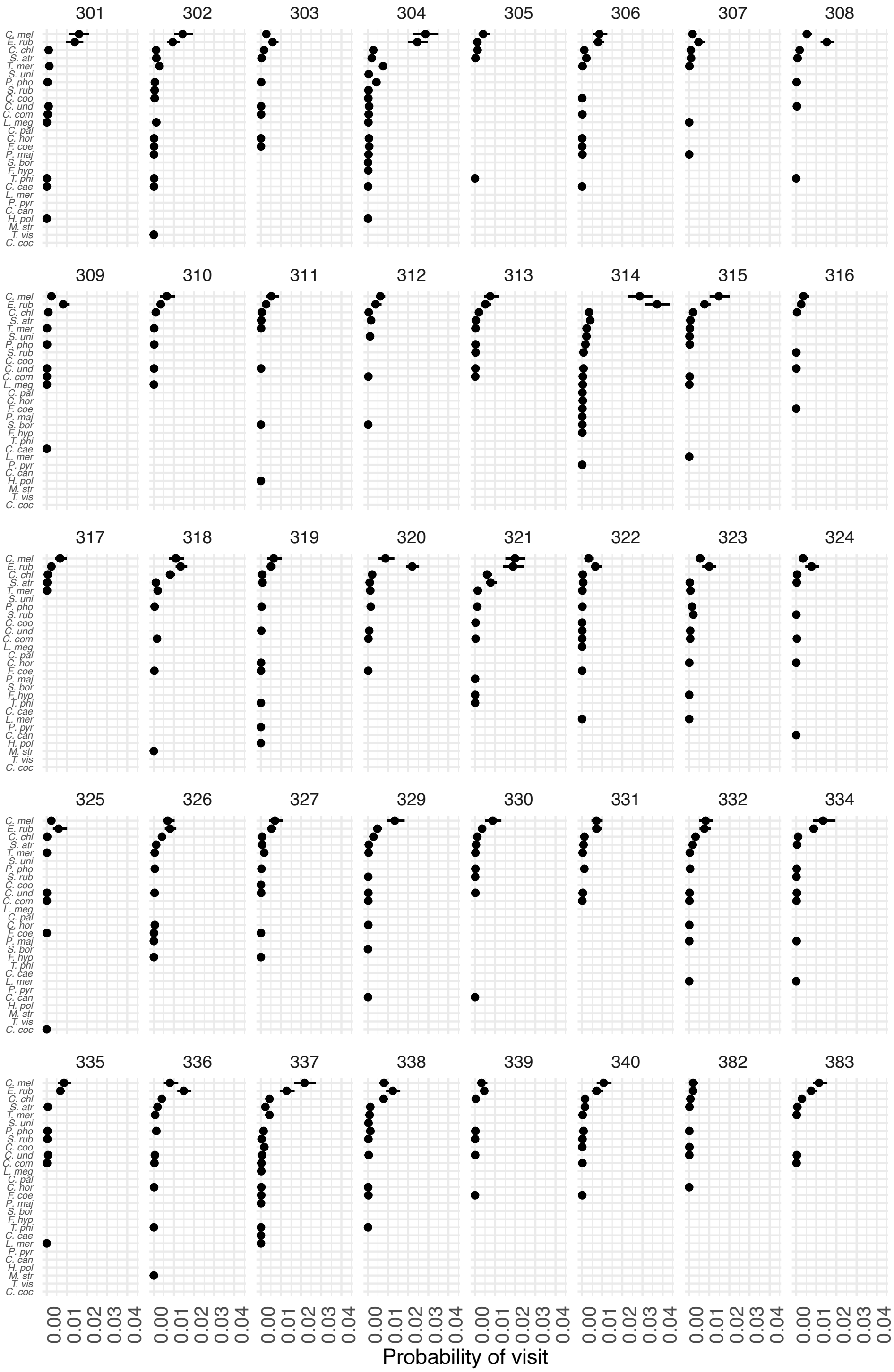

Figure A2.11. Consensus probability of visit from each bird species to each individual plant at El Puntal site, obtained by averaging posterior distributions from DNA barcoding and video cameras. Intervals represent Bayesian 80% confidence intervals.

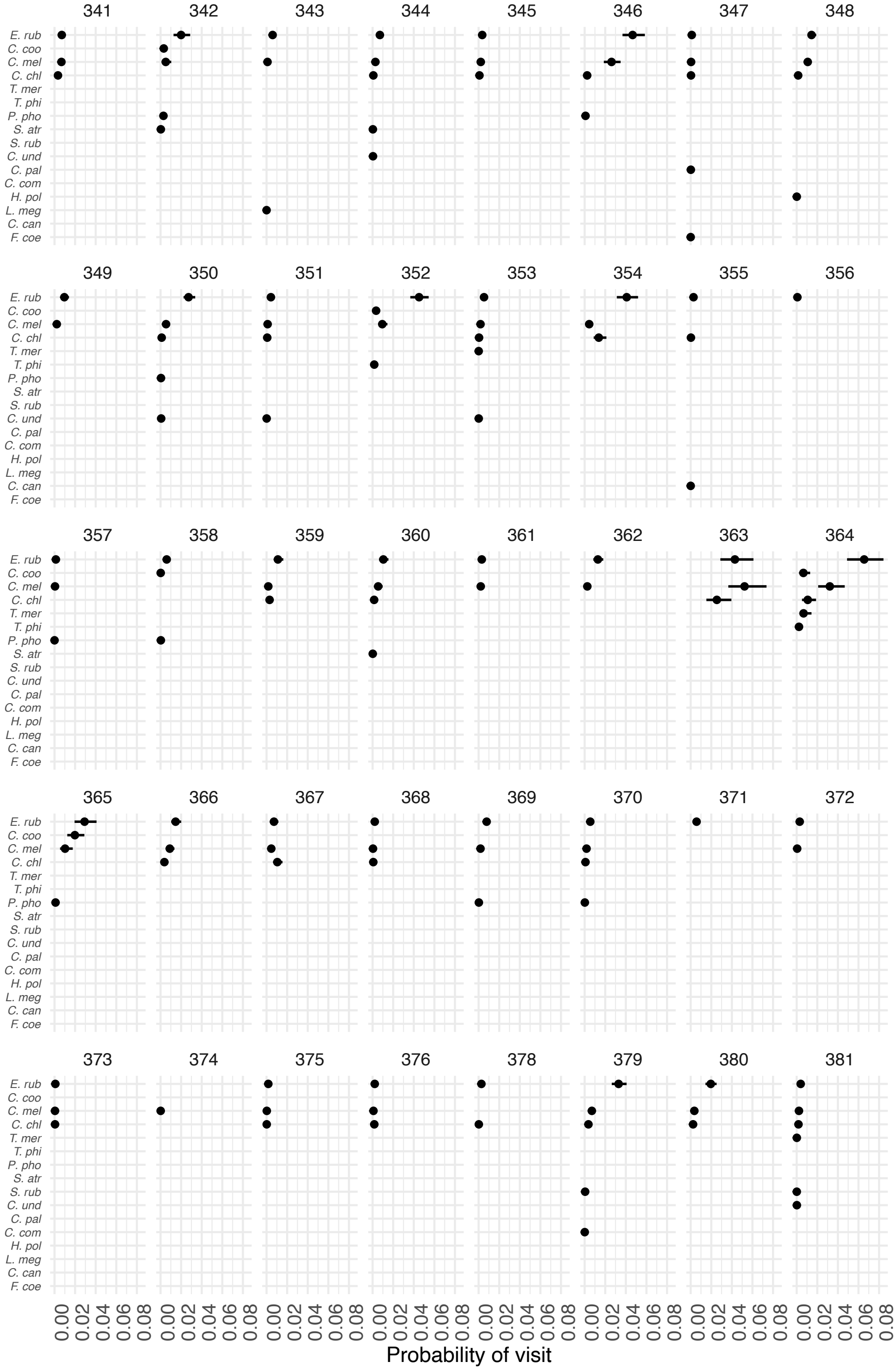

Figure A2.12. Posterior probability of visit from each bird species to each individual plant at Las Madroñas site, estimated from DNA barcoding of bird droppings beneath each plant. Intervals represent Bayesian 80% confidence intervals.

Probability of fruit/seed consumption per visit

To estimate the proportion of bird visits involving fruit or seed consumption we used information on feeding bouts obtained from video recordings (Fig. A2.13). For each bird species we recorded the number of visits involving feeding and those where the bird left the plant without consuming any fruit or seed. To obtain the probability of feeding ($Pfeed_j$) for each bird species we analysed these data using a Bernoulli distribution and a random effect for bird species:

$$Feed_j \sim Bernoulli(Pfeed_j)$$

$$logit(Pfeed_j) = \mu_{feed} + \alpha_j$$

$$\alpha_j \sim N(0, \sigma^2_{feed})$$

μ_{feed} had a weak prior probability Normal(0, 2) on logit scale, corresponding to a very uncertain probability of feeding centred around 0.5. σ_{feed} had the same prior N(0, 2) allowing for different visiting and feeding behaviours among bird species.

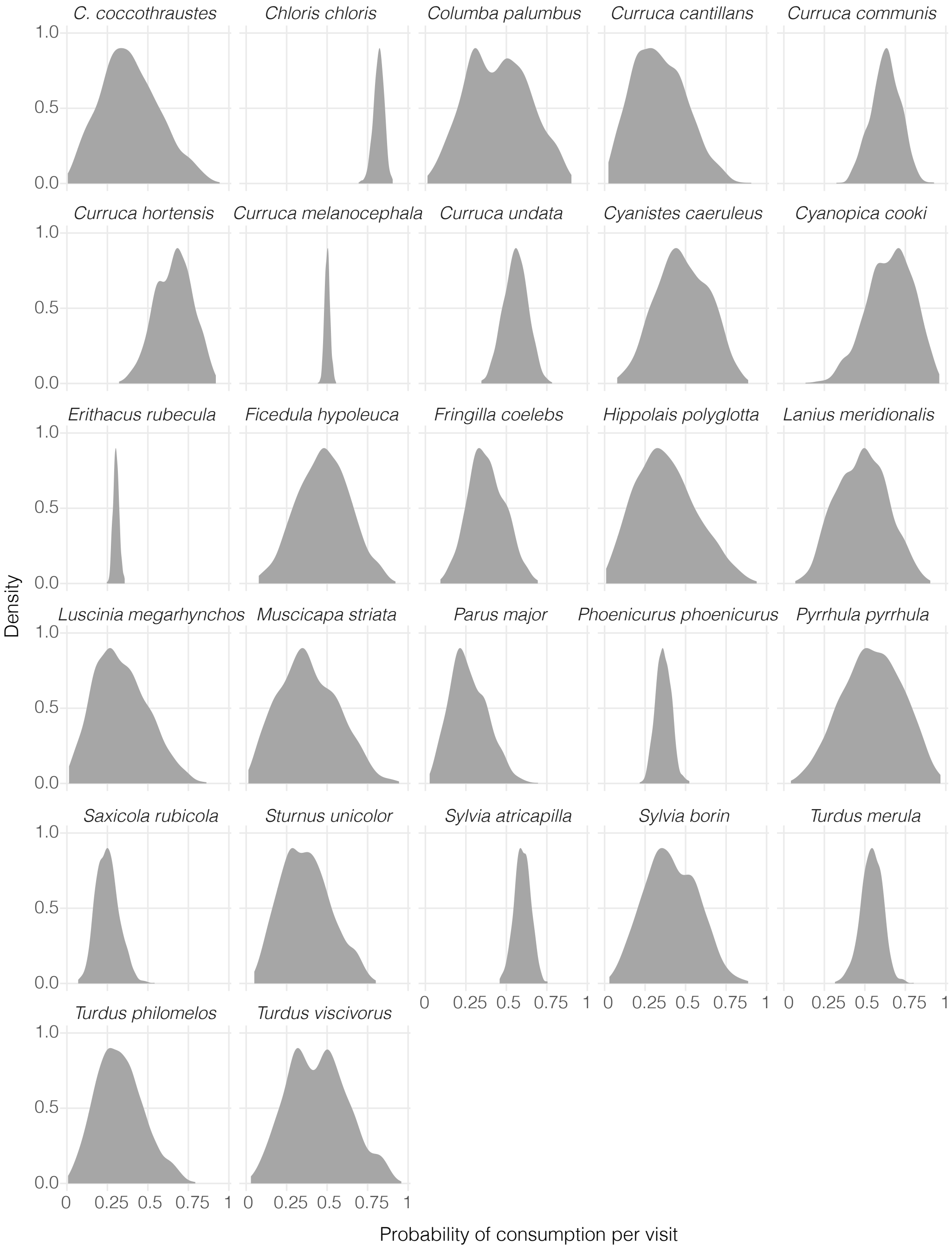

Figure A2.13. Estimated probability for each bird species of consuming at least one fruit or seed when visiting lentisc plants.

Number of fruits/seeds consumed per visit

Once we had estimated the number of visits from each bird species and their probability of feeding in each visit, we estimated the number of fruits or seeds consumed in feeding visits ($Nfruit_j$; Fig. A2.14) using a Negative Binomial distribution with a random effect for bird species and including body mass (from Elton Traits: Wilman *et al.* 2014) as a covariate:

$$Nfruit_j \sim NegBinomial(\lambda_j, \phi_{fruit})$$

$$log(\lambda_j) = \mu_{fruit} + \alpha_j + \beta_{BM}\, log(\text{bodymass}_j)$$

$$\alpha_j \sim N(0, \sigma^2_{fruit})$$

As 'bodymass' predictor was centred around 20 grams value, μ_{fruit} is the expected log number of fruits/seeds consumed by a bird species with 20 grams of body mass, and was assigned a Normal(0.7, 0.3) prior distribution on log scale, corresponding to an expected grand mean of 2 fruits consumed per visit. α_j is the bird species random effect. Its standard deviation σ_{fruit} had a half-Normal prior with standard deviation of 0.5 units. The β_{BM} parameter represents the expected increase in fruit consumption with increasing body mass and had a weakly informative Normal(0.5, 0.5) positive prior since the amount of fruits consumed is generally positively associated to bird size. Finally, the ϕ_{fruit} parameter accommodates overdispersion in the count data (Winter & Bürkner 2021) and had a Gamma(0.01, 0.01) prior distribution.

Since we modelled the probability of consumption independently, here we only used feeding observations where at least one fruit or seed was consumed. Thus, we used a lower truncation value of one. Also, since *Chloris chloris* is an eager seed predator with radically different feeding behaviour, we modelled this species independently to preserve the assumption of exchangeability of the random effects (Kéry & Schaub 2011). In this case we ran an intercept-only truncated negative binomial model with Normal(2, 0.5) prior distribution on log scale, corresponding to a mean of c. 7.4 seeds consumed per visit.

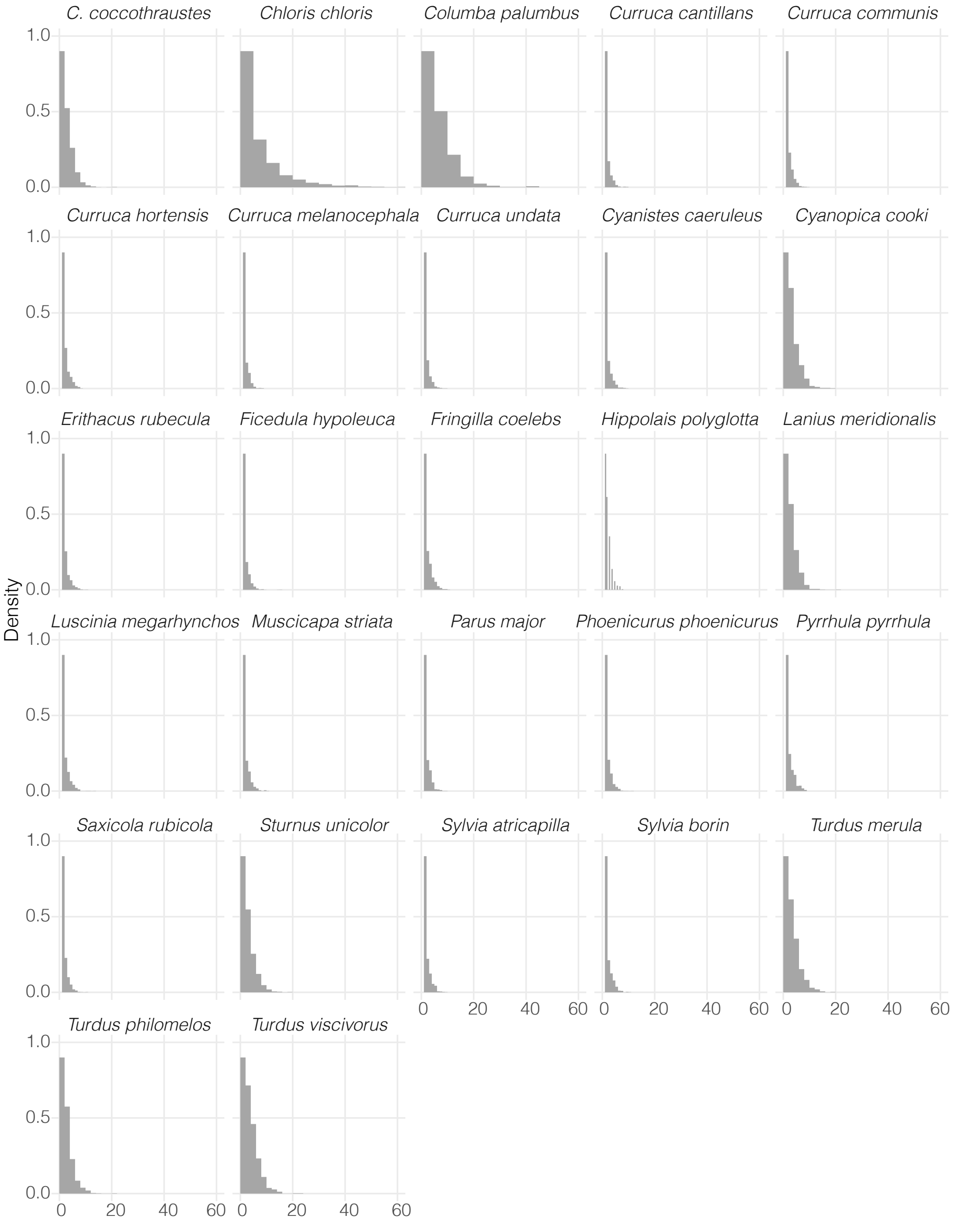

Figure A2.14 Estimated number of fruits consumed per visit by each bird species.

Calculating the quantity component

To calculate the number of fruits/seeds consumed by each bird species from each plant (the quantity component QTY for both effectiveness estimates RPE and SDE), we multiplied all the posterior distributions estimated previously, namely (i) the total number of bird visits at each site *k* ($Nvisit_k$), (ii) the probability of visit from each bird species *j* to each plant *i* ($Pvisit.bird_{ij}$), (iii) the probability that a visit from a bird species *j* involves fruit or seed consumption ($Pfeed_j$), and (iv) the number of fruits/seeds consumed per feeding visit ($Nfruit_j$):

$$QTY_{ij} = Nvisit_k \times Pvisit.bird_{ij} \times Pfeed_j \times Nfruit_j$$

2E.2. Quality component of Resource Provisioning Effectiveness

To estimate the quality of individual plants' reward we calculated the energy acquired per fruit (for pulp consumers) or seed consumed (for granivorous birds). Energy calculations were based on: seed/pulp fresh mass, percentage of water in seed/pulp and the energetic yield factors for seed and pulp dry mass.

Pulp and seed fresh mass of individual plants (Appendix 2C) was converted to dry mass using information available on % water content in seed and pulp reported in nearby *P. lentiscus* populations (Jordano 1984). To estimate the energy rendered per g of dry seed mass, we used a yield factor of 28.14 kJ/g (Khiari *et al.* 2020). Pulp yield energy was estimated based on lipid, carbohydrate and protein percentages (5.5 % proteins, 58.8 % lipids and 25.8 % carbohydrates; Herrera 1987). These percentages were multiplied by standard energy conversion factors for major nutrients in fruits (14.1 kJ/g for proteins, 35.0 kJ/g for lipids and 15.1 kJ/g for carbohydrates; MacLean *et al.* 2003). The resulting energetic yield factor for pulp dry mass was 25.25 kJ/g.

Final quality was then calculated as the product of seed/pulp fresh mass (g), non-water % in seed/pulp, and seed/pulp yield energy factor (kJ/g). Variations in seed and fruit quality between individual plants were therefore based on differences between the fresh mass of pulp and seed.

2E.3. Quality component of Seed Dispersal Effectiveness

Probability of escaping granivorous birds predation

For granivorous birds (*Chloris chloris, Pyrrhula pyrrhula, Fringilla coelebs and Coccothraustes coccothraustes*) we estimated the probability of seeds escaping predation as they are dropped during handling. This is a very rare event, but it happens occasionally. To estimate its frequency we counted the number of intact and destroyed seeds collected in seed traps beneath lentisc plants, and fitted a hierarchical Binomial regression:

$$Ndropped_i \sim Binomial(Nseed_i, Pdrop_i)$$

$$logit(Pdrop_i) = \mu_{drop} + \alpha_i$$

$$\alpha_i \sim N(0, \sigma^2_{drop})$$

where the proportion of intact (dropped) seeds beneath each mother plant, or probability of escaping predation ($Pdrop_i$), is modelled as a random effect with mean μ_{drop} with a weak Normal(-6.9, 2) prior distribution (corresponding to one seed per thousand escaping predation on average) and standard deviation σ_{escape} with a half-Normal(0, 1) prior. This analysis reported a posterior probability of escaping predation of 0.0014 ± 0.0005 (mean ± SE).

Probability of dispersal to different microhabitats

For each bird species we estimated the probability of dispersing seeds to each of the five microhabitats defined (PL: under *Pistacia lentiscus* plants, FR: under other fleshy fruited species, NF: under non-fleshy fruited species, OA: open ground areas, P: under pine trees). For that we used two steps: first we modelled the total number of seeds arriving to each microhabitat, and then we identified the proportion of seeds brought by each bird species using the identifications obtained through DNA barcoding of bird droppings.

To estimate the seed rain density in each microhabitat we modelled the total number of seeds arriving per m^2 using a Negative Binomial distribution:

$$Nseed_s \sim NegBinomial(\eta_s, \phi_s)$$

$$log(\eta_s) = \mu_{FR} + \mu_m + \text{offset}(log(\text{sampling.area}_s))$$

Here, $Nseed_s$ is the total number of seeds collected at each seed trap or transect, μ_{FR} is the average number of seeds arriving per m^2 in the FR microhabitat (taken as intercept), μ_m is the average difference between each microhabitat and FR, and ϕ_s accommodates overdispersion in the count data. μ_{FR} had a weakly informative Normal(3, 2) prior centred around 20 seeds/m^2, and μ_m had a Normal(0, 2) prior, allowing for large differences in seed rain density among microhabitats. ϕ_s had a Gamma(0.01, 0.01) prior. We used an offset to account for different sampling area across microhabitats.

To estimate the proportion of seeds contributed by each bird species *j* to each sampling station *s* in each microhabitat *m*, we used a hierarchical Binomial model:

$$Nseed_{sj} \sim Binomial(Nseed_s, Pseed.bird_{mj})$$

$$logit(Pseed.bird_{mj}) = \beta_{FRj} + \beta_{NFj} + \beta_{OAj} + \beta_{PLj} + \beta_{PPj}$$

$$\begin{pmatrix} \beta_{FRj} \\ \beta_{NFj} \\ \beta_{OAj} \\ \beta_{PLj} \\ \beta_{PPj} \end{pmatrix} \sim N\left(\begin{pmatrix} \mu_{\beta_{FRj}} \\ \mu_{\beta_{NFj}} \\ \mu_{\beta_{OAj}} \\ \mu_{\beta_{PLj}} \\ \mu_{\beta_{PPj}} \end{pmatrix}, \begin{pmatrix} \sigma^2_{\beta_{FRj}} & \rho_{\beta_{FRj}\beta_{NFj}} & \rho_{\beta_{FRj}\beta_{OAj}} & \rho_{\beta_{FRj}\beta_{PLj}} & \rho_{\beta_{FRj}\beta_{PPj}} \\ \rho_{\beta_{NFj}\beta_{FRj}} & \sigma^2_{\beta_{NFj}} & \rho_{\beta_{NFj}\beta_{OAj}} & \rho_{\beta_{NFj}\beta_{PLj}} & \rho_{\beta_{NFj}\beta_{PPj}} \\ \rho_{\beta_{OAj}\beta_{FRj}} & \rho_{\beta_{OAj}\beta_{NFj}} & \sigma^2_{\beta_{OAj}} & \rho_{\beta_{OAj}\beta_{PLj}} & \rho_{\beta_{OAj}\beta_{PPj}} \\ \rho_{\beta_{PLj}\beta_{FRj}} & \rho_{\beta_{PLj}\beta_{NFj}} & \rho_{\beta_{PLj}\beta_{OAj}} & \sigma^2_{\beta_{PLj}} & \rho_{\beta_{PLj}\beta_{PPj}} \\ \rho_{\beta_{PPj}\beta_{FRj}} & \rho_{\beta_{PPj}\beta_{NFj}} & \rho_{\beta_{PPj}\beta_{OAj}} & \rho_{\beta_{PPj}\beta_{PLj}} & \sigma^2_{\beta_{PPj}} \end{pmatrix}\right)$$

The probability that a seed arriving at a given microhabitat is brought by bird species *j* was modelled as a random effect where parameters (β_{FRj}, β_{NFj}, β_{OAj}, β_{PLj}, β_{PPj}) are drawn from a multivariate Normal distribution. $\mu_{\beta FRj}$ had a weak Normal(-3.3, 1) prior (assuming equal prior probability among bird species as the inverse logit of -3.3 ≈ 1/27 bird species), the σ parameters had half-Normal(0, 2) priors, and the correlation matrix among β parameters had LKJ(2) prior distribution.

Then, to estimate the number of seeds dispersed to each microhabitat by each bird species we multiplied the posterior from the first model (total seed rain per microhabitat) with the probability that seeds are brought by each bird species (model above). For each bird species, the relative probability of dispersing seeds to each microhabitat (Fig. A2.15) can finally be calculated as the ratio of the number of seeds dispersed to each microhabitat by the total number of seeds dispersed.

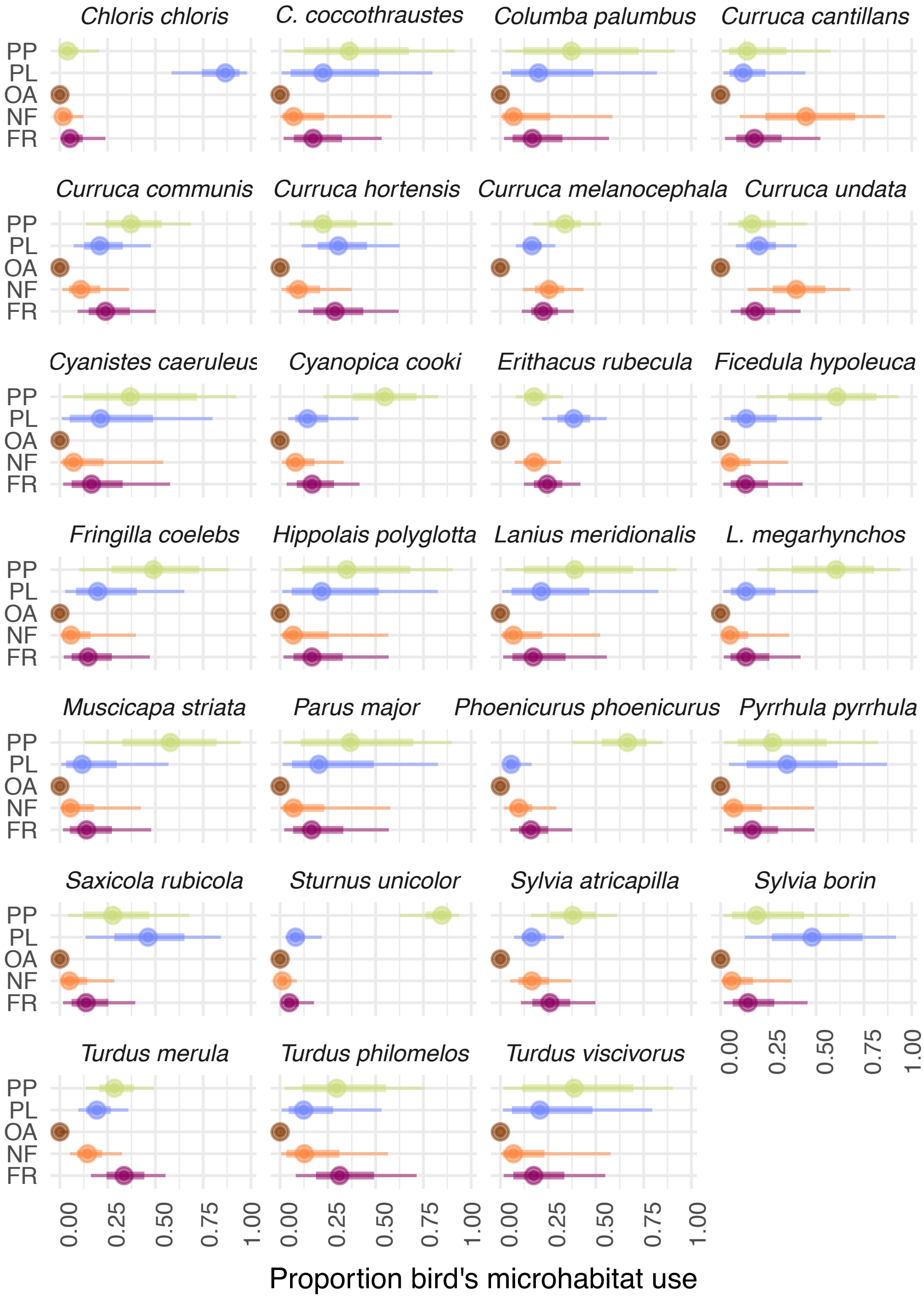

Figure A2.15. Posterior probabilities of dispersal to each microhabitat by each of the 27 bird species consuming *Pistacia lentiscus* fruits or seeds (FR: under fleshy fruited species (non–*Pistacia*), NF: under non–fleshy fruited species, OA: in open ground areas, PL: under *Pistacia lentiscus* stands, PP: under pine trees). Dots denote median probability, and thick and thin bars represent 66% and 95% credible intervals, respectively.

Probability of escaping post-dispersal predation

Rodent predation was quite fast and severe. Two days after installing the experiment, half of the experimental units had received full or partial predation. Within the first month more than 90% of the open units had experienced predation. At the same time, our seedling emergence experiments showed that seedlings started emerging on the 28th day after sowing, which agrees with published evidence of early germination and emergence in the species (García-Fayos & Verdú 1998, Del Campo *et al.* 2014). Therefore we considered the first month after dispersal as the critical period for seeds to be preyed upon before germination.

Our mesh-protected experimental units failed to prevent rodent predation on several occasions, hence we discarded using the data from these controls for the analysis of predation escape. In those control units that effectively repelled rodents, seeds typically remained intact for a long time, suggesting that most seed predation is actually done by rodents.

To estimate the probability of surviving post-dispersal predation in each microhabitat (Fig. A2.16), we thus counted the number of intact (not predated) seeds within the first month after installing the experiment. We used a Binomial distribution:

$$Nintact_u \sim Binomial(10, Pescape_u)$$

$$logit(Pescape_u) = \mu_{escapeFR} + \beta_{escapeNF} + \beta_{escapeOA} + \beta_{escapePL} + \beta_{escapePP} + \alpha_u$$

$$\alpha_u \sim N(0, \sigma^2_{escape})$$

$\mu_{escapeFR}$ represents the probability of escaping predation in the FR (fleshy-fruited) microhabitat, taken as intercept, and was given a weak Normal(-1, 2) prior implying relatively high predation rates (as rodent predation is often higher under dense vegetation; Fedriani & Manzaneda 2005). The β parameters thus represent the differences in predation rates in the other microhabitats (compared to FR), and were given broad Normal(0, 2) prior distributions allowing for large differences between microhabitats. Finally, we included an observation-level random effect (α_u) to account for potentially overdispersed predation rates among experimental units, with a half-Normal(0, 3) prior for σ_{escape}.

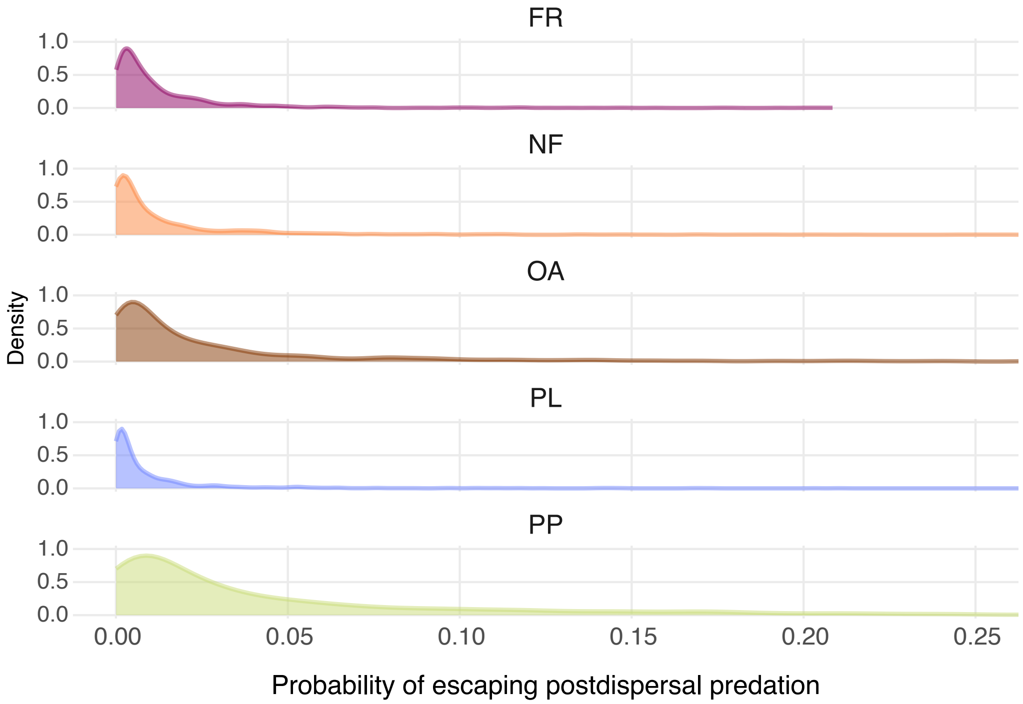

Figure A2.16 Posterior probability of escaping post-dispersal seed predation in each microhabitat.

Probability of seedling emergence and survival

We estimated the probability of emergence and seedling survival through the first summer (up to mid October) using data from two seasons (2018-19 and 2019-20) (Fig. A2.17):

$$Survival_s \sim Bern(Psurv_s)$$

$$logit(Psurv_s) = \mu_{survFR} + \beta_{survNF} + \beta_{survOA} + \beta_{survPL} + \beta_{survPP} + \beta_{2020} + \beta_{survNF2020} + \beta_{survOA2020} + \beta_{survPL2020} + \beta_{survPP2020} + \alpha_e$$

$$\alpha_e \sim N(0, \sigma^2_{surv})$$

Seedling emergence and survival was modelled as a Bernoulli process, with probability depending on microhabitat and season. μ_{survFR}, the intercept parameter, represents the probability of survival on the FR (fleshy-fruited) microhabitat in the first season, and was given a Normal(-6.9, 2) prior (logit scale), corresponding to 0.1% survival (*i.e.*, only 1 in 1000 dispersed seeds would produce a seedling still alive after their first summer). The β parameters accommodate differences in survival probability among microhabitats and seasons, and had Normal(0, 2) priors. Finally, there was a random effect (α_e) to account for replicated measurements within sowing units (each experimental unit had 16 sown seeds). α_e was drawn from a Normal distribution with standard deviation σ_{surv} having half-Normal(0, 1) prior.

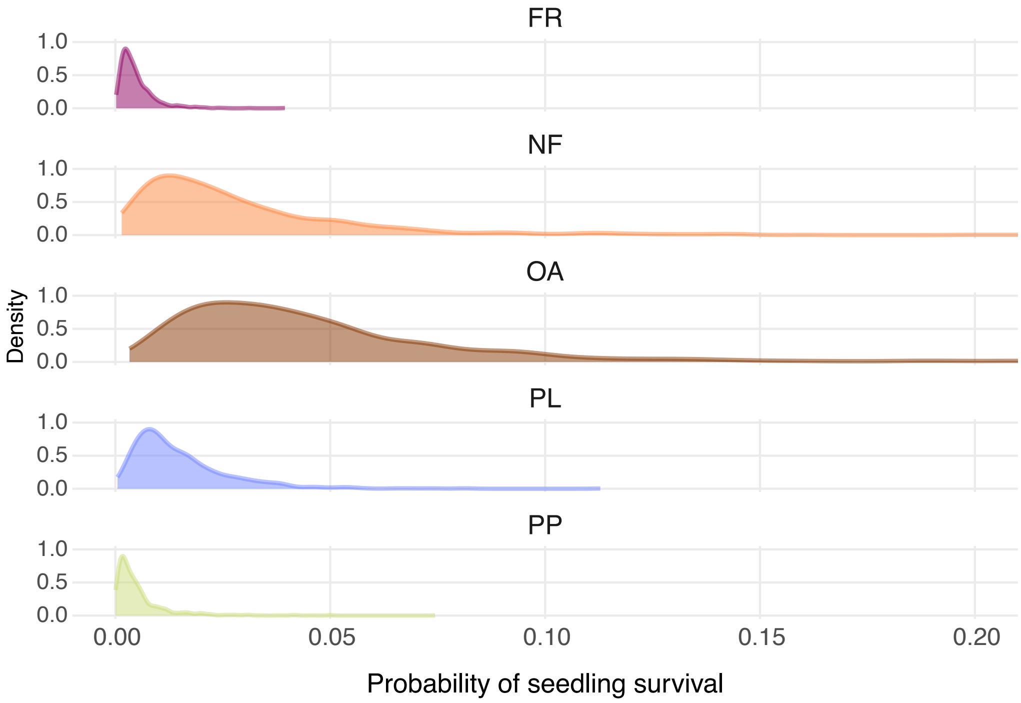

Figure A2.17. Posterior probability of seedling emergence and early survival in each microhabitat, combining results from both 2018-19 and 2019-20 seasons.

Calculating the quality component of SDE

The quality component of seed dispersal effectiveness (SDE) estimates the probability of a seed dispersed by a given bird species to turn into a seedling surviving its first summer (Fig. A2.18). This probability can be calculated as the product of the posterior probabilities obtained above:

- Probability of escaping predation from granivorous birds (*Pdrop*)
- Probability, for each bird species, of dispersing seeds towards each microhabitat (*Pseed.bird*)

- Probability of escaping post-dispersal (rodent) predation in each microhabitat (*Pescape*)
- Probability of emergence and early seedling survival in each microhabitat (*Psurv*)

Note these estimates of SDE quality represent an upper bound of seedling recruitment per dispersed seed since we do not account for the viability of dispersed seeds (González-Varo *et al.* 2019a).

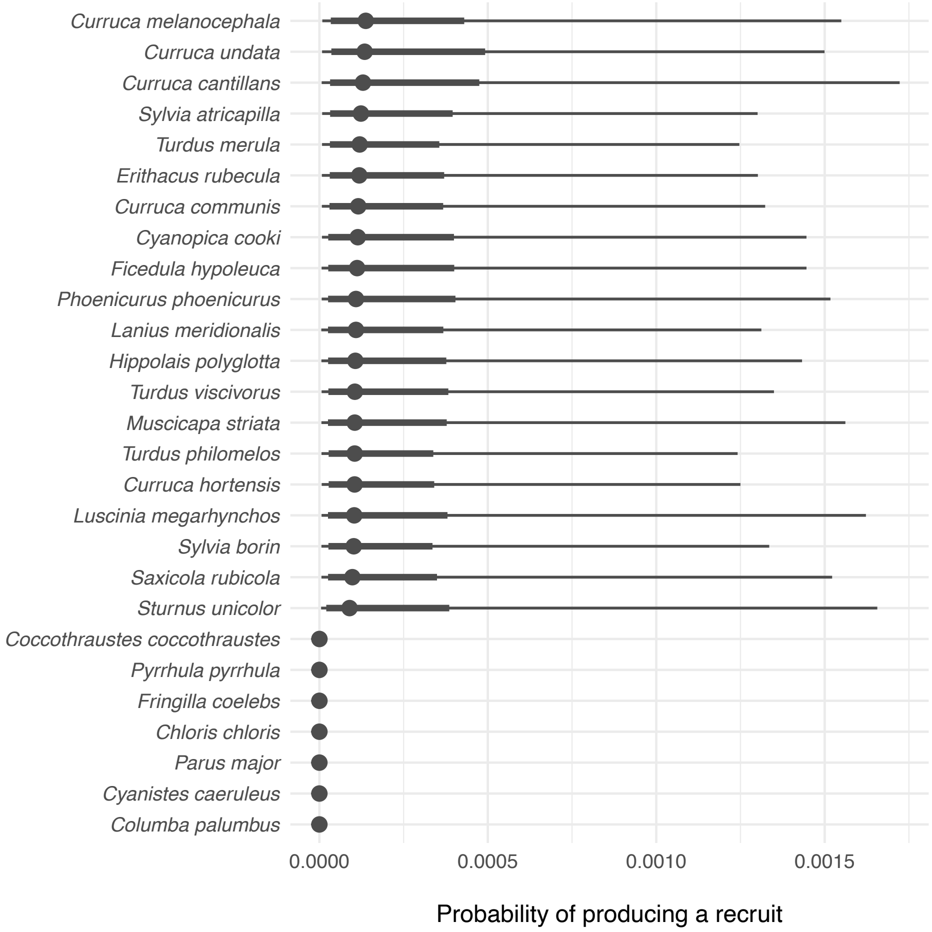

Figure A2.18. The quality component of seed dispersal effectiveness, represented as the probability of seedling recruitment per consumed fruit or seed for each bird species. Bird species appear sorted by decreasing median probability (represented by dots). Intervals represent Bayesian 66% and 95% credible intervals (thick and thin lines, respectively).

2E.4. Complete SDE landscape (including non-legitimate dispersers)

Seed predators and pulp peckers were removed from the Seed Dispersal Effectiveness (SDE) landscape shown in the main text to facilitate visualisation of the quality component and the differences between legitimate dispersers. The following figure A2.19 shows the complete SDE landscape incorporating the *non-legitimate dispersers* (*Chloris chloris*, *Fringilla coelebs, Pyrrhula pyrrhula, Coccothraustes coccothraustes*, *Columba palumbus, Parus major* and *Cyanistes caeruleus*).

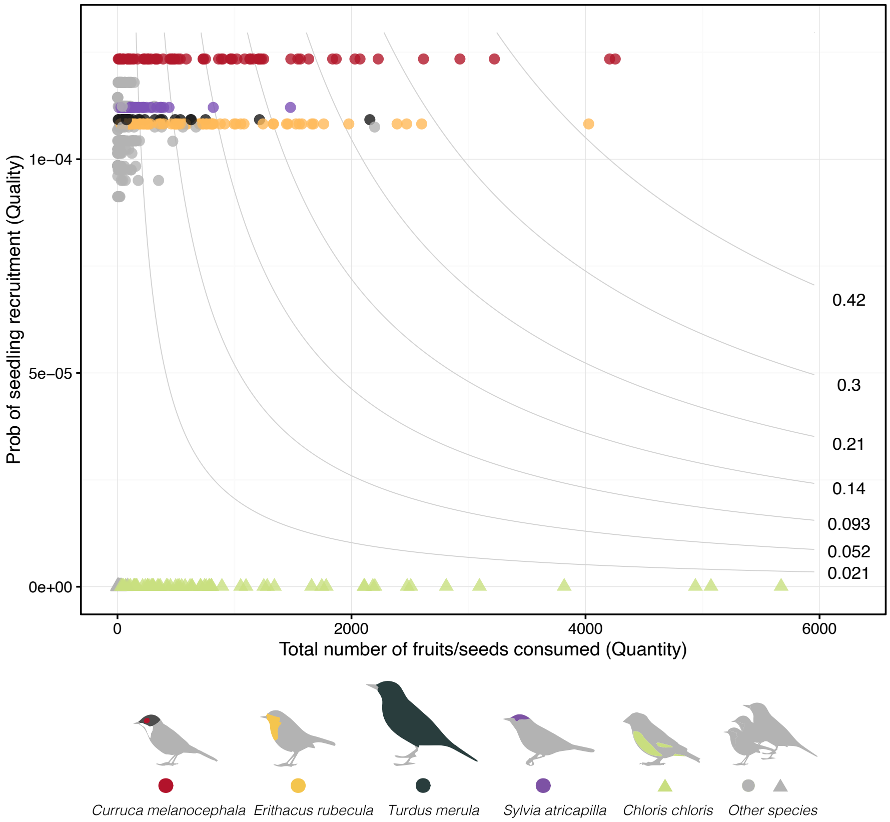

Figure A2.19. Seed dispersal effectiveness landscape (SDE) for individual *Pistacia lentiscus* plants. Each point represents an individual plant pairwise interaction with a given avian frugivore species represented in different colours. The horizontal axis depicts the total number of fruits (or seeds, in the case of the granivorous species) consumed by each bird species in each individual plant and the vertical axis represents the posterior median probability of recruiting a seedling from a fruit ingested by each bird species. The product of the horizontal (Quantity) and vertical (Quality) axis gives the total number of plant recruits for each bird-plant pairwise interaction. Different combinations of quantity and quality can result in equal effectiveness values, as shown by the SDE isolines.

2E.5. Variance partitioning of effectiveness components

To estimate the relative importance of each component (*i.e.*, quantity and quality) on the total effectiveness, we adjusted separate models of effectiveness as a function of each component (all variables were log-transformed). For Seed Dispersal Effectiveness we only considered interactions with legitimate dispersers. The coefficient of determination (R^2) of each model represented the partitioned variance of the total effectiveness. R^2 values were normalised to sum up to 100%.

Appendix 2F. Reciprocity and Asymmetry calculations

2F.1. Analysis of reciprocity

Reciprocity between the reward of individual plants and frugivorous birds was estimated using Pearson correlation coefficients between the log-transformed RPE and SDE values. We aggregated the total rewards offered and received by each individual plant (*i.e.,* adding up the rewards across all bird species interacting with each plant), using the 1000 posterior distribution samples (Fig. A2.20). A high positive correlation between RPE and SDE would indicate high reciprocity: individual plants contributing high resource provisioning (RPE) obtain in turn high dispersal effectiveness (SDE) from their assemblage of frugivores.

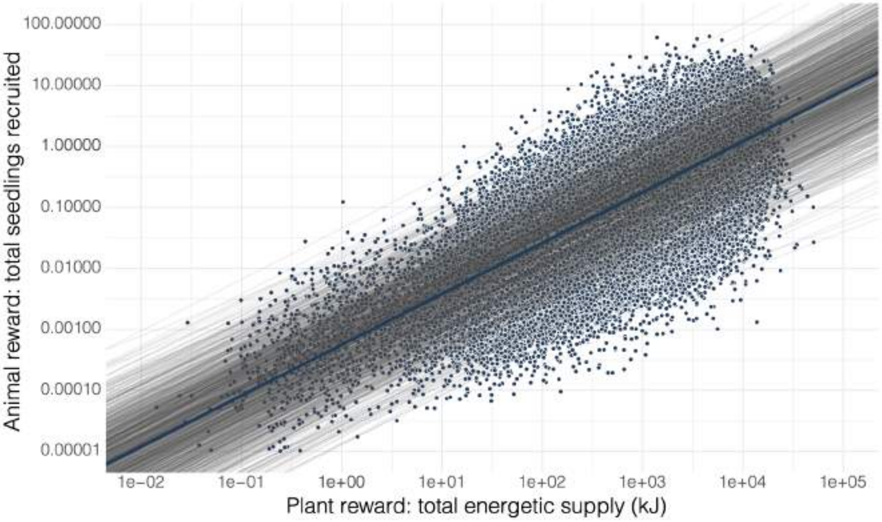

Figure A2.20. Relationship between the total energetic supply provided by individual plants (aggregating all its consumer bird species) and the number of seedlings recruited by each plant (n = 79). Each point represents one of the 1000 posterior distribution probabilities estimated per plant. Grey shaded lines represent the linear trend for each of the 1000 posteriors, and the thicker dark line represents the mean linear trend. Note both axes are in logarithmic scale.

2F.2. Dependence and asymmetry calculations

We calculated mutual dependence (*d*) for each pairwise interaction, so that two dependence values were obtained: measures the proportion of seeds dispersed (SDE) that plant *i* receives from animal species *j* relative to all the seeds dispersed for that plant. In turn, measures the proportion of energy acquired (RPE) that animal species j receives from individual *P. lentiscus* plant *i,* relative to all the energy acquired by that animal (eq. 1). The sum of the dependencies of a given species/individual on all its partners must equal 1.

eq. 1a: $$d_{P_i \rightarrow A_j} = \frac{SDE_{ij}}{\sum_{A=1}^{n} SDE_i}$$, for the dependence of *P. lentiscus* plant *i* on animal species *j*; and

eq. 1b: $$d_{A_j \rightarrow P_i} = \frac{RPE_{ij}}{\sum_{P=1}^{m} RPE_j}$$, for the dependence of animal species *j* on plant *i,*

where *d* is the dependence of plant *i* on animal species *j*, or vice versa; SDE_{ij} is the estimated number of seedlings recruited by plant *i* via frugivore species *j*; RPE_{ji} is the amount of kilojoules plant *i* reported to frugivore species *j*; and *n* and *m* represent the total number of animal species and individual plants, respectively.

Interaction asymmetry (AS) is defined as the difference of animal d_{ji} and plant d_{ij} dependencies divided by the maximum dependence value of these two (Bascompte *et al.* 2006, Vázquez *et al.* 2007)

eq. 2: $$AS_{P_i A_j} = \frac{d_{P_i \rightarrow A_j} - d_{A_j \rightarrow P_i}}{max(d)}$$

AS values can range from -1 to 1, where 0 indicates total symmetry (*i.e.*, both partners depend on each other with the same intensity), values approaching +1 indicate that the plant is more dependent on the animal than *vice versa*, and negative values indicate that the animal is more dependent on the plant than the plant on the animal.

To account for the potential effect of matrix size (*i.e.*, variation in the number of individual plants surveyed) on asymmetry values, we carried out simulations with adjacency matrices including variable numbers of plants (see Appendix 2H). We did not find evidence for asymmetry values being significantly biassed by changes in matrix size. In addition, we compared the observed asymmetry distribution with two different null models (see Appendix 2H). We observed that the highly skewed asymmetry distribution pattern did not differ when animals and plants were allowed to interact randomly following Patefield and Vázquez null models (Patefield 1981, Vázquez *et al.* 2007, Dormann *et al.* 2009).

Appendix 2G. Effects on consumption (quantity component)

2G.1. Proportion of plants' crop consumed

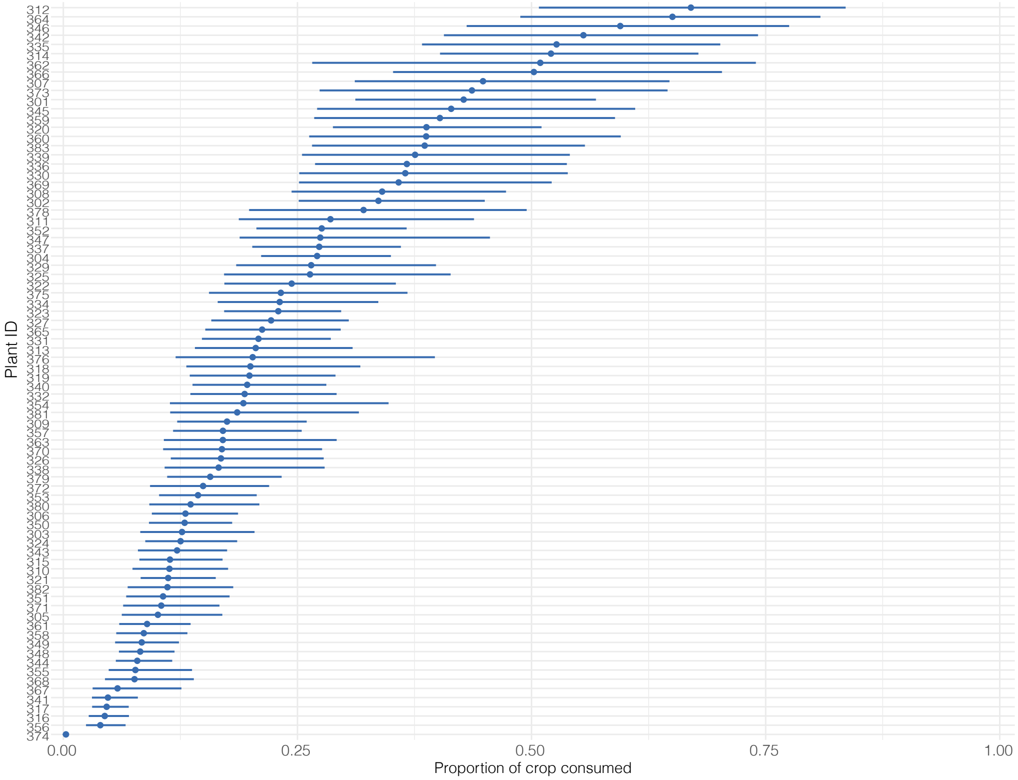

Figure A2.21. Proportion of the initial fruit crop size estimated to be consumed by birds for each individual plant. Error bars denote the 50% credible interval.

2G.2. Birds consumption of available energy

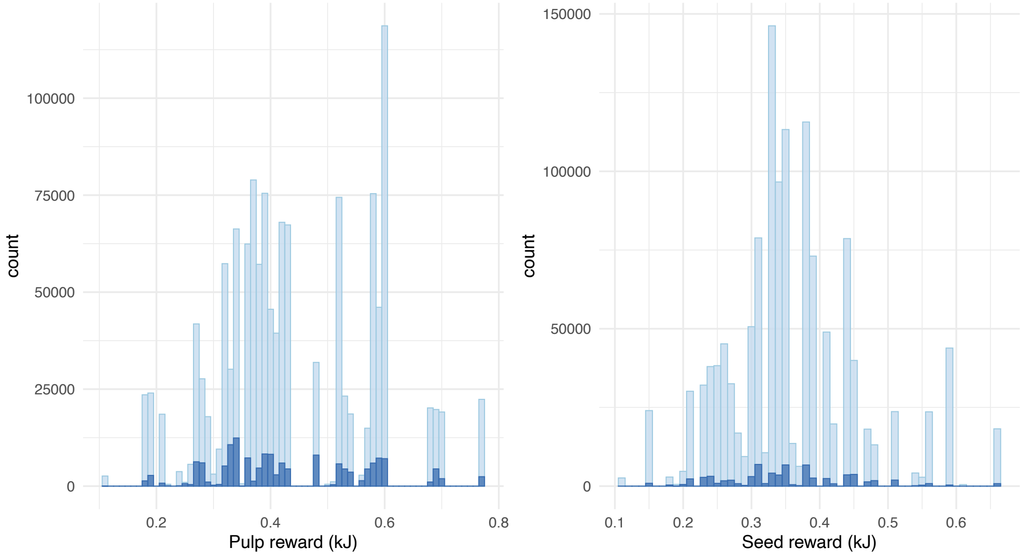

Figure A2.22. Frequency distributions of available "pulp reward" (left) and "seed reward" (right) in kilojoules (kJ) (*i.e.*, counts of individual fruits available at the start of the fruiting season with a given energy content per fruit or seed; light blue bars) and the estimated number of fruits/seeds consumed by birds (dark blue bars; *i.e.*, counts of individual fruits/seeds consumed as a function of their energy content). Pulp "reward" illustrates the potential energy gain for frugivores consuming the fruits and regurgitating or defecating the seed (*e.g.*, legitimate seed dispersers and/or pulp consumers) and seed "reward" indicates the potential energy gain for avian seed predators that discard fruit pulp and consume the seed.

2G.3. Predictors of fruit consumption intensity from individual plants

To test the effect of different predictors (energetic pulp reward, plant canopy area and crop size) on fruit consumption from individual plants, we used a generalised linear model with a negative binomial distribution fitted with glmmTMB R-package (Brooks *et al*. 2017). All continuous predictors were log-transformed. See table A2.5 with model results.

Table A2.5. Summary statistics of the generalised linear model performed to test the effects of plant traits on fruit consumption by legitimate seed dispersers. We used a negative binomial distribution with a log link.

Predictors	Estimate ± SE	p
Intercept	0.181 ± 0.659	0.783
log(Crop Size)	0.277 ± 0.068	<0.001
log(Pulp mass)	0.486 ± 0.192	0.011
log(Plant Area)	0.959 ± 0.099	<0.001
Site	0.751 ± 0.134	<0.001

Appendix 2H. Null models for interaction asymmetry estimates

In order to determine if matrix size was having an effect on the asymmetry distribution values encountered, we repeated the analysis subsampling from the total number of plants. Asymmetry values could be affected by the number of plants selected and sampled in the study because of varying matrix size and shape. We considered three different matrix sizes, of 20, 40 and 60 plants, that were compared to the asymmetry obtained from the 80 plants observed matrix. We performed 1000 permutations for each matrix dimension. Asymmetry in subsampled matrices was not greatly altered (Fig. A2.23). All matrices showed few symmetric interactions. However, when the matrix included fewer plants the frequency of interactions where the animal is more dependent on the plant (*i.e.*, negative asymmetries towards -1) increased while the frequency of interactions where the plant is more dependent on the animal (*i.e.*, positive asymmetry values towards +1) decreased. This change in the sign of asymmetry is expected, given that a reduction in the number of plants available would lead to a greater estimated dependence of the birds on individual plants.

In addition, to test whether the asymmetry distribution encountered deviates from the expected asymmetry in randomly-built matrices, we compared the observed values to those obtained with null model matrices. We randomised fruit consumption following both Patefield and Vázquez null models (n=1000 permutation per model) (Dormann *et al.* 2009). The asymmetry frequency distribution encountered with both null models also maintained a "U" shaped pattern (Fig. A2.24). The Patefield null model increased the total number of unique-pairwise interactions, as it does not constrain connectance, allowing plants to create new links with birds and increasing their interaction degree. The creation of new links also caused an increase in the cases where animals were more dependent on plants. On the contrary, Vázquez null model results did not differ from the observed asymmetry distribution, except on a slight but significantly lower number of interactions for the more dependent avian species. That is, our system presented a higher frequency of interactions in which the animal is more dependent than would be expected when maintaining network connectance and species were allowed to interact randomly.

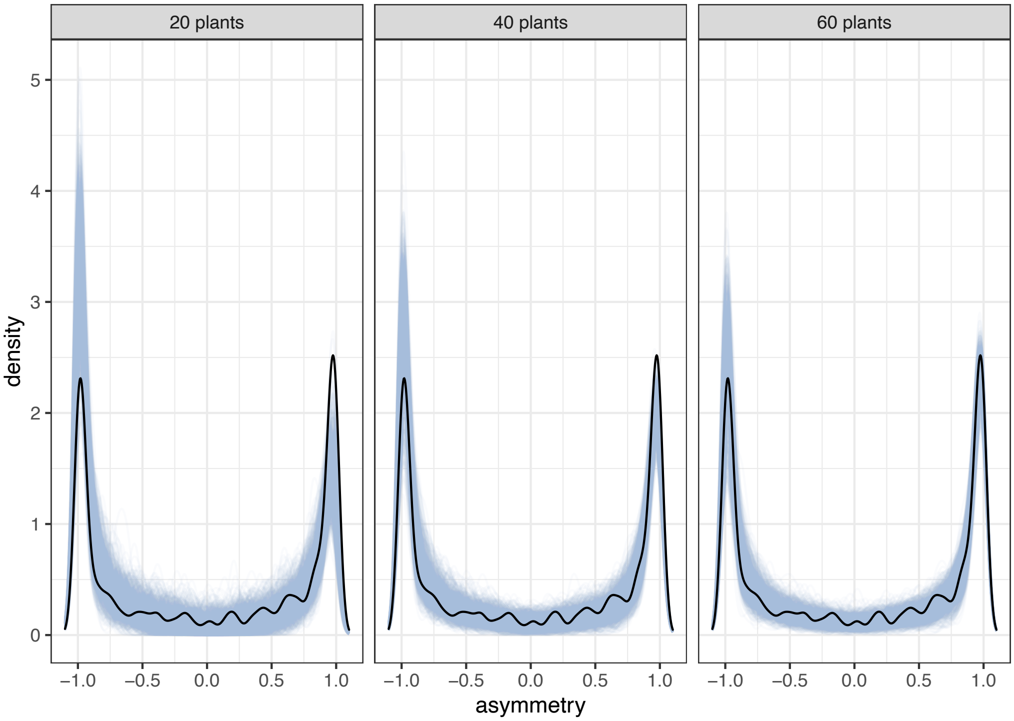

Figure A2.23. Frequency distributions (density function) for interaction asymmetry when using three different matrix sizes (*i.e.*, reducing rows to 20, 40 or 60 individual plants). Thin blue lines represent the 1000 permutations per matrix dimension. Black line represents the median observed asymmetry values.

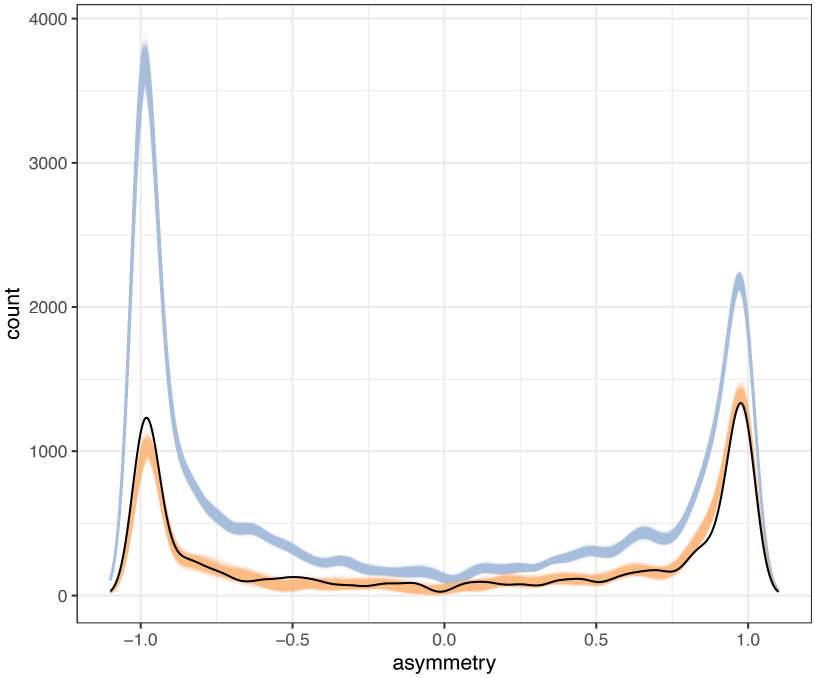

Figure A2.24. Frequency distribution (count data) for interaction asymmetry using counts for null models. The blue line represents the 1000 permutations of the Patefield model; the orange line represents the 1000 permutations of the Vázquez model; black line represents median observed asymmetry values.

Appendix 2I. Software

We used R version 4.1.2 (R Core Team 2021) and the following R packages: bayesplot v. 1.8.1 (Gabry *et al.* 2019, Gabry & Mahr 2021), bayestestR v. 0.11.5 (Makowski, Ben-Shachar & Lüdecke 2019), bipartite v. 2.16 (Dormann, Gruber & Fruend 2008), brms v. 2.16.3 (Bürkner 2017, 2018), DHARMa v. 0.4.5 (Hartig 2022), effect.lndscp v. 0.2.8 (Jordano & Rodríguez-Sánchez 2019), ggdist v. 3.1.1 (Kay 2022), ggpubr v. 0.4.0 (Kassambara 2020), ggridges v. 0.5.3 (Wilke 2021), glmmTMB v. 1.1.2.3 (Brooks *et al.* 2017), here v. 1.0.1 (Müller 2020), knitr v. 1.37 (Xie 2014, 2015, 2021), lme4 v. 1.1.28 (Bates *et al.* 2015), modelbased v. 0.7.2 (Makowski *et al.* 2020), patchwork v. 1.1.1 (Pedersen 2020), plotly v. 4.10.0 (Sievert 2020), rmarkdown v. 2.12 (Xie, Allaire & Grolemund 2018, Xie, Dervieux & Riederer 2020, Allaire *et al.* 2022), summarytools v. 1.0.0 (Comtois 2021), tidylog v. 1.0.2 (Elbers 2020), tidyverse v. 1.3.1 (Wickham *et al.* 2019), vegan (Oksanen *et al.* 2020), and visreg v. 2.7.0 (Breheny & Burchett 2017).

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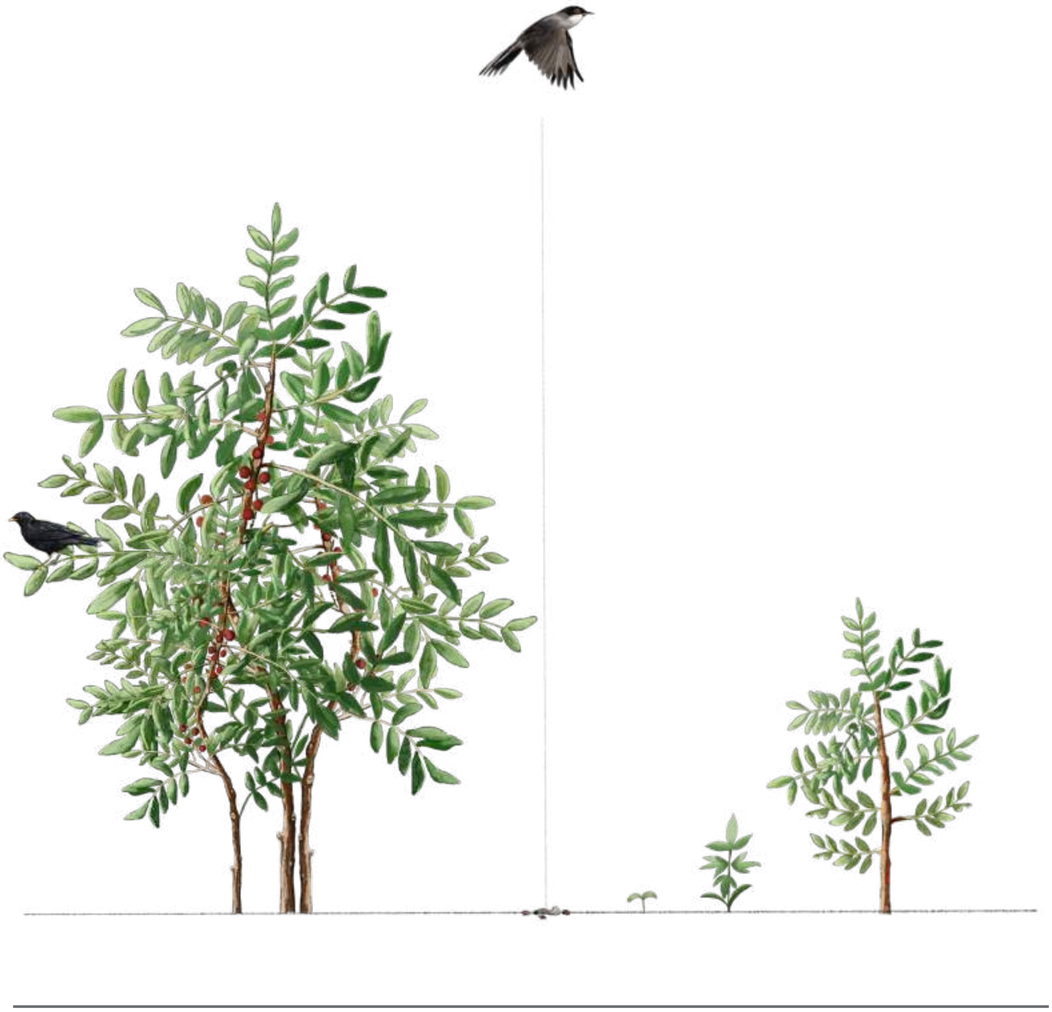
CHAPTER 3

Lasting effects of avian-frugivore interactions on seed dispersal and seedling establishment.



Quintero, E., Arroyo, J., Dirzo, R., Jordano, P., & Rodríguez-Sánchez, F. (2024). Lasting effects of avian-frugivore interactions on seed dispersal and seedling establishment. Journal of Ecology 00: 1-17.

Abstract

1. The consequences of plant-animal interactions often transcend the mere encounter stage, as those encounters are followed by a chain of subsequent stages on the plant's reproductive cycle that ultimately determine fitness. Yet, the dissemination and recruitment stages of animal-mediated seed dispersal are seldom analysed jointly, hindering a full understanding of the ecology of seed dispersal.
2. We analyse the dispersal and recruitment of a fleshy-fruited plant (*Pistacia lentiscus*), from fruit production to seedling survival up to their second year. We link early reproductive investment of individual plants to seedling recruitment and explore the role played by seed viability, the coterie of frugivores and microhabitat seed deposition.
3. The proportion of viable seeds was generally low (mean = 34%) but highly variable among individual plants (range: 0 - 95%). Seed viability did not seem to have a direct effect on individual plant's recruitment.
4. We recorded 28 bird species feeding on *P. lentiscus* fruits or seeds. Their contribution to plant recruitment was mainly determined by their intensity of fruit consumption and probability to disperse viable seeds. Most frugivores presented non-random microhabitat preferences, delivering uneven seed contributions to different sites.
5. Post-dispersal seed predation by rodents was the most limiting phase in *P. lentiscus* recruitment. Yet, microhabitats showing the lowest predation rates received the lowest seed rain. Hence, we found a decoupling of the dissemination and recruitment stages: most seeds do not arrive at the most suitable microhabitats.
6. We estimate *P. lentiscus* plants need to produce c. $5x10^5$ fruits to recruit a single seedling that survives to its second summer in our study site. Its

success as a prevalent species in Mediterranean lowland landscapes relies on its high fecundity and thorough fruit removal and dispersal by a diversified frugivore assemblage, which compensates for the high seed unviability characteristic of this genus.

7. Synthesis - Measuring the delayed, post-dispersal outcomes of animal frugivory interactions may overturn inferences based on consumption observations only. Seed rain patterns are often decoupled from microhabitats' suitability for seedling recruitment. Hence, more integrative studies that encompass the entire plant reproductive cycle (from fruit production to seedling recruitment) are needed to fully understand frugivores' lasting contribution to plant regeneration in natural populations.

Keywords: Doñana National Park, frugivory, microhabitat, *Pistacia lentiscus*, plant regeneration, plant-animal mutualisms, post-dispersal predation, seed viability, seedling recruitment, seedling survival.

Introduction

Plant population regeneration entails a series of demographic stages spanning flowering, fruiting, seed dispersal, seedling emergence, establishment, and subsequent growth (Harper 1977, Wang & Smith 2002). Each of these transition steps has the potential to limit recruitment and population growth (Nathan & Muller-Landau 2000). Natural recruitment in plants can be restricted by two main mechanisms: (i) seed limitation, when not enough seeds arrive at sites suitable for seedling recruitment, and/or (ii) microhabitat suitability, if seeds arrive at sites where seedling recruitment is prevented by physical or biotic factors (Clark *et al.* 1999, Moore & Elmendorf 2006). For animal-dispersed (zoochorous) plants, both the amount of seeds dispersed and the microhabitat where seeds arrive will be ultimately determined by their animal partners (Schupp *et al.* 1989). The arrival stage determines how many seeds reach a particular target microhabitat, depending on the consumption and movement of the different frugivore species. The recruitment stage determines the fraction of this seed rain that transitions to become established seedlings, saplings and, eventually, adults. Despite being crucially intertwined, frugivore studies typically examine just one of these two stages and thus we lack a full appreciation of the limiting transitions and bottlenecks throughout the regeneration process (Harms *et al*. 2000, Howe & Miriti 2000).

Several ecological characteristics of frugivores will determine their effectiveness as seed dispersers and their net contribution to the plants' reproductive cycle: for example, variation in their consumption frequency and fruit handling behaviour, or in their habitat use preferences, which will influence the spatial pattern of seed deposition (Razafindratsima & Dunham 2015, Schupp *et al.* 2010). Frugivores use the landscape heterogeneously (Wenny & Levey 1998), thus determining non-random dispersal by depositing seeds in different microhabitats in proportions not directly determined by their availability in the landscape (*e.g.*, Jordano & Schupp 2000, Lázaro *et al.* 2005). The microhabitat where seeds are deposited is crucial for plant recruitment, since the deposition site often shows important differences in microclimatic conditions, such as soil moisture, nutrient availability, or seed predators' activity (Gómez-Aparicio 2008). Frugivores providing high-quality dispersal will predominantly carry seeds to more suitable locations where seeds have greater probability of escaping predation or experience better microclimatic conditions for seedling establishment and subsequent growth. Hence, the set of animal frugivores with their characteristic feeding and movement behaviour, have lasting effects on individual plants recruitment (Wenny & Levey 1998). Importantly, these effects are delayed relative to the actual occurrence of the plant-frugivore interaction, and probably this has hindered the joint treatment of the dissemination and establishment processes. Few studies have addressed how these sequential effects of animal frugivores (immediate contributions to the seed rain and delayed effects on recruitment) are intertwined during the seed dispersal process, *i.e.*, how frugivore activity may link with the resulting recruitment patterns (*e.g.*, Côrtes *et al.* 2009, Rey & Alcántara 2000, Jordano & Schupp 2000).

In addition to post-dispersal processes, the outcome of the mutualistic interactions in terms of plant recruitment may also be constrained by factors occurring prior to the establishment of the interaction itself. Many plants produce fully-developed fruits containing unviable seeds with no chances of recruitment, for example, due to pre-dispersal seed predation, seed abortion, or parthenocarpy (Jordano 1989). Varying degrees of seed viability can thus determine the final probability of plant recruitment, beyond the number and location of dispersed seeds (González-Varo *et al.* 2019a). In those situations, a complete evaluation of the outcome of mutualistic interactions and individual plants' reproductive, dispersal and regeneration success requires a comprehensive examination of both pre- and post-dispersal stages (Herrera *et al.* 1994, Yang *et al.* 2011).

Here we provide a comprehensive study of the regeneration cycle of *Pistacia lentiscus* L., a widespread plant species in the Mediterranean shrublands, aiming to disentangle the role of avian frugivores and variation in seed viability on early plant recruitment. This plant represents an interesting case study because, on the one hand, it produces an abundant fruit crop (thousands of fruits), interacts with a wide array of frugivores (supergeneralist), and can become locally very abundant, often being the dominant species in Mediterranean lowland shrublands. On the other hand, this species regularly produces relatively large percentages of unviable seeds within the fully-developed fruits (Grundwag 1976), with considerable variation among individual plants, ranging between 10 and 40% (Jordano 1989, Verdú & García-Fayos

1998, González-Varo *et al.* 2019a). This study seeks to elucidate what are the plant's demographic consequences of interacting with different assemblages of frugivores for reproductive success beyond the mere fruit-frugivore contact, and encompassing the following successive stages: fruit consumption (1), pre-dispersal avian predation on seeds (2), dispersal of viable seeds (3), the consequences of differential seed deposition in microhabitats through seed escape from rodent predation (4), seedling emergence (5) and seedling survival until its second summer (6) (Fig. 3.1).

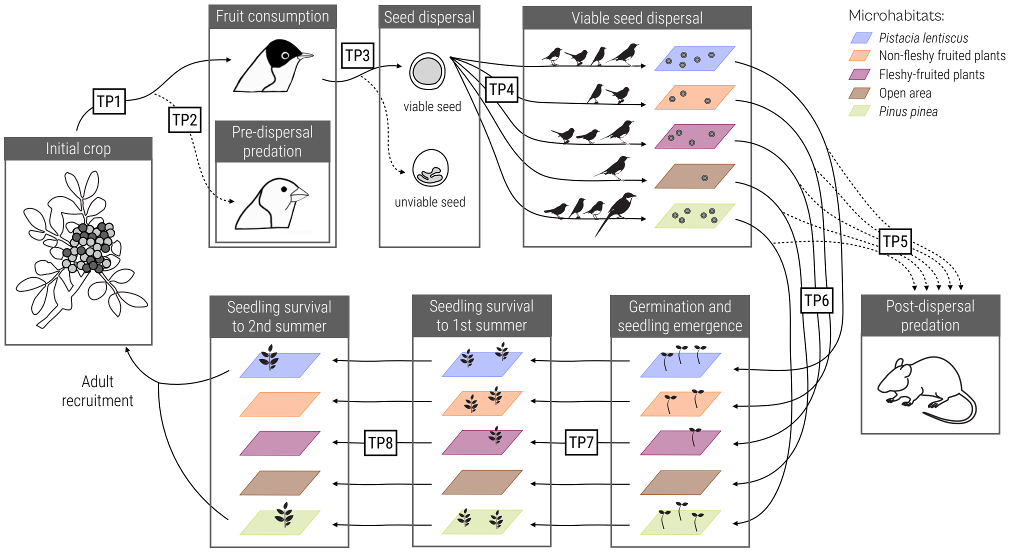


Figure 3.1. Simplified schematic view of the demographic cycle of *Pistacia lentiscus* focusing on its seed dispersal and recruitment stages. The figure shows demographic stages (rectangles) with their associated ecological processes. Continuous lines represent the contribution of propagules to the next demographic phase while dashed lines indicate the loss of propagules. The derived effects of each demographic stage results in variable transition probabilities (TPs) between the successive demographic stages considered in this study. TP1: prob. of fruit consumption, TP2: prob. of seeds escaping finch predation, TP3: prob. of dispersing a viable seed; TP4: prob. of viable seed being dispersed to a certain microhabitat, TP5: prob. of dispersed seeds escaping rodent predation, TP6: prob. of seedlings emerging from surviving seeds, TP7: prob. of seedling surviving its 1st summer and TP8: prob. of seedling surviving its 2nd summer. Coloured rectangles represent distinct microhabitat types that differ in arrival of seeds and recruitment probabilities. Different avian assemblages disperse seeds to different microhabitats as a result of foraging preferences (in TP3). The product of the successive TP values determines the overall probability of recruitment (OPR) for the plant.

Specifically, we address the following main questions: (1) Does a remarkably high incidence of seed unviability combine with high dispersal success and establishment for the (few) viable seeds produced in the demographic cycle of a dominant plant species? In other words, are frugivores, through their consumption, able to compensate for the high unviability rates of *P. lentiscus* seeds and disperse enough viable seeds for plant regeneration? (2) Do frugivores, with their non-random patterns of foraging and heterogeneous landscape use, limit seed arrival to potentially suitable microhabitats? That is, do viable seeds predominantly reach certain microhabitats as a result of differential dispersal mediated by specific frugivore species, and does seed fate differ in these microhabitats? Finally, (3) which are the main limiting demographic transitions in the seed dispersal and recruitment cycle of *P. lentiscus,* and do frugivores, through their direct (dispersal) and indirect (pre- and post-dispersal) effects, play different roles in the plant's demographic stages, beyond their consumption frequencies?

We expect birds will provide complementary dispersal services attending to their different use of the landscape (González-Castro *et al.* 2015, Lavabre *et al.* 2016) leaving a distinct spatial signal that will serve as the starting template for population recruitment to follow (Howe & Miriti 2004, Perea *et al.* 2021). Similarly, we expect microhabitats will differ in their suitability for seed survival, emergence and growth into seedlings (Gómez-Aparicio 2008). This spatial pattern in the seed rain is expected to vary for individual plants depending on the assemblage of frugivore species consuming their fruits and their non-random dispersal service. Understanding the role of frugivorous species on the limitation of plant recruitment will be useful to predict the consequences of the increasingly omnipresent environmental changes and animal fluctuations, driven by anthropogenic impact, for plant regeneration and distribution in nature.

Methods

Study species

Pistacia lentiscus L. (Anacardiaceae) is an evergreen shrub species widely-distributed in the Mediterranean basin (Martínez-López *et al.* 2020, Verdú & García-Fayos 2002). It is found in low and medium altitude Mediterranean shrublands, where it can become dominant, acting as a foundation species (Ellison 2019). This species is dioecious (*i.e.*, separate male and female individuals) and wind-pollinated but relies on animals for the dispersal of its seeds that are covered with a thin fleshy mesocarp forming a drupe. The pulp is very rich in lipids (Herrera 1992) and heavily consumed by frugivorous birds during the fruiting season, from September to March, spanning late summer, autumn and winter. A significant number of the seeds produced are unviable as a result of parthenocarpy (*i.e.*, fruit development without fertilisation), embryo abortion or pre-dispersal seed predation by wasps (Grundwag 1976, Jordano 1989). The wasp *Megastigmus pistaciae* of the superfamily Chalcidoidea oviposits on the fruit, where the larvae will consume the endosperm from within, rendering the seed unviable (Traveset 1993, Verdú & García-Fayos 1998). The frequency of empty seeds varies from year to year, as well as among *P. lentiscus* populations (Jordano 1988b, 1989, Verdú & García-Fayos 1998). Fruits have a red colour when unripe that turns into black when fully ripe (Jordano 1989). Frugivores show a strong preference for black ripe fruits over red fruits (Jordano 1989), since black fruits have a higher percentage of lipids (Trabelsi *et al.* 2012). Fruits that turn black also present significant higher chances of having filled, viable seeds (Jordano 1989). Thus, frugivores are expected to disperse a higher amount of viable seeds but together with a variable fraction of empty seeds. The proportion of unviable seeds dispersed appears to increase along the fruiting season (González-Varo *et al.* 2019a). *Pistacia lentiscus* is considered mainly a bird-dispersed plant (Herrera 1989, Appendix 3A), although fruit consumption by carnivores and ungulates has been reported (Perea *et al.* 2013). Yet, mammal fruit consumption is rare and their contribution to dispersal is probably negligible since they break most seeds during consumption, acting mostly as seed predators (Mancilla-Leytón 2013, Perea *et al.* 2013). In addition, no mammal consumption of *P. lentiscus* fruits was detected in our study sites, hence here we focus on frugivory and seed dispersal by birds.

Sampling design and estimation of initial (pre-dispersal) seed viability

We conducted fieldwork in Doñana National Park, southern Spain, between the years 2019-2021. The ICTS-RBD and the Doñana National Park provided us onsite access authorisations and permit to carry out fieldwork. We monitored a total of 80 female *Pistacia lentiscus* plants along the fruiting season at two Mediterranean scrubland sites: El Puntal (EP; 36° 57' 54.3816" N, 6° 26' 47.1588" W) and Laguna de las Madroñas (LM; 37° 1' 49.2312" N, 6° 28' 19.1604" W).

For each individual plant we measured its cover area, counted the initial crop size with the help of a hand counter (at the beginning of the fruiting period, *i.e.*, September 2019) and estimated the proportion of viable seeds found in their crop. Seed viability was estimated through floatation/sink experiments (Albaladejo *et al*. 2009, González-Varo *et al*. 2019a). We bagged branches at individual plants with a mesh fabric to prevent fruit consumption by birds before sampling for viability analysis. This was necessary to avoid biassed viability estimates caused by birds' selective consumption of ripe black fruits (Jordano 1989). At EP site, branches were bagged in three consecutive periods along the season: early (bagged 21st August and collected 25th September), mid (bagged 25th September and collected the 7th-11th November) and late period (bagged 11th November and collected 23rd December). The amount of fruits per branch was variable (mean number of fruits per plant = 146, range = 13-595; see grouped sample size for each plant on top of Fig. 3.2). Differences in viability between periods were not significant (Appendix 3B). At LM site, bags were placed once on individual plants' branches on the 29th August, and collection date varied between plants (between 2nd October and 25th November), depending on when fruits ripened. At the LM site, the mean number of seeds collected per plant was 131 (range = 12-503; see Fig. 3.2). We aggregated fruits collected in the different sampling periods to calculate seed unviability of each plant. In addition, to determine the causes of seed unviability (*i.e.*, parthenocarpy, abortion or wasp predation), we dissected a minimum of 30 floating (unviable) seeds of each plant (mean number of seeds per plant = 47). We modelled the proportion of viable seeds produced by each plant using a Bayesian logistic regression where the logit probability of producing viable seeds had a wide prior Normal(0, 2).

Frugivory interaction sampling

To estimate the number of fruits consumed by different avian species from individual plants we combined two non-invasive techniques: DNA-barcoding and video footage (Quintero *et al*. 2022). For the first method, we collected faecal samples and regurgitated seeds by placing seed traps beneath *P. lentiscus* plants at both sites. One to two seed traps of 0.22 m2 were installed per individual plant and operated during the full fruiting season (September 2019 to February 2020). We extracted animal DNA present on the samples' surface, amplified and sequenced it to determine the avian species identity (for a detailed protocol see González-Varo *et al*. 2014 and Quintero *et al.* 2023 for modifications). A total of 2691 faecal and regurgitated samples were collected for DNA-barcoding, of which 93% were analysed with a 94% identification success rate.

In addition, we installed continuous recording cameras in front of 40 focal plants at EP site to record avian visitation and consumption behaviour. Each plant was recorded 9 times over the course of the fruiting season from September to January, rendering 19 hours recorded per plant on average (range = 18-20). Cameras recorded 3970 animal visits; with species reliably identified for 91% of the visits.

Combining data extracted from both methodologies we calculated the total number of fruits consumed by avian frugivores on individual plants during the entire fruiting season (see Quintero *et al.* 2023 for details). Briefly, we multiplied the posterior distributions obtained from four Bayesian models of: (1) the total number of bird visits (using DNA-barcoding data), (2) the probability of each bird species visiting individual plants (using both DNA-barcoding and camera data), (3) the probability that a bird visit involves fruit or seed consumption (using camera data), and (4) the number of fruits or seeds consumed per visit with feeding event (using camera data). Additionally, the proportion of fruits consumed was calculated by dividing the estimated number of fruits that birds consumed by the plants' crop size.

The number of fruits consumed by frugivores was corrected by pre-dispersal seed predation, since four bird species (finches from Fringillidae family) were observed acting mainly as seed predators, breaking the seed coat in half and feeding on the embryo. By relating the number of predated and undamaged seeds found in seed traps and attributed to granivores, we estimated that c. 0.14% of the seeds consumed by these granivores actually escaped predation (80% CI = 0.08 - 0.2%; Quintero *et al.* 2023).

Bird dispersal of viable seeds

Dispersed seed viability was estimated during the DNA extraction phase for frugivore identification (González-Varo *et al.* 2019a). After adding the extraction buffer mix to the samples and incubating them at 50°C for 75 minutes, we checked the seed floatability in the supernatant inside the microcentrifuge tubes. We used a hierarchical Bayesian logistic regression to estimate the proportion of viable seeds consumed by each bird species. The probability of birds dispersing viable (versus unviable) seeds had an informative prior based on the fact that birds consume mostly black fruits (98% of the consumed fruits versus 2% of red fruits) and black fruits are generally more viable (75% viable compared to 27% viable red fruits) (Quintero *et al.* 2023 and Appendix 3B; see also Jordano 1989, González-Varo *et al.* 2019a). We used a Normal(1, 1) prior distribution on the logit scale, corresponding to c. 0.73 probability that bird-dispersed seeds are viable.

We estimated the number of viable seeds dispersed by each bird species from each plant as the product of each bird's posterior probability of dispersing viable seeds and the estimated total number of fruits consumed from each plant by each bird species. In a few cases (n = 7 out of 80 plants) the estimated number of viable fruits consumed by birds surpassed the estimated amount of viable seeds produced by the plant. The viable fruit consumption surplus was then redistributed between the other plants based on their availability of viable seeds and bird consumption intensity at each plant.

Post-dispersal seed fate and seedling recruitment

To assess the subsequent consequences of seed dispersal by different frugivores on plant recruitment, we selected five microhabitats at EP site that were deemed to potentially differ in seed deposition and fate (Jordano & Schupp 2000, Lavabre *et al.* 2016): under *P. lentiscus* female conspecifics (PL), under other fleshy fruited species (FR), under non-fleshy fruited species (NF), under pine trees (*Pinus pinea*; PP) and open ground areas (OA). We estimated the cover of each microhabitat using ten 30-m long vegetation transects randomly distributed across the EP site. Microhabitat cover percentages were calculated using the R package 'vegetools' (Rodríguez-Sánchez 2016).

To estimate the density of *P. lentiscus* seed rain we placed seed traps in all microhabitats except in open area (OA), where we used 17 1-m wide transects that we monitored weekly (biweekly in 5 out of 17 censuses) adding up to 3506 m in length. For the PL microhabitat we used the 40 seed trays of 0.22 m^2 located beneath the 40 focal plants at EP site, while for FR, NF and PP microhabitats we placed two seed trays totalling 0.168 m^2 at 15 sampling points per microhabitat. The identity of the animal disperser from collected seed samples was inferred using DNA-barcoding, using the same protocol as above. We estimated the number of *P. lentiscus* seeds dispersed by each frugivore species to each microhabitat in two steps (Quintero *et al.* 2023). First, we used a Bayesian Negative Binomial regression to estimate the total number of *P. lentiscus* seeds arriving at each microhabitat. We used an offset to account for different sampling areas across microhabitats, and considered the total extent of each microhabitat at the EP site (4.1 ha) to estimate the total seed rain per microhabitat. Second, we used a Bayesian binomial model to estimate the proportion of seeds dispersed by each frugivore at each microhabitat, based on frugivore assignments derived from DNA barcoding. Finally, the number of *P. lentiscus* seeds dispersed to each microhabitat by each frugivore was obtained as the product of both posterior distributions (number of seeds arriving at each microhabitat, and the estimated proportion of seeds brought by each frugivores to each microhabitat).

To assess the intensity of post-dispersal seed predation by rodents in each of the five microhabitats we performed seed-offering experiments at EP site starting in January of 2019. We located six replicated seed predation stations per microhabitat, where each replicate consisted of a petri dish containing 10 viable seeds that were monitored daily and then gradually spaced over time. We estimated the probability of dispersed seeds to escape rodent predation during the first 30 days, when seedlings start emerging, through a Bayesian binomial model (Quintero *et al*. 2023).

Finally, we measured seedling emergence and survival for two years using seed sowing experiments at EP site. We conducted this experiment twice, one starting in January 2019 and the other in October 2019. At each microhabitat we installed six germination stations the first season (2018-19), and seven the second season (2019-20). In each station we sowed 16 viable *P. lentiscus* seeds protected with wire mesh to prevent predation, herbivory, debris and trampling. The experimental stations were monitored approximately every fortnight for the first four months and monthly thereafter.

We modelled separately seedling emergence and seedling survival after their first and second summer using a hierarchical Bayesian model with Bernoulli distribution. Sample sizes were: 1040 seeds for the seedling emergence model, 126 seedlings for the model of seedlings survival through the first summer, and 32 seedlings for modelling survival up to the 2nd summer. All models had microhabitat and fruiting season as fixed effects while germination station was set as a random factor to account for lack of independence within sowing units. We used relatively informative priors for the average seedling emergence and survival on the logit scale: for emergence we used a Normal(-1.8, 2) prior centred around ~15% emergence, for seedling survival through the first summer a Normal(-1.4, 2) prior corresponding to 20% survival, and for seedling survival through the second summer a Normal(-0.8, 2) prior corresponding to 30% survival (based on information from Amat *et al*., 2015; Trubat *et al*., 2011). Other parameters had large prior distributions: Normal(0, 2) for the microhabitat and season effects, and half-Normal(0, 1) prior for the germination station random effect.

Diversity analysis of seed rain

To evaluate the seed dispersal service performed by each frugivore species we performed diversity analyses of the spatial seed rain deposition. For each bird species we considered the number of seeds collected at each of the n = 102 sampling units at EP site and calculated diversity using Hill numbers for the first three orders (Jost 2007, Chao *et al.* 2014b). Diversity in this case translates into the effective number of sites receiving seeds brought by a given frugivore species. First order (q = 0) Hill number indicates the site richness (*i.e*., the number of sites that received at least one seed), second order (q = 1) is the exponential Shannon diversity index which not only accounts for the number of sites receiving seeds but also for the relative abundance of seeds across sites; and third order (q = 2) is the inverse of the Simpson's diversity index, which places higher weight to the evenness of seed relative abundances across sites.

Bottlenecks in transitions between demographic stages and total recruitment

Finally, we reconstructed the complete recruitment cycle of individual *P. lentiscus* plants, from fruit production up to 2nd-year seedling recruitment, to identify the major demographic bottlenecks as well as the contribution of different frugivores and microhabitats at the scale of individual plants. In particular, we calculated the following transition probabilities (TPs): (TP1) probability of fruit consumption by birds, (TP2) probability of seeds escaping bird predation and being dispersed, (TP3) probability of a dispersed seed being viable, (TP4) probability of seeds arriving at specific microhabitats, (TP5) probability of seeds escaping rodent predation, (TP6) probability of seedling emergence at each microhabitat, (TP7) probability of seedlings surviving their first summer and (TP8) probability of seedlings surviving their second summer (Fig. 3.1). When estimating the number of propagules arriving at each demographic stage for the five distinct microhabitats, we directly started after TP2 (seeds that escaped bird predation and got dispersed). The overall probability of recruitment (OPR) at each microhabitat was calculated as the product of the full posterior distributions of the step-specific probabilities for seed/seedling transitions after seed arrival (TP5 to TP9).

Data Analysis

We performed all analyses with R version 4.3.0 (R Core Team 2023). For the Bayesian analyses we used Stan (Stan Development Team, 2023) and brms v. 2.19.0 (Bürkner 2017). For model checking we used DHARMa v. 0.4.6 (Hartig 2022) and DHARMa.helpers v. 0.0.1 (Rodriguez-Sanchez 2023). For the diversity analyses we used hillR v. 0.5.1 (Li 2018) and vegan v. 2.6.4 (Oksanen *et al.* 2022). For data management and visualisation we used tidyverse v. 2.0.0 (Wickham *et al.* 2019) combined with ggdist v. 3.3.0 (Kay 2022). For a complete list of all packages used please refer to Appendix 3H.

Results

Pre-dispersal seed viability

The viability of the seeds produced by *P. lentiscus* plants was generally low (mean = 34%; SD = 19%) in congruence with previous studies (Jordano 1988b, Verdú & García-Fayos 1998). There was strong variation in seed viability between individual plants, LM population being more variable than EP (Fig. 3.2, Table A3.2). Unviability causes also varied substantially between individual plants, with abortion having the highest incidence (38%), followed by parthenocarpy (25%) and wasp predation (2-4%) (Table A3.2).

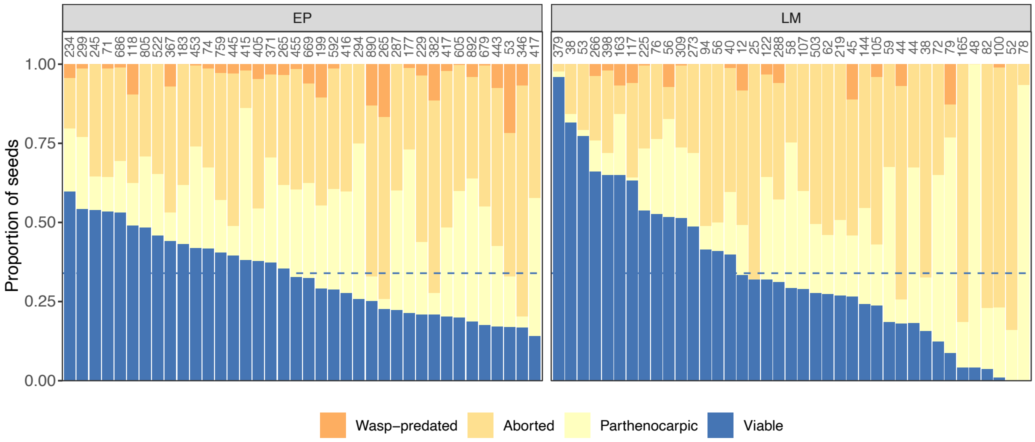


Figure 3.2. Bar graph showing the proportion of the four types of seeds found in individual plants. The two panels represent two *Pistacia lentiscus* populations (EP, LM) and each bar represents an individual plant. Numbers above indicate sample size (number of fruits sampled) and dashed lines represent mean seed viability at each population.

Viable fruit consumption and dispersal

We recorded a total of 28 bird species consuming *P. lentiscus* fruits. Five of the bird species detected were new in respect to previous literature (Table A3.1). Birds consume both unripe and ripe fruits, yet with marked preference for ripe ones, and thus may disperse either viable or unviable seeds. We detected 526 unique pairwise interactions between frugivores and individual plants, which represent 24% of all the potential connections. Most interactions, however, were dominated by just three species, *Curruca melanocephala, Erithacus rubecula* and the seed predator *Chloris chloris*. These three species were responsible for more than 85% of all the fruits consumed (see Quintero *et al.* 2023 for more frugivory interaction details).

Overall, *P. lentiscus* seeds represented 70% of the fruits consumed by birds during the study period of 2019-2020 (Table 3.1). Birds in which the prevalence of *P. lentiscus* seeds was highest (>85%) were mostly partial frugivores or seed predators, denoting a higher preference for *P. lentiscus* in their fruit diet. On the other hand, species such as *Cyanopica cooki* or *Sylvia atricapilla* showed lower prevalence of *P. lentiscus* seeds in their diets, indicating reliance on other fruiting resources.

The viability of dispersed seeds found in the seed traps was 29.5% (n = 1892 dispersed seeds). The estimated probability of a dispersed seed being viable was highest when fruits were consumed by summer migrants (median = 0.57, 80% CI = 0.34 - 0.80) and lowest when consumed by winter migrants (median = 0.46, 80% CI = 0.22 - 0.84) (Table 3.2, Appendix 3C). Plants with larger crops dispersed more seeds, regardless of their viability. In other words, large crop sizes did not favour the dispersal of more viable over unviable seeds (Appendix 3D).

Seed rain among microhabitats

All sampling points at EP received at least one seed of *Pistacia lentiscus*, indicating seed dispersal was widespread and abundant. The spatial distribution of seed rain was however uneven, with seed abundance differing by two orders of magnitude across sampling points (Fig. 3.3A). Seed rain density was positively related with the number of frugivore species contributing seeds to each location ($r = 0.71$, p-value < 0.001)

Different bird species produced contrasting patterns of seed rain (Fig. 3.3B; Appendix 3E). The most abundant bird species (*Curruca melanocephala* and *Erithacus rubecula*) ensured widespread seed rain across the landscape, contributing seeds to

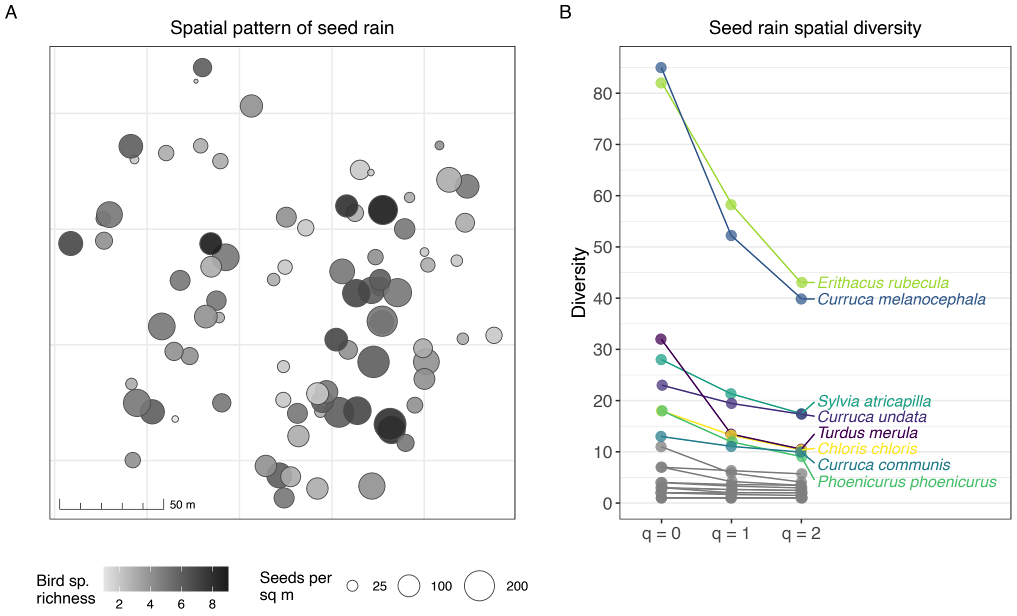


Figure 3.3. A) Spatial pattern of *P. lentiscus* seed rain performed by birds. Dots indicate locations of seed traps monitoring seed rain in the El Puntal study plot. Shading intensity in points denotes bird species richness found at each sampling point and circle size denotes seed rain density (seeds per m^2). Open area (OA) microhabitat is not represented in this figure as it was sampled using transects rather than fixed seed trays (see Methods). B) Diversity profile of the spatial pattern of seed rain generated by each bird species. Diversity is estimated using Hill numbers which represent the effective number of sites receiving seeds (out of 102 sampling points in total), *i.e.*, a proxy of the spatial "spread" of dissemination performed by the frugivore assemblage. When q = 0, diversity is equal to the number of sites receiving at least one seed dispersed by that bird species (richness); q = 1 is the exponential Shannon's index and q = 2 is the inverse Simpson's index. The higher the q value, the more weight given to the evenness of seed abundances across sampling points.

more than 80% of all seed traps. The following three most consuming species (*Sylvia atricapilla*, *Turdus merula* and *Curruca undata*) dispersed seeds to more than 20% of the sampling points (Fig. 3.3B, Table 3.1). Considering the evenness in their seed deposition pattern revealed further differences among bird species. Diversity (D) scores for each bird species in Fig. 3.3B represent the effective number of sites receiving seeds, and the higher the degree (q), the higher the importance of evenness in seed relative abundances across sampling points. Therefore, the more pronounced the slopes in Fig. 3.3B, the more uneven the relative seed contribution across sites for that bird species. Thus, *E. rubecula* and *C. melanocephala* disperse seeds to a large number of sampling points (>80%) but some sites receive much fewer seeds than others, causing the drop in their respective diversity for q > 0. Likewise *T. merula* is the third species contributing seeds to more sites (D = 32 for q = 0) but its seed deposition was markedly concentrated at certain sampling sites leading to low diversity (D = 10.5) for q = 2. In contrast, *Curruca undata* and *Sylvia atricapilla*, which disperse seeds to fewer sites than *T. merula*, achieve a more even, widespread seed rain than the latter species (Fig. 3.3B).

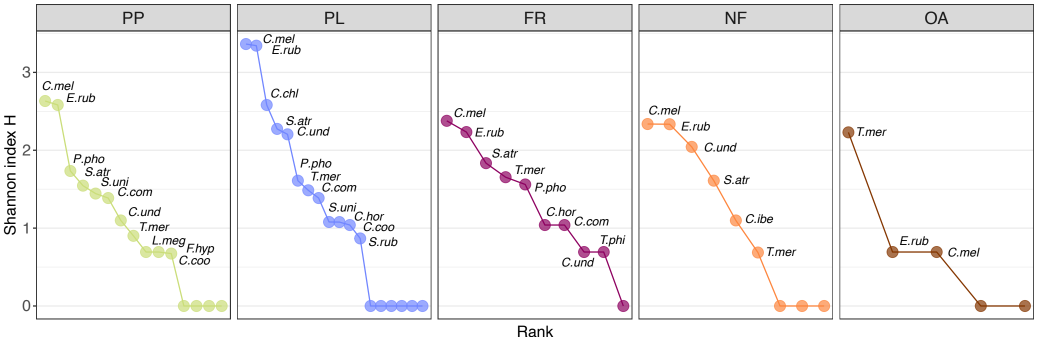


Figure 3.4. Cumulative contribution of birds to the diversity (Shannon index) of *Pistacia lentiscus* seed rain across sampling points for each microhabitat. Higher diversity values indicate higher spread of the seed dissemination among sites (more even distribution), while lower values suggest higher concentration of seeds across fewer sites. Panels are ordered by decreasing probability of bird's depositing seeds at each specific microhabitat. Microhabitats codes: PL = under female *Pistacia lentiscus* plants, FR = under other fleshy fruited species, NF = under non-fleshy fruited species, PP = under pine trees, OA = open areas. Animal species codes in alphabetical order: *C.chl = Chloris chloris, C.com = Curruca communis, C.coo = Cyanopica cooki, C.hor = Curruca hortensis, C.ibe = Curruca iberiae, C.mel = Curruca melanocephala, C.und = Curruca undata, E.rub = Erithacus rubecula, F.hyp = Ficedula hypoleuca, L.meg = Luscinia megarhynchos, P.pho = Phoenicurus phoenicurus, S.atr = Sylvia atricapilla, S.rub = Saxicola rubicola, S.uni = Sturnus unicolor, T.mer = Turdus merula, T.phi = Turdus philomelos*. Unlabelled points indicate other avian species in the frugivore assemblage contributing to just one site in that specific microhabitat in which case Shannon index is 0.

The analysis of seed rain across microhabitats also revealed contrasting differences in seed abundance and frugivores' contributions. Sites covered by *Pinus pinea* (PP) and *P. lentiscus* (PL) received the largest seed densities (122 seeds/m^2, 80% CI = 98 – 158, and 88 seeds/m^2, 80% CI = 79–101, respectively), also contributed by the largest number of frugivores (15 and 18 species, respectively) (Fig. 3.4, Appendix

Table 3.1. Metrics defining frugivores' role in *Pistacia lentiscus* seed dispersal and recruitment. First four columns refer to P (proportion) indicating the relative contribution of that bird species to the total service provided by all birds at EP site. Bird species are listed in descending order by the proportion of seedlings recruited. Numbers indicate the median of the corresponding posterior distributions while numbers in brackets indicate 80% credible interval. The last two columns refer to the general role of birds in the population. 'Traps with seeds (%)' indicates the percentage of seed traps receiving at least one *P. lentiscus* seed dispersed by a bird species. 'Prevalence of *P. lentiscus* in seed rain (%)' indicates the percentage of *P. lentiscus* seeds found in bird droppings out of the total number of different seeds dispersed by bird species.

Bird species	P of fruits consumed	P of seeds dispersed	P of viable seeds dispersed	P of seedlings recruited	Traps with seeds (%)	Prevalence of *P. lentiscus* in seed rain (%)
Curruca melanocephala	0.3 [0.1–0.5]	0.5 [0.5–0.5]	0.5 [0.5–0.6]	0.5 [0.4–0.6]	85.0	65.5
Erithacus rubecula	0.2 [0.09–0.4]	0.3 [0.3–0.3]	0.2 [0.2–0.3]	0.2 [0.2–0.4]	82.0	85.0
Sylvia atricapilla	0.04 [0.02–0.1]	0.03 [0.02–0.04]	0.03 [0.02–0.05]	0.08 [0.05–0.1]	28.0	37.5
Turdus merula	0.04 [0.01–0.1]	0.06 [0.05–0.07]	0.04 [0.03–0.05]	0.04 [0.03–0.05]	32.0	71.2
Phoenicurus phoenicurus	0.01 [0.005–0.04]	0.02 [0.01–0.02]	0.02 [0.01–0.03]	0.03 [0.01–0.05]	18.0	94.4
Curruca communis	0.008 [0.003–0.02]	0.009 [0.006–0.01]	0.02 [0.01–0.03]	0.03 [0.02–0.04]	13.0	45.9
Sturnus unicolor	0.004 [9e-04–0.01]	0.01 [0.007–0.01]	0.03 [0.02–0.04]	0.02 [0.008–0.06]	11.0	41.5
Cyanopica cooki	0.005 [0.002–0.02]	0.005 [0.003–0.008]	0.008 [0.004–0.01]	0.02 [0.01–0.03]	7.0	16.0
Curruca undata	0.009 [0.004–0.02]	0.03 [0.02–0.04]	0.03 [0.02–0.04]	0.01 [0.007–0.02]	23.0	100.0
Curruca hortensis	0.003 [0.001–0.01]	0.006 [0.004–0.01]	0.01 [0.006–0.02]	0.009 [0.005–0.02]	7.0	66.7
Saxicola rubicola	0.002 [9e-04–0.007]	0.003 [0.002–0.005]	0.007 [0.003–0.01]	0.009 [0.003–0.02]	4.0	87.5
Luscinia megarhynchos	0.001 [4e-04–0.005]	0.001 [4e-04–0.003]	0.002 [5e-04–0.005]	0.004 [0.002–0.009]	3.0	100.0
Ficedula hypoleuca	4e-04 [1e-04–0.001]	0.001 [4e-04–0.003]	0.002 [6e-04–0.005]	0.001 [5e-04–0.003]	3.0	100.0
Turdus philomelos	4e-04 [1e-04–0.002]	0.001 [5e-04–0.003]	0.002 [5e-04–0.005]	0.001 [4e-04–0.002]	3.0	50.0
Sylvia borin	3e-04 [8e-05–0.001]	0.001 [5e-04–0.003]	0.002 [6e-04–0.005]	7e-04 [2e-04–0.002]	1.0	60.0
Chloris chloris	0.3 [0.08–0.6]	0.02 [0.01–0.02]	0.02 [0.01–0.03]	7e-04 [1e-04–0.002]	18.0	85.7
Curruca iberiae	5e-05 [1e-05–2e-04]	0.004 [0.002–0.01]	0.008 [0.003–0.02]	1e-04 [5e-05–2e-04]	4.0	66.7
Muscicapa striata	3e-05 [5e-06–2e-04]	4e-04 [8e-05–0.001]	7e-04 [1e-04–0.003]	8e-05 [3e-05–2e-04]	1.0	100.0
Fringilla coelebs	0.002 [6e-04–0.006]	8e-04 [3e-04–0.002]	0.001 [3e-04–0.003]	6e-06 [2e-06–2e-05]	2.0	100.0
Pyrrhula pyrrhula	7e-05 [2e-05–3e-04]	5e-04 [1e-04–0.001]	8e-04 [2e-04–0.003]	3e-07 [9e-08–7e-07]	1.0	100.0

3E). In contrast, open area (OA) received very low seed densities (median 0.08 seeds/ m^2, 80% CI = 0.069-0.083) brought by only three bird species, with most seed rain contributed by *T. merula* (Fig. 3.4). The most frequent consumers (*C. melanocephala* and *E. rubecula*) were the main contributors of seed dispersal to all microhabitats, except open areas. Some species also showed marked preference for specific microhabitats, such as *Sturnus unicolor* for pine trees, or *Curruca undata* for non-fleshy plants or *Chloris chloris* for P. *lentiscus* plants.

Subsequent consequences of seed dispersal

Post-dispersal seed fate (Fig. 3.1) varied among microhabitats, however these differences were not pronounced (Table 3.2; Fig. A3.4). TPs refer to the probabilities that a propagule reaching a specific demographic stage will survive the ecological process acting at that stage; thus, these are stage-specific transition probabilities. First, the probability of surviving post-dispersal rodent predation was very low in all microhabitats (median = 0.008; 80% CI = 0.001 – 0.07), but slightly higher under pine trees (median = 0.02) and open areas (median = 0.01). Seedling emergence for viable seeds was around 8% (80% CI = 0.04 – 0.18). Seeds arriving to open areas had the highest probability of emergence (median = 0.17) and seeds falling under fleshy-fruited species the lowest (median = 0.05). Seedling survival through their first summer was slightly higher than the previous transition stages (median = 0.25; 80% CI = 0.09 – 0.53), being highest under non-fleshy fruited plants. Lastly, the probability of surviving until their second summer was around 20% (80% CI = 0.02 – 0.54), being significantly lower in open areas (median = 0.02). In the end, the overall probability of recruitment (OPR) after seed arrival was similar among microhabitats (median = 1.9×10^{-5}; 80% CI = 1.3×10^{-6} – 2.6×10^{-4}), as the effects of different post-dispersal stages partially cancelled each other. Seeds arriving under pine trees had the highest probabilities of recruitment, yet these probabilities considerably overlap with those of seeds arriving under fleshy-fruited species, the lowest quality microhabitat (Table 3.2).

Table 3.2. Transition probabilities (TPs; see Fig. 3.1) and overall probability of recruitment (OPR) in different microhabitats. Numbers in brackets denote 80% credibility intervals. TP is the probability that a propagule gets from the previous stage to the subsequent stage (*e.g.*, probability that a seed that escaped seed predation emerges as a seedling). The OPR is the cumulative probability of recruitment across all successive stages; it was calculated by multiplying the full distribution of the posterior probabilities at each demographic stage after seed arrival (*i.e.*, not considering the first row).

	Fleshy-fruited species (FR)	**Non fleshy-fruited species (NF)**	**Open ground areas (OA)**	***Pistacia lentiscus* female plants (PL)**	**Pine trees (PP)**
P seed arrival at EP site (TP4)	0.19 [0.15 – 0.24]	0.5 [0.44 – 0.58]	0.00018 [0.00015 – 0.00022]	0.28 [0.24 – 0.33]	0.019 [0.015 – 0.026]
P escape seed predation (TP5)	0.0068 [0.0012 – 0.034]	0.0054 [0.00051 – 0.042]	0.013 [0.0015 – 0.096]	0.0037 [0.00036 – 0.029]	0.023 [0.0023 – 0.15]
P seedling emergence (TP6)	0.051 [0.027 – 0.086]	0.091 [0.05 – 0.16]	0.17 [0.094 – 0.25]	0.087 [0.049 – 0.15]	0.052 [0.028 – 0.097]
P seedling survival to 1st summer (TP7)	0.12 [0.085 – 0.21]	0.52 [0.32 – 0.73]	0.3 [0.21 – 0.42]	0.26 [0.14 – 0.38]	0.14 [0.061 – 0.26]
P seedling survival to 2nd summer (TP8)	0.16 [0.048 – 0.34]	0.11 [0.019 – 0.34]	0.023 [0.0064 – 0.069]	0.45 [0.26 – 0.63]	0.4 [0.18 – 0.65]
Overall Probability of Recruitment	5.5×10^{-6} [$6.5 \times 10^{-7} - 4.1 \times 10^{-5}$]	2.3×10^{-5} [$1.1 \times 10^{-6} - 3.3 \times 10^{-4}$]	1.3×10^{-5} [$9.8 \times 10^{-7} - 1.4 \times 10^{-4}$]	3.4×10^{-5} [$2.7 \times 10^{-6} - 3.1 \times 10^{-4}$]	5.3×10^{-5} [$4.2 \times 10^{-6} - 4.7 \times 10^{-4}$]

Transitions between demographic stages and total recruitment

Seedling recruitment experienced a 6-order magnitude decay from ripe fruit production stage to the seedling survival through their second summer stage. From nearly a million fruits initially produced by the 40 studied plants at EP site, we estimate that only a few seedlings (median = 1.6, 80% CI = 0.2 – 10.1) were recruited and survived through their second summer (Appendix 3F and 3G). Individual plants' probability of recruitment was quite even, although four individual plants had 2-3 times higher probability of recruiting than the others (Appendix 3F). Recruitment of individual plants was not correlated with the proportion of viable seeds found in the crop ($r = 0.16$, $p = 0.32$), suggesting that higher plant investment in viable seeds does not directly translate into higher recruitment. Overall, we estimate that the median number of fruits required to recruit a 2-year-old seedling was 514,000 fruits. For context, crop sizes in our focal plants ranged from 3,500 to 119,000 fruits.

Of all the demographic transitions studied, post-dispersal predation by rodents was the most limiting stage, followed by seedling emergence (Fig. 3.5 and Table 3.2). With the exception of Fringillidae (mainly *Chloris chloris,* which destroyed almost all seeds consumed), bird species' contribution to recruitment was directly related to their fruit consumption intensity ($r = 0.81$, p-value < 0.001 for all birds species, $r = 0.99$, p-value < 0.001 for only legitimate dispersers, Fig. 3.5A and Table 3.1).

When examining total recruitment at EP site, based on seed rain density at the population level rather than crop production of the 40 focal plants, *Pistacia lentiscus* (PL) and non-fleshy fruited species (NF) emerged as the microhabitats where most seedlings were recruited (median number of seedlings in PL = 11, 80% CI = 1 – 98; median number of seedlings in NF = 10, 80% CI = 1 – 119; Fig. 3.5B). This is largely explained by the large area covered by both microhabitats at EP site (45% NF and 22% PL) and their good overall quality in terms of recruitment probabilities (Table 3.2). Open area, despite being potentially suitable, showed virtually no recruitment (median number of seedlings = 1.3×10^{-3}; 80% CI = 1×10^{-4} – 0.02, Fig. 3.5B) because of the limited arrival of seeds. Pine trees (PP) are very scarce in our study site (1% cover) and received relatively few seeds, yet the lower rodent predation in this microhabitat led to relatively high recruitment considering its reduced extent. We estimate that for every million seeds arriving to pine trees, this microhabitat would be able to recruit 15 seedlings (80% CI = 1.4 –123), while fleshy fruited plants would roughly manage to recruit 2 seedlings (80% CI = 0.2 –14). Overall, we estimate the

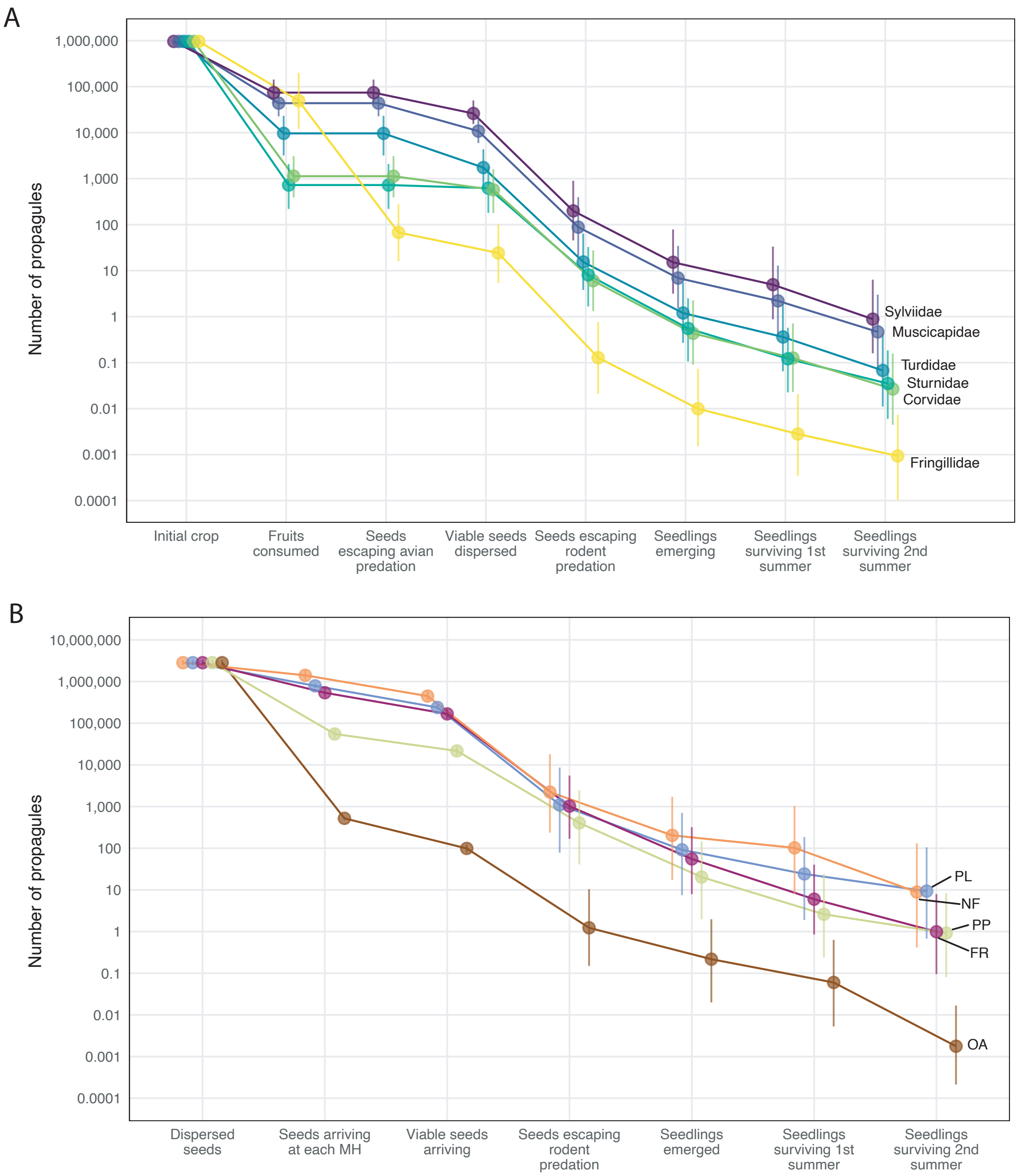


Figure 3.5. Decline in the number of propagules of *Pistacia lentiscus* (fruits or seeds depending on the demographic stage) along the seed dispersal and recruitment process. Panel A shows the contribution of different bird families along the recruitment process for the 40 plants studied at EP site. Panel B shows the demographic transition for seeds dispersed at different microhabitats for the whole *P. lentiscus* population at EP site. Each point represents the median of the posterior distributions and bars represent 80% CI. Note the log-scale in y-axis.

total number of recruits surviving the second summer was 47 (80% CI = 9 – 249), coming from ~2.8 million seeds dispersed (80% CI = 2.5 - 3.3 million) for a total area of 4.1 ha.

Discussion

Here we examine the sequential stages from fruit production to seedling recruitment of a fleshy-fruited plant to obtain an integrative view of plants' reproductive cycle (Schupp & Fuentes 1995). This integrative approach enabled us to disentangle the role of frugivores, seed dispersal, and microhabitat deposition along the demographic transitions of a seed until establishing as a second-year seedling. We have found a decoupling of the dissemination and recruitment processes: despite the overall high fecundity of *P. lentiscus* plants and the large array of frugivores dispersing its seeds, which ensure a widespread and relatively abundant seed rain, most seeds arrive at suboptimal microhabitats for seedling recruitment. As a result, individual plants must produce large amounts of fruits to ensure minimal recruitment.

Causes and consequences of seed viability on plant recruitment success

Plants widely differed in the amount of viable seeds they produced. The causes for this variation are not clearly understood, however they seem to be related to a combination of plants' individual life history, pollen limitation and water allocation (Jordano 1988b, Verdú & García-Fayos 1998). Higher investment in viable seeds did not seem to directly increase individual plant's recruitment, probably because other factors and processes are also mediating in recruitment success. Having unfilled seeds is hypothesised to have evolved to reduce seed lost to pre-dispersal predation (Traveset 1993, Fuentes & Schupp 1998, Verdú & García-Fayos 2001). The production of large fruit crops, even if unviable, can also contribute to attracting higher amounts of dispersers, which also explains the benefit of retaining parthenocarpic and aborted fully-developed fruits in the crop. Yet we found no evidence in the two study populations that larger fruit crops resulted in an increased percentage of viable seeds dispersed relative to unviable seeds dispersed. Noteworthy, this study did not follow the identity of individual plants' seeds after dispersal, but inferred average seed fate at the population-level. Besides seed viability, individual differences in seed size likely affects post-dispersal success (predation, germination and seedling survival; Alcántara & Rey 2003). Further research that tracks maternal seed identity through post-dispersal stages will help to understand the effect of seed viability investment on recruitment

We found slightly lower viability in seeds dispersed compared to the initial viability of plants' crop. This was unexpected as bird species positively select black fruits of *P. lentiscus* that have higher viability rates than red fruits (see also Jordano 1989). This preference may be offset by the fact that birds consume both the more abundant, unripe fruits and the fully ripe fruits (either with viable seeds or not). It is also possible that dispersed seeds decrease their viability when exposed to harsh climatic conditions such as marked changes in temperature, moisture and heat exposure (Franchi *et al*., 2011). *Pistacia lentiscus* seeds are sensitive to very high temperatures (Salvador & Lloret 1995) and rarely form seed banks because of their short seed longevity (García-Fayos & Verdú 1998). This explanation is consistent with the fact that seeds dispersed by *Turdus merula*, which deposit most seeds at exposed open areas, showed the lowest viability (Appendix 3B).

Frugivore and microhabitat roles in seedling recruitment

Pre- and post-dispersal processes of plants are often studied in isolation. Integrative studies that connect frugivore consumption with seedling recruitment are much less frequent (*e.g*., Herrera *et al*. 1994, Jordano & Herrera 1995, Schupp & Fuentes 1995, Jordano & Schupp 2000, Rey & Alcántara 2000, Côrtes *et al*. 2009, Donoso *et al*. 2016). Here we managed to estimate the recruitment success of individual plants from seed production to seedling survival, assessing the delayed consequences of pre-dispersal (*e.g*., seed viability, frugivore predation) and post-dispersal stages (frugivore-mediated seed rain, microhabitat-associated rates of seed predation or seedling survival) on plant overall recruitment success. Moreover, our analysis allowed assessing the contribution of each frugivore species to final recruitment.

Our results suggest that bird species' contribution to *P. lentiscus* recruitment is stable along demographic transitions. The number of seedlings recruited through the interaction with birds was directly related to their quantity of fruit consumption. The major exception are Fringillids, which shifted from playing an important role in fruit removal to destroying nearly all the seeds consumed, contributing only marginally to recruitment (Heleno *et al*. 2011). The fact that animals' recruitment service is mainly guided by consumption (the frequency component) indicates redundancy in their dispersal service (Quintero *et al.* 2023, Rehling *et al.* 2023).

Despite ample functional redundancy among frugivores, their dispersal services were complementary in some important aspects, such as temporal and spatial patterns. Birds present at the beginning of the fruiting season (trans-Saharan migrants) dispersed a greater amount of viable seeds than summer migrants (in congruence with González-Varo *et al.* 2019a), thus increasing their relative contribution to recruitment (Table 3.1, Fig. A3.1). In addition, although the main disperser species deposited seeds in all microhabitats, bird species differed in their contribution to different microhabitats most likely as a consequence of preferences for good perching sites for resting (Athiê & Dias 2016). These bird preferences translated into microhabitat differences in the amount of seed rain and the diversity of bird species contributing to it. For example, most of the (few) seeds arriving at open areas are brought by a single bird species, *Turdus merula*. Hence, this disperser must play an important role in colonising new spaces, and its eventual local disappearance could have important consequences on *P. lentiscus'* colonisation ability and plant community structuring (González-Varo *et al*. 2017, Campo-Celada *et al*. 2022, Isla *et al*. 2023). The overall spatial clumping of the seed rain was concordant with many previous studies (*e.g.*, Clark *et al.* 1998, Arnell *et al.* 2021). The unevenness found in seed deposition sites together with microhabitat preferences illustrate how bird species' differences in site fidelity and landscape use generate a patchy template on which plant regeneration takes place. This heterogeneous and non-random seed deposition leads to the creation of orchards (aggregation clusters of heterospecific seeds and seedlings; Lázaro *et al.* 2005), having lasting consequences on local plants' recruitment. We expect the spatial and temporal complementary role of frugivores' on dispersal will be differently manifested in other plant populations and habitats with varying animal-disperser abundances and microhabitat compositions (García-Rodríguez *et al.* 2022).

Differences in microhabitat quality for arriving seeds along the regeneration cycle were discordant; high-quality patches in some stages became low-quality patches in others, as previously reported in several studies (Herrera *et al.* 1994, Jordano & Herrera 1995, Schupp 1995, Clark *et al.* 1999, Gómez-Aparicio 2008). These differences have been attributed to both biotic and abiotic factors such as plant-plant competition/facilitation, fungal pathogens, insects, temperature, humidity or soil nutrients (Fricke *et al.* 2014, Traveset *et al.* 2003). High seed densities at fleshy-fruited microhabitats may enhance the call-effect for rodent predation and increase plant-plant competition. In contrast, open areas, a microhabitat typically avoided by birds (Jordano & Schupp 2000, Alcántara & Rey 2003), lead to low seed predation due to lack of shelter for rodents (Fedriani & Manzaneda 2005), but high seedling mortality due to higher water and irradiance stress (Amat *et al.* 2015).

Spatial discordance between seed rain and recruitment has been repeatedly reported (Houle 1992, Jordano & Herrera 1995, Schupp & Fuentes 1995, Rey & Alcántara 2000; but see García *et al*. 2005). In this study we found that microhabitat differences in the transition probabilities between demographic stages partially cancelled each other, leading to small differences in the overall probability of recruitment (OPR) among microhabitats. However, variations between microhabitats were large enough to detect small spatial discordances, depicted by the differences in the shape and crossings of the decaying cumulative curves among microhabitats (Fig. 3.5B, Fig. A3.4). In the end, recruitment was mostly determined by the initial number of seeds arriving at each microhabitat, which was again determined by the microhabitat preferences of bird frugivores.

Demographic bottlenecks and recruitment success

Post-dispersal seed predation emerged as the main limiting demographic transition in *P. lentiscus* regeneration (also reported in González-Varo *et al*. 2019a), followed by seedling emergence, in congruence with similar studies carried out in Mediterranean species (Gómez-Aparicio 2008).

The overall probabilities of recruitment (OPR) for *P. lentiscus* at our study site were similar, although a bit lower, than those found for other Mediterranean plants (Herrera *et al.* 1994, Jordano & Herrera 1995, Rey & Alcántara 2000, Traveset *et al.* 2003, Gómez-Aparicio 2008). Even if the OPR were low and overlapped between microhabitats, some microhabitats differed in their median probability by as much as 10-fold, with pine trees (PP) showing considerably larger suitability for recruitment than fleshy-fruited (FR) or open areas (OA). These differences, if accumulated over time, can have important consequences for landscape regeneration. In fact, evidence from the literature supports significantly lower recruitment of *P. lentiscus* in open areas compared to beneath tree canopies (Verdú & García-Fayos 1996a, García-Fayos & Verdú 1998), including pine trees (Maestre *et al.* 2004). Additionally, recruitment can be dependent on population maturity and establishment. Our study site is a densely vegetated shrubland, dominated by reproductive adults of *P. lentiscus*, while young saplings of this plant are harder to find. García-Fayos & Verdú (1998) also found recurrent low densities of *P. lentiscus* seedlings in closed shrublands.

The quantity of avian fruit consumption was the key determinant of plants' recruitment success, while qualitative differences among frugivores and microhabitats played a minor role. This underscores the importance of a reliable and abundant seed dispersal service for recruitment, and its susceptibility to eventual fluctuations in frugivore abundances. We anticipate that the identity of frugivores and their seed deposition patterns will become important in different habitats, under natural succession or anthropogenic disturbances scenarios, where recruitment success will be dependent on the distribution of available microhabitats in the landscape (Wenny & Levey 1998, Rost *et al*. 2009, García-Rodriguez *et al.* 2022).

Conclusion

Our investigation of the recruitment process of *Pistacia lentiscus*, from fruit production to seedling recruitment, unveiled a key role of frugivores and their non-random microhabitat use on individual plant recruitment success. Frugivore effects include both immediate interaction outcomes during fruit consumption and, importantly, delayed effects lasting after seed dissemination.

Pistacia lentiscus overcomes its high seed unviability by investing in large crops and representing a staple nutritious resource to many bird species, which disperse vast amounts of seeds. We found that different bird species provide largely overlapping, but also complementary, dispersal services. Bird species markedly differ in the amount of fruits they consume and in the proportion of viable seeds they disperse, related to the timing of their arrival during the fruiting season. Functional redundancy in birds' overall post-dispersal quality makes *P. lentiscus* particularly robust to the loss of minor consuming species and therefore more resilient to ecosystem disturbances (Loiselle *et al.* 2007).

This study supports previous literature highlighting the importance of consumption frequency for plant recruitment (*e.g.*, Vázquez *et al*. 2005, Rehling *et al.* 2023). However, behind the major effects of interaction frequency, the differences detected in spatial patterns of seed deposition by birds and the suitability of microhabitats for future plant recruitment underscore the importance of evaluating post-dispersal consequences of plant-animal frugivory interactions. Our results illustrate the complexity of the recruitment process in which there is an interplay between plants' investment in viable seeds, bird consumption and dispersal service, and the suitability of the microhabitat to which seeds arrive. The loss of certain avian species with specific phenologies (*e.g.*, frugivorous wintering migrants; see Campo-

Celada *et al*. 2022) and preferences for fruit consumption and land-use may impact plant recruitment in the most suitable microhabitats, having lasting consequences in plant regeneration and vegetation physiognomy.

Acknowledgements

We are grateful to Jorge Isla, Blanca Arroyo, Marta Peláez and Luisa Genes for fruitful discussions that helped improve this manuscript. We thank the logistics and facilities support form ICTS-RBD Doñana and the Doñana National Park for onsite access authorisations during the fieldwork. The 'Molecular Ecology Laboratory' (LEM–EBD–CSIC; ISO9001:2015 and ISO14001:2015 certifications) provided logistical support for molecular analyses. We thank two anonymous reviewers and editors for helpful comments on the previous version of this manuscript.

Funding information: EQ received support from "la Caixa" Foundation (ID 100010434), under agreement LCF/BQ/DE18/11670007 and from a Fulbright fellowship co-funded by Junta de Andalucía. EQ, PJ and FRS received support from LifeWatch ERIC-SUMHAL project (LIFEWATCH-2019-09-CSIC-13), with FEDER-EU funding, and grant PID2022-136812NB-I00 funded by MCIN/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR. FRS and PJ were additionally supported by VI Plan Propio de Investigación, Universidad de Sevilla (VI PPIT-US), FEDER 2014-2020 and Consejería de Economía, Conocimiento, Empresas y Universidad, Junta de Andalucía (grant US-1381388, Universidad de Sevilla).

APPENDICES CHAPTER 3

Appendix 3A. *Pistacia lentiscus* frugivore assemblage

Appendix 3B. Variation in pre-dispersal seed viability along the season

Appendix 3C. Post-dispersal seed viability

Appendix 3D. Relation between plants' crop size, seed viability and seed dispersal by frugivores

Appendix 3E. Microhabitat seed-deposition and quality

Appendix 3F. Seedling recruitment by plant and bird species

Appendix 3G. Stage transitions in seedling recruitment by individual plants

Appendix 3H. Software

The data and code generated for Chapter 3 are archived in the Zenodo digital repository: https://doi.org/10.5281/zenodo.8289087 and the GitHub repository: https://github.com/elequintero/MS_P.lentiscus_demography.

Appendix 3A. *Pistacia lentiscus* frugivore assemblage

Herrera, C. M. (1984). A study of avian frugivores, bird-dispersed plants, and their interaction in mediterranean scrublands. *Ecological Monographs,* 54(1), 1–23.

Jordano, P. (1989). Pre-dispersal biology of *Pistacia lentiscus* (Anacardiaceae): Cumulative effects on seed removal by birds. *Oikos*, 55(3), 375–386.

Izhaki, I., Walton, P. B., & Safriel, U. N. (1991). Seed shadows generated by frugivorous birds in an Eastern Mediterranean scrub. *The Journal of Ecology*, 79(3), 575.

Parejo-Farnés, C., Albaladejo, R. G., Camacho, C., & Aparicio, A. (2018). From species to individuals: Combining barcoding and microsatellite analyses from non-invasive samples in plant ecology studies. *Plant Ecology,* 219(10), 1151–1158.

González-Varo, J. P., Arroyo, J. M., & Jordano, P. (2019). The timing of frugivore-mediated seed dispersal effectiveness. *Molecular Ecology*, 28(2), 219–231.

Acosta-Rojas, D. C., Jiménez-Franco, M. V., Zapata-Pérez, V. M., De La Rúa, P., & Martínez-López, V. (2019). An integrative approach to discern the seed dispersal role of frugivorous guilds in a Mediterranean semiarid priority habitat. *PeerJ*, 7, e7609.

Costa, J. M., Ramos, J. A., Timóteo, S., Silva, L. P. da, Ceia, R. S., & Heleno, R. H. (2020). Species temporal persistence promotes the stability of fruit–frugivore interactions across a 5-year multilayer network. *Journal of Ecology*, 108(5), 1888–1898.

Table A3.1. Bird species detected feeding on *Pistacia lentiscus* fruits in different studies and with different methodologies.

[1] - Three faecal samples with *P. lentiscus* seeds were found in open area microhabitat attributed to this species. However, since no samples were found under focal plants of *P. lentiscus,* we were unable to estimate their visitation rates, feeding frequency or fruits per visit, which prevented us from estimating their fruit consumption. Therefore, this species has not been considered in this or the previous study. Furthermore, given the anecdotic presence in the seed rain (just three faecal samples), the role of this species in *P. lentiscus* dispersal and recruitment must be negligible.

[2] - Samples found under *Pistacia lentiscus* female plants but with no *P. lentiscus* seed.

[3] - Two *Sturnus* species - *S. unicolor* and *S. vulgaris*.

* - Species only detected in present thesis.

	Present study	Present study	Herrera 1984	Jordano 1989	Izhaki *et al.* 1991	Parejo-Farnés *et al.* 2018	González-Varo *et al.* 2019	Acosta-Rojas *et al.* 2019	Costa *et al.* 2020
Methods:	DNA-barcoding	Cameras	Mist-nets	Mist-nets and focal obs.	Focal obs.	DNA-barcoding	DNA-barcoding	DNA-barcoding	Mist-nets
Curruca melanocephala	●	●	●	●	●	●	●	●	●
Erithacus rubecula	●	●	●	●	●		●	●	●
Sylvia atricapilla	●	●	●	●	●	●	●		●
Turdus merula	●	●	●	●	●	●		●	●
Phoenicurus phoenicurus	●	●		●	●		●		
Curruca communis	●	●	●	●			●		●
Sturnus unicolor	●	●		●[3]					
Cyanopica cooki	●	●		●					
Curruca undata	●	●		●				●	●
Curruca hortensis	●	●		●			●		
Saxicola rubicola	●	●		●					
Luscinia megarhynchos	●	●		●			●		
Ficedula hypoleuca	●	●		●			●		●
Turdus philomelos	●	●		●	●	●	●	●	
Sylvia borin	●	●	●	●		●	●		●
Chloris chloris	●	●		●					
Curruca iberiae	●	●		●					
Muscicapa striata	●	●		●			●		●
Fringilla coelebs	●	●		●					
*Pyrrhula pyrrhula**	●	●							
*Alectoris rufa**	●[1]								
Columba palumbus	●[2]			●					
Turdus viscivorus		●						●	
*Lanius meridionalis**		●							
*Coccothraustes coccothraustes**		●							
Parus major		●		●					
Cyanistes caeruleus		●		●					
*Hippolais polyglotta**		●							
Phoenicurus ochruros				●	●			●	
Phylloscopus collybita				●					
Regulus ignicapillus				●					
Pycnonotus barbatus					●				
Number of species:	22	26	6	25	8	5	11	7	9

Appendix 3B. Variation in pre-dispersal seed viability along the season

Site	Viability	Abortion	Parthenocarpy	Wasp predation
EP	0.33 ± 0.13	0.38 ± 0.12	0.25 ± 0.13	0.04 ± 0.05
LM	0.35 ± 0.24	0.38 ± 0.22	0.25 ± 0.22	0.02 ± 0.03

Table A3.2. Proportion of viable seeds and causes of unviability (abortion, parthenocarpy and wasp predation). Average across individuals ± standard deviation.

To examine potential differences in seed viability between the three collection periods at EP site during 2019-2020, we fitted a generalised linear mixed model (GLMM) with a beta-binomial error distribution and logit link function using glmmTMB (Brooks *et al.* 2017). We used plant's viability in response to the collection period (early, mid, late) and used plant ID as a random intercept. We did not observe any significant difference between periods.

```
Parameter   | Log-Odds |  SE |  95% CI        |   z   |   p
----------------------------------------------------------
(Intercept)|   -0.71  | 0.15 | [-1.00, -0.41]| -4.70 | < .001
mid-season |    -0.03 | 0.20 | [-0.43,  0.36]| -0.17 | 0.865
late-season|    -0.33 | 0.22 | [-0.76,  0.09]| -1.54 | 0.122

Parameter             |Coefficient |  95% CI
----------------------------------------------------------
SD (Intercept: plant_id) | 0.24    | [0.06, 0.96]
```

Appendix 3C. Post-dispersal seed viability

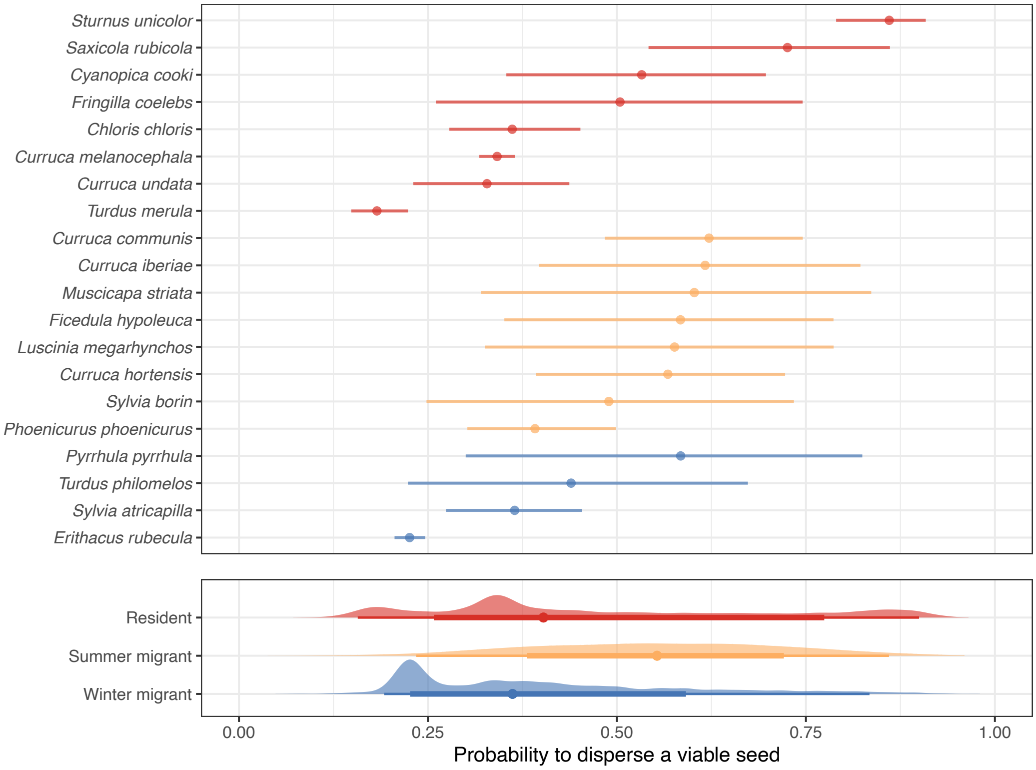


Figure A3.1. Posterior probability of seeds being viable when dispersed by different bird species. Points represent medians. Horizontal bars above denote 80% credibility interval. Bars below denote 0.66 (thick line) and 0.95 (thin line) credibility intervals.

Appendix 3D. Relation between plants’ crop size, seed viability and seed dispersal by frugivores

We hypothesised that seed dispersal would be positively affected by crop size and that when only considering viable seed dispersal, the effect of crop size might be higher, as larger crops attract more birds, which could be dispersing proportionally more viable seeds and so being in a large crop size would be more advantageous for viable seeds.

We fitted two generalised linear models of the number of dispersed seeds with a negative binomial distribution (using glmmTMB, Brooks *et al.* 2017) and used log-converted crop size and population site as fixed effects. We did not detect differences in regression slopes between crop size and seed dispersal using all seeds (slope = 0.74 ± 0.08 SE) or only viable seeds (slope = 0.72 ± 0.07 SE). Hence we did not find evidence that larger crop sizes favour the dispersal of viable seeds in a larger proportion.

Model 1 – Dispersal of seeds regardless viability

```
Parameter     |Log-Mean| SE   |  95% CI       | z    | p
----------------------------------------------------------
(Intercept)   |  0.29   | 0.65 | [-0.98, 1.57] | 0.45 | 0.652
crop [log]    |  0.74   | 0.08 | [ 0.59, 0.89] | 9.66 |< .001
site [Puntal] |  0.48   | 0.19 | [ 0.10, 0.85] | 2.50 | 0.012
Marginal R squared = 0.67
```

Model 2 – Dispersal of only viable seeds

```
Parameter  |Log-Mean| SE   |   95% CI          |  z    |  p
----------------------------------------------------------
(Intercept)  |-1.00 | 0.64 | [-2.25, 0.25]      | -1.57 | 0.116
crop [log]   | 0.72 | 0.07 | [ 0.57, 0.87]      |  9.64 | < .001
site [Puntal]| 0.64 | 0.19 | [ 0.27, 1.01]      |  3.37 | < .001
Marginal R squared = 0.68
```

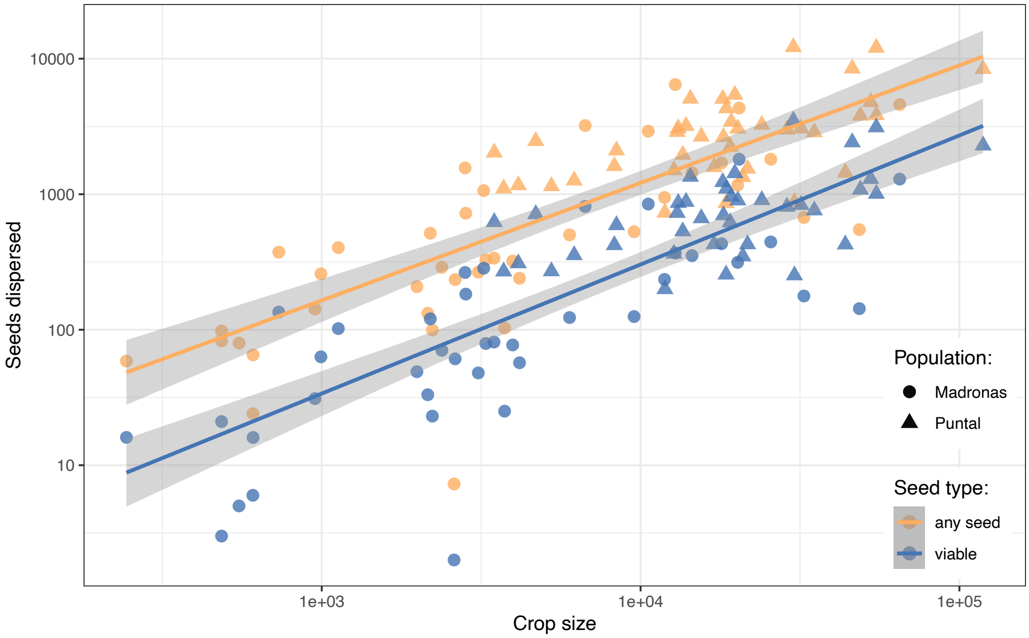


Figure A3.2. Relation between the crop size of individual plants and the amount of seeds dispersed by birds. Colours denote seed type (all seed types included vs. only viable seeds included) and shape denotes the two studied populations (LM and EP). Note both axes are in log-scale. The trend lines represent the linear positive relation between both variables and the shaded area represents 95% confidence interval, according to Model 1 and Model 2 fitted above.

Appendix 3E. Microhabitat seed-deposition and quality

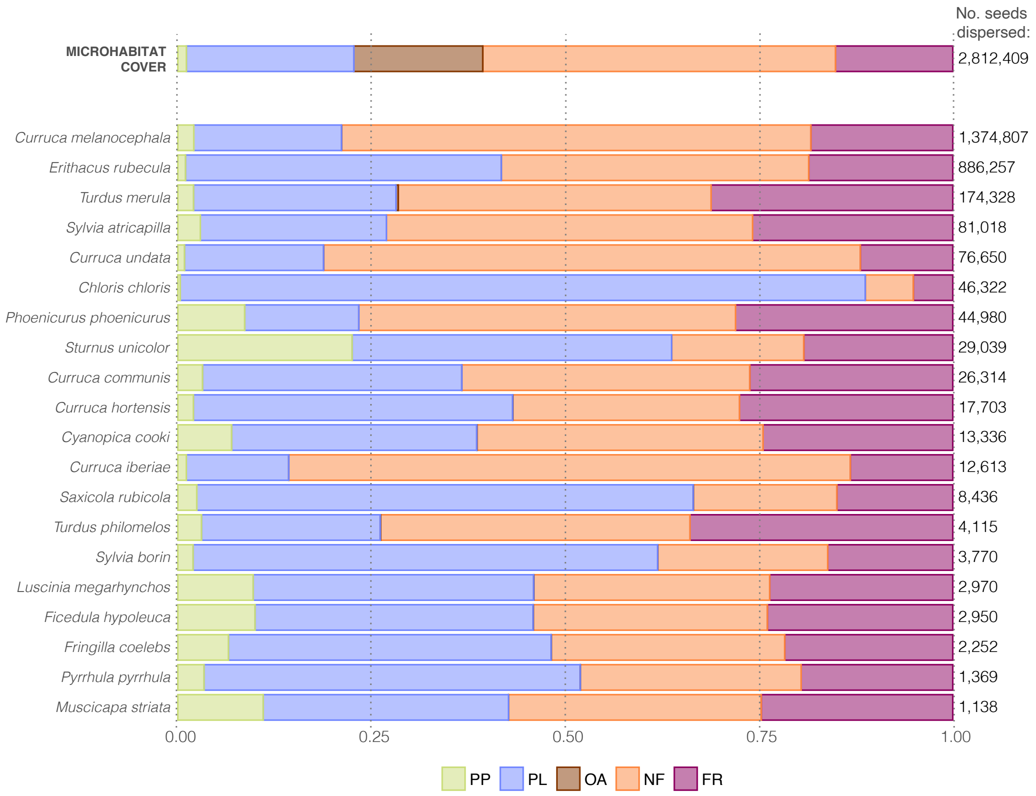


Figure A3.3. Proportion of microhabitat cover at EP site and the probability that seeds dispersed by each bird species fall in one of these microhabitats. Numbers in the right of each bar indicate the total estimated number of *P. lentiscus* seeds dispersed by each bird species in the study site. Microhabitat codes: under female *Pistacia lentiscus* plants (PL), under other fleshy fruited species (FR), under non-fleshy fruited species (NF), under pine trees (PP) and in open areas (OA).

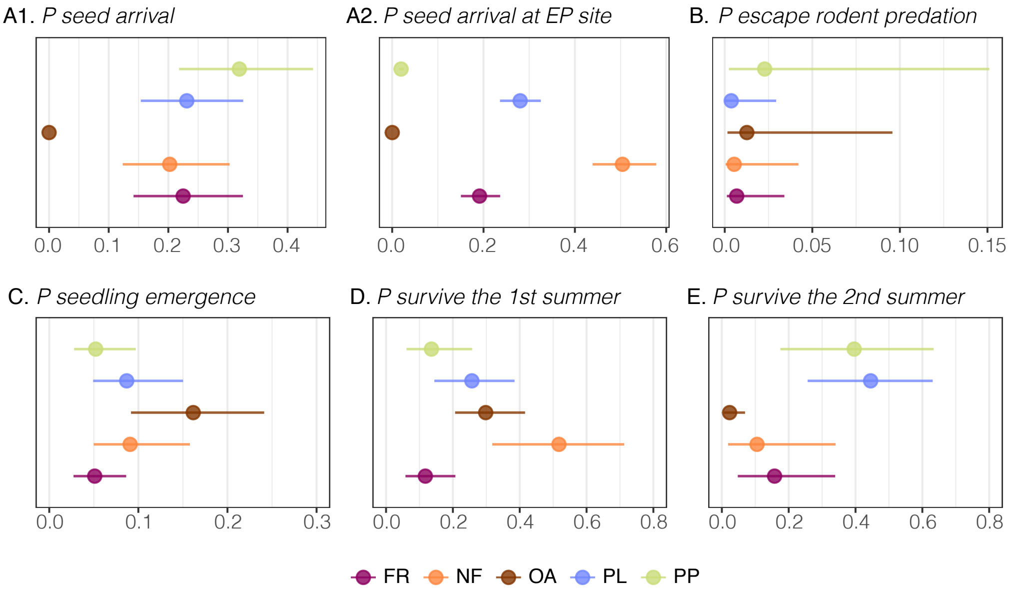


Figure A3.4. Transition probabilities (TPs) for seedling recruitment in different microhabitats: (A1) seed arrival to a certain microhabitat (per square metre, *i.e.*, assuming equal microhabitat abundance), (A2) seed arrival considering microhabitat relative abundances at EP site, (B) seeds escaping post-dispersal predation, (C) seedling emergence, (D) seedling survival to the 1st summer and (E) seedling survival to the 2nd summer. Error bars denote 80% credibility intervals.

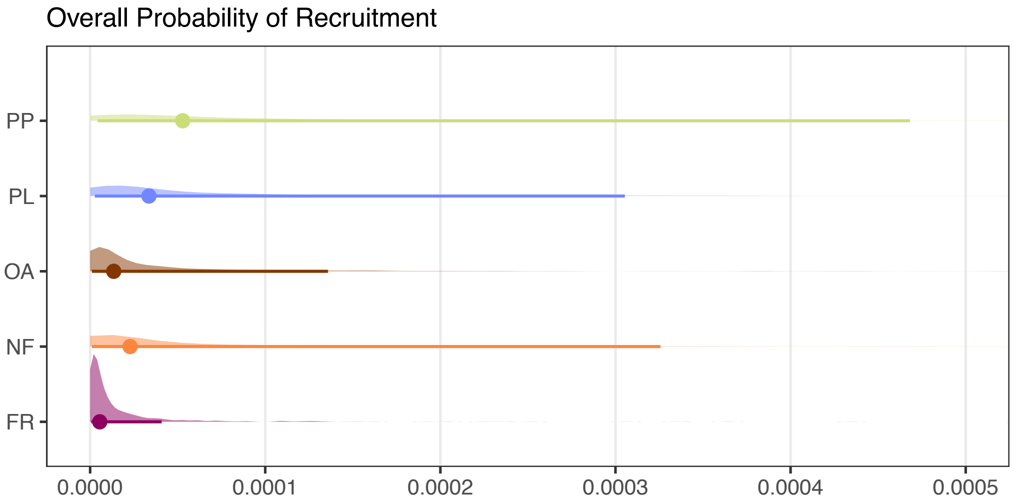


Figure A3.5. Overall probabilities of recruitment (OPRs) for seedling recruitment in different microhabitats. Error bars denote 80% credibility intervals.

Appendix 3F. Seedling recruitment by plant and bird species

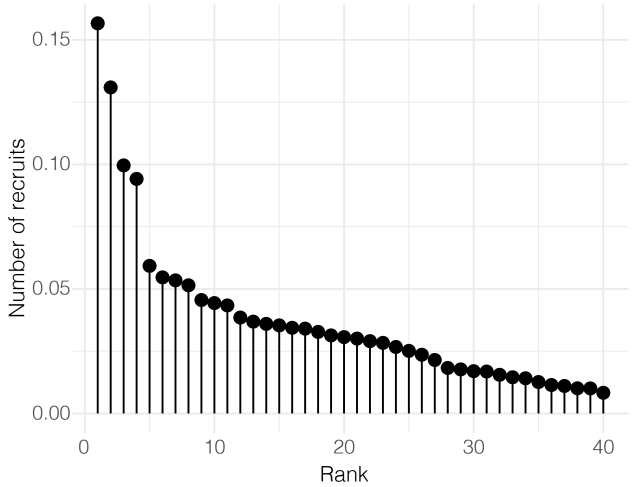


Figure A3.6. Individual plants (dots) ranked according to the expected number of recruits attained (seedlings surviving their second summer), given realised fruit consumption at EP site during fruiting season 2019-20. Values represent the median of their respective posterior distributions.

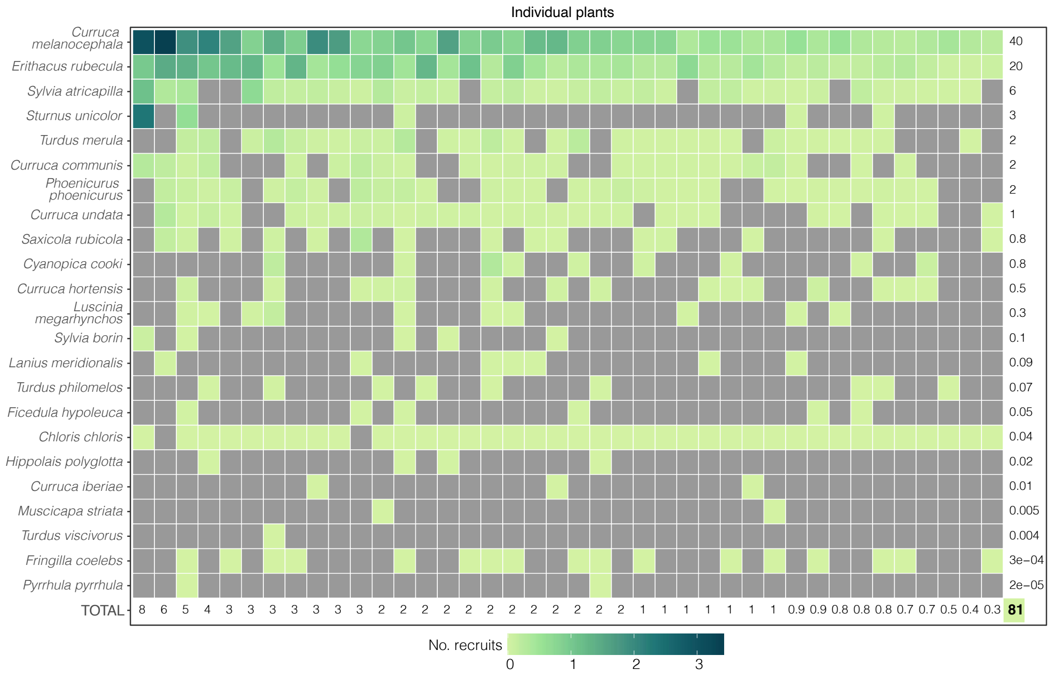


Figure A3.7. Estimated number of seedlings that plants could recruit per every 1,000,000 fruits produced by interacting with their respective assemblage of frugivorous birds. Animals (rows) and plants (columns) are ordered by the total number of recruits (number of seedlings surviving 2nd summer). Total recruits are indicated at the right-end of the panel for animals and at bottom for plants.

Appendix 3G. Stage transitions in seedling recruitment by individual plants

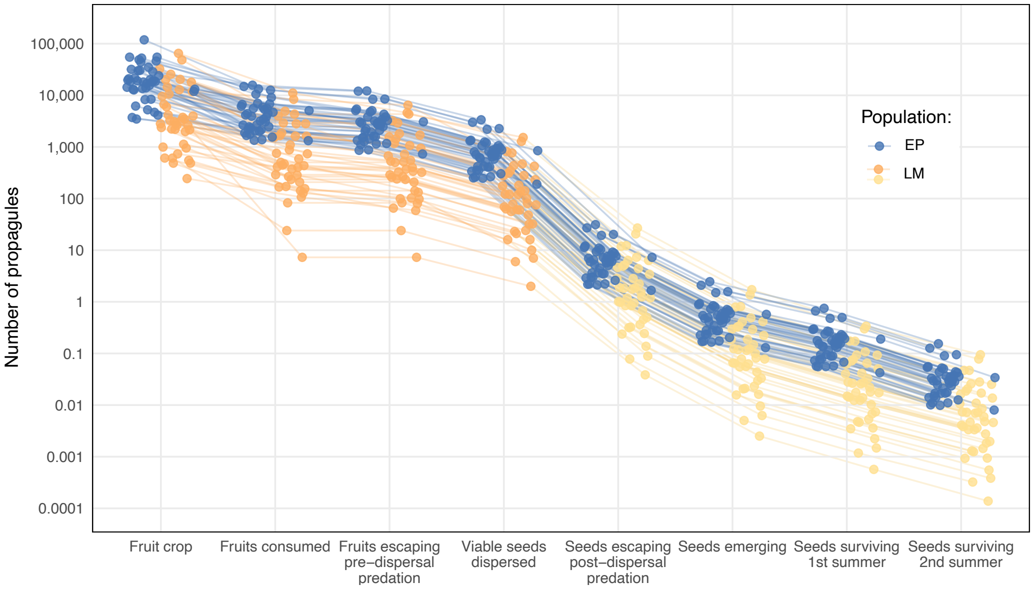


Figure A3.8. Decrease in the number of propagules of individual *Pistacia lentiscus* plants (fruits, seeds or seedlings depending on the demographic stage) along the seed dispersal and recruitment process. Each point represents the median of posterior distributions. Colours indicate the population where plants belong. The last four demographic stages at Laguna de las Madroñas (LM) site are dimmer to indicate that these numbers are inferred from post-dispersal consequences at El Puntal (EP) site.

Table A3.3. Median transition probabilities (Prob.) between demographic stages for any given plant at EP site, with 80% credibility interval. TPs number correspond to those in Figure 3.1 in the main text. The prob. of viable seeds to arrive to different microhabitats (TP4) is present in the first row of Table 3.2.

	Stage transition	Median	80% CI low	80% CI high
TP1	Prob. fruit consumption	0.23	0.08	0.59
TP2	Prob. escape pre-dispersal predation	0.78	0.36	0.97
TP3	Prob. viable seed dispersal	0.27	0.14	0.51
TP5	Prob. escape rodent predation	0.009	0.002	0.038
TP6	Prob. seedling emergence	0.08	0.05	0.12
TP7	Prob. seedling survive 1st summer	0.34	0.19	0.57
TP8	Prob. seedling survive 2nd summer	0.22	0.07	0.43

Appendix 3H. Software

We used R version 4.3.0 (R Core Team 2023) and the following R packages: arm v. 1.12.2 (Gelman & Su 2021), assertr v. 2.8 (Fischetti 2021), bayestestR v. 0.12.1 (Makowski, Ben-Shachar & Lüdecke 2019), brms v. 2.19.0 (Bürkner 2017, 2018, 2021), data.table v. 1.14.2 (Dowle & Srinivasan 2021), DHARMa v. 0.4.6 (Hartig 2022), DHARMa.helpers v. 0.0.1 (Rodríguez-Sánchez 2023), effects v. 4.2.2 (Fox 2003, Fox and Hong 2009, Fox & Weisberg 2018, 2019), ggalt v. 0.4.0 (Rudis, Bolker & Schulz 2017), ggdist v. 3.1.1 (Kay 2022), ggpubr v. 0.4.0 (Kassambara 2020), ggrepel v. 0.9.1 (Slowikowski 2021), ggspatial v. 1.1.7 (Dunnington 2022), glmmTMB v. 1.1.3 (Brooks *et al.* 2017), grateful v. 0.1.11 (Rodríguez-Sánchez, Jackson & Hutchins 2022), here v. 1.0.1 (Müller 2020), hillR v. 0.5.1 (Li 2018), kableExtra v. 1.3.4 (Zhu 2021), knitr v. 1.39 (Xie 2014, 2015, 2022), lme4 v. 1.1.29 (Bates *et al.* 2015), modelbased v. 0.8.5 (Makowski *et al.* 2020), parameters v. 0.18.2 (Lüdecke *et al.* 2020), patchwork v. 1.1.1 (Pedersen 2020), plotly v. 4.10.0 (Sievert 2020), rcartocolor v. 2.0.0 (Nowosad 2018), RColorBrewer v. 1.1.3 (Neuwirth 2022), renv v. 0.17.2 (Ushey 2023), rmarkdown v. 2.14 (Xie, Allaire & Grolemund 2018, Xie, Dervieux & Riederer 2020, Allaire *et al*. 2022), rstan v. 2.21.5 (Stan Development Team 2022), scales v. 1.2.0 (Wickham & Seidel 2022), sessioninfo v. 1.2.2 (Wickham *et al*. 2021), shinystan v. 2.6.0 (Gabry & Veen 2022), summarytools v. 1.0.1 (Comtois 2022), tidylog v. 1.0.2 (Elbers 2020), tidyverse v. 1.3.1 (Wickham *et al.* 2019), vegan v. 2.6.4 (Oksanen *et al.* 2022), vegetools (Rodríguez-Sánchez 2006), viridis v. 0.6.2 (Garnier *et al.* 2021).

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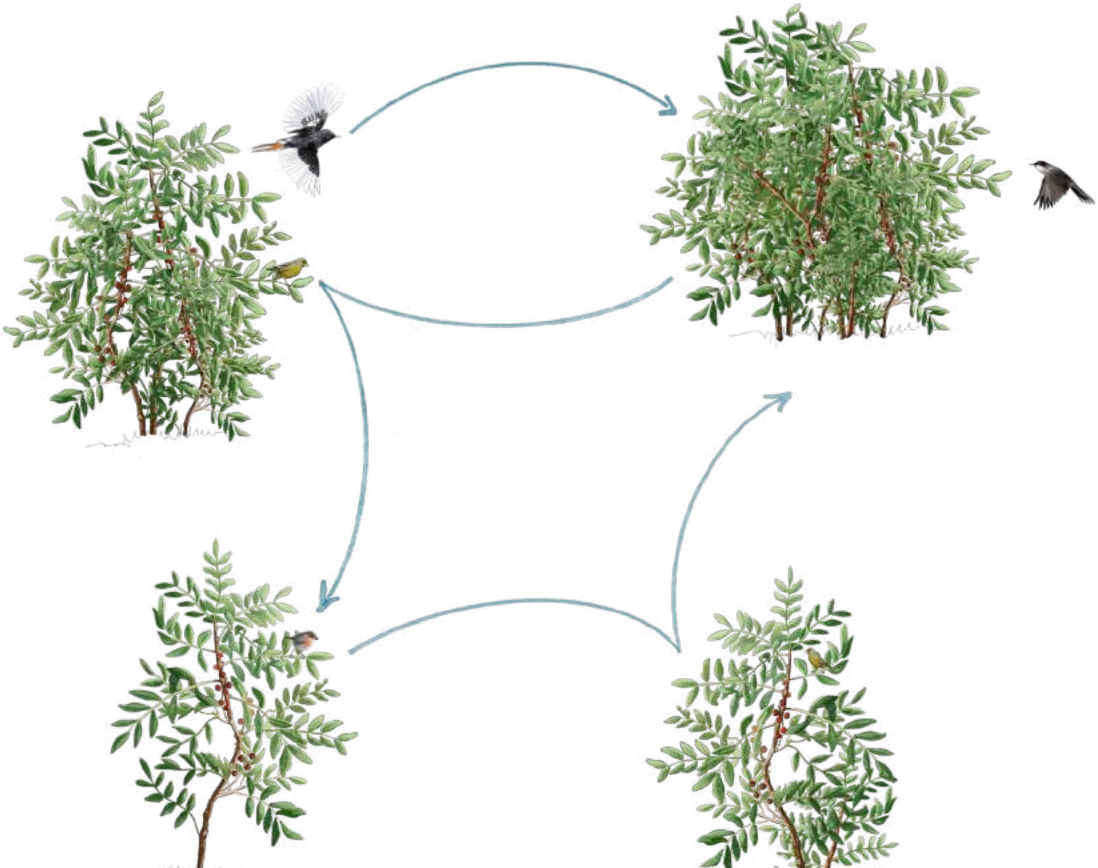
CHAPTER 4

Downscaling mutualistic networks from species to individuals reveals consistent interaction niches and roles within plant populations.



Quintero, E., Arroyo-Correa, B., Isla, J., Rodríguez-Sánchez, F., & Jordano, P. Downscaling mutualistic networks from species to individuals reveals consistent interaction niches and roles within plant populations. Submitted, 2024.

Abstract

The study of mutualistic interactions among species has received considerable attention over the past 30 years. However, less is known about the structure of individual interaction configurations within species. Recently, individual-based networks have begun to garner more attention, as they represent the fundamental scale at which ecological interactions are assembled. We compiled 44 empirical individual-based networks on plant-animal seed dispersal mutualism, encompassing 995 plant individuals across 28 species from different regions worldwide. We compare the structure of individual-based networks to that of species-based networks and by extending the niche concept to interaction assemblages, we explore levels of individual plant specialisation. We examine how individual variation influences network structure and how plant individuals "explore" the interaction niche of the population. Both individual-based and species-based networks exhibited high variability in network properties, leading to a lack of marked structural and topological differences between them. Our results reveal low to medium specialisation, with European populations exhibiting higher generality compared to American and Asian populations. Within populations, frugivores' interaction allocation among plant individuals was highly heterogeneous, with one to three frugivore species dominating interactions in most populations. Regardless of plant species or geographical region, plant individuals displayed similar interaction profiles across populations, with only a few individuals playing a central role and exhibiting high diversity in the interaction assemblage. Our results emphasise the importance of downscaling from species-based to individual-based networks to understand the structuring of any given ecological community and provide an empirical basis for the extension of niche theory to complex interaction networks.

Keywords: individual-based networks, interaction niche, frugivory, mutualism.

Significance statement

Ecological interactions in nature occur between individual partners rather than species, and their outcomes determine fitness variation. By examining among-individual variation in interaction niches, we can bridge evolutionary and ecological perspectives to understand interaction biodiversity. This study investigates individual plant variation in frugivore assemblages worldwide, exploring how individual plants "build" their interaction profiles with animal frugivores. Surprisingly, the structure of networks composed of individuals was indistinguishable from networks composed of species. Independently of species or region, interaction frequencies among frugivore partners was highly skewed, with a small subset of species providing most interactions. Additionally, within populations, only a few plants played a key role in attracting a high diversity of frugivores, making them central to the overall network structure.

Introduction

Species are a fundamental unit of study in most ecological research, resulting in numerous theoretical and methodological approaches to assess how their interactions support ecosystem functions. Food web theory and, recently, network ecology based on graph theory emerged as a useful framework to study these multi-species interactions simultaneously and assess the complexity of natural ecosystems (Solé & Valverde 2004, Fortuna & Bascompte 2008, Fontaine *et al.* 2011). Starting with food webs (Cohen 1978), network theory expanded its versatility to other ecological interaction modes such as mutualisms (Jordano 1987a, Memmott 1999). Since then, abundant literature has revealed emergent and global properties of ecological networks, highlighting surprisingly similar architecture in the way they are assembled (McCann *et al.* 1998, Mora *et al*. 2018). Among ecological networks, mutualistic networks represent mutually beneficial interactions, and their structure and topology have been extensively explored (Bascompte & Jordano 2007). Plant-animal mutualistic networks are highly heterogeneous (*i.e.*, most species have few interactions while a minority of species are much more connected) and nested (*i.e.*, specialists interact with subsets of the species with which generalists interact), leading to asymmetric dependences among species (Jordano *et al.* 2003, Bascompte & Jordano 2007). Yet, it is not clear to what extent these properties percolate to networks at lower levels of organisation, such as those composed of individual interactions.

Although interaction patterns are usually summarised at the species level, ecological interactions actually occur as encounters between individuals rather than species. For instance, while we may say that blackbirds consume and disperse raspberries (*Turdus merula*-*Rubus idaeus*), it is actually certain individual raspberry plants that interact with individual blackbirds within a local population. By missing this individual-level resolution we miss two important opportunities: 1) the ability to effectively link individual trait variation with interaction outcomes (fitness effects) and thus connect ecological and evolutionary perspectives; and 2) to bridge the gap between niche theory and complex interaction networks, i.e, to assess how individual-based interactions scale up into complex interaction webs.

Classical studies of frugivory interactions for animal-mediated seed dispersal have been plant-focused (*e.g.*, Snow & Snow 1988), and provide a most useful framework to zoom-in into species interactions of particular plant species and the coterie of animal frugivores they interact with. Thus, by considering individual-based networks, in which one set of nodes is composed of plant individuals, and the other set is composed of animal species (*i.e.*, individual-species network), we can examine individual variation in "interactions build-up", as well as its subsequent implications, in *e.g.* fitness (Rodríguez-Rodríguez *et al.* 2017). This is helpful not just for building a proper bridge between interaction ecology and demographic consequences (*e.g.*, Quintero *et al.* 2023), but also for bridging network ecology with evolutionary consequences (Guimarães *et al.* 2011, Segar *et al.* 2020).

Network structure may not be consistent across hierarchical scales of organisation (Tur *et al.* 2014, Wang *et al.* 2021). To begin with, the similarity in the set of partners available to individuals of the same species will be higher than that to different species. That is, the physical and phenological traits of conspecific individuals tend to be more similar than those among species (Siefer *et al.* 2015), discouraging major forbidden interactions (but see Albert *et al.* 2010, González-Varo & Traveset 2016). Thus, we might expect networks composed of individuals to exhibit architectural and structural properties different to those found in species networks; yet, this remains an underexplored question.

Downscaling the study of interactions to individuals allows us to observe how the variation among individuals in their partner use is distributed in the population (Fig. 4.1A-B). Since its origins, the niche concept has provided an ideal framework for studying individual variation in resource use (Grinnell 1917, Van Valen 1965, Bolnick *et al.* 2003). Even so, most previous work has focused on antagonistic interactions such as predator-prey trophic niches (Bolnick *et al.* 2003, Araujo *et al.* 2011, Costa-Pereira *et al.* 2018, 2019). It was only until very recently that niche theory was applied for understanding individual variation in mutualistic interactions (Tur *et al.* 2014, Albrecht *et al.* 2018, Phillips et al. 2020, Koffel *et al.* 2021, Arroyo-Correa *et al.* 2023, Gómez *et al.* 2023b). For this study, we rely on the concept of 'interaction niche' as the space defined by the set of species with which a population can interact (Fig. 4.1C) (Ponisio *et al.* 2019).

Interaction probabilities between plant individuals plants and animal species (*i.e.*, probability of interspecific encounter, PIE; Chase & Knight 2013) are influenced by a myriad of factors such as population abundances, accessibility of resources, individual preferences or physiological needs (*e.g.,* optimal foraging theory concept) as well as required matching in traits and phenology (Guimarães 2020). It was Darwin's idea that individual variation acts as the necessary raw material for natural selection (Bolnick *et al*. 2011). Intraspecific trait variability, neighbourhood attributes and spatio-temporal context drive animal preferences for certain plant individuals,

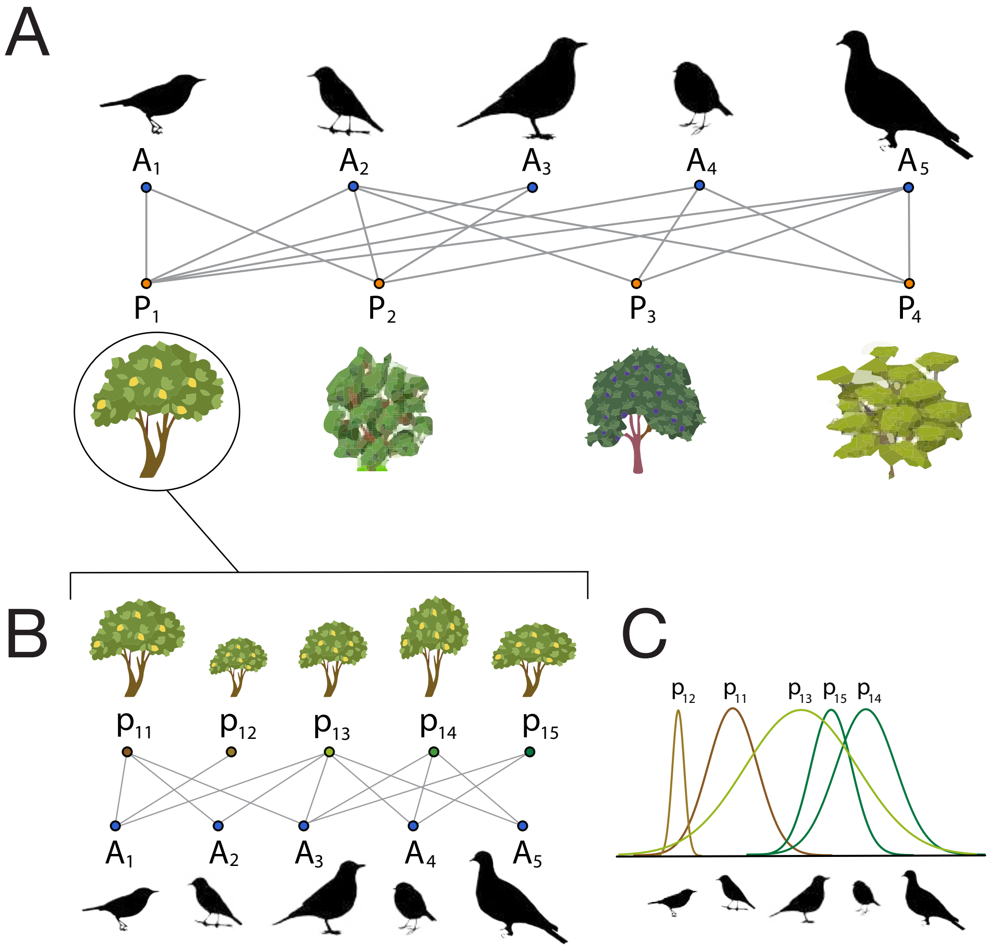


Figure 4.1. A) Schematic graph of an example, species-based, interaction network between four ornithochorous plant species (P_1 - P_4) and the frugivore assemblage with five animal species (A_1 - A_5) (top). B) A zoom in on the individual-based network of plant species P_1 depicting the interactions of plant individuals (p_{11} - p_{15}) with five animal species, exemplifying the study focus of this paper. C) Different plant individuals interact with frugivore assemblages of variable diversity, illustrating their individual interaction niches (exemplified by the five coloured niche utilisation curves within the inset).

which will govern the establishment of interactions between plant individuals and their mutualistic partner species (Sallabanks 1993, Snell *et al.* 2019, Isla *et al.* 2023). These differences among plant individuals in interactions will be ultimately translated into outcome variations, acting as raw material for coevolution (Thompson 1999). In mutualistic systems such as pollination or seed dispersal, variation in the patterns of interaction or exploitation of niches (partners) can play a determining role, as mutualists directly affect the reproductive outcome of individuals, influencing fitness variation, population dynamics and trait selection.

Quantifying variation in individual interaction niche-partitioning can shed light on the coexistence and stability of mutualistic communities. For instance, individuals in a population can behave as specialists or generalists when exploiting their interaction niche, and this may influence how these individuals are affected by interspecific competition and how partner diversity is promoted, determining, *e.g.*, degree distributions in interaction networks (Bascompte & Jordano 2014). The extent to which individuals behave as specialists or generalists in a population can be elucidated by partitioning niche variation into its between- (BIC) and within-individual (WIC) components. This approach can prove useful to predict niche-shifts or niche expansion (Roughgarden 1972, Bolnick *et al.* 2007). The levels of individual specialisation in the individual-based networks can be estimated as the proportion of the total niche width in the population (TNW; total partner diversity) due to within-individual variation (WIC; average partner diversity of individuals). Thus, the distribution of frugivore-partner species richness and interaction allocation among plant individuals can be highly variable in local populations (*e.g.*, Jordano & Schupp 2000, Guerra *et al.* 2017, Miguel *et al.* 2018, Jácome-Flores *et al.* 2020, Quintero *et al.* 2023). By studying the levels of plant individual specialisation and how frugivores allocate their interactions among plants, we aim to understand how variation in mutualistic interactions takes place within plant populations (Fig. 4.1).

The patterns we observe when species interact arise from the way in which their individuals interact (Guimarães 2020). A variety of node-level metrics for complex networks can provide insight into an individual's strategy within its population (Dormann 2011, Poisot *et al.* 2012). Several studies have used node-level metrics to characterise individuals' positioning in the network, informing us about their role and significance in their population (*e.g.,* Gómez & Perfectti 2012, Guerra *et al.* 2017, Rodríguez-Rodríguez *et al.* 2017, Crestani *et al.* 2019, Vissoto *et al.* 2022, Arroyo-Correa *et al.* 2023, Isla *et al.* 2023). However, most of these studies have used a single or several metrics separately to understand the interaction profile of individuals and for single populations. By using a selection of node-level metrics combined, we aim to characterise interaction profiles of plant individuals with frugivore species and the distribution and frequencies of roles among and within populations in different geographic regions. If plant individuals from different populations across biome types and geographic regions present modes of interaction associated with their life-history, we could expect their individuals to exhibit similar interaction profiles, markedly different from those of individuals of other species and/or regions. Conversely, if phylogeny or context-dependent effects were not determinant in the way plant individuals interact with their frugivore partners, we could expect a consistency in individual interaction profiles distributions for all populations, irrespectively of geographic location or biome type.

The overarching goal of this study is to investigate the role played by individuals in the assembly of complex ecological networks of species interactions, determining their structuring and functioning. To do so, we combine network theory and niche theory to characterise the interaction profile of plant individuals in mutualistic seed dispersal systems across different geographic regions (Europe, Asia and America). We outline three main objectives: 1) to examine whether networks composed of individuals exhibit different architectural and structural properties than those found in species-based networks, 2) to understand how variation in frugivory interactions takes place at the plant population level by quantifying individual niche-partitioning and frugivore interaction allocation, and 3) to characterise interaction profiles of plant individuals with frugivore species and assess the distribution and frequencies of roles among the population.

Methods

Dataset acquisition and curation

We compiled studies on frugivory ecological networks with publicly-available data, both at the species and the individual plant levels. Species-based networks were gathered from 41 published studies at the community scale (see Table A4.1). For individual-based networks, which are scarcer, we compiled phyto-centric studies (plant-based), with quantitative information on frugivore visitation on plant individuals within populations. We combined published studies with unpublished datasets, gathering data for 20 different study systems. Some of the studies selected presented more than one network from different communities (in species-based studies) or populations (in individual-based studies). These datasets document interactions between plant species and animal frugivore species (in species-based studies) or interactions between plant individuals of a single species and animal frugivore species (in individual-based studies). Data are provided as adjacency matrices, where rows represent plant species (or individuals) and columns represent animal species, with matrix elements a_{ij} indicating interaction frequency (visitation frequency to plants). In order to ensure networks sufficiently sampled to robustly characterise their structure and interaction profiles, we only kept those that were reasonably complete. We checked for sampling coverage of individual-based networks using iNext R package (Hsieh *et al*. 2016) (Table A4.2). To do so, we converted matrix data to an incidence frequency-data and considered plant individuals as sampling units and the number of frugivore species detected at each plant (species richness; $q = 0$). We discarded networks in which the number of interacting nodes (plants and frugivore species) was less than 15 (n = 11 networks). Our final dataset consisted of 105 networks with an average size of 380 potential links or cells (range = 55 - 2904) and 90 unique interactions (range = 21 - 419). Forty-four were individual-based networks and 61 were species-based networks (Table A4.1).

Individual-based networks were carefully curated and standardised by sampling effort on plant individuals(time and/or area). To do that, we divided the observed interaction intensity (*e.g.*, number of visits) by the amount of time observed and/or the area sampled. When possible we referred the interaction value to the coarsest level, that is, frugivore visitation events, otherwise number of fruits consumed. Once all individual-based networks were standardised by sampling effort, we scaled both individual-based and species-based networks by dividing the weight of each pairwise interaction by the total number of interactions in the matrix (grand total standardisation; Quintero *et al*., 2022). Therefore, the interaction values (matrix cells) represent the relative frequency of a plant individual (in individual-based networks) or a plant species (in species-based networks) interacting with a given frugivore species, and the sum of all relative frequencies equals one.

Network-level metrics

For both the individual and species-based networks, we calculated several network-level metrics, using R packages bipartite (Dormann *et al.* 2008) and igraph (Csárdi & Nepusz 2006). We selected a representative set of metrics that had suitable biological interpretation and were not highly correlated (Variance Inflation Factor < 3) and/or strongly affected by the number of species/individuals sampled or overall network size (Fig. A4.1, Fig. A4.2).

Selected network-level metrics were:

1. Connectance *(topology).* This metric gives the proportion of realised over potential links in the network. Calculated as the sum of realised links (unweighted) divided by the number of cells in the matrix. Values range from 0 (no links) to 1 (fully connected networks where all nodes interact among them) (Dunne *et al.* 2002).
2. Weighted nestedness wNODF *(structure)*. Informs on the way interactions are organised. A highly nested structure is one in which nodes with fewer connections tend to interact with a subset of highly connected nodes that in turn interact with the highly connected ones (Bascompte *et al.* 2003). Values of 0 indicate non-nestedness, those of 100 perfect nesting (Almeida-Neto & Ulrich 2011).
3. Assortativity *(topology)*. This metric indicates the level of homophily among nodes in the graph. It ranges from -1 to 1, when high it means that nodes tend to connect with nodes of similar degree; when low, nodes of low-degree connect with nodes of high-degree (disassortative) (Barabasi 2016, Newman 2002).
4. Modularity *(structure - clustering)*. Reflects the tendency of a network to be organised nodes from other modules (Bascompte & Jordano 2014). Ranges from 0 (no clusters) in distinct clusters, where nodes within a module interact more among them than with to 1 (highly compartmentalised network) (Newman 2006).
5. Eigenvector centralization (centrality). This metric quantifies how centralised or decentralised the distribution of eigenvector centrality scores is across all nodes in a network (Freeman *et al.* 1979). The eigenvector centrality of a given node in a network is a measure of the influence of that node, taking into account both the node's direct connections and the connections of its neighbours. Nodes with high eigenvector centrality are connected to other nodes that are also central, giving them a higher score (de Oliveira Lima *et al.* 2020). Therefore, the network-level eigenvector

centralization provides a measure of the extent to which a few nodes dominate the network in terms of influence. In a network with low centralization, the centrality scores are relatively evenly distributed among the nodes, suggesting a more decentralised structure where many nodes contribute to the overall connectivity of the network, and therefore to the interaction services. On the other hand, a network with high centralization indicates that only a small number of nodes have a higher centrality, suggesting a more centralised structure where a few nodes play a crucial role in the network's overall connectivity. We normalised this measure to ensure that the centralization value is relative to the maximum centralization for a network of a given size.

6. Alatalo interaction evenness *(interaction diversity).* A metric to measure evenness in interaction distribution (Alatalo 1981, Müller *et al.* 1999). This metric is based on Hill numbers (Hill 1973) and it is calculated as the modified Hill's ratio: $({}^{2}D - 1)/({}^{1}D - 1)$. It uses the diversity (D) of order 1 in the denominator ($q = 1$; exponential of Shannon index; expH') and diversity of order 2 in the numerator ($q = 2$; inverse Simpson's index). We chose this metric over traditional measure of interaction evenness (Pielou's or Shannon's evenness) because it was less correlated with connectance, also, being based on Hill numbers, gives a better description of interaction diversity (see Fig. A4.2). Its correlation with Pielou's interaction evenness was $r = 0.66$, $p > 0.01$.

Niche specialisation

We estimated populations' niche specialisation using the Shannon approximation of the WIC/TNW index for discrete data (Roughgarden 1972, Bolnick *et al*. 2002). In this case, we define as a niche-resource the available coterie of visiting frugivore species in a given population. This index computes the relative degree of individual specialisation as the proportion of total niche width (TNW) explained by within-individual variation (WIC). Total niche width (TNW) is calculated as the total diversity of frugivore species visiting the plant population, using Shannon index. The within-individual variation (WIC) is calculated as the average Shannon diversity of frugivores for each plant individual, weighted by the relative proportion of all frugivore interactions in the population that are used by each individual. Finally, WIC is divided by TNW. Values closer to 1 indicate a population composed of generalist individuals that are using most of the population niche. On the contrary, values closer to 0 indicate a population of specialist individuals using small subsets of the population niche, with large differences in resource-use among them. To test differences in individual specialisation (*i.e.*, WIC/TNW) between different bioregions (n = 3) we fitted a mixed-effects linear model with a normal distribution where the study was present as a random factor (Bates *et al.* 2015).

Node-level metrics

To characterise plant individuals' interaction profiles in their populations, we computed a set of node-level indices for each plant individual using R package bipartite (Dormann *et al.* 2008). Additionally, we calculated average niche overlap using Bray-Curtis dissimilarity index (vegan R package; Oksanen *et al.* 2022). Again, we selected a representative set of metrics that had a suitable biological interpretation for assessing the individuals' interaction profiles and were not highly correlated nor affected by the number of individuals sampled (Fig. A4.6, Fig. 4.7).

Selected node-level metrics were:

1. Normalised degree *(interaction diversity).* Represents the richness of partners for a given node and is scaled relative to the rest of nodes in the network. Ranges from 0 to 1, where a plant individual would score 1 if it interacts with all the frugivore species available (Dormann 2011).

2. Species specificity index *(interaction diversity).* Informs about the variation in the distribution of interactions with frugivore species partners. It is estimated as a coefficient of variation of interactions for each plant individual, normalised to range between 0 and 1 (Julliard *et al.* 2006, Poisot *et al.* 2012). High values indicate higher variation in dependence on frugivore species. Plants with high dependence on few or a single frugivore species yield values close to 1, and plants that distribute their interactions equally with many frugivore species show low values.

3. Normalized species strength *(interaction intensity).* Quantifies the dependence of the community on a given node (Dormann 2011). It is calculated as the sum of the dependencies of each frugivore species (*i.e.*, the fraction of all visits to a given plant individual coming from a particular frugivore species) (Bascompte *et al.* 2006).

4. Weighted closeness *(node position).* This metric provides an index of the magnitude to which a given node has short connection paths to all other nodes in the network (Opsahl *et al.* 2010). It is influenced by the intensity and number of links and indicates to what extent a node is in the "centre" of the connections of the graph. This metric is calculated on the unipartite projection of the individual-based network for the plant individuals, in which links between plant individuals represent the number of frugivore interactions shared. In this context, the weighted closeness of a plant individual is estimated as the inverse of the sum of all path lengths (link weights) between this plant individual and all other plant individuals in the unipartite network. Therefore, plant individuals with higher values of weighted closeness are strongly connected with more plant individuals in the population through shared frugivore species.
5. Mean interaction overlap using Bray-Curtis index *(node similarity).* This measure of interaction overlap informs on the average similarity in frugivore use between pairs of plant individuals. This metric indicates how different the frugivore assemblage of a given plant individual is compared to the rest of the population (*e.g.*, Gómez *et al.* 2010). Higher values (*i.e.*, higher overlap) indicate a higher similarity in interaction assemblage for a given plant individual with respect to other individuals in the population.

Data analysis for network metrics

In order to determine variation distribution in network structuring and topology we performed two principal component analyses (PCA), one at network-level and other at node-level. Previous studies have used PCA for comparing network metrics (*e.g.*, Sazima *et al.* 2010, Medeiros 2018, Mora *et al.* 2018, Burin 2021, Acevedo-Quintero *et al.* 2023). For comparing network metrics at two resolution scales (species-based and individual-based) we performed a PCA including both groups of networks and their values for selected network-level metrics. This procedure resulted in an ordination of both species-level and individual-based networks in relation to the multivariate space defined by network-level metrics. For comparing the plant individuals' interaction profiles, the second PCA was performed using the set of node-level metrics estimated for plant individuals within their population (*i.e.*, individual-based networks). This resulted in an ordination of elements (plant individuals) in relation to the multivariate space defined by node-level metrics. Thus, such PCAs provide an exploratory analysis of how networks (species-based and individual-based networks; first PCA analysis) or plant individuals (individual-based networks; second PCA analysis) span the multivariate space of network metrics: in such ordination, the location of each network or individual characterises its structural properties or interaction profiles, respectively. We calculated 95% confidence data ellipses for different network types in both PCAs (individual-based vs. species-based in the first PCA, and for different geographical regions in the second PCA). For the second PCA we also calculated an overall 95% data ellipse for normal distributed data using all individual plants and quantified the number of outlying plant individuals relative to their own network (*i.e.*, population).

For a complete list of all packages used please refer to Appendix 4F.

Results

Structure of individual versus species-based networks

We assembled a total of 44 individual-based plant-frugivore networks and compared them with 61 species-based networks using six network metrics (connectance, nestedness, modularity, assortativity, centralization and interaction evenness, see Methods). Networks at different resolution scales presented similar structural properties, overlapping across the multivariate PCA space (Fig. 4.2). All metrics varied considerably, with a remarkable overlap at both resolution scales (see Fig. A4.3). Noticeably, interaction evenness was higher in individual-based networks than in species-based networks (interaction evenness: ind-based = 0.67, sp-based = 0.59; Fig. A4.3).

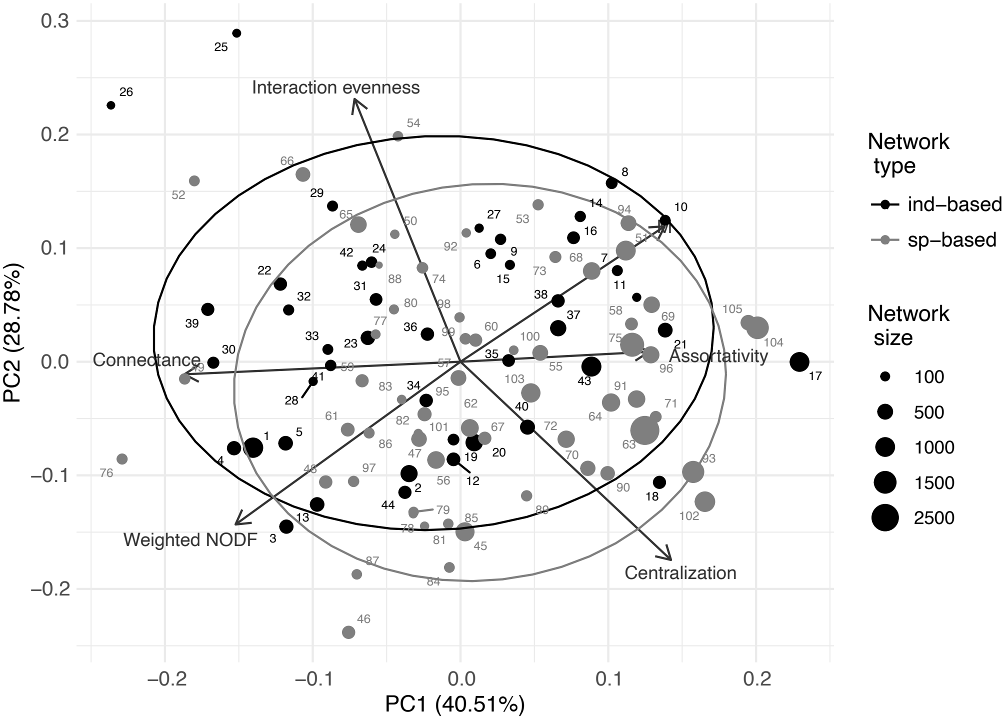


Figure 4.2. Principal Component Analysis of individual and species-based frugivory networks based on six different networks metrics. Each point represents a plant-frugivore network and the number indicates study identification (see Table A4.1). The dot size is proportional to network size (number of cells in the interaction matrix) and the colour represents the resolution scale of the network. Ellipses of 95% confidence for normal data distribution are depicted for each network type.

Plant individuals' specialisation in interaction niche

Most plant populations studied presented slight to medium levels of individual specialisation (mean WIC/TNW = 0.61; range = 0.28 - 0.90). Individual-based networks from America and Asia (India) showed a higher proportion of more specialised plant individuals, whereas European/Mediterranean populations presented higher levels of individual generalisation (Fig. 4.3; difference Europe-America = 0.23, p-value >0.01). Plant populations interacting with higher numbers of frugivore species had a wider interaction niche (TNW, *i.e.*, Shannon diversity index), but not necessarily higher levels of individual specialisation (WIC/TNW) (Fig A4.4).

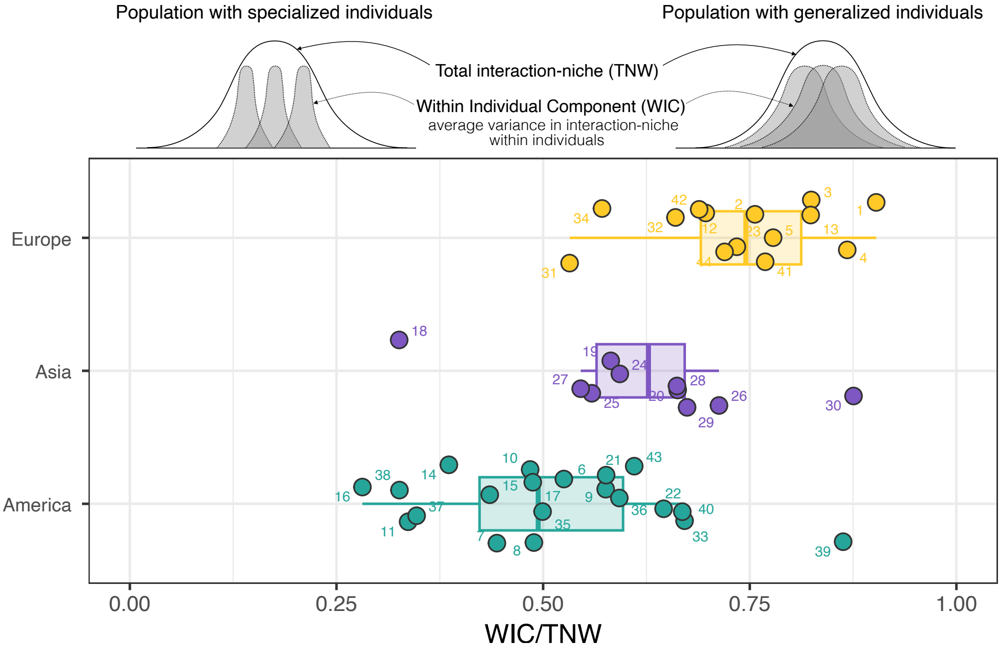


Figure 4.3. Values of population specialisation (WIC/TNW) for all plant individual-focused studies (n = 44 networks). The total niche width (TNW) refers to the interaction niche of the population (*i.e.*, interactions with frugivore species) and the within-individual component (WIC) is the average variation in the use of this interaction niche found within individual plants. Each point in the graph represents an individual-based network (see Table A4.1 for each network metadata) and colours represent the continent of the study site. At the top of the graph, a schematic representation of two plant populations: the one on the left presents a population composed of highly specialised individuals (low WIC; low WIC/TNW) and the one on the right presents a population composed of highly generalised individuals (high WIC; high WIC/TNW). Values of WIC/TNW closer to 1 represent populations with generalised individuals where plants use most of the available interaction niche. On the other extreme, values closer to 0 indicate populations with specialised individuals that use a smaller subset of the available interaction niche (in this case plants do not tend to interact with the same frugivore species).

Frugivore interactions within plant populations

Notably, just a reduced subset of frugivore species in most networks (generally between one and three) usually accumulated most of the interactions, while the rest of frugivore species contributed a minor proportion. On average less than 20%

of frugivores contributed more than half of the interactions, regardless of the total number of frugivore species in the population (SD = 9.6%, Fig. A4.5). The frugivores that contributed most interactions also tended to interact with a higher number of plant individuals (Spearman's rho = 0.81, p-value < 0.01, Fig. 4.4). Remarkably, frugivore species with smaller contributions interacted with a variable proportion of plant individuals, such proportion being higher in European networks and lower in American networks (Fig. 4.4). Frugivores' body mass was not correlated with interaction contribution (rho = -0.05) nor with the proportion of plant individuals interacted (rho = -0.11).

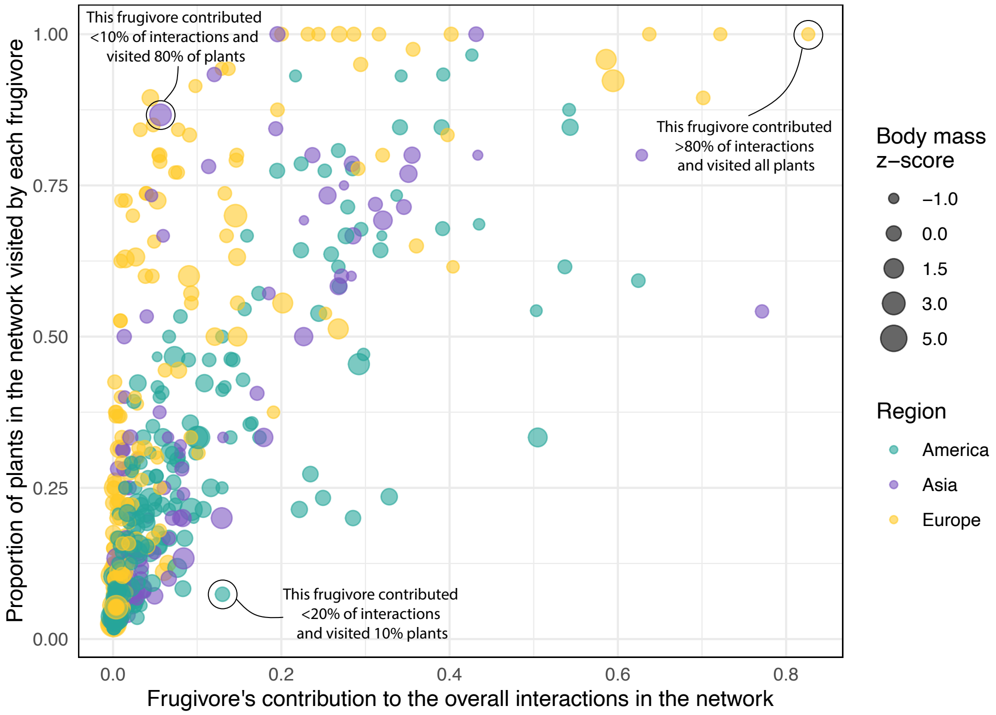


Figure 4.4. Relationship between the overall contribution to the total number of interactions by each frugivore species (*i.e.*, link weights in the individual-based networks) and the proportion of plant individuals with which they interacted (*i.e.*, animal degree). Each point represents a frugivore species in their respective plant population (individual-based network). Point colour indicates the geographic region and point size represents the frugivore's body mass relative to its population (z-score).

Plant individuals' interaction profiles

The 44 individual-based networks included a total of 995 plant individuals: 452 (from 9 species) from America, 170 (10 species) from Asia and 373 (9 species) from Europe. The principal component analysis based on all the individual plants' interaction profiles did not produce distinct clusters by region or population. Instead, individuals from different populations (*i.e.*, individual-based networks) spread across the multidimensional space, suggesting ample within-population heterogeneity in plant individuals' interaction profiles (Fig. 4.5). The first principal component (PC1), explaining more than half of the variation, was mainly related to interaction degree and specialisation index, thus capturing individual variation in frugivore richness and composition. The second component (PC2) explained 22% of the variation and was correlated with niche overlap and interaction strength; these metrics are related to plant individuals' interaction patterns in relation to their conspecifics and affected by interaction frequency (link weight). The PC2 distinguished plant individuals based on their frugivore assemblage composition, where plant individuals with more unique frugivore assemblages were positioned in the bottom area of the PCA , while many plants with highly-overlapping frugivore assemblages within their populations were positioned towards the upper area. The third component (PC3; 14% variation explained; Table A4.4) was strongly related to weighted closeness, a measure of how strongly and well connected individuals are within the network (*i.e.*, central).

Overall, individual plants from Europe tended to have more similar frugivore assemblages (higher niche overlap), while plants from America presented less overlapping and more specialised individual assemblages (Fig. 4.5). Few plants were highly central in the interaction network (high weighted closeness) and important for frugivore dependence (high species strength) (*i.e.*, points in the bottom-left area of the multivariate space). Most individual plants showed uneven dependencies on frugivore species and/or medium-high frugivore overlap with other plants in the population. Yet plants with strong dependencies on one or few frugivore species tended to show lower overlap with other individuals in the frugivore assemblage, suggesting a trade-off between partner specialisation and partner sharing (upper-right Fig. 4.5).

The 95% CI ellipse for all individuals (black line ellipse in Fig. 4.5) illustrates a broad overlap among species, irrespective of biome or frugivore assemblage composition, *i.e.*, points for individual plants broadly admixed relative to the multivariate network metrics space. Notably, highly central plants, that account for a high proportion of interactions within their populations, represent outliers in their networks and most networks presented one or few individuals with these network role (*i.e.*, outside the overall 95%CI ellipse; median = 1 individual per network, min-max range = 0–5 individuals per network); this represented an average of the 7% of the individuals within populations (range min-max = 0% and 33%). Out of 44 networks (*i.e.*, plant populations), 36 had outlier individuals and out of 28 plant species, 26 had outlier individuals (see Fig. A4.8). These outlying individuals displayed a notable association with high scores in weighted closeness (see 3D representation in Fig. A4.9).

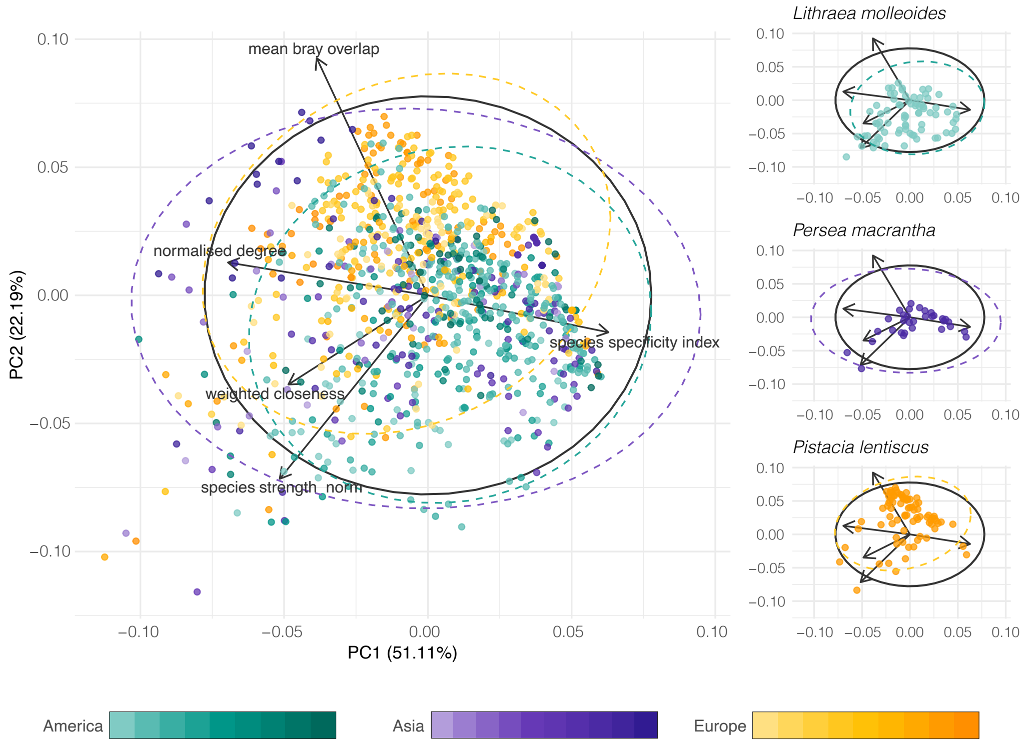


Figure 4.5. PCA for node-level metrics. Each point represents an individual plant. Point colour indicates the geographic region and different shades to refer to different plant species within each region. Ellipses represent 95% CI for all individual plants (in black) and each continent (in colour) (see Fig. A4.9 for a 3D visualisation of PCA results). Panels on the right are a subset of PCA for three plant species.

Discussion

Our study provides new insights on how downscaling from species to individuals reveals new aspects of ecological interaction assembly, such as the consistency of structural properties of networks across biological organisation scales, how mutualistic partner allocation takes place in different populations around the world, as well as the similarity in the interaction profiles within populations regardless the species or context we observe.

Downscaling in ecological resolution

The structure of plant-animal mutualistic networks revealed fundamental heterogeneity in structure across networks and scales. We did not reveal major deviations in the assembly patterns of interactions as we zoomed in the scale of resolution- from the hierarchically higher species scale to a lower, individual-based scale. Previous efforts to explore network architecture when downscaling the ecological resolution, found shifts in the structuring of pollination networks (Tur *et al.* 2014, Wang *et al.* 2021). Both studies observed that individual-based networks were less connected and that individuals were more specialised than species. Their approach for testing the effect of scale change in network structure is however different from the one we use, as these studies focused on a specific community at a specific time and used the same methodology for sampling both resolution scales. In contrast, our approach considers communities and populations from different parts of the world at different times and sampled with different methodologies, capturing as much variation as possible to examine differences and similarities in general interaction patterns. We only observed slight differences across scales in interaction evennes. The resulting higher overall interaction evenness in individual-based networks can be explained by a lack of major forbidden links, which allows frugivores to potentially interact with most individual plants and distribute their interactions more homogeneously when compared to interactions established with a more heterogeneous set of species partners. We argue that the addition of new species or individuals with new traits to a network provides new link possibilities, yet in the case of species-based networks these potential interactions will need to undergo stronger trait and phenological matching filters than individual-based networks (Sazatornil *et al.* 2016). Simply put, a given frugivore species may interact with a broader range of partners within a plant population than when interacting with the full range of available plant *species* in a plant community. The former set imposes less restricted limitations to interactions by including much more homogenous conspecific partners (despite intraspecific variation; see, González-Varo & Traveset 2016). In this way, downscaling from species to individuals fundamentally alters the probabilistic distribution of interactions among partners (Poisot *et al*. 2016). Besides differences in the analytical approach between our study and those of Tur *et al*. 2014 and Wang *et al*. 2021, we also look at different mutualistic systems. The specialisation levels of pollination and frugivory systems likely differ due to the nature of both ecological interactions (Wheelwright & Orians 1982, Jordano 1987a). For instance, structural limitations such as morphological adjustment between the corolla and mouthparts in pollination systems usually require stronger morphological coupling and are perhaps more favoured (to avoid hybridization) than the adjustment between fruits and the mouthparts of the consumers in seed-dispersal systems (Wheelwright & Orians 1982, Jordano 1987a), making pollination networks more specialised (see, *e.g*., Mello *et al.* 2011). Aside from minor differences in certain network metrics, the overall topology and structure of frugivory networks at different resolution scales was not sufficient to make clear distinctions. The absence of discriminatory patterns to determine the scale could indicate the existence of underlying effects that control ecological network configurations. We argue that numerical effects (*i.e*., organism abundances) are likely at the base of these emergent properties, governing interaction strength distribution across nodes and asymmetric interactions (Vázquez *et al*. 2007, Schleuning *et al.* 2011, Guimarães 2020).

Exploitation of the interaction niche

Individuals' interaction niches were narrower than that of their populations, supporting that individual specialisation in mutualisms is substantial and common in nature (Bolnick *et al.* 2003). Plant individuals' specialisation levels were similar to specialisation levels reported in other animal taxa (Araujo *et al*. 2011). Interestingly, the degree of individual specialisation markedly varied across regions, with European populations being more generalised than South American populations, yet with most plant species showing WIC/TNW ratios >50 %, which indicates moderate generalisation among individual plants. Furthermore, frugivores' interaction allocation among individual plants also varied among regions. Broader and more overlapping frugivore assemblages in Mediterranean regions versus higher specialisation and variability in tropical and subtropical networks (higher frequency of study cases with WIC/TNW ratios <50 %) can be attributed to differences both in taxonomic diversity and redundancy levels in seed dispersal service, with frugivore assemblages being substantially more diversified and complementary as we descend in the latitudinal gradient (Fleming 1987, Willig *et al*. 2003). Although the latitude–niche breadth hypothesis does hold for food webs in terrestrial systems (Cirtwill *et al*. 2015), it could be influencing specialisation patterns of mutualistic plant-animal in frugivory systems. The degree of fruit dependence in the animal's diet may influence individual specialisation; tropical birds are more likely to be obligate frugivores, heavily relying on fruits, while temperate birds present more generalised diets with less dependency on fruits as their primary food source (Dalsgraad *et al*. 2017). Species range size may also be behind differences encountered across regions, as it has been demonstrated to increase populations' niche breadth (Galiana *et al.* 2023). Different levels of individual specialisation can have implications for population stability and niche expansion (Van Valen 1965). According to the niche variation hypothesis, populations with higher levels of individual specialisation will be more prone to niche expansion (Araujo *et al*. 2011). Niche shifts and expansion have become exceptionally important for adaptation to changing climate conditions (Hällfors *et al*. 2023) as well as changes in frugivore assemblages and fluctuating abundances (Campo-Celada *et al*. 2022). Therefore, the variation we found among geographical regions in frugivore assemblage specialisation will have a likely impact on the adaptation of plant-frugivore mutualistic interaction niche in current and future scenarios of global change.

Differential contribution by frugivores to plant consumption was widespread in all populations, supporting previous studies and providing more evidence on how just a few species, even within diversified assemblages, perform most of the mutualistic interactions (Fig. A4.5, Rother *et al*. 2016, Guerra *et al*. 2017, Isla *et al*. 2023, Rehling *et al.* 2023, Thiel *et al*. 2023). These interaction patterns will result in asymmetric dependencies between plant individuals and frugivore species, where the main frugivore species show low specificity for specific plants, while most plant individuals rely just on the main frugivore's service (Quintero *et al*. 2023). Asymmetric dependency between partners also emerges at species-species interaction level (Vázquez & Aizen 2014, Bascompte *et al*. 2006); further downscaling into individual-individual interactions would help elucidate if asymmetry remains consistent across scales. Finally, although frugivore body mass did not prove to be a good indicator of their contribution to interactions (although see Valenzuela-Ospina & Kattan 2021), it may play a role in seed dispersal effectiveness due to its positive correlation with the number of fruits consumed per visit or the frequency of long-distance seed dispersal events (Snow & Snow 1988, Jordano *et al.* 2007, Godínez-Álvarez *et al.* 2020).

Individual plants' interaction profile

Individual plants showed widespread interaction patterns, regardless of the population or species, as revealed by a rather homogeneous multivariate structure of interaction metrics, lacking distinct groupings in the multivariate space. This resulted in a heterogeneous positioning of individual plants across the multivariate space, pointing to the existence of fundamental architectural patterns in the assemblage of mutualistic interactions that are not strongly constrained by phylogeny or geographic location but rather by the interplay between traits and numerical effects (Jordano 1987a, Carnicer *et al.* 2009, Albrecht *et al.* 2018, Guimarães 2020). We observed a consistent distribution of plant roles within populations, in which a large majority of individuals act in an average manner, a smaller number of individuals stand out for their specialisation and only very few individuals stand out for their high diversity, frugivores' dependence and central role in interactions. This pattern reinforces previous findings in food webs, which revealed the presence of a core group of species fulfilling similar ecological roles, alongside peripheral species exhibiting idiosyncratic profiles (Mora *et al.* 2018). It is likely that within frugivory networks these key individuals present unique phenotypic traits, such as abundant fruit crops or advantageous locations that make them reliable to many frugivores (Snell *et al.* 2019, Isla *et al.* 2023).

Although some of the plant species considered in this study were generalists within their community, individuals in their population also showed narrower and specialised interaction niche breadths (Guerra *et al.* 2017), illustrating how ecological generalists are in fact heterogeneous collections of relative generalist and specialist individuals (Bolnick *et al.* 2007, Arroyo-Correa *et al.* 2023). This admixing illustrates how even ecologically generalist plant species actually emerge as a combination of individual plants with broad interaction generalisation and other individuals with higher interaction specificity.

Concluding remarks

We found consistent patterns of interaction assembly across biological scales using a set of biologically informative network metrics. On top of the absence of a clear hierarchy differentiation in network structure between individuals and species, we found that almost every network included a similar representation of individual profiles, evidencing a common backbone in the way interactions are organised (Mora *et al*. 2018). Conducting future analyses on interaction modes or motifs of individual-based networks may provide us with new insights, as these approaches have proven effective in distinguishing networks between and within ecological systems (Mora *et al.* 2018, Michalska-Smith *et al*. 2021, Pichon *et al.* 2023).

Intraspecific variation was at the core mutualistic interactions configuration, driven by the widespread interaction profiles of frugivore species with individual plants. High levels of intraspecific variation have been shown to confer greater stability to mutualistic systems (Arroyo-Correa *et al.* 2023). By zooming in on ecological interactions this study provides valuable insights into how mutualism interactions are structured at the individual level and reveal underlying patterns of role assignment within populations and across geographical regions.

Acknowledgements

We are grateful to Miguel Jácome, Gemma Calvo, Pablo Homet, Pablo Villaba and Juan Miguel Arroyo for support in data collection and processing with some of the individual networks which was fundamental for this manuscript, as well as, discussion and comments for improving the quality of this work. We are also grateful to Daniel Stouffer for his help and discussion on the network centralization metric. We are also grateful to Daniel Stouffer for his help and discussion on network centralization metric. We thank the logistic and facilities support form ICTS-RBD Doñana and the Doñana National Park for the access authorizations to collect data from many of the individual networks here presented. Funding from LIFEWATCH-2019-09-CSIC-13 project and PID2022-136812NB-I00, Spanish Ministry of Science and Innovation, as well as funding from the VI Plan Propio de Investigación of the Universidad de Sevilla (VI PPIT-US) supported this research (PJ). EQ was supported by "la Caixa" Foundation fellowship LCF/BQ/DE18/1167000. FRS was supported by VI PPIT-US and grants US-1381388 from Universidad de Sevilla/Junta de Andalucía/FEDER-UE and grant CNS2022-135839 from Ministerio de Ciencia e Innovación.

APPENDICES CHAPTER 4

Appendix 4A. Network characteristics and sampling effort

Table A4.1. List of networks providing the identification code used in figures, the type (ind: individual-based, sp: species-based), the focal plant species in the case of individual-based networks, the country where network was sampled, the number of plants and number of frugivores present in the network, the network size (potential interactions), the number of unique interactions (realised interactions), the name of the population where sampling took place in case of several populations for the same study, and the reference of the study from which the network was extracted.

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
1	ind	*Pistacia lentiscus* (Anacardiaceae)	Spain	40	27	1080	392	El Puntal	Camera traps and DNA-barcoding	Quintero, E., Rodríguez-Sánchez, F., & Jordano, P. (2023). Reciprocity and interaction effectiveness in generalised mutualisms among free-living species. Ecology Letters, 26(1), 132–146.
2	ind	*Pistacia lentiscus* (Anacardiaceae)	Spain	40	16	640	134	Laguna de las Madroñas	DNA-barcoding	
3	ind	*Juniperus phoenicea* (Cupressaceae)	Spain	35	10	350	137	Colonizacion	Camera traps and DNA-barcoding	Isla, J., Jácome-Flores, M., Arroyo, J. M., & Jordano, P. (2023). The turnover of plant–frugivore interactions along plant range expansion: Consequences for natural colonization processes. Proceedings of the Royal Society B: Biological Sciences, 290(1999), 20222547.
4	ind	*Juniperus phoenicea* (Cupressaceae)	Spain	35	10	350	154	Ojillo	Camera traps and DNA-barcoding	
5	ind	*Juniperus phoenicea* (Cupressaceae)	Spain	35	11	385	148	El Marqués	Camera traps and DNA-barcoding	
6	ind	*Lithraea molleoides* (Anacardiaceae)	Argentina	13	10	130	37	Los Hornillos	Focal observations	Vergara-Tabares, D. L., Blendinger, P. G., Tello, A., Peluc, S. I., & Tecco, P. A. (2022). Fleshy-fruited invasive shrubs indirectly increase native tree seed dispersal. Oikos, 2022(2).
7	ind	*Lithraea molleoides* (Anacardiaceae)	Argentina	14	10	140	33	Las Calles	Focal observations	
8	ind	*Lithraea molleoides* (Anacardiaceae)	Argentina	14	13	182	46	La Poblacion	Focal observations	
9	ind	*Lithraea molleoides* (Anacardiaceae)	Argentina	13	12	156	41	San Javier	Focal observations	

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
10	ind	*Lithraea molleoides* (Anacardiaceae)	Argentina	12	11	132	29	Las Rabonas	Focal observations	Vergara-Tabares, D. L., Blendinger, P. G., Tello, A., Peluc, S. I., & Tecco, P. A. (2022). Fleshy-fruited invasive shrubs indirectly increase native tree seed dispersal. Oikos, 2022(2).
11	ind	*Lithraea molleoides* (Anacardiaceae)	Argentina	11	7	77	25	Los Molles	Focal observations	
12	ind	*Laurus nobilis* (Lauraceae)	Spain	18	17	306	87		Focal observations	Rodríguez-Sánchez, F. (2010). An integrative framework to investigate species responses to climate change: Biogeography and ecology of relict trees in the Mediterranean. PhD Thesis. Universidad de Sevilla, Spain.
13	ind	*Prunus mahaleb* (Rosaceae)	Spain	19	20	380	211		Focal observations	Jordano, P. (1995). Frugivore-Mediated Selection on Fruit and Seed Size: Birds and St. Lucie's Cherry, Prunus Mahaleb. Ecology, 76(8), 2627–2639. Jordano, P., & Schupp, E. W. (2000). Seed Disperser Effectiveness: The Quantity Component and Patterns of Seed Rain for Prunus mahaleb. Ecological Monographs, 70(4), 591-615.
14	ind	*Euterpe edulis* (Arecaceae)	Brazil	17	9	153	31	Restinga	Focal observations	Friedemann, P., Côrtes, M. C., de Castro, E. R., Galetti, M., Jordano, P., & Guimarães Jr, P. R. (2022). The individual-based network structure of palm-seed dispersers is explained by a rainforest gradient. Oikos, 2022, e08384.
15	ind	*Euterpe edulis* (Arecaceae)	Brazil	15	7	105	25	Lowland	Focal observations	
16	ind	*Euterpe edulis* (Arecaceae)	Brazil	30	8	240	50	Premontane	Focal observations	
17	ind	*Cecropia glaziovii* (Urticaceae)	Brazil	27	37	999	124		Focal observations	Jordano, P. 2024. Material for the course: Curso Posgraduação Frugivoria 2016, UNESP Rio Claro, Brazil.

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
18	ind	*Heynea trijuga* (Meliaceae)	India	24	11	264	48		Focal observations	Gopal, A., Mudappa, D., Raman, T. S., & Naniwadekar, R. (2020). Forest cover and fruit crop size differentially influence frugivory of select rainforest tree species in Western Ghats, India. Biotropica, 52(5), 871-883.
19	ind	*Myristica dactyloides* (Myristicaceae)	India	25	7	175	45		Focal observations	
20	ind	*Persea macrantha* (Lauraceae)	India	32	21	672	186		Focal observations	
21	ind	*Henriettea succosa* (Melastomataceae)	Brazil	18	22	396	77		Focal observations	Crestani, A. C., Mello, M. A. R., & Cazetta, E. (2019). Interindividual variations in plant and fruit traits affect the structure of a plant-frugivore network. Acta Oecologica, 95, 120-127.
22	ind	*Prestoea decurrens* (Arecaceae)	Ecuador	31	9	279	100		Camera traps	Lamperty, T., Karubian, J., & Dunham, A. E. (2021). Ecological drivers of intraspecific variation in seed dispersal services of a common neotropical palm. Biotropica, 53(4), 1226–1237.
23	ind	*Corema album* (Ericaceae)	Spain	24	15	360	129		Camera traps	Villalva, P., Arroyo-Correa, B., Calvo, G., Homet, P., Isla, J., Mendoza, I., Moracho, E., Quintero, E., Rodríguez-Sánchez, F., & Jordano, P. (2023). FRUGIVORY CAMTRAP: A dataset of plant-animal interactions recorded with camera traps. https://doi.org/10.20350/DIGITALCSIC/15623
24	ind	*Bursera penicillata* (Burseraceae)	India	15	11	165	43		Focal observations	Ramaswami, G., Somnath, P., & Quader, S. (2017). Plant-disperser mutualisms in a semi-arid habitat invaded by Lantana camara L. Plant Ecology, 218, 935-946.
25	ind	*Erythroxylum monogynum* (Erythroxylaceae)	India	12	6	72	29		Focal observations	
26	ind	*Flacourtia indica* (Salicaceae)	India	13	5	65	33		Focal observations	

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
27	ind	*Flueggea leucopyrus* (Phyllanthaceae)	India	10	8	80	22		Focal observations	Ramaswami, G., Somnath, P., & Quader, S. (2017). Plant-disperser mutualisms in a semi-arid habitat invaded by Lantana camara L. Plant Ecology, 218, 935-946.
28	ind	*Canthium coromandelicum* (Rubiaceae)	India	10	8	80	30		Focal observations	
29	ind	*Santalum album* (Santalaceae)	India	14	10	140	38		Focal observations	
30	ind	*Ziziphus oenopolia* (Rhamnaceae)	India	15	13	195	102		Focal observations	
31	ind	*Chamaerops humilis* (Arecaceae)	Spain	39	6	234	76	Matasgordas	Footprint traps	Jácome-Flores, M. E. et al. 2020. Interaction motifs variability in a Mediterranean palm under environmental disturbances: the mutualism–antagonism continuum. Oikos 129: 367–379.
32	ind	*Chamaerops humilis* (Arecaceae)	Spain	24	6	144	57	Martinazo	Footprint traps	
33	ind	*Miconia irwinii* (Melastomataceae)	Brazil	15	9	135	59		Focal observations	Guerra, T. J. et al. 2017. Intraspecific variation in fruit–frugivore interactions: effects of fruiting neighborhood and consequences for seed dispersal. Oecologia 185: 233–243.
34	ind	*Juniperus macrocarpa* (Cupressaceae)	Spain	26	11	286	72		Camera traps	Villalva, P., Arroyo-Correa, B., Calvo, G., Homet, P., Isla, J., Mendoza, I., Moracho, E., Quintero, E., Rodríguez-Sánchez, F., & Jordano, P. (2023). FRUGIVORY CAMTRAP: A dataset of plant-animal interactions recorded with camera traps. https://doi.org/10.20350/DIGITALCSIC/15623
35	ind	*Prosopis flexuosa* (Fabaceae)	Argentina	26	9	234	72	Araya-San Ignacio	Camera traps	Miguel, M.F., Jordano, P., Tabeni, S. and Campos, C.M. (2018), Context-dependency and anthropogenic effects on individual plant–frugivore networks. Oikos, 127: 1045-1059.
36	ind	*Prosopis flexuosa* (Fabaceae)	Argentina	28	10	280	84	El Bonito	Camera traps	

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
37	ind	*Prosopis flexuosa* (Fabaceae)	Argentina	54	10	540	112	El Doménico	Camera traps	Miguel, M.F., Jordano, P., Tabeni, S. and Campos, C.M. (2018), Context-dependency and anthropogenic effects on individual plant–frugivore networks. Oikos, 127: 1045-1059.
38	ind	*Prosopis flexuosa* (Fabaceae)	Argentina	35	8	280	61	MaB Ñacuñán Reserve Protected 1	Camera traps	
39	ind	*Prosopis flexuosa* (Fabaceae)	Argentina	29	9	261	99	MaB Ñacuñán Reserve Protected 2	Camera traps	
40	ind	*Schinus terebinthifolia* (Anacardiaceae)	Brazil	26	16	416	93		Focal observations	Vissoto, M., Vizentin-Bugoni, J., Sendoya, S. F., Gomes, G. C., & Dias, R. A. (2022). Plant height and spatial context influence individual connectivity and specialization on seed dispersers in a tree population. Oecologia.
41	ind	*Phillyrea angustifolia* (Oleaceae)	Spain	10	16	160	72	El Puntal	DNA-barcoding	Unpublished data. SUMHAL project. IP: Pedro Jordano - 2021-2022
42	ind	*Phillyrea angustifolia* (Oleaceae)	Spain	9	12	108	41	Laguna de las Madroñas	DNA-barcoding	
43	ind	*Marcgravia longifolia* (Marcgraviaceae)	Peru	24	43	1032	127		Focal observations	Thiel, S., Willems, F., Farwig, N., Rehling, F., Schabo, D. G., Schleuning, M., Shahuano Tello, N., Töpfer, T., Tschapka, M., Heymann, E. W., & Heer, K. (2023). Vertically stratified frugivore community composition and interaction frequency in a liana fruiting across forest strata. Biotropica, 55, 650–664
44	ind	*Osyris lanceolata* (Santalaceae)	Spain	19	14	266	62		DNA-barcoding	Unpublished data. SUMHAL project. IP: Pedro Jordano - 2021-2022

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
45	sp		Spain	25	36	900	228	Cazorla	Focal observations	Olesen JM, Bascompte J, Dupont YL, Elberling H, Rasmussen C, Jordano P. (2011). Missing and forbidden links in mutualistic networks. Proceedings of the Royal Society B-Biological Sciences 278: 725-732.
46	sp		Spain	16	17	272	120	Hato Ratón	Mist-netting	
47	sp		Spain	17	28	476	130	Spain	Focal observations	García-Castaño, J.L. (2001). Consecuencias demográficas de la dispersión de semillas por aves y mamíferos frugívoros en la vegetación mediterránea de montaña. PhD Thesis. Universidad de Sevilla, Spain.
48	sp		Papua New Guinea	31	9	279	119		Focal observations	Beehler B. (1983) Frugivory and polygamy in birds of paradise. Auk, 100, 1-11.
49	sp		South Africa	16	10	160	110		Focal observations	Frost P.G.H. (1980) Fruit-frugivore interactions in a South African coastal dune forest. In: Acta XVII Congressus Internationalis Ornithologici (ed. Noring R), pp. 1179-1184. Deutsche Ornithologische Ges., Berlin, Germany.
50	sp		Spain	12	7	84	40		Mist-netting	Guitián J. (1983) Relaciones entre los frutos y los passeriformes en un bosque montano de la cordillera Cantabrica occidental. PhD Thesis. Universidad de Santiago, Spain.
51	sp		Brazil	35	29	1015	146		Focal observations	Galetti M. & Pizo M.A. (1996) Fruit eating birds in a forest fragment in southeastern Brazil. Ararajuba, Rev. Brasil. Ornitol., 4, 71-79.
52	sp		Mexico	5	27	135	86		Focal observations	Kantak, G.E. (1979). Observations on some fruit-eating birds in Mexico. Auk, 96, 183-186.
53	sp		England	11	14	154	47		Focal observations	Snow, B.K. & Snow, D.W. (1988). Birds and berries. T. and A.D. Poyser, Calton, England.
54	sp		Japan	15	8	120	38		Focal observations	Noma, N. & Yumoto, T. (1997). Fruiting phenology of animal-dispersed plants in response to winter migration of frugivores in a warm temperate forest on Yakushima Island, Japan. Ecological Research, 12, 119-129.

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
55	sp		Australia	71	7	497	142		Focal observations	Crome, F. H.J. (1975). The ecology of fruit pigeons in tropical Northern Queensland. Aust Wildl Res, 2, 155-185.
56	sp		Trinidad and Tobago	50	14	700	234		Focal observations	Snow B.K. & Snow D.W. (1971) The feeding ecology of tanagers and honeycreepers in Trinidad. Auk, 88, 291-322.
57	sp		United States	7	21	147	50		Focal observations	Baird, J.W. (1980). The selection and use of fruit by birds in an Eastern forest. Wilson Bulletin, 92, 63-73.
58	sp		Kenya	8	30	240	69	Interior little disturbed	Focal observations	Menke, S., Böhning-Gaese, K. & Schleuning, M. (2012). Plant-frugivore networks are less specialized and more robust at forest-farmland edges than in the interior of a tropical forest. Oikos, 121, 1553-1566.
59	sp		Kenya	7	38	266	104	Edge little disturbed	Focal observations	
60	sp		Kenya	8	34	272	88	Interior highly disturbed	Focal observations	
61	sp		Kenya	8	39	312	115	Edge highly disturbed	Focal observations	
62	sp		Brazil	15	49	735	143		Focal observations	Pizo, M.A. (2004). Frugivory and habitat use by fruit-eating birds in a fragmented landscape of southeast Brazil. Ornitologia Neotropical, 15, 117-126.
63	sp		Kenya	33	88	2904	419		Focal observations	Schleuning M, Bluthgen N, Florchinger M, Braun J, Schaefer HM, Bohning-Gaese K. 2011. Specialization and interaction strength in a tropical plant-frugivore network differ among forest strata. Ecology 92: 26-36.
64	sp		Brazil	49	16	784	131		Focal observations	Castro, E.R.D. (2007). Fenologia reprodutiva do palmito Euterpe edulis (Erecaceae) e sua influência na abundância de aves frugívoras na floresta atlântica. PhD Thesis. Instituto de Biociencias. Universidade Estadual Paulista "Juio de Mesquita Filho" Rio Claro, SP, Brazil.

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
65	sp		Brazil	13	45	585	183		Focal observations	Correia, J.M.S. (1997). Utilização de espécies frutíferas de mata Atlântica na alimentação da avifauna da reserva biológica de Poço das Antas, RJ. MSc Thesis. Instituto de Biologia. UNB, Brazil.
66	sp		Brazil	13	30	390	145		Focal observations	Alves, K.J.F. (2008). Composição da avifauna e frugivoria por aves em um mosaico sucessional na mata Atlântica. MSc Thesis. Instituto de Biociencias. Universidade Estadual Paulista, Julio de Mesquita Filho, Rio Claro, SP, Brazil.
67	sp		Brazil	9	30	270	92		Focal observations	Athie, S. (2009). Composição da avifauna e frugivoria por aves em um mosaico de vegetação secundária em Rio Claro, região centro-leste do estado de São Paulo. MSc Thesis. Universidade Federal de São Carlos, Brazil.
68	sp		Brazil	25	28	700	90		Focal observations	Ferreira Fadini, R. & De Marco Jr., P. (2004). Interações entre aves frugívoras e plantas em um fragmento de mata atlântica de Minas Gerais. Ararajuba, 12, 97-103.
69	sp		Brazil	26	22	572	79		Focal observations and mist-netting	Hasui, Erica. (1994). O papel das aves frugivoras na dispersao de sementes em um fragmento de floresta semidecidua secundaria em Sao Paulo, SP. MSc thesis. USP Sao Paulo, Brazil.
70	sp		Brazil	22	20	440	67		Focal observations	Silva, R. F. d. M. (2011). Interações entre plantas e aves frugívoras no campus da Universidade Federal Rural do Rio de Janeiro. In: Instituto de Florestas. Universidade Federal do Rio de Janeiro Rio de Janeiro, Brazil.
71	sp		Brazil	12	15	180	32	15-year-old restored plot	Focal observations	Ribeiro da Silva, F., Montoya, D., Furtado, R., Memmott, J., Pizo, M.A. and Rodrigues, R.R. (2015), The restoration of tropical seed dispersal networks. Restor Ecol, 23: 852-860.
72	sp		Brazil	23	29	667	129	25-year-old restored plot	Focal observations	
73	sp		Brazil	14	14	196	35	57-year-old restored plot	Focal observations	

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
74	sp		Brazil	6	28	168	50		Focal observations	Robinson, V. (2015). Interações entre aves frugívoras e plantas em um fragmento de mata atlântica de Minas Gerais. In: Instituto de Biociencias. Universidade Estadual Paulista "Julio de Mesquita Filho" Rio Claro, SP, Brazil.
75	sp		Brazil	30	58	1740	240		Focal observations	Rodrigues, S. B. M. (2015). Rede de interações entre aves frugivoras e plantas em una área de mata Atlântica no sudeste do Brasil. Universidade Federal de Sao Carlos, Campus Sorocaba Sorocaba, SP, Brazil.
76	sp		New Zealand	18	8	144	102		Focal observations	Burns, K.C. (2013). What causes size coupling in fruit-frugivore interaction webs? Ecology, 94, 295-300.
77	sp		Poland	8	12	96	38	site_11	Focal observations	Albrecht, J., Bohle, V., Berens, D. G., Jaroszewicz, B., Selva, N., & Farwig, N. (2015). Variation in neighbourhood context shapes frugivore-mediated facilitation and competition among co-dispersed plant species. Journal of Ecology, 103(2), 526–536.
78	sp		Poland	7	11	77	32	site_13	Focal observations	
79	sp		Poland	9	13	117	42	site_15	Focal observations	
80	sp		Poland	8	13	104	36	site_30	Focal observations	
81	sp		Poland	8	10	80	29	site_35	Focal observations	
82	sp		Poland	8	10	80	30	site_36	Focal observations	
83	sp		Poland	8	11	88	33	site_102	Focal observations	
84	sp		Poland	10	13	130	42	site_111	Focal observations	
85	sp		Poland	8	15	120	57	site_112	Focal observations	
86	sp		Poland	9	16	144	41	site_203	Focal observations	
87	sp		Poland	6	20	120	43	site_301	Focal observations	
88	sp		Poland	5	11	55	21	site_314	Focal observations	
89	sp		Poland	8	19	152	56	site_315	Focal observations	

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
90	sp		Brazil	22	17	374	78		Focal observations	Andrade, P., Mota, J. & Carvalho, A. (2011). Mutual interactions between frugivorous birds and plants in an urban fragment of Atlantic Forest, Salvador, BA. Revista Brasileira de Ornitologia, 19, 63-73.
91	sp		Puerto Rico	34	20	680	95		Focal observations	Yang, S., Albert, R. & Carlo, T.A. (2013). Transience and constancy of interactions in a plant-frugivore network. Ecosphere, 4(12): 147.
92	sp		Brazil	14	6	84	22		Mist-netting	Garcia, Q.S., Rezende, J.L.P. & Aguiar, L.M.S. (2000). Seed dispersal by bats in a disturbed area of Southeastern Brazil. Revista de Biología Tropical, 48, 125-128.
93	sp		Peru	77	18	1386	196		Mist-netting	Gorchov, D.L., Cornejo, F., Ascorra, C.F. & Jaramillo, M. (1995). Dietary overlap between frugivorous birds and bats in the peruvian amazon. Oikos, 74, 235-250.
94	sp		Costa Rica	35	14	490	95		Mist-netting	Palmeirim, J.M., Gorchov, D.L. & Stoleson, S. (1989). Trophic structure of a neotropical frugivore community: is there competition between birds and bats? Oecologia (Berl.), 79, 403-411.
95	sp		Costa Rica	35	14	490	119		Mist-netting	Lopez, J.E. & Vaughan, C. (2004). Observations on the Role of Frugivorous Bats as Seed Dispersers in Costa Rican Secondary Humid Forests. Acta Chiropterologica, 6, 111-119.
96	sp		Ecuador	43	15	645	97		Animal feces (mist-netting and transects)	Heleno, R.H., Olesen, J.M., Nogales, M., Vargas, P. & Traveset, A. (2013). Seed dispersal networks in the Galapagos and the consequences of alien plant invasions. Proc Biol Sci, 280, 20122112.
97	sp		Mexico	22	7	154	47	Tropical montane cloud forest fragment	Mist-netting	Hernandez-Montero, J.R., Saldana-Vazquez, R.A., Galindo-Gonzalez, J. & Sosa, V.J. (2015). Bat-fruit interactions are more specialized in shaded-coffee plantations than in tropical mountain cloud forest fragments. PLoS ONE, 10, e0126084.
98	sp		Mexico	19	6	114	34	Shaded-coffee plantation	Mist-netting	

Net no.	Type	Focal plant species	Country	Plants	Frugivores	Net size	Unique interactions	Population site	Sampling method	Reference
99	sp		Brazil	24	7	168	50		Mist-netting	Passos, F.C., Silva, W.R., Pedro, W.A. & Bonin, M.R. (2003). Frugivoria em morcegos (Mammalia, Chiroptera) no Parque Estadual Intervales, sudeste do Brasil. Revista Brasileira de Zoologia, 20, 511-517.
100	sp		Brazil	13	7	91	30		Mist-netting	Pedro, W.A. (1992). Estrutura de uma taxocenose de morcegos da reserva do Panga (Uberlandia, MG), com enfase nas relações troficas em Phyllostomidae (Mammalia: Chiroptera). MSC thesis. Universidade Estadual de Campinas Campinas, SP, Brazil.
101	sp		Panama	17	20	340	86		Mist-netting	Poulin, B., Wright, S.J., Lefebvre, G. & Calderon, O. (1999). Interspecific synchrony and asynchrony in the fruiting phenologies of congeneric bird-dispersed plants in Panama. Journal of Tropical Ecology, 15, 213-227.
102	sp		Brazil	56	20	1120	104		Mist-netting	Sarmento, R., Alves-Costa, C.i.P., Ayub, A. & Mello, M.A.R. (2014). Partitioning of seed dispersal services between birds and bats in a fragment of the Brazilian Atlantic Forest. Zoologia (Curitiba, Impresso), 31, 245-255.
103	sp		Germany	30	31	930	189		Focal observations	Stiebel, H. & Bairlein, F. (2008). Frugivorie mitteleuropäischer Vögel I: Nahrung und Nahrungserwerb. Vogelwarte, 46, 1-23.
104	sp		Bolivia	36	41	1476	127	Forest edge	Focal observations	Saavedra, F., Hensen, I., Beck, S. G., Böhning-Gaese, K., Lippok, D., Töpfer, T., & Schleuning, M. (2014). Functional importance of avian seed dispersers changes in response to human-induced forest edges in tropical seed-dispersal networks. Oecologia, 176, 837-848.
105	sp		Bolivia	20	23	460	52	Forest interior	Focal observations	

Table A4.2. Sampling coverage in individual-based networks with lower- and upper-confidence limits of sample coverage (95%) (SC LCL, SC UCL, respectively).

Net no.	Focal plant species	Sampling coverage	SC LCL	SC UCL
1	*Pistacia lentiscus*	0.99	0.99	1.00
2	*Pistacia lentiscus*	0.96	0.93	0.99
3	*Juniperus phoenicea*	0.99	0.97	1.00
4	*Juniperus phoenicea*	0.99	0.98	1.00
5	*Juniperus phoenicea*	1.00	0.99	1.00
6	*Lithraea molleoides*	0.87	0.78	0.96
7	*Lithraea molleoides*	0.94	0.85	1.00
8	*Lithraea molleoides*	0.92	0.86	0.98
9	*Lithraea molleoides*	0.91	0.82	1.00
10	*Lithraea molleoides*	0.87	0.74	1.00
11	*Lithraea molleoides*	0.93	0.82	1.00
12	*Laurus nobilis*	0.98	0.95	1.00
13	*Prunus mahaleb*	1.00	0.99	1.00
14	*Euterpe edulis*	0.91	0.80	1.00
15	*Euterpe edulis*	0.89	0.79	0.99
16	*Euterpe edulis*	0.96	0.92	1.00
17	*Cecropia glaziovii*	0.88	0.84	0.93
18	*Heynea trijuga*	0.96	0.91	1.00
19	*Myristica dactyloides*	0.98	0.94	1.00
20	*Persea macrantha*	0.98	0.96	1.00
21	*Henriettea succosa*	0.87	0.81	0.93
22	*Prestoea decurrens*	0.98	0.97	1.00
23	*Corema album*	0.98	0.95	1.00
24	*Bursera penicillata*	0.94	0.87	1.00
25	*Erythroxylum monogynum*	0.97	0.91	1.00
26	*Flacourtia indica*	1.00	0.97	1.00
27	*Flueggea leucopyrus*	0.84	0.72	0.95
28	*Canthium coromandelicum*	0.91	0.82	1.00
29	*Santalum album*	0.88	0.79	0.96
30	*Ziziphus oenopolia*	0.97	0.94	1.00
31	*Chamaerops humilis*	1.00	0.99	1.00
32	*Chamaerops humilis*	1.00	0.99	1.00
33	*Miconia irwinii*	1.00	0.98	1.00
34	*Juniperus macrocarpa*	0.97	0.94	1.00
35	*Prosopis flexuosa*	1.00	0.98	1.00
36	*Prosopis flexuosa*	0.97	0.93	1.00
37	*Prosopis flexuosa*	0.97	0.95	0.99
38	*Prosopis flexuosa*	0.95	0.92	0.99
39	*Prosopis flexuosa*	0.99	0.97	1.00
40	*Schinus terebinthifolia*	0.96	0.92	0.99
41	*Phillyrea angustifolia*	0.95	0.90	0.99
42	*Phillyrea angustifolia*	0.94	0.88	1.00
43	*Marcgravia longifolia*	0.82	0.75	0.88
44	*Osyris lanceolata*	0.90	0.84	0.97

Appendix 4B. Comparison of networks at different resolution scales

To compare networks focused at the population level (individual-based) and the community level (species-based) we calculated several network descriptors. We then used these descriptors to build a PCA-derived multivariate space defined by their correlation structure, so that the location of each network is defined by a combination of both topological (*e.g.,* degree, connectance) and structural (*e.g.*, nestedness, modularity) descriptors. In this way, networks closely located in this multivariate space would have more similarities in the combination of metrics (and thus topology and structure) than networks located in different parts of the space.

Network-level metrics

With the aim of visualising families of metrics that describe similar aspects of the bipartite networks, we computed an agglomerative hierarchical clustering (HC) analysis for all the metrics (function hclust in stats R package, R Core Team 2023).

We selected metrics indicative of biological properties of the networks, aiming to reduce redundancy in their meaning and avoiding high correlation with network size. Since we aim at finding structural differences among networks with different resolution scales we tried to avoid metrics strongly affected by sampling design, species diversity and study region characteristics (*e.g.* tropical vs. temperate regions), such as web asymmetry, Shannon diversity or links per species. Both the cluster analysis and the correlation analysis help us select network-level metrics that are interpretable in biological terms while trying to avoid highly correlated metrics. The selected network-level metrics allow us to discern differences in the topological properties of individual-based and species-based networks.

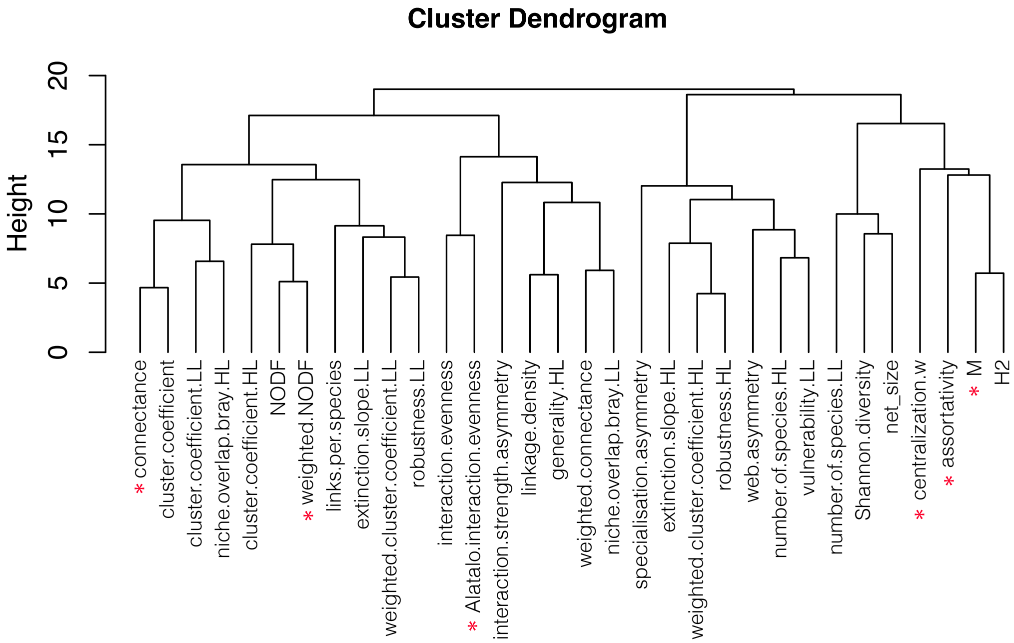


Figure A4.1. Hierarchical clustering analysis results for all the network metrics calculated. Metrics with * are the selected ones.

We checked for correlation among the selected metrics and with network size (Fig. A4.2). We did not find strong effects of correlation with network size (medium/low correlation). The highest correlations were between centralization and interaction evenness, and weighted NODF and modularity. All variables have a VIF < 3.15. Variance Inflation Factor (VIF).

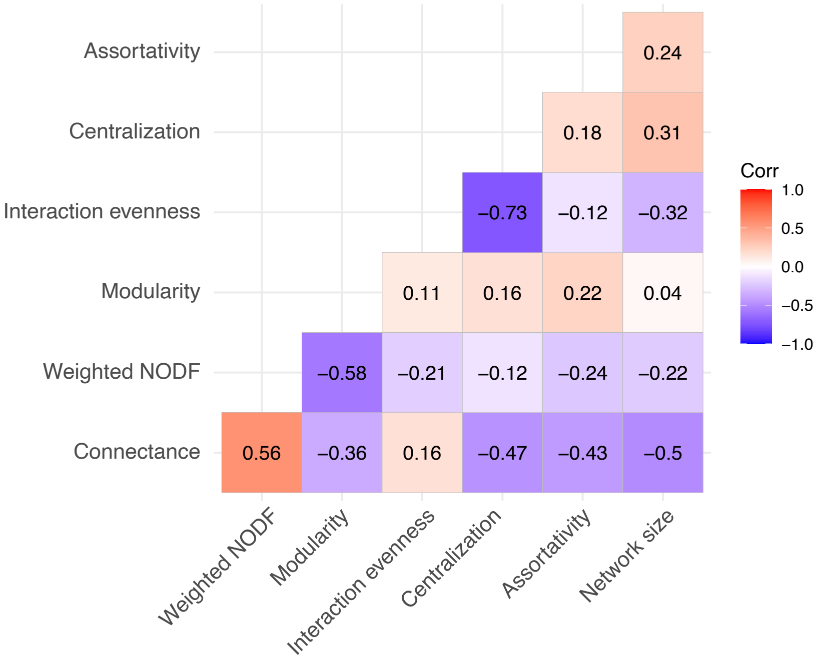


Figure A4.2. Correlation plot between selected network-level metrics for PCA analysis.

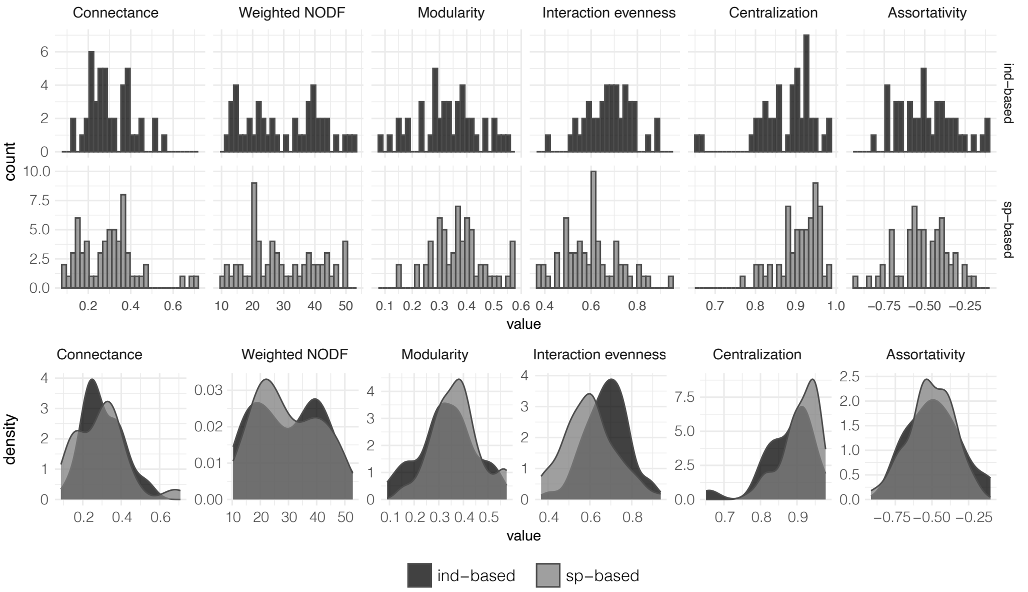


Figure A4.3. Distribution of network-level metrics of frugivory networks at different scales of resolution. Species-based networks are represented in grey (n = 61) and individual-based networks in black (n = 44). Upper two rows show histograms for each network type, and the lower row shows density plot for both network types facilitating comparison.

PCA analysis for comparing networks at different resolution scale

Table A4.3. Principal Component Analysis results for network-level metrics.

	PC1	PC2	PC3	PC4	PC5	PC6
Importance of components:						
Eigenvalue	1.56	1.31	0.91	0.74	0.54	0.42
Proportion of Variance	0.41	0.29	0.14	0.09	0.05	0.03
Cumulative Proportion	0.41	0.69	0.83	0.92	0.97	1
PC loadings:						
Connectance	-0.54	-0.03	0.1	-0.56	0.56	-0.26
Weighted NODF	-0.44	-0.42	-0.23	-0.27	-0.71	-0.05
Modularity	0.4	0.35	0.36	-0.69	-0.31	0.1
Alatalo interaction evenness	-0.21	0.67	-0.1	0.19	-0.25	-0.63
Centralization	0.41	-0.51	0.25	0.01	0.01	-0.71
Assortativity	0.37	0.03	-0.86	-0.3	0.15	-0.11

Appendix 4C. Population specialisation (TNW ~ WIC)

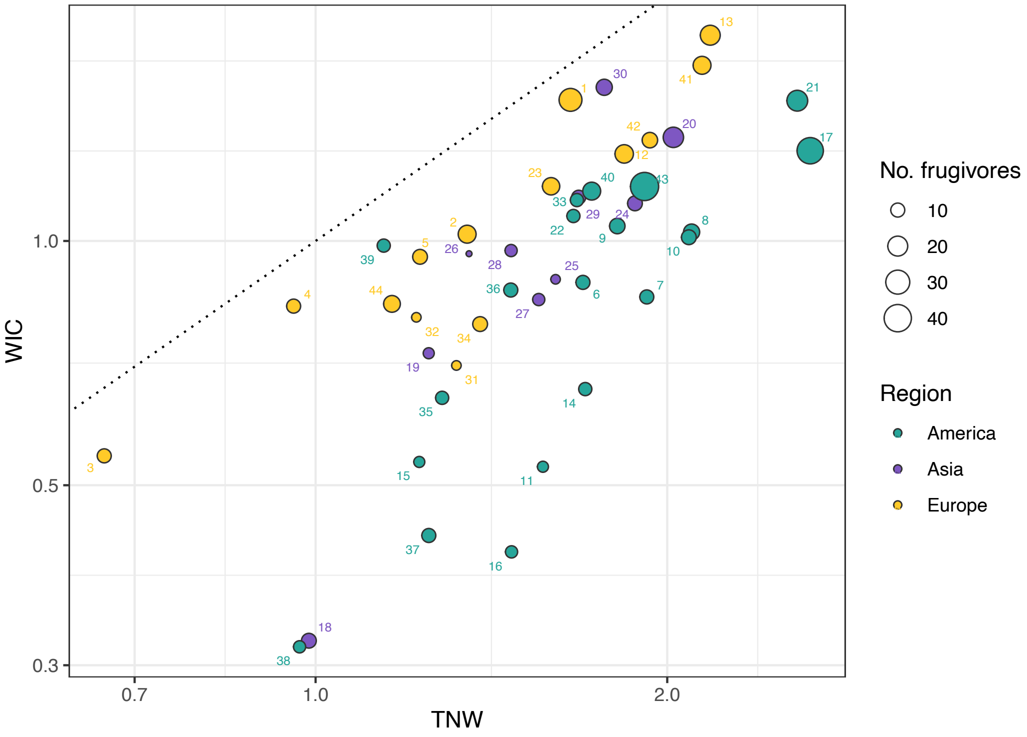


Figure A4.4. Total niche width (TNW) versus Within Individual Component (WIC) for individual-based frugivory networks. Point size is proportional to the number of frugivore species in the network, point colour indicates the geographic region and number the network id (see Table A4.1). Note the log-scale in both axes. The dotted line represents a 1:1 ratio, in which the WIC would be equal to the TNW indicating individual niche widths that encompass the whole population niche width. The closer the networks are to the line, the higher WIC/TWN (*i.e.*, lower individual specialisation). Networks including many frugivore species tend to have a wider interaction niche (TNW), but not necessarily higher levels of individual specialisation (WIC/TNW, *i.e.*, far from the 1:1 line).

Appendix 4D. Interaction curves by frugivores

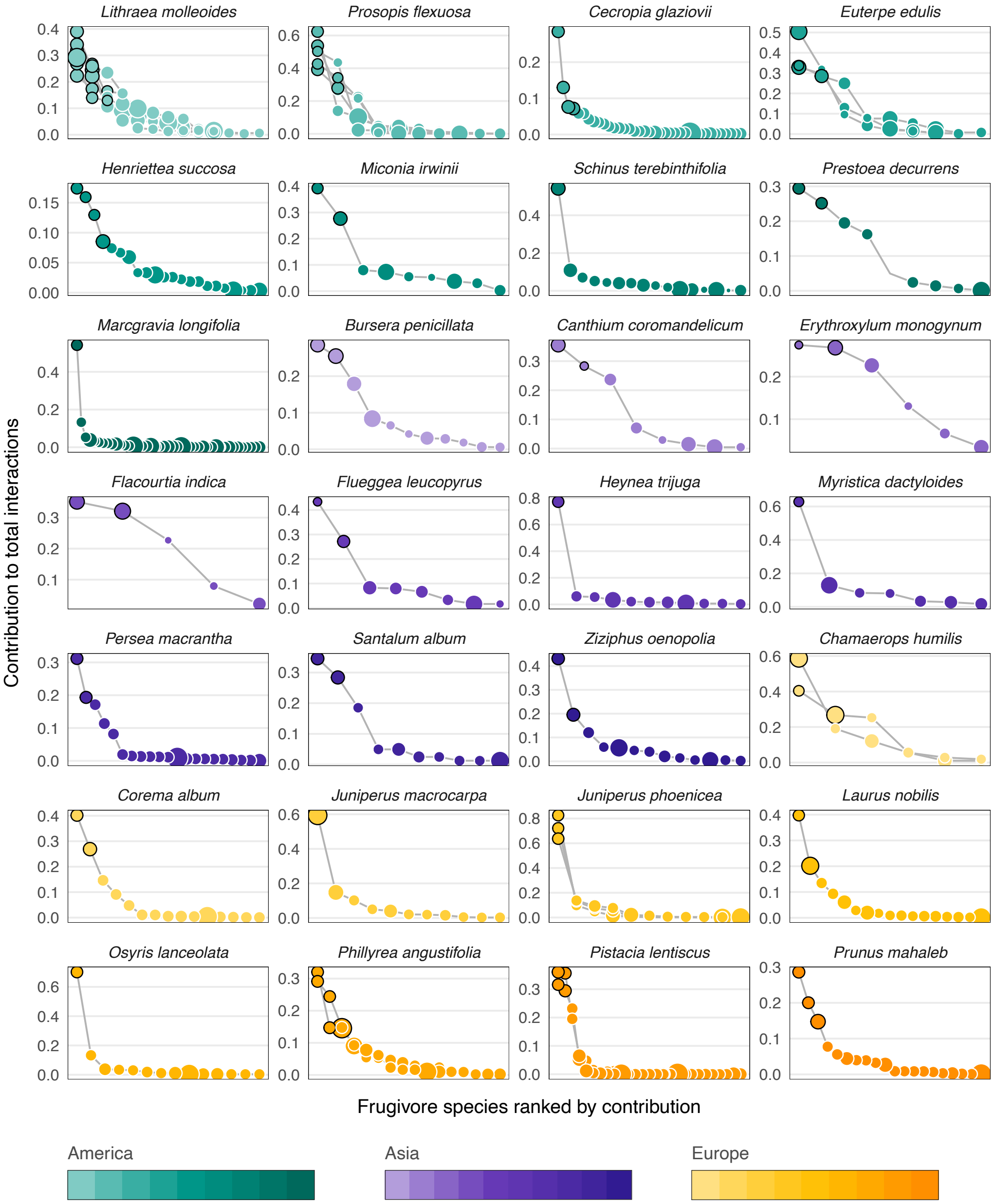


Figure A4.5. Relative contribution of each frugivore species (dots) to the total interactions of each plant species. Frugivores are ordered by contribution ranking. Plant species with more than one population/network present several curves. Colours for each species correspond with different geographical regions (teal = America, purple = Asia, orange = Europe) and different colour shades differentiate plant species, these correspond with colours in the individual-based networks PCA plot (Fig. 4.5). Dots size represents frugivore body mass relative to the population (z-score) and black outlines in dots indicate those frugivore species whose aggregate contributions account for at least 50% of the interactions.

Appendix 4E. Plant individuals' interaction profiles.

Node-level metrics

Same as with network-level metrics, we tried to select node-level metrics were not strongly correlated. All variables had a maximum VIF of 2.85 (VIF < 3).

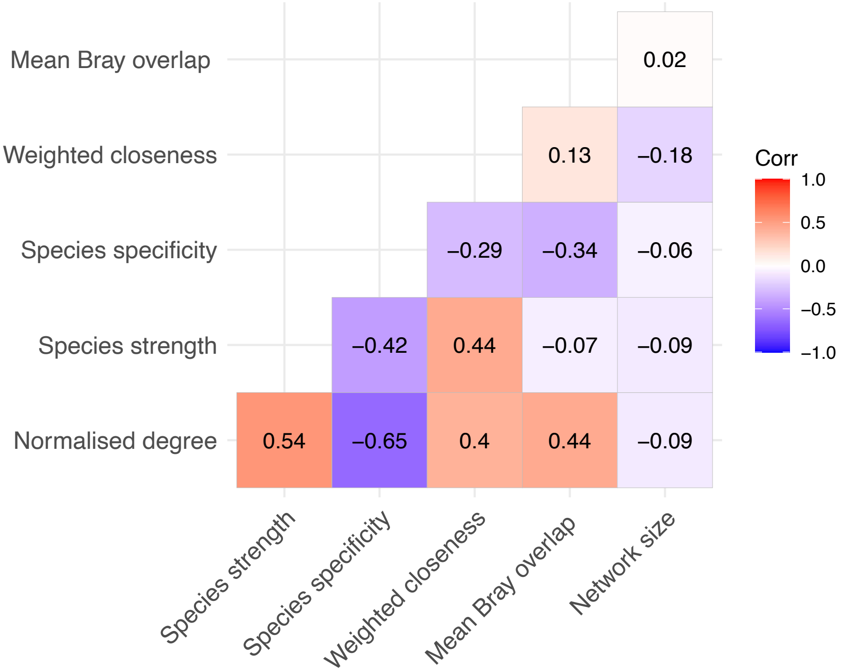


Figure A4.6. Correlation plot between selected node-level metrics.

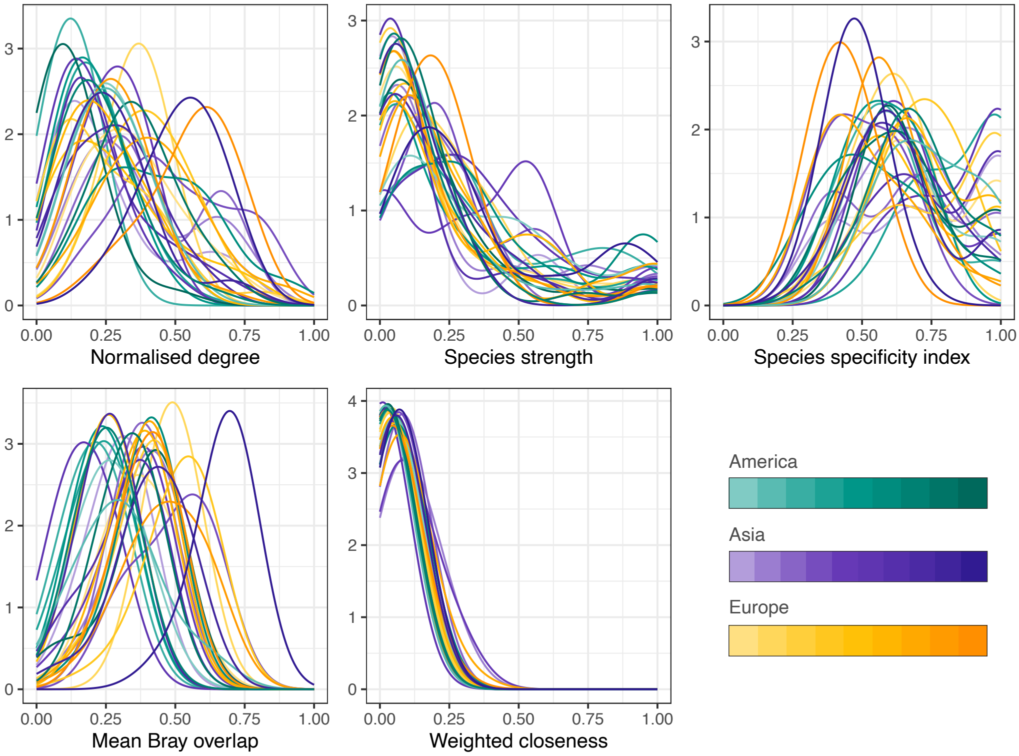


Figure A4.7. Density plots of selected node-level metrics estimated for plant individuals within their networks. Colour indicates a different geographical region (teal = America, purple = Asia, orange = Europe) and different colour shades differentiate plant species.

PCA analysis for comparing plant individuals' interaction profiles

Table A4.4. Principal Component Analysis results node-level metrics.

	PC1	PC2	PC3	PC4	PC5
Importance of components:					
Eigenvalue	1.60	1.05	0.84	0.64	0.48
Proportion of Variance	0.51	0.22	0.14	0.08	0.05
Cumulative Proportion	0.51	0.73	0.87	0.95	1.00
PC loadings:					
Normalised degree	-0.56	0.10	-0.19	-0.21	0.77
Species strength normalised	-0.42	-0.58	-0.23	-0.51	-0.42
Species specificity index	0.51	-0.12	0.39	-0.70	0.29
Weighted closeness	-0.39	-0.28	0.84	0.24	0.02
Mean Bray overlap	-0.31	0.75	0.22	-0.38	-0.38

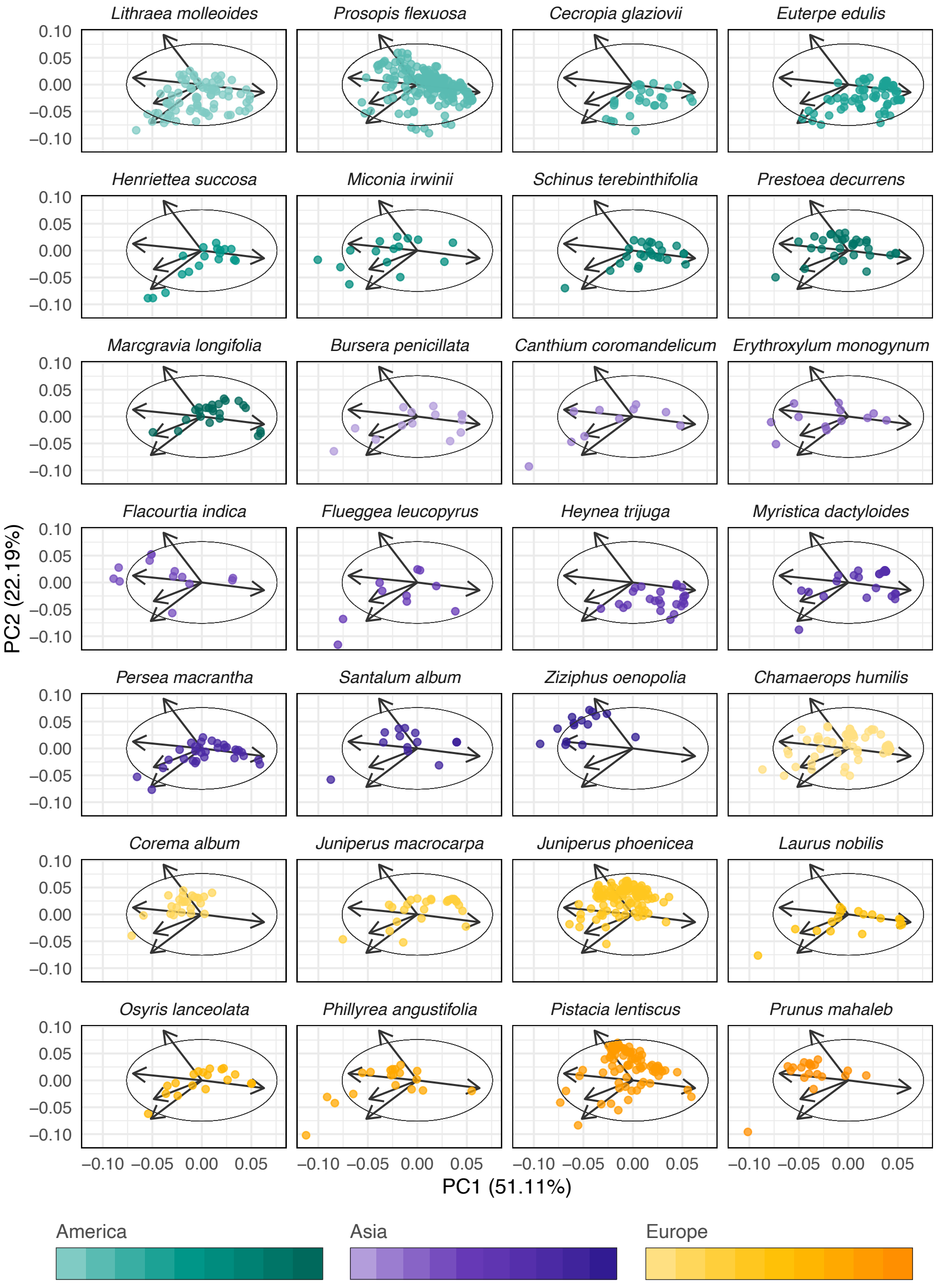


Figure A4.8. Principal Component Analysis for node-level metrics of individual plants in their respective networks. PCA multivariate space is faceted by plant species to facilitate display of plant individuals distribution in the multivariate species and the identification of outlying individuals. Note that some species present more than one population (*i.e.*, more than one network, see Table A4.1). Colour indicates a different geographical region (teal = America, purple = Asia, orange = Europe) and different colour shades differentiate plant species. See Fig. 4.5 for information on what node-level metric represents each of the five arrows.

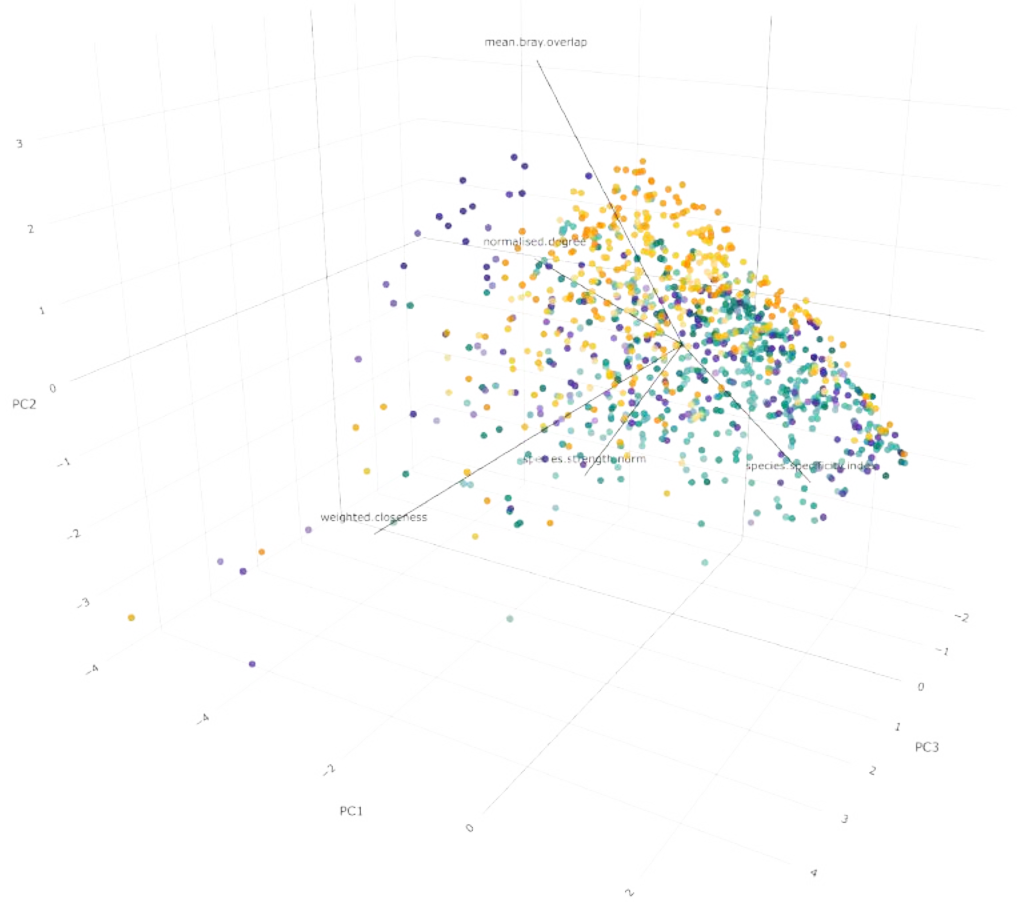


Figure A4.9. Three dimensional representation of Principal Component Analysis for node-level metrics of individual plants in their respective networks (Fig. 4.5) (interactive graph link: https://plotly.com/~elequinter/1/). This representation evidences how PC3 is primarily influenced by weighted closeness and how the outlying individuals in the two-dimensional point cloud, that exhibit idiosyncratic interaction profiles, are also strongly associated with PC3.

Appendix 4F. Software citations

We used R version 4.2.0 (R Core Team 2022) and the following R packages: bipartite v. 2.17 (Dormann, Gruber & Fruend 2008, Dormann *et al*. 2009, Dormann 2011), car v. 3.1.0 (Fox & Weisberg 2019), cluster v. 2.1.3 (Maechler *et al.* 2022), DHARMa v. 0.4.5 (Hartig 2022), fmsb v. 0.7.3 (Nakazawa 2022), ggcorrplot v. 0.1.4 (Kassambara 2022), ggforce v. 0.3.3 (Pedersen 2021), ggfortify v. 0.4.16 (Tang, Horikoshi & Li 2016, Horikoshi & Tang 2018), ggpubr v. 0.4.0 (Kassambara 2020), ggrepel v. 0.9.1 (Slowikowski 2021), ggsci v. 2.9 (Xiao 2018), gllvm v. 1.4.3 (Niku *et al.* 2019, 2021, 2023, van der Veen *et al.* 2021, 2022), glmmTMB v. 1.1.3 (Brooks *et al.* 2017), grateful v. 0.1.11 (Rodríguez-Sánchez, Jackson & Hutchins 2022), gridExtra v. 2.3 (Auguie 2017), here v. 1.0.1 (Müller 2020), hillR v. 0.5.1 (Li 2018), igraph v. 1.3.2 (Csardi & Nepusz 2006), iNEXT v. 3.0.0 (Chao *et al.* 2014, Hsieh, Ma & Chao 2022), knitr v. 1.39 (Xie 2014, 2015, 2022), lme4 v. 1.1.29 (Bates *et al*. 2015), MASS v. 7.3.56 (Venables & Ripley 2002), modelbased v. 0.8.5 (Makowski *et al.* 2020), patchwork v. 1.1.1 (Pedersen 2020), plotly v. 4.10.0 (Sievert 2020), rcartocolor v. 2.0.0 (Nowosad 2018), reshape2 v. 1.4.4 (Wickham 2007), RInSp v. 1.2.5 (Zaccarelli, Mancinelli & Bolnick 2013), rmarkdown v. 2.14 (Xie, Allaire & Grolemund 2018, Xie, Dervieux & Riederer 2020, Allaire *et al.* 2022), scales v. 1.2.0 (Wickham & Seidel 2022), SIBER v. 2.1.8 (Jackson & Parnell 2023), tidylog v. 1.0.2 (Elbers 2020), tidyverse v. 1.3.1 (Wickham *et al*. 2019), tnet v. 3.0.16 (Opsahl 2009), vegan v. 2.6.2 (Oksanen *et al.* 2022).

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GENERAL DISCUSSION

Research on mutualisms has resulted in remarkable advancements in recent years. The emergence of new and more robust methods to study and approach them, coupled with abundant research on interaction configurations and outcomes from the ecological and evolutionary perspectives, has greatly advanced this process. Ecologists are now beginning to assess their complexity more efficiently, grasping with more detail their implications in ecosystems. For example, studies are now starting to make refined predictions under future global scenarios (*e.g.*, González-Varo *et al.* 2021a, Fricke *et al.* 2022) as well as highlighting their role in conservation and restoration (*e.g.*, Gilarranz *et al*. 2015, Genes & Dirzo 2022). This PhD thesis focused on interpreting the basic structure of interaction networks by assessing how their complexity emerges from the actual interactions by individual partners in nature, building up to multi-species interaction networks in an ecosystem (Thompson 2009). We consider the specific case of generalised mutualisms among free-living species (seed dispersal by animal frugivores) because it illustrates an extreme case of mutualism evolution along a gradient of interaction specificity (Bronstein 1994). Specifically, our main goal is to understand the role in this context of super-generalist species, *i.e.*, those constituting the central core of large interaction networks (Guimarães *et al.* 2011, Lewinsohn & Cagnolo 2012). We first consider issues related to sampling protocols and data merging approaches to document these extremely diversified networks. In a second step we delve into the details of the mutualistic interaction in terms of reciprocal effects between partners and how the pooling of individual-based interactions conform to species-specific patterns of interaction. Next we focus on interaction outcomes to assess the consequences of mutualistic/antagonistic interactions in terms of plant recruitment for a super-generalist species. Finally we explore how topological (*e.g.*, degree, connectance) and structural (nestedness, modularity) invariants in interaction modes across individuals involved in these mutualisms emerge across different ecosystem type or higher taxonomic adscription.

Advances in sampling methods and data combining approaches

Thanks to the enormous diversification of methods used to record interactions since the pioneer approaches based on focal observations (Howe & Vande Kerckhove

1980, Snow & Snow 1988), it is now possible to sample frugivory interactions based on most appropriate methodologies given the natural system, time and budget available. The emergence of new and more sophisticated techniques such as molecular tools and image-based approaches have opened up new possibilities for passive and automated sampling. These methods are highly advantageous as they minimise the need for extensive fieldwork, while enabling the collection of large amounts of data over space and time. Yet, new methodologies also come with new challenges. In the last few years, numerous papers and issues in journals have published articles on how to deal with new techniques for monitoring interactions, highlighting limitations and providing recommendations (*e.g.*, Kays *et al.* 2020, Tercel *et al.* 2021). We show how categorising methodologies based on the stage at which the focus is set during the plant-frugivore interaction (*i.e.*, visitation, transport or deposition; Schupp *et al.* 2017), is helpful to identify the unique information they provide for different processes (*e.g.*, trait selection at visitation, diet and gut passage time at transport or plant demography processes at deposition).

Given the plethora of sampling methods, more studies are now venturing in data merging approaches. There is growing recognition of the benefits of combining data from various sources to achieve more robust and accurate estimates (Bosch *et al.* 2009, Heleno *et al.* 2022, Chapter 1). Several studies have therefore underscored the importance of effectively merging data to maximise the potential of these combined datasets, providing guidance and cautionary advice (Xing & Fayle 2021, Brimacobe *et al.* 2023, Cuff *et al.* 2023). The examples we provide in Chapter 1 on different data merging approaches serve as a groundwork for future studies seeking to combine data in the most efficient manner. We show how any combination of methods yielded better results in terms of completeness and representability. These examples showcase the potential benefits and outcomes of merging datasets from various sources.

As we continue to refine our methods and explore new avenues of research, we will uncover more and rarer frugivory interactions. It is certain that in the years to come, the recording of interactions will continue to develop and improve fast, given the impressive advances in technology and tools such as artificial intelligence. However, these technological advances should not diminish the importance and usefulness of apparently rudimentary methods such as field observations, since the latter are the ones that allow us to see and perceive ecological encounters with all our senses, encouraging us to develop meaningful hypotheses.

Mutualistic service exchange: high reliability in interaction frequency causes high reciprocity, but maintains asymmetric dependences

Focusing on two local populations of *Pistacia lentiscus* plants and their frugivore community, we showed in Chapter 2 how the effectiveness of the mutualistic service for both plants and animals was mainly guided by the quantity component (fruit consumption). Our findings align with other studies where the quantity component also expresses the highest variation, supporting that interaction frequency alone can act as a good surrogate of effectiveness in many cases (Vázquez *et al*. 2005, Rehling *et al*. 2023). Yet, evidence available so far has not found a consistent global pattern that determines whether quantity or quality predominates as the primary component explaining the greatest variance in mutualistic effectiveness (Nevo *et al*. 2023). For example, studies performed in systems composed of frugivores with higher functional complementarity have highlighted the importance of quality in determining mutualistic effectiveness (*e.g*., González-Castro *et al.* 2015, García-Rodríguez *et al*. 2022, Gómez *et al*. 2022). We hypothesise that in highly generalised mutualisms where there is low specialisation in the services provided between partners, the quantity component may exhibit more variability than the quality component (Vázquez *et al*. 2005), driving the effectiveness of interactions. In such generalised systems, the outcomes of interactions would be less constrained by trait-matching and would primarily be influenced by numerical factors due to large variance across species, such as resource abundances, as well as context-dependent effects, such as matching in phenology. For example, drivers of such numerical effects are represented by our results on how plant size and crop abundance have a positive effect on frugivore attraction, a well-known and highly reported relation on literature (Weiner & Solbrig, 1984, Sallabanks 1993, Ortiz-Pulido *et al*. 2007, Schupp *et al*. 2019).

The strong dominance of the quantity component for determining effectiveness led to a high reciprocity in the rewards exchanged between interacting partners, pointing to a stable and fair two-way transfer in the exchange of mutualistic services. High reciprocity translates into a reliable mutualism, in which higher investment by any partner will be fairly rewarded. A reciprocal system can be expected to foster the reinforcement of interactions over time, perpetuating cooperation among individuals and species. We anticipate large differences in quality between partners can compromise reciprocity in a mutualistic system, posing challenges to achieve balance in service exchange. Such unbalancing can be manifested in systems with highly heterogeneous frugivore assemblages, where the varying qualities of different partners can cause major variations in final mutualistic effectiveness (González-Castro *et al*. 2015, García-Rodríguez *et al*. 2022). Another unbalancing scenario is the presence of antagonists that act as disruptors in the mutualism, further compromising reciprocity (Jácome-Flores *et al*. 2020). Yet, even in the presence of high seed-predator activity, our system still exhibited a high overall reciprocity between individual plants and their frugivore assemblage. Further studies examining breakdowns in mutualisms (*e.g*., Sachs & Simms 2006, Chomicki & Renner 2017) will provide valuable insights into the evolution of cooperation among free-living species.

Regardless of reciprocity, our study system exhibited high asymmetry in dependence between interacting partners. Asymmetrical dependence between partners has been repeatedly reported in generalised mutualisms at different hierarchical scales (*e.g*., Herrera 1984b, Jordano 1987a, Bascompte *et al*. 2006, Guimarães *et al*. 2006, Guerra & Pizo 2014, Chomicki *et al*. 2020).

Role of frugivores in seedling recruitment and microhabitat suitability for seed deposition

Bridging the gap between the frugivore activity and seedling establishment is a complex task that requires extensive field data to study the various demographic transitions that propagules undergo (Harper 1977, Wang & Smith 2002). Filling this gap allows a full assessment of interaction outcomes in mutualisms among free-living species. Thus far, some studies have investigated the decline of propagules and stage transition probabilities throughout the recruitment process of plant populations (Herrera *et al*. 1994, Jordano & Herrera 1995, Rey & Alcántara 2000, Traveset *et al*. 2003, Jordano *et al*. 2004, Gómez-Aparicio 2008, Rother *et al*. 2013). In Chapter 3 we show how studying the variation in frugivores' landscape use and their non-random seed dispersal patterns, it is possible to attribute their distinct contribution to recruitment (*e.g*., Godínez-Álvarez *et al*. 2002, Godínez-Álvarez & Jordano 2007, Brodie *et al*. 2009, Donoso *et al*. 2016, Rehling *et al*. 2023) as well as estimating their relative contribution along different stages of the recruitment process. Although birds exhibited heterogeneous landscape use, we did not observe a reversal of contributive roles throughout the demographic process, with the exception of seed predators that exert high consumption but anecdotal recruitment. Functional redundancy in birds' overall post-dispersal quality makes *P. lentiscus* particularly robust to the loss of minor consuming species and therefore more resilient to ecosystem disturbances (Zamora 2000, Loiselle *et al*. 2007, Donoso *et al*. 2017). These results further underscore the importance of numerical effects, such as high fruit abundance and copious seed dispersal serviced by a diversified avian frugivore assemblage, in the recruitment of *Pistacia lentiscus*.

Despite the finding that seedling recruitment is primarily determined by bird consumption, we detected a decoupling between seed dissemination and recruitment processes: most seeds arrive to suboptimal microhabitats for seedling recruitment. In contrast to the study of Wenny & Levey in 1998 which found that bellbirds performed a direct seed dispersal to most suitable microhabitats (open gaps), the seed dispersers of *P. lentiscus* carry a significant amount of seeds to less favourable microhabitats, as observed in other dispersal systems (*e.g.,* Brodie *et al*. 2009, Razafindratsima & Dunham 2015). This decoupling emphasises the role of certain frugivore species in spatial recruitment, resulting from conflicts between the best quality spots for different dispersal stages (Schupp 1995). Notably, species such as *Sturnus* and *Turdus* are likely to play an important role in transporting *P. lentiscus* seeds to more suitable microhabitats such as pine trees and open areas and also have the potential to facilitate the colonisation of new habitat patches (González-Varo *et al.* 2017, 2023, Isla *et al.* 2023) where the intensity of seed predation is lower (Verdú & García-Fayos 1996b).

Downscaling into ecological interactions assembly: from species to individuals

Zooming in the scale for studying mutualisms can provide valuable information on the configuration of interactions as illustrated in Chapter 4. Plant-animal mutualistic networks did not show deviations in structure when downscaling from the species to individuals. We rather found a fundamental heterogeneity in interaction assembly across frugivory networks worldwide. While reusing networks created by different researchers has limitations because of the different sampling approaches (see Brimacombe *et al.* 2023), it is noteworthy we were unable to detect significant differences given both network types (individual-based and species-based) used a variety of sampling methods. This lack of differentiation across biological scales could indicate the existence of underlying effects that control the way in which ecological networks are assembled (Guimarães 2020). For example, simple multiplicative effects of local species abundances in determining the probabilities of interspecific encounters (Hurlbert 1971). We argue numerical effects, such as organism abundances, are likely at the base of these emergent properties, governing asymmetric interaction strength distribution across nodes (Jordano 1987a, Vázquez *et al.* 2007, Schleuning *et al.* 2011).

Every individual in a population "builds" its interactions with a distinct set of partner species, for example, a fruiting tree being visited– and its seeds dispersed– by, say, just four species out of the 14 frugivore species recorded to visit this tree species in its local population. Thus, each individual has a distinct interaction profile resulting from ranking the relative importance of different partner species (*e.g.*, animal frugivore species) in their total interactions (see, *e.g.*, Rother *et al.* 2016). When examining the interaction profiles of individual plants across different studies compiled from all around the world, we observed consistent configuration patterns irrespective of the species or region to which the population belonged. Comparing the individual-based network of *P. lentiscus* with other individual-based networks from different plant families and regions, we observed a similar distribution of interaction profiles among individuals. Most noteworthy was the presence of few individuals across most populations exhibiting a highly central role in the network by having a highly dependent and diversified assemblage. These individuals comprised less than 10% of sampled individuals on average (one on each population in the case of *P. lentiscus*). This pattern aligns with previous findings in food webs, which identified a core group of species fulfilling similar ecological roles, alongside peripheral species exhibiting unique interaction profiles (Mora *et al.* 2018). It is likely that within frugivory networks these generalist individuals (scoring high weighted closeness, *i.e.*, centrality) present unique phenotypic traits, such as abundant fruit crops or advantageous locations that make them reliable to many frugivores (Snell *et al.* 2019) ultimately related to size, age or fecundity hierarchies (Schmitt *et al.* 1987, Buston & Cant 2006) in plant populations.

Applying the niche concept to mutualistic interactions proved useful to determine the levels of individual specialisation in the populations (Tur *et al.* 2014, Phillips *et al.* 2020, Koffel *et al.* 2021, Arroyo-Correa *et al.* 2023, Gómez *et al.* 2023b). Individuals' interaction niches are narrower than those of their populations, supporting the well-known fact that specialisation is substantial and common in nature (Bolnick *et al.* 2003), even in generalised mutualisms. Specialisation levels varied across regions, with European populations being more generalised than South American populations. We argue that taxonomic diversity, functional redundancy, fruit diet specialisation and species' range size may be behind encountered regional differences (Fleming 1987, Willig *et al.* 2003, Dalsgaard *et al.* 2017).

When shifting our focus to frugivore species we observed a consistent and widespread differential contribution to interactions across all populations. Most plant populations sampled presented one to three frugivore species responsible for most visits, regardless of the richness of the frugivore assemblage. This consistency in frugivore interaction patterns was irrespective of the region or species under examination, further evidencing the existence of underlying common characteristics in the way generalised mutualistic interactions are organised. This generalised result across frugivory systems underscores the role of reliable frugivore species for the maintenance of the seed dispersal service (*e.g.,* Guerra *et al.* 2017, Vissoto *et al*. 2022, Isla *et al*. 2023, Rehling *et al.* 2023).

The super-generalist strategy

Super-generalist species play a unique role in ecosystems by supporting a significant portion of the local diversity (Guimarães *et al.* 2011). These super-generalists, though few in number, have important implications for the structuring of interaction networks as they connect semi-independent groups of species; that is, they are the nodes that glue together other nodes that otherwise would remain disconnected, within isolated modules (Olesen *et al*. 2007). Thus these species define the core of the network and crucially contribute to network cohesiveness in these generalised mutualisms. These characteristics lead us to wonder how this strategy evolved and how super-generalists impact the coevolution process among mutualistic species. For example, Cosmo *et al*. 2023 showed how the introduction of a super-generalist (honeybees) in a local pollination network increased the contribution of indirect effects on several other species, reducing their fitness and hindering coevolution.

Getting to know how super-generalist species establish their interactions at the species and individual level can provide valuable information on their strategy. In this PhD thesis we show how *Pistacia lentiscus* supports a highly diversified frugivore assemblage in their community while maintaining asymmetrical interaction dependence but keeping high reciprocity in the value of the mutualistic exchange. Measuring reciprocity and dependence asymmetry can be important to understand the evolution of mutualism. In fact, Lomáscolo *et al*. 2019 suggest that rather than interaction frequency, it is the symmetry of interaction strength that favours coevolution. Yet a characteristic feature of most studies of mutualism has been the consideration of just one of the partner groups, omitting in most cases any treatment of reciprocity and/or interaction asymmetry (Bronstein 1994).

Results of this thesis evidence that abundance parameters are a key feature of the success of super-generalist organisms (Fort *et al*., 2016). We show how *Pistacia lentiscus,* by investing a lot in the production of large and accessible crops, becomes a staple nutritious resource to many resident and migrant bird species, which disperse vast amounts of seeds, overcoming its high seed unviability, and ensuring successful recruitment.

Future perspectives

Further deepening the scale at which we study ecological interactions will enrich our knowledge on the interaction configuration among mutualistic partners. Since ecological processes occur at the individual level and natural selection operates at this scale, examining interactions among individuals within species can be enlightening. Moreover it allows establishing an actual bridge between ecology and evolution in the analysis of species interactions (see Melián *et al*. 2018). For example, in our study case, further identifying frugivores at the individual level would allow us to investigate if the reciprocal or asymmetrical patterns encountered persist across scales, and how the properties we observe at the species-level emerge (Clark *et al*. 2011, Arroyo-Correa *et al*. 2023). Genetic approaches such as microsatellites or SNPs to identify individuals within frugivore species (Parejo-Farnés *et al*. 2018) or the maternal genotyping of seeds collected from captured individuals with mist-nets (see Godoy & Jordano 2001) provide a promising avenue for exploring individual-individual frugivory interactions.

Furthermore, framing mutualism through the lens of a Biological Market Theory (BMT; Noë & Hammerstein 1995, Kiers *et al*. 2003) can enhance our understanding of the exchange of services between fleshy-fruited plants and frugivores within a population or community. Frugivory mutualisms can be understood as a trade market, where plants offer fruits as advertisement for the nutrition they offer, and animals choose these resources among various potential candidates, existing variation in preferences, competition and many context-related factors (a consumer-resource interaction; Holland *et al*. 2005). Biological Market Theory can aid to characterise and quantify the role of choice among partners, as they engage in different trading strategies that vary in the quality or quantity of the resources they provide (Noë & Kiers 2018). Combining mutualism with market theory can inspire the formulation of new hypotheses and predictions regarding the resource exchange strategies across individuals and species.

Ultimately, studying frugivory in a broader context can help us gain a better understanding of the dynamics of mutualistic relations. For example, studying how the diet of facultative frugivores versus obligate frugivores gets affected by the abundance of other nutritional resources and determines their investment into the mutualism (*e.g.*, González-Varo *et al*. 2021b); or how other plant biological interactions such as pre-dispersal predation or herbivory interfere in the outcome of frugivory seed dispersal (*e.g*., Moreira *et al.* 2019, Morrison *et al*. 2020). Recent analytical methods such as multilayer networks provide a powerful tool to approach this high diversity of biological interactions (De Domenico *et al*. 2013, Garrido *et al*. 2023, De Domenico 2022). Furthermore, the characterisation of interaction modes or motifs within networks have been revealed to have a strong potential for differentiating species' role (Mora *et al*. 2018) and interaction types (*i.e.*, antagonism vs. mutualism, Pichon *et al.* 2023). All these perspectives require synthetic approaches, analogous to those explored in this PhD thesis, including thorough documentation and inventory of interactions biodiversity, consideration of the two interaction partners and reciprocal effects, gauging interaction outcomes, and explicit consideration of the actual biological scale at which interactions occur in nature.

Conclusions

1. Integrating interaction data from different sources is a challenging task. We propose alternative combination approaches, both qualitative, easier to implement but resulting in information loss, and quantitative, more demanding but achieving higher resolution. The optimal data integration strategy will depend on the data type available and study goals.
2. *Pistacia lentiscus* exhibits the characteristics of a super-generalist species in the Mediterranean shrubland, supplying fruits and seeds that are consumed by a diverse frugivore assemblage of 28 bird species. This is probably among the highest local diversity of frugivores reported for a Mediterranean plant.
3. Interaction frequency (number of fruits consumed) can be a good surrogate of effectiveness in generalised mutualisms where the resources (fruits) are abundant, the quality of reward is not highly variable and partner specialisation is not high, as is the case of *Pistacia lentiscus* and its frugivore community.
4. We expect high reciprocity in many mutualistic systems, where higher investment translates into higher benefits for both interacting partners, especially when the quantitative component drives interaction effectiveness. Despite reciprocity, we found high asymmetry in partner dependence: individual plants rely mostly on a few abundant disperser species but these, in turn, do not depend strongly on specific individual plants, matching results from species-level analyses of frugivory mutualisms.
5. Our comprehensive assessment of *Pistacia lentiscus*' reproductive cycle allowed us to assess the delayed consequences of the seed dispersal mutualism and estimate the contribution of avian frugivores to individual plant recruitment. Such contribution was mainly determined by their intensity of fruit consumption and the probability to disperse viable seeds. Nevertheless, we detected an uncoupling between avian seed dispersal and microhabitat

quality, so that most seeds do not arrive at the most suitable microhabitats for seedling recruitment. This highlights the key role of different bird species for recruitment in heterogeneous landscapes.

6. We estimate *P. lentiscus* plants need to produce around half a million fruits to recruit a single seedling that survives to its second summer in our study site. Post-dispersal seed predation by rodents, followed by seedling emergence, were the most limiting stages. We argue that *P. lentiscus*' success in Mediterranean lowlands stems from its high fecundity and thorough seed dispersal by a diversified frugivore assemblage, compensating for high seed unviability and other demographic limitations.
7. Individual-based networks are fundamental to understanding how individual plants in natural populations structure their interactions with mutualistic partners. Our results reveal that the structure of individual-based mutualistic networks is very similar to that of species-based networks. The lack of structural differentiation between these two hierarchical scales suggests the existence of underlying, unifying mechanisms in the organisation of ecological interactions.
8. Applying niche theory to individual-based frugivory networks reveals consistent low to medium levels of individual specialisation in natural populations. The distribution of frugivory interactions among individual plants was highly skewed, with few frugivore species dominating most interactions in all populations.
9. Plant individuals explore similar interaction network profiles across populations despite belonging to different species or geographical regions. Only a few individuals played a central role in the structuring of frugivory interactions within each population.
10. Our results suggest that super-generalist species may evolve within diversified assemblages when they combine sets of traits that make them accessible to generalised, specialised and partial frugivores, building interactions of high reciprocity. The study case of *P. lentiscus* highlights the significance of traits such as fruit accessibility, high fruit production, extended fruiting season and high nutritious reward.

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Agradecimientos

Todos los finales son duros, pero bonitos a la vez, a la hora de escribir esta sección me vienen a la memoria todos los recuerdos de golpe y desordenados. Así pues, intentaré hacer un breve repaso de estos cinco años y pico que ha durado esta tesis, para agradecer a las personas que han recorrido este camino junto a mí, que me han cuidado, inspirado o afectado de algún modo especial, y que me han servido de faro para seguir avanzando.

Primero que nada, mi inmensa gratitud a los dos 'pedazo' de directores de tesis que me han guiado. Su apoyo incondicional, su conocimiento infinito y su orientación han sido imprescindibles para llevar a cabo este proyecto. Pedro, gracias por tu confianza inicial y darme la oportunidad de empezar un trabajo de máster, mandarme a Brasil con gente maravillosa y querer continuar trabajando juntos en una tesis. No conozco mayor entusiasta y amante de la naturaleza, tu pasión por la ciencia se transmite y contagia fácilmente. Una mente brillante, nunca falta de ideas y impresionante conocimiento en temas tan sorprendentemente diversos (pueden ir desde las métricas de redes más complejas, hasta las nuevas telas con las que visten a la virgen de la Esperanza). Gracias por tu apoyo inicial, por venir mucho al campo con nosotros y enseñarnos tanto. He aprendido muchísimo de tu visión, siempre sin perder de vista el panorama completo, ves más allá y sabes ver la importancia en cada pequeño resultado. Paco, esta tesis habría sido muy diferente si no hubieses aceptado a embarcarte en esta aventura. Recuerdo cuando te pedí ser director que me dijiste, "me encantaría Elena, pero no sé que estaré haciendo de aquí a unos meses, podría estar conduciendo un camión". Y menos mal que no, porque si no tanto la ciencia como yo habríamos perdido muchísimo. Gracias por implicarte tan a fondo en este proyecto y por haber aportado tanto. La visión más Bayesiana de la ciencia, honesta y con atención a los detalles siempre importantes que creo encontraré en mi vida. De ti he aprendido muchísimo y estoy muy agradecida por tu paciencia e inmensa dedicación. Por inculcarme la importancia de la ciencia abierta, por querer seguir mejorando siempre. Y más allá de lo profesional, agradecerte todo el apoyo moral y alegría que transmites. Sois los dos un gran referente para mí.

A mis compañeros Jorge y Blanca, gracias a los cuales esta tesis ha sido tan llevadera y tan divertida. Sois dos mentes geniales, con muchísima pasión por la ecología y miles de buenísimas ideas. Pero no sólo sois dos científicos impresionantes, sois personas excepcionales, de corazón. Jorge, haber podido compartir contigo todo el camino de inicio a fin ha sido una suerte. Compartir largas temporadas de trabajo, nuestras penurias y alegrías, las aventuras en el campo, descubrir diferentes zonas de Doñana juntos, cuando la marisma estaba preciosa y veíamos patos por cientos. Gracias por haberme enseñado de cantos de aves. Pero, sobre todo, por ser un buen amigo y por la confianza. Blanca, la mejor incorporación al grupo de doctorandos que se puede desear. Eres genial, una mente súper rápida y comprensiva, un conocimiento botánico enorme y una máquina de R y análisis. Haber podido compartir contigo varias salidas al campo ha sido divertidísimo. La energía tan buena que transmites, tu humor que se contagia y siempre con anécdotas y cosas nuevas que contar. Gracias por estar siempre ahí, dispuesta a ayudar y a escuchar. Me llevo dos amigos para toda la vida.

Y siguiendo por el grupo, agradecer a Miguel y Gemma por la acogida más cariñosa en Triana que se podía esperar. Miguel, gracias por incluirme en tu vida trianera tan fácilmente cuando llegué, en todos vuestros planes y por estar siempre ahí para cualquier cosa. Gemma, quien iba decir que después serías tú la que más cerca estaría, gracias por cuidar tanto de nosotros, por estar siempre pendiente de todos y por el apoyo incondicional. Sois una pareja genial. A JuanMi, por la cantidad de trabajo de laboratorio, miles y miles de muestras barcodeadas, y por querer mejorar siempre el trabajo. Gracias por enseñarme con paciencia y detalle todo el protocolo, esa perfección y cuidado en el hacer las cosas es encomiable. A todos los miembros del Integrative Ecology Group, a los que llegaron y ya se fueron, aquellos con los que he coincidido más tiempo o los que acaban de llegar. Gracias a poder compartir tiempo con cada uno, me llevo una pequeña parte de vosotros. A las postdoc más trabajadoras, Irene y Ana, que no paran quietas ni un segundo, por sus ánimos. A Cris, Alfredo, Elena, Edu, Pablo Homet, Eva, Pablo Villaba, Iago y Dani, por vuestra ayuda y por hacer los días de trabajo tan entretenidos y llevaderos. Gracias a los brasileños que más tiempo se han quedado y se han hecho un huequito en nuestras vidas: a Lica y Caio. A los estudiantes de Erasmus, James y Lewis, por su asistencia en campo. A Antonio Orta por tragarse junto a mí infinitas horas de videos de lentisco y por ser tan entusiasta de las aves. A Geno Schupp, por ese maravilloso effectiveness framework, por escuchar mis primeros resultados e inspirar mis inicios en el mundo de la frugivoría.

También quiero agradecer al personal más variado de la EBD. Desde el administrativo, en especial a Sonia y Olga por estar siempre dispuestas a ayudar con la mejor de sus sonrisas, con los enrevesados y siempre cambiantes papeles o 14A. Al personal de centralita por recibirnos cada día y estar pendiente de todo, gracias en especial a Manuela por las charlas de salida de la EBD que te sacan del trabajo, a Francisco por su cortesía y a Pedro que tiene todo siempre listo y preparado en horario de las 6.30am. Por supuesto a Soraya, la más nerviosa, por su cariño, el cachondeo y el palique en el despacho. A Luis Torres por ayudarme con la instalación del DeepMeerkat en el servidor y a Riky por venir a volar el dron en los dos lentiscares. A las instalaciones de la ICTS por permitirnos realizar el trabajo de campo y a los guardas y limpiadoras de la reserva. En especial a Alejandro, por enseñarme el mejor camino a Madroñas, por sacarnos de más de un atasco en la arena, por estar siempre dispuesto a ayudar y por su curiosidad por nuestro trabajo de campo.

Agradecer a Rodolfo Dirzo, por invitarme a su laboratorio en Stanford, por la acogida más calurosa que podía esperar y por la rapidísima integración en su maravilloso grupo. I want to thank all the people that were in the lab during my stay, particularly to Luísa, Jenni, Marta, Tyler, Chinmay, Amanda and Anna, for creating a wonderful work environment, teas and coffees after lunch and still wanting to continue making plans after work. To Lulú and Anwar, for taking care of me upon my arrival, welcoming me into their home and family and making me feel part of it. Big thanks to the friends I made there, Paco, David, Marija, Karl, Daniel and Kaden, for the many plans, moral support and amazing hikes and camping in the endless beauty of California's nature.

Y como no, agradecer a la piedra angular de estos cinco años de tesis, a los amigos de la EBD (o no EBD) que hacen que la vida y la tesis en Sevilla valga tanto la pena, por su apoyo constante, todos los planes, desayunos, tapeos, cervezas, escapadas a la playa, a buscar linces, casas rurales y sobretodo las risas y las sonrisas. Empezando por María José, Lina y Dani, el mejor equipo de apoyo; gracias por vuestros ánimos, por estar siempre ahí y creer en mí, sois los amigos más increíbles. A los que empezamos el doctorado a la vez, Sara, Vero, Berni e Isa por compartir avances y conversaciones de pasillo o cervezas en la Alameda. A Paula, por los bonitos dibujos que ilustran estos capítulos y por más cafelitos al sol. A Manu y Juan, los dos mejores asturianos de la península. A Javi, Rubén, Irene Paredes, Víctor, Jorge Monje, Pedro, Eneko, Marina, Juan de Dios, Maria Lucena, Fer, Dani Kleiman, Bia, Christoph, Ale, Sarai, Rosa, por los miles de planes, fiestas y casas rurales. A Josephine, por su energía arrolladora y ser una amiga incondicional por lejos que estés. A los mas nuevos (que cinco años dan para mucho y la tasa de renovación es muy alta), a Rafa e Irene, Lorena, Lucía, siguiendo por un grupo bien grandote de gente, que no me quiero dejar a nadie, por venir con tanta energía y alegrar las reuniones. También al recién formado equipo coolegas: Perea, MJ, Lucía, Jorge, Gemma, Blanca y Pablo, por los buenos ratitos en campo y de cervezas, y por seguir avanzando en nuestros proyectos, y como sabiamente dicen por ahí, siempre con perspectiva humana.

Y siguiendo por Madrid, a Marga y Ale, mis biólogas favoritas, dos mujeres fuertes, la mar de apañás, por los ánimos y por el intento de vernos en cada oportunidad posible. A mis amigos de Tres Cantos; Ana, Bea, Clara, Marta, Meri, Nuria, Patri, Oscar, Sandra y Silvia. Las diez personas más maravillosas que han estado ahí desde el inicio de los tiempos, hemos crecido juntos y, aunque la distancia nos separa, sé que siempre estarán ahí, el núcleo duro.

A toda mi familia por extensión, por su cariño, a mis primos y las parejas por hacer la llegada a Sevilla desde el 2018 tan fácil y acogedora.

A mis padres que siempre me han respaldado en todos los modos posibles y me han dado las herramientas necesarias para enfrentar los problemas. Por enseñarme que las cosas hay que ganárselas con esfuerzo y trabajo. Porque, además, parte de esta tesis también os la debo a vosotros, por vuestro trabajazo minucioso midiendo, contando, pesando y abriendo miles y miles de frutos de lentiscas. A mi hermana Mayte, porque nunca te faltan palabras de ánimo o ganas para conseguir que las cosas salgan. Por apoyarme en todo momento, por nuestras payasadas y planes cuando nos vemos o por las llamadas a distancia, que aunque aleatorias en el tiempo siempre me alegran el día.

A mis abuelos en especial, les dedico esta tesis, pues ellos me han cuidado, me han criado y han hecho de mis padres los que son. Con sus personalidades tan distintas, su afecto e infinito cariño. Os tengo siempre muy presentes.

Por último, acabar agradeciendo a Enrico, seguramente la persona que más de cerca ha vivido esta tesis, que me ha apoyado siempre en los momentos difíciles. Has compartido todas mis penas y alegrías como si fuesen tuyas. Por tu apoyo siempre incondicional, por cuidarme tanto. Por escuchar con paciencia y por interesarte por mi trabajo. Grazie per cucinare così bene e deliziosamente! Porque compartir la vida y aprender juntos es una maravilla.

Impreso
en Sevilla,
12 de febrero de 2024.