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Partitioning the relative contribution of one-phase and two-phase seed dispersal when evaluating seed dispersal effectiveness

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Summary

1. Seed dispersal effectiveness (SDE) is a conceptual framework that aims at quantifying the contribution of seed dispersal vectors to plant fitness. While it is well recognized that diplochorous dispersal systems, characterized by two successive dispersal steps performed by two different vectors (Phase I = primary seed dispersal and Phase II = secondary seed dispersal) which are common in temperate and tropical regions, little attention has been given to distinguishing the relative contribution of one-phase and two-phase dispersal to overall SDE. This conceptual gap probably results from the lack of a clear methodology to include Phase II dispersal into the calculation of SDE and to quantify its relative contribution.

2. We propose a method to evaluate the relative contribution of one-phase and two-phase dispersal to SDE and determine whether two seed dispersers are better than one. To do so, we used the SDE landscape and an extension of the SDE landscape, the Phase II effect landscape, which measures the direction and magnitude of the Phase II dispersal effect on overall SDE. We used simulated and empirical data from a diplochorous dispersal system in the Peruvian Amazon to illustrate this new approach.

3. Our approach provides the relative contribution of one-phase SDE (SDE1) and two-phase SDE (SDE2) to overall SDE and quantifies how much SDE changes with the addition of Phase II dispersal. Considering that the seed dispersal process is context dependent so that Phase II depends on Phase I, we predict the possible range of variation of SDE according to the variation of the probability of Phase II dispersal. In our specific study system composed of two primate species as primary dispersal vectors and different species of dung beetles as secondary dispersal vectors, the relative contribution of SDE1 and SDE2 to overall SDE varied between plant species. We discuss the context dependency of the Phase II dispersal and the potential applications of our approach.

4. This extension to the conceptual framework of SDE enables quantitative evaluation of the effect of Phase II dispersal on plant fitness and can be easily adapted to other biotic and/or abiotic diplochorous dispersal systems.

Key-words: diplochory, primary seed dispersal, secondary seed dispersal, seed dispersal effectiveness, seed burial, seed survival

Introduction

Seed dispersal effectiveness (SDE) is a conceptual framework that aims at quantifying the contributions of seed dispersal vectors to plant fitness. SDE is estimated by multiplying a quantity component (number of seeds dispersed) by a quality component (probability of a dispersed seed becoming a mature tree), but other variables such as seedling recruitment or survival are generally used as proxies (Schupp 1993; Schupp, Jordano & Gómez 2010). The SDE definition implies considering the whole multi-staged process of plant regeneration, from seed dispersal to seedling recruitment, growth and survival. That is one of the reasons why the authors of a conceptual review of SDE preferred to rename SDE as 'seed dispersal effectiveness' (Schupp, Jordano & Gómez 2010) instead of the previously proposed term 'seed disperser effectiveness' (Schupp 1993). While the latter emphasizes the effects of distinct vectors on plant recruitment and still is of course a valid concept, the former highlights the possibility to assess the overall dispersal effectiveness that a plant experiences from multiple dispersers (Schupp, Jordano & Gómez 2010) and provides scope for many more applications.

Indeed, the seed dispersal process rarely involves a single dispersal vector and also rarely results from a single step. Diplochory is increasingly recognized as a common seed dispersal process in temperate and tropical regions (Vander Wall & Longland 2004). It is defined as the dispersal of seeds by two different dispersal vectors in two successive steps called Phase I and Phase II (Fig. 1a; Vander Wall & Longland 2004) but also accommodates cases of dispersal in more than two steps. Phase I (primary dispersal) contributes to the movement of seeds away from the parent plant,

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(a)

- **Phase I:** primary seed dispersal
- **Phase II:** secondary seed dispersal
- **Pb1:** proportion of primary dispersed seeds without Phase II
- **Pb2:** proportion of primary dispersed seeds with Phase II
- **SDE:** seed dispersal effectiveness
- **SDE1:** effectiveness of seed dispersal in one phase
- **SDE2:** effectiveness of seed dispersal in two phases by two different vectors
- **eSDE1:** expected SDE when Pb2 = 0 (and Pb1 = 1)
- **eSDE2:** expected SDE when Pb2 = 1 (and Pb1 = 0)

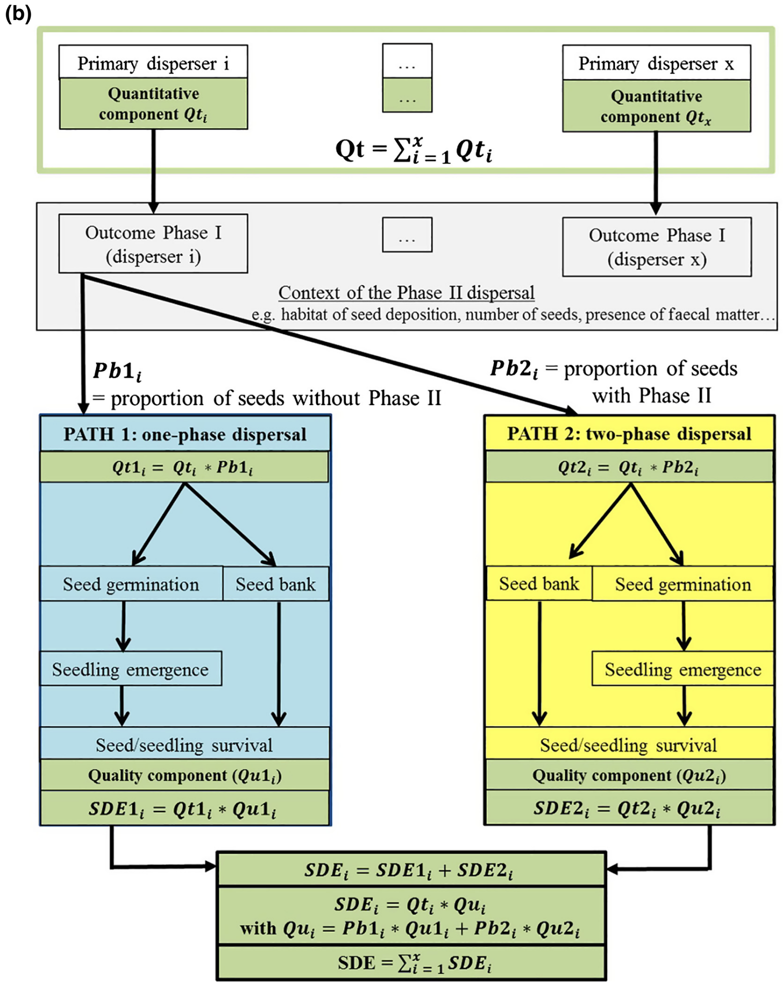

Fig. 1. (a) Definitions and (b) use of the parameters to assess the relative contribution of one-phase (SDE1) and two-phase seed dispersal (SDE2) to seed dispersal effectiveness (SDE). The quantity of seeds dispersed by primary disperser vectors is represented by Qt, the quantity component of SDE. Each seed disperser vector *i* is characterized by specific physical/physiological/behavioural traits that will determine the outcome of the primary dispersal: where and how the seeds will be dispersed. The characteristics of primary seed deposition are considered to be the context in which Phase II occurs. This context will determine the probability of occurrence of Phase II (Pb2, varying from 0 to 1). The outcome of Phase II (e.g. seed burial or not) can be considered, in turn, as the context for the subsequent events determining final seed/seedling survival (quality component of SDE = Qu). It is assumed that seed/seedling predation as well as mortality due to other factors may occur at each step but are not represented in the diagram. Green squares represent the variables to be considered when evaluating SDE. SDE is the observed SDE in the system and is the sum of the SDE_i. SDE_i is the sum of $SDE1_i$ (SDE resulting from a one-phase dispersal, path 1 represented in blue) and $SDE2_i$ (SDE resulting from a two-phase dispersal, path 2 represented in yellow).

while Phase II (secondary dispersal) is usually defined as the additional horizontal or vertical movement of seeds to another site (Vander Wall & Longland 2004). Previous studies showed that the actions of secondary seed dispersers such as ants (Passos & Oliveira 2002; Pizo, Passos & Oliveira 2005; Gallegos, Hensen & Schleuning 2014), dung beetles (Feer 1999; Andresen & Feer 2005; Beaune *et al.* 2012) or scatter-hoarding rodents (Forget *et al.* 2002; Jansen *et al.* 2012; Pérez-Ramos *et al.* 2013) can positively or negatively affect seed germination, seedling recruitment and survival. The horizontal movement of seeds may reduce the clustering of seedlings (Lawson, Mann & Lewis 2012), and seed burial may better protect seeds from predation, but may also hinder seedling emergence when burial is too deep (Shepherd & Chapman 1998; Forget *et al.* 2002; Andresen & Levey 2004; Beaune *et al.* 2012).

While the SDE concept is increasingly used to compare the effectiveness of different seed dispersal vectors or to assess the SDE resulting from multiple dispersers (e.g. Wenny & Levey 1998; Martins 2006; Spiegel & Nathan 2007; Calviño-Cancela & Martín-Herrero 2009; Pérez-Ramos *et al.* 2013), little attention has been given to distinguish the relative contribution of Phase I and Phase II to overall SDE (but see Böhning-Gaese, Gaese & Rabemanantsoa 1999; Roth & Wall 2005; Christianini & Oliveira 2010; Ruiz *et al.* 2010). This might be due to the fact that the SDE conceptual framework does neither formally explain how to include Phase II dispersal into the calculation of SDE nor provide a way to quantify its contribution, a gap that we propose to fill in this paper. One essential step to include Phase II into the calculation of SDE is to consider the chain of events of the dispersal process, Phase II being dependent on Phase I. This means that, to determine the relative contribution of one-phase and two-phase dispersal to overall SDE, we should compare the effectiveness of one-phase dispersal (SDE1) to the effectiveness of two-phase dispersal (SDE2) (Fig. 1). To do that, we can use the SDE landscape proposed by Schupp, Jordano & Gómez (2010). This SDE landscape consists of a two-dimensional space where x and y axes represent the quantity and quality components of SDE, respectively, and where elevational contours represent isoclines of effectiveness. This landscape enables visualization of the variation of SDE according to different dispersal contexts (e.g. different seed dispersers or different years). In many dispersal systems, some seeds are only dispersed through Phase I dispersal and their SDE is represented by SDE1, while other seeds are both primarily and secondarily dispersed and their SDE is represented by SDE2. Here, we suggest comparing SDE1 to SDE2. Using this framework, it will be possible to visualize how many seeds benefit from dispersal in one phase and dispersal in two phases as well as the quality of both processes. This enables partitioning of the relative contribution of SDE1 and SDE2 to overall SDE, SDE being equal to SDE1 + SDE2 (Fig. 1b).

It is largely recognized that the seed dispersal process is context dependent (Balcomb & Chapman 2003; Schupp 2007; Loayza, Loiselle & Rios 2011; Perea *et al.* 2013). Phase II is no exception to the rule since the probability of its occurrence (Pb2, Fig. 1b) is partly determined by the characteristics of Phase I such as the habitat of seed deposition or the presence of faecal matter (Fig. 1; Andresen 2002; Culot *et al.* 2011). These characteristics thus constitute the context in which Phase II occurs. Therefore, it is interesting to visualize how SDE varies according to Pb2 ($0 \leq \text{Pb2} \leq 1$) and link this variation to the characteristics of Phase I.

A final step to determine how much better (or worse) two seed dispersers are compared to one is the evaluation of the magnitude of change of SDE following the addition of Phase II dispersal. To do that, we suggest an extension of the SDE landscape, the Phase II effect landscape. We show that the direction and magnitude of the effect of Phase II dispersal depends on both the proportional change of dispersal quality from one-phase to two-phase dispersal and on the probability of secondary seed dispersal.

In this paper, we propose a method to (i) integrate the secondary dispersal phase when calculating SDE values, (ii) partition the relative contribution of one-phase and two-phase dispersal to SDE, (iii) determine the range of variation of SDE according to the frequency of Phase II dispersal and (iv) graphically represent the direction and magnitude of the effect of Phase II dispersal on SDE. To illustrate this new approach, we use simulated and empirical data from a diplochorous dispersal system in the Peruvian Amazon involving two primate species as primary dispersers and dung beetles as secondary dispersers. We hope to encourage researchers to apply this new approach to other diplochorous dispersal systems.

Methods

STUDY AREA AND SYSTEM

The study took place at the *Estación Biológica Quebrada Blanco* (EBQB) located in north-eastern Peru (04°21′S, 73°09′W). Our study area consisted of the home range of one mixed-species group of tamarins (see below) comprising *c.* 36 ha of primary forest and *c.* 4 ha of an abandoned pasture, regenerating since 2000. More information about the study area can be found in Culot *et al.* (2010). Field data were collected during a total of 28 months (two 2-month periods and four 6-month periods), with each month represented at least twice: June–July 2004, January–February 2005, September 2005–February 2006, June–November 2006, March–August 2007 and December 2007–May 2008.

We studied a mixed-species group (Heymann & Buchanan-Smith 2000) composed of 4–10 moustached tamarins, *Saguinus mystax* Spix, and 3–6 saddleback tamarins, *Saguinus nigrifrons* Geoffroy[1] . Details on their ecology can be found elsewhere (e.g. Garber 1993; Peres 1993; Nickle & Heymann 1996; Heymann, Knogge & Tirado Herrera 2000). Both species disperse the seeds of a large number of plant species (Knogge & Heymann 2003; Culot *et al.* 2010). Dung beetles are the main secondary seed dispersers in our study system. Tamarin faeces attract a community of 25 dung beetle species able to bury seeds at a depth ranging from 0·5 to 11 cm (Culot *et al.* 2011).

DATA COLLECTION

In order to register the primary seed dispersal events, we followed the mixed-species group 4 days per week, 3–4 weeks per month, from early morning (when they left a sleeping site) until late afternoon (when they entered a sleeping site). One day per week, we characterized all observed defecations by their seed disperser (*S. nigrifrons* or *S. mystax*) and the seed species they contained. We marked all seeds ≥0·4 cm length found in fresh tamarin defecations, which represents more than 85% of species consumed (Knogge 1998). We marked the seeds *in situ* by attaching a 25-cm white nylon thread ending with a 5-cm piece of coloured and numbered raffia. More details on the methodology used to follow seed fate in the field can be found in Appendix S1. In total, we marked 981 seeds belonging to at least 105 plant species in 606 defecations. For the present analysis, we used a subset of 267 seeds belonging to five plant species with the largest sample size (more than 20 seeds marked) that had been regularly checked for at least 1 year: *Buchenavia viridiflora* Ducke (Combretaceae, $N = 58$), *Byrsonima poeppigiana* A. Juss. (Malpighiaceae, $N = 79$), *Inga loretana* J. F. Macbr. (Fabaceae, $N = 81$), *Parkia panurensis* Benth ex H. C. Hopkins (Fabaceae, $N = 23$) and *Paullinia* sp. L. (Sapindaceae, $N = 26$). We only used the occurrence of seed burial (vertical movement) as measure of secondary seed dispersal. We did not consider the seeds' possible horizontal movements for the following reasons: (i) seed density in tamarin faeces is low (Knogge & Heymann 2003), and therefore, the clumping of seeds is unlikely to limit recruitment, and (ii) horizontal movements of seeds by dung beetles are usually short in our system (Culot *et al.* 2009). We thus assume that in our system the main possible effect of dung beetle activity on seed/seedling survival is through seed burial. We checked marked seeds after 4 days to determine the proportion of seeds without secondary dispersal (Pb1) and with secondary dispersal (Pb2, see Fig. 1b). Then, we checked the seeds once a month for at least 1 year to determine the seedling emergence time of each species and seed/seedling survival. In order to compare the dispersal quality of all species at the same life stage, we considered, as our quality component, the survival of seeds and seedlings within 1 month after the last observed seedling emergence of each species (quality component Qu1 and Qu2 corresponding to the quality of dispersal in one phase and two phases, respectively, Fig. 1b). The seeds that did not emerge as seedlings but were still alive within this period were considered successful events since

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[1] Previously *Saguinus fuscicollis nigrifrons* (see Matauschek, Roos & Heymann 2011) for the taxonomic change.

they can still germinate. We are aware that our measure of seed dispersal quality has some limitations since we did not control for the effect of the microhabitat of seed deposition on seed/seedling mortality or on Phase II probabilities. However, we consider that these limitations do not affect the main conclusions of this paper nor its methodological considerations.

DATA ANALYSES

We performed the analyses and made all figures using R 2.15.2 software (R Development Core Team 2014). We evaluated SDE through the estimation of the quantity and quality components (Fig. 1).

Relative contribution of one-phase and two-phase dispersal to SDE

The integration of Phase II in the calculation of SDE and the partitioning of SDE into one-phase (SDE1) and two-phase dispersal (SDE2) was made according to Fig. 1b. The quantity component (Qt) is the sum of the extrapolated number of seeds dispersed by each primary seed disperser *i* per year. We estimated the number of seeds by multiplying the number of seeds defecated per day by the total number of fruiting days of each plant species comprised within the period of consumption by the tamarins. We then subdivided the quantity of seeds dispersed by each primary disperser (Qt_i) into $\mathrm{Qt1}_i$ and $\mathrm{Qt2}_i$ according to the dispersal probability of seeds in one ($\mathrm{Pb1}_i$) or two phases ($\mathrm{Pb2}_i$) so that: $\mathrm{Qt1}_i = \mathrm{Qt}_i * \mathrm{Pb1}_i$ and $\mathrm{Qt2}_i = \mathrm{Qt}_i * \mathrm{Pb2}_i$ and $\mathrm{Pb1}_i + \mathrm{Pb2}_i = 1$. We defined the quality component as the probability that seeds survive as a seed or a seedling within 1 month after the last observed seedling emergence when the seeds are dispersed in one phase ($\mathrm{Qu1}_i$) or two phases ($\mathrm{Qu2}_i$). Therefore, the overall quality component (Qu_i) is equal to $\mathrm{Pb1}_i * \mathrm{Qu1}_i + \mathrm{Pb2}_i * \mathrm{Qu2}_i$. The relative contribution of one-phase dispersal to SDE_i is the dispersal effectiveness of seeds dispersed in only one phase ($\mathrm{SDE1}_i = \mathrm{Qt1}_i * \mathrm{Qu1}_i$), while the contribution of two-phase dispersal to SDE_i is the dispersal effectiveness of seeds dispersed in two phases ($\mathrm{SDE2}_i = \mathrm{Qt2}_i * \mathrm{Qu2}_i$) (Fig. 1b). SDE_i is thus the sum of $\mathrm{SDE1}_i$ and $\mathrm{SDE2}_i$. The overall SDE is therefore the sum of all SDE_i (Fig. 1b). We calculated SDE_i and all its components for both tamarin species. Then, for all other analyses, we pooled the data of both species together since we were interested in the mixed-species group as dispersal vector. In this manner, we represented the relative contribution of SDE1 and SDE2 to overall SDE for five plant species in the SDE landscape.

Although not illustrated by our results, our approach can also handle with systems involving successive secondary dispersal events such as the multiple recaching of seeds by scatter-hoarding rodents. To do that, one can partition Pb2 according to the number of seed re-caching ($\mathrm{Pb2R}(y)$, with *y* the number of times the seeds were recached so that $\mathrm{Pb2} = \sum_{y=0}^{n} \mathrm{Pb2R}(y)$ with *n* the maximum number of times the seeds were recached) and associate a quality component, $\mathrm{Qu2R}(y)$, for each of these cases. Therefore, $\mathrm{SDE2}_i$ can be partitioned into $\sum_{y=0}^{n} \mathrm{Qt}_i * \mathrm{Pb2R}(y) * \mathrm{Qu2R}(y)$.

Potential range of variation of SDE according to Phase II probability

In order to represent the potential range of variation of SDE values due to the frequency of Phase II, we plotted the observed SDE together with eSDE1 and eSDE2 (Fig. 1a). SDE corresponds to the SDE value in the context of our study (Pb2 measured in the field) in which the quality component Qu is equal to Pb1 * Qu1 + Pb2 * Qu2 . eSDE1 is the expected SDE under the assumption that 0% of seeds are secondarily dispersed (Pb2 = 0), *that is* when all seeds are only dispersed in one-phase (Pb1 = 1, Fig. 1b). Likewise, eSDE2 is the expected SDE under the assumption that 100% of seeds are secondarily dispersed (Pb2 = 1), *that is* when all seeds are dispersed in two phases (Pb1 = 0). In other words, eSDE1 corresponds to the SDE1 path of Fig. 1b but considering Pb1 = 1, while eSDE2 corresponds to the SDE2 path of Fig. 1b considering Pb2 = 1. The value of SDE can thus theoretically vary between eSDE1 and eSDE2. It is important to note that the overall quantitative component remains constant since it is initially defined by the primary seed dispersal phase: only a proportion of primarily dispersed seeds (varying from 0 to 1) will be secondarily dispersed.

The Phase II effect landscape: determining the direction and magnitude of the effect of Phase II on SDE

We built an extension of the SDE landscape, the Phase II effect landscape, to determine the direction and magnitude of Phase II dispersal on SDE. How much SDE is changed with a Phase II dispersal (SDE change) can be calculated as follows:

$$\text{SDE change} = \frac{\text{SDE} - \text{eSDE1}}{\text{eSDE1}}. \qquad \text{eqn 1}$$

Given that:

$$\text{SDE} = \text{SDE1} + \text{SDE2},$$

$$\text{SDE1} = \text{Qt} * \text{Pb1} * \text{Qu1},$$

$$\text{SDE2} = \text{Qt} * \text{Pb2} * \text{Qu2},$$

and Pb1 = 1 in eSDE1, by definition (see above and Fig. 1a),

$$\text{eSDE1} = \text{Qt} * 1 * \text{Qu1}.$$

Therefore, we can rewrite equation (1) as follows:

$$\begin{aligned}\text{SDE change} &= \frac{(\text{Qt} * \text{Pb1} * \text{Qu1}) + (\text{Qt} * \text{Pb2} * \text{Qu2}) - (\text{Qt} * \text{Qu1})}{\text{Qt} * \text{Qu1}}\\ &= \frac{\text{Qt} * (\text{Pb1} * \text{Qu1} + \text{Pb2} * \text{Qu2} - \text{Qu1})}{\text{Qt} * \text{Qu1}}\\ &= \frac{\text{Pb1} * \text{Qu1} + \text{Pb2} * \text{Qu2} - \text{Qu1}}{\text{Qu1}}.\end{aligned}$$

Given that Pb1 + Pb2 = 1, and thus Pb1 = 1 − Pb2,

$$\begin{aligned}\text{SDE change} &= \frac{(1 - \text{Pb2}) * \text{Qu1} + \text{Pb2} * \text{Qu2} - \text{Qu1}}{\text{Qu1}}\\ &= \frac{\text{Qu1} - \text{Pb2} * \text{Qu1} + \text{Pb2} * \text{Qu2} - \text{Qu1}}{\text{Qu1}}\\ &= \frac{\text{Pb2} * \text{Qu2} - \text{Pb2} * \text{Qu1}}{\text{Qu1}}.\\ &= \frac{\text{Pb2} * (\text{Qu2} - \text{Qu1})}{\text{Qu1}} \qquad \text{eqn 2}\end{aligned}$$

Therefore, we can say that the magnitude and direction of the effect of a Phase II dispersal on SDE values depends on the Phase II dispersal probability (Pb2) and the proportional change of dispersal quality from a one-phase dispersal to a two-phase dispersal ((Qu2-Qu1)/Qu1) (eqn 2). Therefore, the change of SDE with Phase II dispersal does not depend on the initial quantity of seeds dispersed but on which proportion of them was secondarily dispersed. Using this approach, we built the Phase II effect landscape as a two-dimensional space where the values on *x* and *y* axes, respectively, represent the Phase II dispersal probability (Pb2) and the proportional change of dispersal quality ((Qu2-Qu1)/Qu1). Similarly to the SDE landscape, elevational contours represent isoclines of how much SDE changes with the addition of a Phase II dispersal and are given by multiplying the values of the *x* and *y* axes. We first used simulated data to illustrate how SDE changes according to Pb2, Qu1 and Qu2. To do that, we chose three probability values (low = 0·1, medium = 0·5 and high = 0·9) of Pb2, Qu1 and Qu2. We calculated the proportional change of dispersal quality from a one-phase dispersal to a two-phase dispersal ((Qu2-Qu1)/Qu1) for all possible combinations of values and plotted them as a function of Pb2. Then, we used empirical data of five plant species from our study system as an example.

Results

RELATIVE CONTRIBUTION OF ONE-PHASE AND TWO-PHASE DISPERSAL TO SDE

The relative contribution of SDE1 and SDE2 to SDE varies between plant species. The SDE landscape shows that two-phase dispersal accounts for a smaller proportion of overall dispersal than one-phase dispersal (lower quantity values) in all five plant species, but results in higher seed/seedling survival (higher quality values) in two of the five species (Fig. 2a). SDE2 represents 8·2% of SDE for *P. panurensis*, 10·4% for *B. viridiflora*, 13·6% for *B. poeppigiana*, 30·2% for *Paullinia* sp. and 45·4% for *I. loretana*. The relative contribution of SDE1 and SDE2 differs slightly depending on the dispersing tamarin species (Fig. 2b).

POTENTIAL RANGE OF VARIATION OF SDE ACCORDING TO PHASE II PROBABILITY

The SDE landscape enables visualization of the range of potential values of SDE according to seed burial probability as well as the position of the observed SDE in the range of these potential values for the five species tested (Fig. 3). A positive effect of seed burial is shown by higher values of SDE and eSDE2 relative to eSDE1, while a negative effect is shown by lower values. Seed burial probability (Pb2) is 0·36 for *I. loretana* and accounts for an increase of 17·4% of SDE relative to eSDE1. An increase in seed burial probability due to a different context could hypothetically increase SDE of *I. loretana* by 47·8% in relation to eSDE1 (Fig. 3). Similarly, seed burial

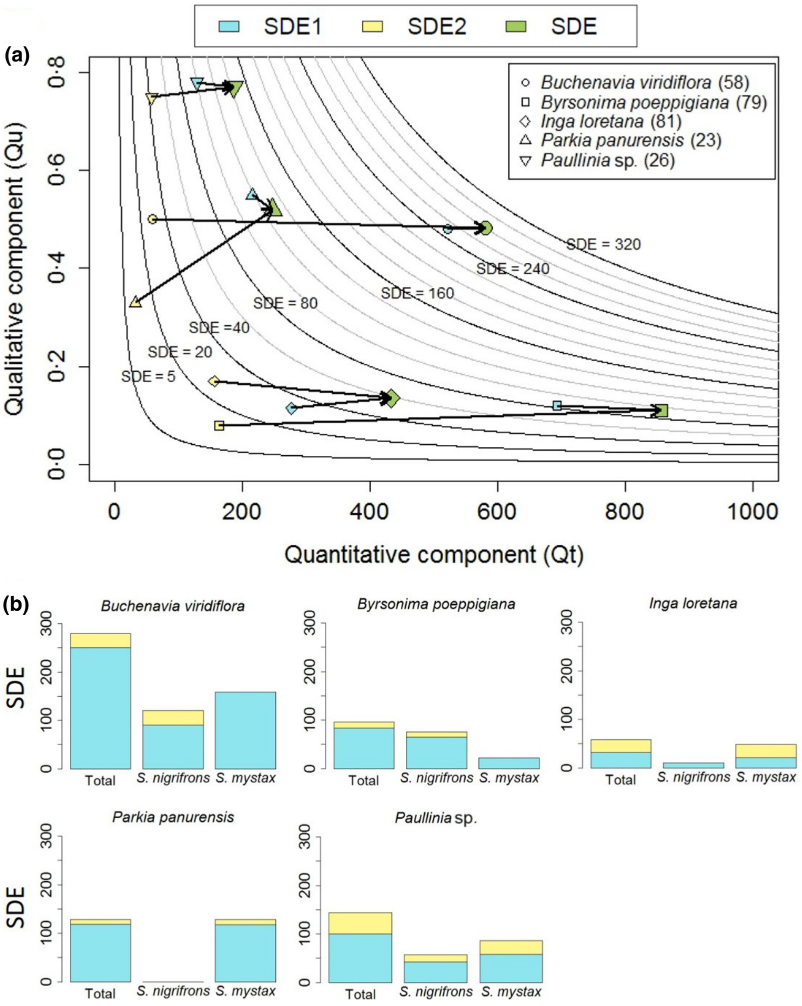

Fig. 2. Seed dispersal effectiveness (SDE) for five plant species primarily dispersed by *Saguinus nigrifrons* and *Saguinus mystax* and secondarily dispersed by dung beetles. Relative contribution of one-phase dispersal (SDE1, in blue) and two-phase dispersal (SDE2, in yellow) to total SDE (in green) represented (a) in the SDE framework and (b) in the bar plot. (a) SDE framework representing the partitioning of the relative contribution of SDE1 (in blue) and SDE2 (in yellow) to total SDE (in green) for the mixed-species group of tamarins. Arrows link SDE1 and SDE2 to overall SDE of each species, SDE being the sum of SDE1 and SDE2. Isoclines represent all combinations of quantity and quality values that yield the same SDE as SDE = quantity × quality (Qt*Qu). The quantity component (Qt) is represented by the extrapolated number of seeds dispersed based on the number of seeds defecated per day multiplied by the total number of fruiting days of each plant species. The quality component (Qu) is represented by the probability that the seeds survived as seeds or as seedlings within 1 month after the last observed seedling emergence. (b) Relative contribution of SDE1 and SDE2 to total SDE and to SDE resulted from the dispersion by *Saguinus nigrifrons* and *Saguinus mystax*.

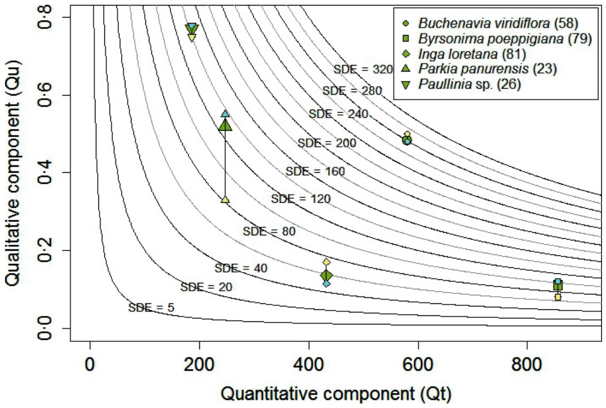

Fig. 3. Seed dispersal effectiveness (SDE) landscape and the consequences of the context dependency of Phase II dispersal on SDE for five plant species, when the quantitative component is held constant. Isoclines represent all combinations of quantity and quality values that yield the same SDE as SDE = quantity × quality (Qt*Qu). The quantity component (Qt) is represented by the extrapolated number of seeds dispersed based on the number of seeds defecated per day multiplied by the total number of fruiting days of each plant species. The quality component (Qu) is represented by the probability that the seeds survived as seeds or as seedlings within 1 month after the last observed seedling emergence. SDE is represented by green points and corresponds to SDE1 + SDE2 (Fig. 1b). eSDE1 (blue points) corresponds to the expected SDE if 0% of the seeds were buried, while eSDE2 (yellow points) corresponds to the expected SDE if 100% of the seeds were buried. Black whiskers represent the range of the potential values of SDE for all possible seed burial probabilities (Pb2). The sample size of each plant species is indicated in parentheses.

probability (Pb2) is 0·10 for *B. viridiflora* and accounts for an increase of 0·63% of SDE relative to eSDE1. An increase in seed burial probability due to a different context can hypothetically increase SDE of *B. viridiflora* by 4·2% in relation to eSDE1 (Fig. 3). The seed burial probability of *B. poeppigiana* (0·16), *P. panurensis* (0·13) and *Paullinia* sp. (0·31) accounts for the decrease of 8·3, 5·5 and 1·3% of SDE relative to eSDE1. An increase in seed burial probability due to a different context can hypothetically decrease SDE of *B. poeppigiana* by 33·3%, *P. panurensis* by 40·0%, and *Paullinia* sp. by 3·8% in relation to eSDE-I (Fig. 3).

THE PHASE II EFFECT LANDSCAPE: DETERMINING THE DIRECTION AND MAGNITUDE OF THE EFFECT OF PHASE II ON SDE

The contribution of Phase II dispersal to the overall SDE is highly variable between plant species, and the Phase II effect landscape helps in visualizing the direction and magnitude of this effect (Fig. 4). Theoretically, SDE values can be decreased by 100% when $Qu2 = 0$ and $Pb2 = 1$ or be infinitely increased by a Phase II dispersal when $Qu1 = 0$ and Qu2 and $Pb2 > 0$. In addition, the strongest effect of Phase II dispersal is achieved when Qu1 is low and Qu2 is medium or high (Fig. 4a). Out of the five species tested, seed burial has positive effect on two species and a negative effect on three. Overall, the amplitudes of the positive effects are larger than the amplitude of the negative effects (Fig. 4b).

Discussion

We propose a method to integrate Phase II dispersal when evaluating SDE, which enables to determine the relative contribution of one-phase and two-phase dispersal processes to SDE. Moreover, we introduce an extension of the SDE landscape, the Phase II effect landscape, which enables quantifying and comparing the effect of Phase II dispersal on SDE between plant species and therefore determining whether and to which extent two seed dispersers are better than one. This method highlights the quantitative differences between plant species of the effect of Phase II on plant fitness and can be easily adapted to other biotic and/or abiotic diplochorous dispersal systems. In the following, we first shortly discuss the results of this approach for our specific study system. Then, given our aim to propose a new approach quantifying the contribution of Phase II dispersal to SDE, we will discuss the range of applications of this methodology.

Our study system highlighted a relatively low contribution of two-phase dispersal to overall SDE (from <10% to slightly more than 40%), which is probably due to the relatively low secondary seed dispersal probability for tamarin faeces compared to systems including other primary dispersers. We expect that in other systems, two-phase dispersal contributes more to SDE than in our system. This could be the case when primary dispersers produce larger amounts of dung (e.g. howler monkeys) that attract a higher number and diversity of dung beetles or when defecated seeds and/or aril or pulp remaining attached to these are attractive to secondary dispersers such as ants and scatter-hoarding rodents. For instance, a first estimation based on data available in the literature indicated that the contribution of two-phase dispersal reaches 64% for seeds primarily dispersed by howler monkeys and secondarily dispersed by dung beetles in a Peruvian rain forest (Andresen 1999). These values are however small compared to what can be observed in a system with seeds primarily dispersed by birds and secondarily dispersed by ants in which 99% of SDE comes from SDE2 (data based on seed germination success) (Passos & Oliveira 2002). A similar tendency is observed in temperate regions where scatter-hoarding rodents are often responsible for removing more than 90% of seeds (Roth & Wall 2005) and for which dispersal quality is higher than previously thought (Jansen *et al.* 2012 for the Neotropics; but see Pérez-Ramos *et al.* 2013 in temperate regions). Further comparisons are difficult due to the scarcity of this kind of quantitative data. We thus hope that our method will encourage respective studies.

Determining the relative contribution of one-phase and two-phase dispersal to SDE opens to many interesting applications. Calculation of eSDE1 and eSDE2 enables the quantification of the potential range of variation of SDE according to secondary seed dispersal probability. It can thus be useful to evaluate possible changes of plant fitness in scenarios in which secondary seed dispersal probability is enhanced or reduced by the dispersal context such as geographic area, year, habitat, primary seed dispersers or frugivore community. The context

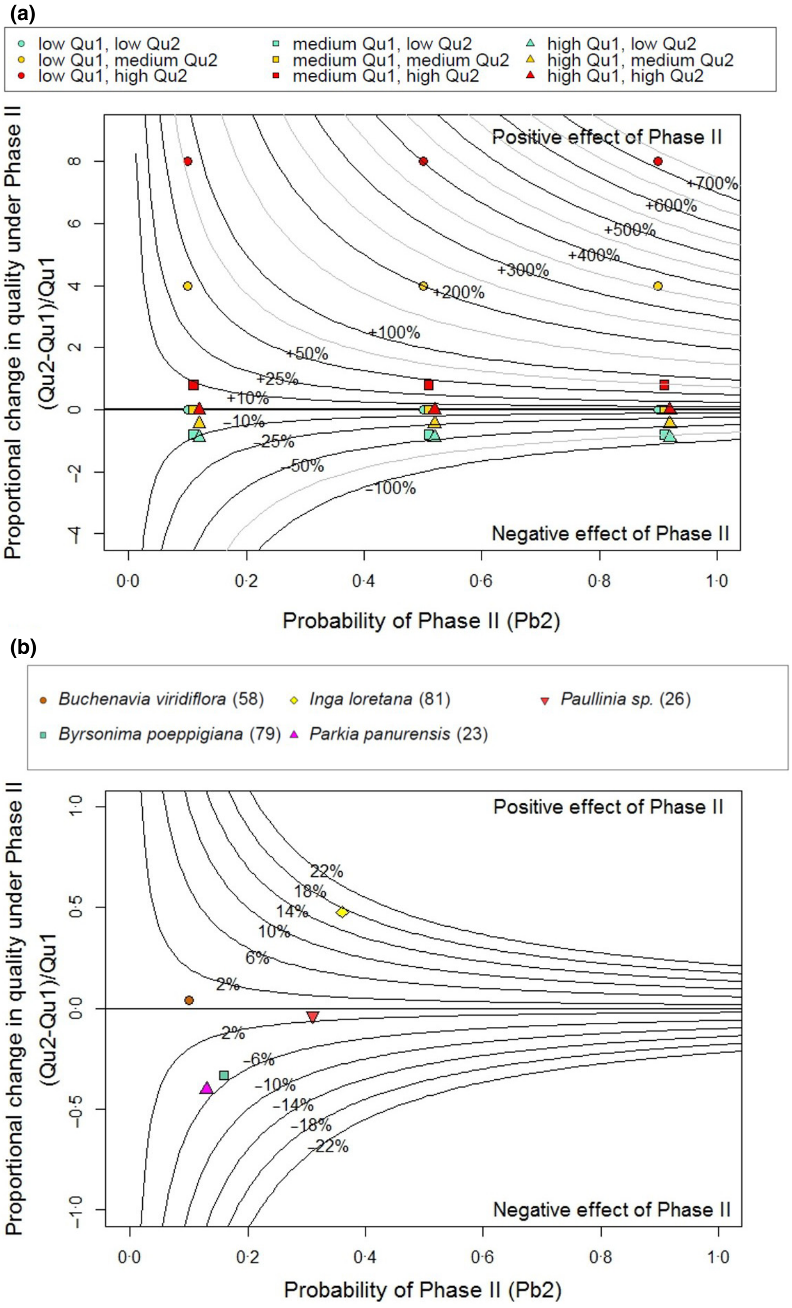

Fig. 4. Phase II effect landscape for (a) simulated data and (b) for empirical data of five plant species. How much seed dispersal effectiveness (SDE) is increased or decreased by Phase II dispersal is obtained by multiplying the probability of Phase II (Pb2) by the proportional change of dispersal quality component under Phase II dispersal ((Qu2-Qu1)/Qu1). In order to be more intuitive, this value was multiplied by 100 to obtain the percentage of increase or decrease of SDE with Phase II dispersal. Isoclines represent all combinations of *x* and *y* values that yield the same increase (positive values) or decrease (negative values) of SDE with Phase II dispersal. For simulated data, low values of Pb2, Qu1 and Qu2 correspond to 0·1, medium values to 0·5 and high values to 0·9 (a). The sample size of each plant species of empirical data is indicated in parentheses (b).

can affect overall seed dispersal patterns (Perea *et al.* 2013) but also the community of secondary seed dispersers. This can lead to drastic modifications of the structure of the seed dispersal network, likely to affect plant fitness, as already demonstrated in pollination networks (Goméz, Perfectti & Jordano 2011). Likewise, changes of the frugivore community can drastically alter not only seed removal and spatial patterns of seed dispersal but also patterns of secondary dispersal unless vectors are redundant in the seed dispersal service they provide. Two species are functionally redundant in their seed dispersal services if they achieve the same SDE (Calviño-Cancela & Martín-Herrero 2009; McConkey & Brockelman 2011). The inclusion of Phase II dispersal into the calculation of SDE allows for a more accurate evaluation of the potential for functional redundancy among species.

The Phase II effect landscape also enables the quick visualization of the effect of secondary seed dispersal on plant fitness by indicating the direction and magnitude of changes of SDE when compared with a one-phase dispersal system. The Phase II effect is the strongest when the seed dispersal quality of one-phase dispersal is low and the quality of two-phase dispersal is medium or high. It is useful to note that if plant species present qualitative components such as Qu1 = 0 and Qu2 = 0, the Phase II effect is null but cannot be represented in the Phase II effect landscape since Qu2-Qu1 cannot be divided by 0. Likewise, if Qu1 = 0 and Qu2 > 0, the Phase II effect cannot be represented but means that the value of SDE is indefinitely higher than the value of SDE1, which is equal to zero, by definition. Otherwise, all possible differences between Qu1 and Qu2 can be used in the Phase II effect landscape without needing data about the quantitative component.

The Phase II effect landscape has several interesting applications. For instance, it would be possible to compare the direction and magnitude of the Phase II effect when seeds are dispersed by different primary dispersers. Indeed, since the characteristics of the primary dispersal phase can not only affect the probability of occurrence of the secondary dispersal phase but also its outcome (seed burial depth for example), the Phase II effect is likely to depend on the Phase I dispersal system in which it is involved. For instance, contrary to seeds dispersed in large faeces (Feer 1999; Andresen & Levey 2004), seeds dispersed by tamarins in a small amount of faecal matter attract dung beetles in such a way that they bury them at shallower depths (mean = 3·5 cm, max = 11 cm; Culot *et al.* 2009, 2011), which decreases the probability of hindering seedling emergence (Koike *et al.* 2012). Consequently, the probability that some tamarin-dispersed seed species be strongly negatively affected by burial by dung beetles might be lower than in larger mammal-dispersed species.

Another application of the Phase II effect landscape would be to compare, for a plant species dispersed by a specific primary dispersal vector, the Phase II effect provided by different secondary seed dispersers (e.g. ants vs. dung beetles). This new approach can also be used to evaluate the cascading effects of anthropogenic perturbations, such as habitat fragmentation and defaunation, on SDE. Indeed, human alteration of habitat and fauna can indirectly affect SDE through changes in frugivore, seed predator and herbivore communities (Kurten 2013), but also through changes in dung beetle communities relying on mammal dung for feeding and nesting (Nichols *et al.* 2009; Culot *et al.* 2013). The impact of a change of dung beetle communities and/or seed predator and herbivore communities on SDE could be detected through the changes of Pb2 and (Qu2-Qu1)/Qu1 components of the Phase II effect landscape. Finally, when the secondary seed dispersers are scatter-hoarding rodents, one can compare two-phase seed dispersal processes (SDE2) that differ in the number of recaching events to test whether this affects seed/seedling survival. By partitioning Pb2 into the probabilities of recaching, a determined number of times, one can also calculate the contribution of two-phase dispersal differing in the number of recaching events.

Comparisons with studies in temperate zones would be helpful to better elucidate the benefits of seed burial. Indeed, phase II dispersal might affect seed/seedling survival and SDE differentially from what we observed in the tropics where seed predation is the main cause of mortality (Hulme 2002). In temperate regions, climatic conditions change more harshly from one season to another and can be responsible for high seed mortality (García 2001; Drescher & Thomas 2013; Joët, Ourcival & Dussert 2013). It would thus be interesting to compare the net benefit of seed burial between temperate and tropical regions and to analyse the consequences for plant ecology and evolution.

The SDE and Phase II effect landscapes can be used in any ecosystem with diplochorous systems involving any biotic and/or abiotic dispersal vectors. They can also be easily extended to pollination systems in which multiple dispersal events of pollen grains might also be observed (Hoyle & Cresswell 2006). In the same line, one can use this new approach to determine the contribution of pollen vs. seed-mediated dispersal to gene flow considering pollination as Phase I and seed dispersal as Phase II. In conclusion, the study of the ecological role of diplochorous dispersal systems can be done using the phase II effect landscape as a complementary tool to SDE assessment in order to disentangle the effect of one-phase and two-phase dispersal to SDE.

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Data accessibility

Details about the methodology used to follow the seed fate was uploaded as online supporting information (Appendix S1). Empirical data used to calculate the quality and quantity components are available from the Dryad Digital Repository (Culot, Huynen & Heymann 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Methodology used to follow the seed fate in the field.