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REVIEW

Going against the flow: a case for upstream dispersal and detection of uncommon dispersal events

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SUMMARY

1. Dispersal and colonisation are key processes determining species survival, and their importance is increasing as a consequence of ongoing habitat fragmentation, land-use change and climate change. Identification of long-distance dispersal events, including upstream dispersal, and of the dispersal mechanisms and resulting spatial dispersal patterns involved provides much-needed information for conservation in an era of rapid environmental change.
2. However, quantifying contemporary dispersal among populations is far from straightforward. We used the relatively well-defined, typically linear, spatial structure of streams, rivers and their associated riparian and aquatic plant populations to illustrate this. We performed a literature review on studies where dispersal and its directionality (upstream versus downstream) were explicitly quantified.
3. Upstream dispersal was detected in the majority (75%) of examined stream and riparian plant species and mediated mainly by waterfowl, but also by other animals and wind. However, upstream movements are generally less frequent than downstream. Upstream dispersal can occur in excess of tens and sometimes even hundreds of kilometres.
4. Most of the reviewed studies suffer from important methodological limitations that generate difficulties in detecting uncommon dispersal events. Major limitations include use of molecular ecological analyses based on unrealistic assumptions, and the inability to separate seed from pollen flow. On the basis of these findings, we outline a flexible research design using DNA-based assignment methods that allows quantification of contemporary dispersal in future studies. We suggest four key improvements: (i) assignment of propagules and/or seedlings; (ii) use of spatial models to inform sampling design; (iii) reducing the influence of unsampled populations and (iv) combined use of nuclear and uniparentally inherited DNA markers to separate gene flow (including pollen and sperm) in general from propagule-mediated dispersal. In combination with direct measurements of seed dispersal these facilitate empirical quantification of dispersal and the detection of uncommon dispersal events, allowing more realistic assessment of spatial population dynamics, relevant for sedentary and relatively immobile organisms.

Keywords: assignment methods, DNA-based dispersal estimates, drift paradox, nonstandard dispersal events, seed dispersal

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Introduction

Populations of almost all species depend critically on dispersal for long-term persistence (Howe & Smallwood, 1982; Nathan & Muller-Landau, 2000; Nathan, 2001; Levin *et al.*, 2003). The role of dispersal ability in determining the fate of populations, and even species, is increasing in importance due to pressures arising from habitat fragmentation, land-use change and climate change. For sedentary organisms or organisms with low mobility, such as plants and many (non-flying) invertebrates, the dispersal ability of an individual may consist of one dispersal phase (e.g. the seed or egg phase) in their life-cycle when propagules are transported by one or more external vectors (van der Pijl, 1982; Bilton, Freeland & Okamura, 2001; Bohonak & Jenkins, 2003; Gardner & Engelhardt, 2008; Mari *et al.*, 2014; Fraaije *et al.*, 2015a). While such dispersal events are of critical importance for the spatial population dynamics and (regional) persistence of species (e.g. Bilton *et al.*, 2001; Brederveld *et al.*, 2011; Sondermann *et al.*, 2015), as well as ecological restoration (e.g. Aavik *et al.*, 2013), they are notoriously difficult to detect (Higgins, Nathan & Cain, 2003; Nathan *et al.*, 2003).

Dispersal is of particular importance for organisms inhabiting discrete, patchy and dynamic habitats, such as freshwater ecosystems (which are often referred to as ‘islands in a sea of land’; Malmqvist, 2002). Both the composition of populations (size, demography, genetic composition) and the spatial distribution of populations across the landscape (position, extent, connectivity) are dependent on successful dispersal events and vary dynamically over time. As a consequence, populations of many species are in a spatiotemporally dynamic, rather than equilibrium, state (Hanski, 1998; Freckleton & Watkinson, 2002, 2003; Siol, Wright & Barrett, 2010) and a process-based understanding of dispersal is required to adequately manage and protect such species in a rapidly changing environment. Yet, while we are increasingly able to apply sophisticated mathematical modelling tools to predict regional level consequences of changes in landscapes (e.g. Artzy-Randrup & Stone, 2010; Mari *et al.*, 2014; Sondermann *et al.*, 2015), empirical quantification of dispersal events remains elusive.

Sedentary or relatively immobile organisms in lotic freshwater ecosystems, such as plants and many invertebrates, are often assumed to disperse mainly via water flow (hydrochory; e.g. Bilton *et al.*, 2001; Boedeltje *et al.*, 2004; Soons, 2006; Sarneel *et al.*, 2011, 2014; Soomers *et al.*, 2013). However, it is often predicted that this would result in unidirectional dispersal only to downstream populations (Honnay *et al.*, 2010), which is highly unlikely given current species distributions (the so-called ‘drift-paradox’, *sensu* Müller, 1982; Anholt, 1995). It is clear from observations and modelling studies that upstream populations need to be recolonised to prevent species from becoming extinct within a given catchment (Anholt, 1995; Humphries & Ruxton, 2002; Levine, 2003). In line with this, the predicted decline in plant species diversity in river headwaters which would follow purely downstream dispersal is rarely observed (e.g. Nilsson *et al.*, 1989; Van Looy *et al.*, 2006; Honnay *et al.*, 2010; but see Riis, Sand-Jensen & Larsen, 2001). Clearly, for their long-term regional survival freshwater species depend on upstream dispersal in some form, although such dispersal events may be infrequent. Indeed, critical upstream dispersal events are likely to occur through ‘nonstandard’ vectors. Nonstandard vectors are defined as vectors other than the ‘standard’ dispersal means as is apparent from morphological adaptations of the organism (*cf.* Higgins *et al.*, 2003).

For plants and invertebrates with low mobility, several mechanisms for upstream dispersal have been proposed. Most importantly, propagules (e.g. seeds, fragments or eggs) can be transported by animals (zoochory; e.g. Green & Figuerola, 2005) or wind (anemochory; e.g. Soons, 2006; Vanschoenwinkel *et al.*, 2008). The main difference between plants and invertebrates is that there are some non-flying invertebrate taxa with adult life stages that can actively move upstream by small-scale movements along the streambed (Humphries & Ruxton, 2002; Elliott, 2003). For invertebrates, modelling studies have also suggested that random diffusion of propagules in the water column can overcome the downstream movement of the water current (advection) under some conditions (Speirs & Gurney, 2001; Humphries & Ruxton, 2002; Pachepsky *et al.*, 2005). Such cases, however, may be limited to systems with very low current velocity, such as in lowland rivers (Speirs & Gurney, 2001). Therefore, it is unlikely that this mechanism accounts for regional persistence in dynamic systems where local populations are frequently extirpated (e.g. due to major flood disturbances), as is the case for many aquatic and riparian plant species.

Moreover, recent work has demonstrated that the dynamics in realistic dendritic models of lotic systems diverge importantly from predictions based on linear approximations (Sarhad, Carlson & Anderson, 2014). Thus, exactly how the drift-paradox is resolved for sedentary organisms in and along lotic freshwater systems remains an open question (e.g. Tero *et al.*, 2003;

Liu, Wang & Huang, 2006; Markwith & Scanlon, 2007; Chen *et al.*, 2009; Pollux *et al.*, 2009; Mari *et al.*, 2014; Triest & Fénart, 2014). This underscores the need for empirical quantification of dispersal, regardless of the mechanisms, in realistic settings so that we may evaluate and predict the impacts of human-induced alterations in environmental conditions (e.g. habitat availability and changes in hydrodynamics).

Here, we investigate the drift-paradox for aquatic and riparian plant species growing in and along streams and rivers, as a case study to explore the importance of (uncommon or nonstandard) dispersal events for spatial population dynamics. Both aquatic and riparian plant communities have been shown to be strongly influenced by water-mediated dispersal (Merritt, Nilsson & Jansson, 2010; Nilsson *et al.*, 2010; Fraaije *et al.*, 2015b), although some riparian species have clear adaptations to other dispersal vectors (e.g. riparian trees such as *Acer* and *Populus*, which are dispersed by both water and wind). The relatively simple spatial configuration of riparian ecosystems not only facilitates this exploration but also already clearly illustrates the empirical challenges that need to be overcome to implement evidence-based dispersal estimates in management and conservation practices at the landscape scale (Verhoeven *et al.*, 2008). In this paper, we took advantage of available dispersal literature on riparian and (riverine) aquatic plants and evaluated the empirical evidence for the nonstandard, but critically important process of upstream dispersal. We found very few quantitative estimates, in part due to methodological limitations of existing studies. The limitations of current methods employed are discussed and we suggest a framework for obtaining quantitative estimates of dispersal frequencies and distances.

Current evidence for upstream dispersal

To evaluate the existing body of empirical results that provide evidence for upstream dispersal, we conducted a literature survey in ISI Web of Knowledge (Thomson Reuters) on 15 February, 2015, using the search term '*(hydrochor* OR 'water dispers*' OR river) AND plant**', which resulted in 26299 hits. This result was firstly refined with the term '*upstream dispersal*', resulting in 45 papers. As molecular tools are often used to assess the genetic structure of (meta)populations we also refined our original search result with the term '*genetic**', resulting in 734 papers. Of these partially overlapping sets of papers, we selected the studies that met the following criteria: (i) primary empirical research presenting data on dispersal and/or gene flow of aquatic or riparian plant species; and (ii) study systems with unidirectional water flow, not prone to tidal movements. This resulted in a set of 100 papers which we analysed further (see Table S1 in Supporting Information).

Dispersal can be observed and quantified by 'direct methods' in the field, using trapping or tracking techniques, including new stable isotope techniques (Carlo, Tewksbury & Martinez del Río, 2009; Carlo *et al.*, 2013). These direct methods have been applied to stream, river and riparian species (e.g. Goodson *et al.*, 2003; Boedeltje *et al.*, 2004; Engström, Nilsson & Jansson, 2009; Sarneel *et al.*, 2014), but as in most cases the source of the trapped seeds could not be identified, such data could not be used to obtain information on direction, frequency, and distance of dispersal. Only one of the 100 studies investigated upstream dispersal directly by trapping seeds (1 mm mesh cloth suspended in the air) in the field (Kohri, Kamada & Nakagoshi, 2011). However, due to the spatial positions of adult plants and seed traps, upstream dispersal could only be inferred in very few cases. In general, dispersal events are very difficult to detect using any such direct methods when considering longer distances (Wang & Smith, 2002; Nathan *et al.*, 2003), and to solve this problem the use of molecular methods to infer dispersal ('indirect methods') has been advocated (Ouborg, Piquot & Van Groenendael, 1999; Bilton *et al.*, 2001; Broquet & Petit, 2009; Aavik *et al.*, 2013). Molecular methods can, for instance, be used to assign individuals to their population of origin or to estimate genetic differentiation among populations as a measure of gene flow (Broquet & Petit, 2009).

Frequency of upstream dispersal

Out of the 100 selected papers, 25 papers explicitly mention that upstream dispersal plays a role in their study system (Table S1). Seven of these focus on species from aquatic habitat, while 18 focus on riparian species. The relative frequency of upstream dispersal versus downstream dispersal was addressed in 12 studies. Only one of these focussed on a purely aquatic species (Pollux *et al.*, 2009), suggesting a need to investigate this group more thoroughly. Two of the 12 studies report that downstream dispersal dominates over upstream without further quantification (Van Looy *et al.*, 2009; Kikuchi, Suzuki & Sashimura, 2011), while one states that upstream and downstream migration rates did not differ (Imbert & Lefèvre, 2003). Nine studies provided quantitative data on the directionality of dispersal. Of these studies, three found 100% downstream dispersal (Arens *et al.*, 1998; Jacquemyn *et al.*, 2010; Mitsui, Isagi & Setoguchi, 2010). The other six studies showed that the frequency of upstream dispersal varies greatly among study systems, ranging from low frequencies of 2.6%, 21% and 27% of the identified migrants (DeWoody, Nason & Smith, 2004; Liu *et al.*, 2006; Pollux *et al.*, 2009), through more or less symmetrical migration rates (47% upstream; Werth & Scheidegger, 2014), to higher upstream dispersal frequencies of 58% and 76% (Tero *et al.*, 2003; Honnay *et al.*, 2009). In fact, even within a study system upstream dispersal frequencies may vary greatly among years (45% versus 76%; Honnay *et al.*, 2009). Overall, upstream dispersal appears to be a relatively widespread phenomenon among species, as it was observed in nine out of 12 studies. However, upstream dispersal was observed (much) less frequently than downstream dispersal in most cases (also nine of the same 12 papers). Of the 12 studies, 11 studies used assignment methods (Table 1, type 2.4) and one applied coalescent genealogy analysis (Table 1, type 2.3).

Table 1 Different types of evidence for upstream dispersal in aquatic and riparian plants. Each method was evaluated for its ability to infer dispersal direction, distance and whether it can separate dispersal from gene flow in general. In addition, the inferential logic and weaknesses of the method are presented. Examples of studies using these methods are given in Table 2.

Method	Direction	Distance	Dispersal versus gene flow	Logic	Weaknesses
Direct					
Propagule trapping & inverse modelling	partial	partial	yes	Based on spatially referenced seed densities (traps) and points of origin (mapped adults); statistical models can be used to infer dispersal.	• Data collection restricted to short distances. • Risk of underestimation of dispersal distance
Vector tracking	yes	partial	yes	Direct record of vector movements reveals potential dispersal routes, including directions and distances.	• Estimates the potential for dispersal, no actual quantification. • Restricted to tractable (mostly animal) dispersal vectors.
Vector suitability experiments	no	no	yes	Demonstrates that the vector has the potential to disperse plant propagules.	• Estimates the potential for dispersal, no actual quantification.
Species range monitoring	partial	partial	yes	Range expansion involves dispersal, if such expansion occurs estimates of dispersal rates can be derived.	• Requires long-term data series or historical records.
Indirect (molecular)					
Correlate diversity with distance along river	no	no	no	Prevalent downstream dispersal and genetic drift lead to increased genetic diversity downstream, lack of such patterns suggest upstream dispersal.	• Assumes long term (genetic) equilibria. • Reverse pattern could also result from same hypothesis when downstream populations are genetic sinks connected to large upstream populations
Genetic differentiation (F_{st}, AMOVA)	no	partial	partial	Lack of genetic differentiation suggests widespread and frequent dispersal.	• Could confuse contemporary with historical gene flow. • Assumes long term equilibria. • Separation of seed and pollen flow requires cpDNA markers.
Coalescent genealogy analysis	yes	yes	partial	Based on a population model (e.g. population sizes and growth rates) gene flow rates can be inferred (also asymmetric migration rates; directionality).	• Assumed (complex) population model strongly influences conclusions. • Assumes random mating in populations • Assumes long term equilibria. • Separation of seed and pollen flow requires cpDNA markers.
Assignment methods	yes	yes	partial	Individual seed(ling)s can be assigned probabilistically to population of origin based on population allele frequencies.	• Seedbanks may lead to erroneous assignments when unsampled • Separation of seed and pollen flow requires cpDNA markers.

Table 2 Summary of the observed or inferred upstream dispersal distances and vectors, as well as the study design for 18 studies providing quantitative data on upstream dispersal.

Study	Studied species	Habitat	Study system	Distance*	Proposed upstream dispersal vector	Sample size†	Methodology‡
Detected upstream dispersal events				Observed distances			
Tero *et al*., 2003	*Silene tatarica* (perennial)	Riparian	River, Europe, boreal	10–19 (21) km	Human, reindeer, waterfowl	7 populations, 24–30 samples	2.4
DeWoody *et al*., 2004	*Boltonia decurrens* (perennial)	Riparian	2 Rivers, North America, temperate	15–285 (285) km	Water, animals	14 populations, 18–96 samples	2.4
Liu *et al*., 2006	*Myricaria laxiflora* (perennial)	Riparian	River, Asia, temperate	70–270 (473) km	None proposed	9 populations, 25–30 samples	2.4
Honnay *et al*., 2009	*Erysimum cheiranthoides* (annual)	Riparian	River, Europe, temperate	2–32 (32) km	Human, (water)birds,	16 populations, 20–25 samples	2.4
Pollux *et al*., 2009	*Sparganium emersum* (perennial)	Aquatic	Small river, Europe, temperate	10–70 (86) km	Fish, waterfowl	9 populations, 12–39 samples	2.4
Werth & Scheidegger, 2014	*Myricaria germanica*	Riparian	2 Rivers, Europe, temperate	<1–33 (196) km	Wind, animals	31 populations, 9–56 samples	2.3, 2.4
Kohri *et al*., 2011	*Elaeagnus umbellata* (perennial)	Riparian	River, Asia, temperate	<1 km	Frugivorous birds, mammals	N.A.	1.1
Wang *et al*., 2011	*Ageratina adenophora* (perennial)	Riparian	River tributaries, rail lines and roads, Asia, temperate	19 km yr^{-1}	Human, wind, animals	200 sites	1.4
Inferred upstream dispersal (based on spatial genetic structure)				Range of inter-population distances tested			
Imbert & Lefèvre, 2003	*Populus nigra* (perennial)	Riparian	River, Europe, temperate	1–77 km	None proposed	22 populations, 22–30 samples	2.2
Markwith & Scanlon, 2007	*Hymenocallis coronaria* (perennial)	Aquatic	4 river shoals, North America, temperate	1–90 km	Waterfowl, mammals	5, 4 and 5 populations, 10–30 samples	2.2
Pollux *et al*., 2007	*Sparganium emersum* (perennial)	Aquatic	River, Europe, temperate	3–42 km	Animals	8 populations, 20–40 samples	2.1
Chen *et al*., 2009	*Potamogeton malaianus* (perennial)	Aquatic	River, Asia, temperate	50–925 km	Birds, fish	10 populations, 22 samples	2.2
Chen *et al*., 2010	*Ceratopteris pteridoides* (annual)	Aquatic	River, Asia, temperate	62–777 km	Birds	8 populations, 30 samples	2.2
Honnay *et al*., 2010	*Rorippa sylvestris* (perennial)	Riparian	River, Europe, temperate	<1–16 km	Waterfowl, fish, mallards	12 populations, 20 samples	2.2
	Erysimum cheiranthoides (annual)	Riparian	River, Europe, temperate	<1–16 km	Waterfowl, fish, mallards	12 populations, <20 samples	2.2
	Sisymbrium austriacum (perennial)	Riparian	River, Europe, temperate	<1-16 km	Waterfowl, fish, mallards	14 populations, <20 samples	2.2
Hu *et al*., 2010	*Fraxinus mandshurica* (perennial)	Riparian	River and tributaries, Asia, temperate	4–13.2 km	Wind	8 populations, 30–41 samples	2.2
Ren, Li & Ding, 2010	*Conyza sumatrensis* (annual/biennial)	Riparian	River, Asia, temperate	70–540 km	Humans	11 populations, 20–30 samples	2.2
Chen *et al*., 2012a	*Zizania latifolia (perennial)*	Aquatic	River, Asia, temperate	2–506 km	Birds, fish	7 populations, 15–20 samples	2.1
Chen *et al*., 2012b	Isoetes sinensis (perennial)	Aquatic	River, Asia, temperate	<1 km	Humans	7 populations, 10 samples	2.1

*Distances at which the upstream dispersal has taken place as proposed by the authors. The maximum spatial extent of the study area is given between brackets.

†The number of samples taken per population are given.

‡Methodology used in the studies, following the classification provided in Table 1.

Modes and distances of upstream dispersal

Of the 100 selected papers, 18 papers provided quantitative data on the distances of upstream dispersal and most mention potential vectors (Table 2, Table S1). Seven of these focus on species from aquatic habitat, while 13 riparian species were investigated. Overall, these 18 papers suggest an array of potential dispersal vectors that could mediate dispersal upstream, including animals [17 times; mostly waterfowl (6×) but also other bird species (4×), fish (4×) and mammals (4×)], but also humans (5×) and wind (6×). In some cases, water also acted as an upstream dispersal vector: during downstream floods backwaters can develop, which can cause floating propagules to move upstream (DeWoody *et al.*, 2004; Chen *et al.*, 2009). There was no difference between aquatic and riparian species in this respect, except that anemochory was not suggested as a vector for aquatic species. Generally, these observations support existing evidence that waterfowl play an important role in the dispersal of freshwater wetland plants (Green, Figuerola & Sánchez, 2002; Kleyheeg, 2015; Kleyheeg *et al.*, 2015), even in flowing waters, with other vertebrates (including humans) being of secondary importance. In general, wind dispersal appears to play a more pronounced role in more terrestrial wetland (riparian) plant species, as demonstrated earlier (Soons, 2006).

Upstream dispersal was detected over distances from less than 1 km up to an impressive 285 km (Fig. 1; Table 2). Direct observations included two different methods of assessing upstream dispersal: analysis of historical geographic distribution data revealed an upstream population expansion rate of 19 km yr^{-1} in an invasive weed (Wang *et al.*, 2011), and seed defecation by frugivorous birds was observed in seed traps 0.21 and 0.36 km upstream of the fruit source (Kohri *et al.*, 2011). Indirect evidence based on molecular methods detected upstream dispersal over much longer distances than these direct observations (Fig. 1; Table 2). Using assignment methods, upstream dispersal was inferred over tens (Tero *et al.*, 2003; Honnay *et al.*, 2009; Pollux *et al.*, 2009; Werth & Scheidegger, 2014) and up to several hundreds of kilometres (DeWoody *et al.*, 2004; Liu *et al.*, 2006). In these studies, the observed maximum distances of upstream dispersal were often close to the maximum extent of the sampled area (Table 2). This suggests that upstream dispersal may occur over longer distances than have so far been recorded, as several of the current estimates were limited by an upper bound imposed by the spatial extent of the sampling (Table 2). Strikingly, observed long-distance dispersal events generally far exceed wind dispersal potential as estimated from mechanistic models for these species (Fig. 1; Table S2), while they are well within the reach of distances covered by migrating waterbirds (Fig. 1; Table S2). Typically, distances travelled by waterbirds within their home ranges are much shorter (Kleyheeg, 2015), but the potential dispersal achieved by migrating waterbirds is likely to be a 'nonstandard' vector with disproportionate consequences for the regional persistence of species.

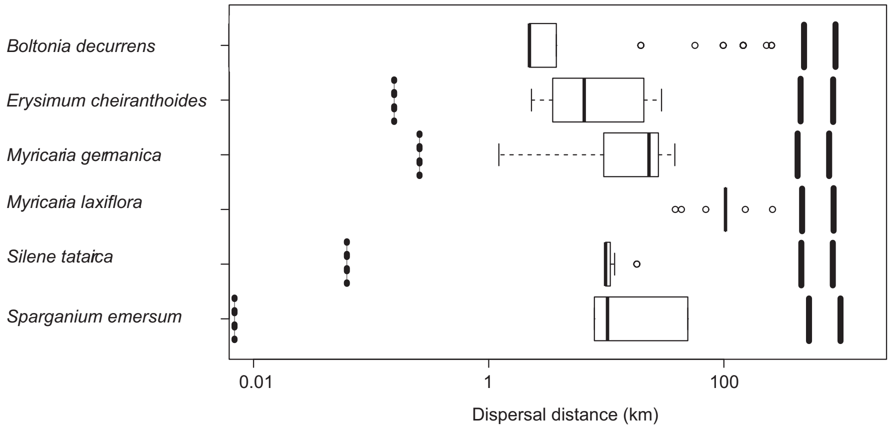

Fig. 1 Boxplots of the observed upstream dispersal distances for aquatic (*S. emersum*) and riparian (the others) plant species, based on assignment methods as available in the current literature (Table 2). The boxes represent the 25th and 75th percentiles of the data, the thick lines in the boxes the medians. The whiskers cover 1.5 times the interquartile range, while more extreme outliers are rendered as open dots. For comparison, we also indicated the theoretical median and 95-percentile dispersal distances for waterfowl-mediated dispersal (calibrated for migrating mallards, solid lines) and the 99.9-percentile dispersal distances for wind-mediated dispersal (dotted lines) as calculated in Table S2.

Several studies using other molecular methods than assignment methods also suggested the occurrence of upstream gene flow (Table 2). These studies all indicate that upstream dispersal plays a role within their study system, the extent of which ranged from <1 km in the smallest study area to 925 km in the largest (based on distribution of sampled populations; Table 2). However, they were unable to detect the individual dispersal events that underlie the observed genetic structure.

Limitations of commonly applied methods to detect dispersal events

While in the general literature upstream dispersal may be suggested to play an important role in the regional persistence of aquatic and riparian plants, the previous section shows that very few papers provide data for its quantification. On the basis of this observation, we here identify some important methodological limitations and pitfalls in the approaches that have been used to detect upstream dispersal (Table 1). We pay particular attention to the quantification of the direction and distances of dispersal. One critical limitation that is common to all the reviewed approaches is that the spatial extent of sampling will determine the maximum dispersal distances that can be inferred. Despite this critical effect, decisions on sampling design have often been made on an *ad-hoc* basis (Balkenhol *et al.*, 2009; Anderson *et al.*, 2010). We address this issue, and propose the use of better informed sampling designs, in the next section on opportunities for future research.

Direct methods

The major advantages of direct methods are that they focus on the actual movement of seeds and other propagules and not on gene flow (Table 1, type 1) and they provide information on the vector mediating dispersal. However, for the quantification of dispersal, especially over longer distances, there are some important limitations.

Propagule trapping (mostly seed trapping; Table 1, type 1.1) is a method commonly applied to estimate dispersal (Bullock & Clarke, 2000). Unless the trapped seeds are marked in such a way that their source can be traced (e.g. using stable isotopes; Carlo *et al.*, 2009, 2013), the main limitation of this method is that estimates of dispersal direction and distance of trapped seeds need to be inferred using statistical models based on the rule-based assignment of trapped seeds to potential parents (Ribbens, Silander & Pacala, 1994; Clark *et al.*, 1999; Jansen *et al.*, 2014). The uncertainties associated with this approach may be large, especially in species with relatively frequent long-distance dispersal events and in situations, where a relatively small proportion of all potential parent individuals can be mapped (e.g. common species). Only in special cases can the direction of dispersal be unequivocally established: for instance, where the seed traps are located upstream from the most upstream established population (Kohri *et al.*, 2011). Another important limitation of trapping is that it is limited to short dispersal distances [up to hundred(s) of meters at most], due to rapid increases in sampling effort with distance (Bullock & Clarke, 2000; Bullock, Shea & Skarpaas, 2006). This makes it unsuitable to detect dispersal events exceeding ca. 100 m.

Direct tracking of individual propagules (visual, radio, radar, GPS) has not been applied in any of the selected studies. Given the small size of most propagules, it is exceedingly difficult to track individual propagules. Where animals (mostly vertebrates) are the vector of upstream dispersal, the tracking techniques can also be applied to the movements of the animals involved (Table 1, type 1.2). If animal movement data are combined with evidence of their ability to transport viable propagules (e.g. gut passage experiments; Table 1, type 1.3) and data on uptake and deposition rates, this demonstrates the potential for the animal to disperse propagules and allows estimation of dispersal direction, distance and possibly even frequency. While dispersal estimation using this method is promising, the effort required to collect data on all components of the dispersal process and combine this into a spatially explicit dispersal model is large and there are few studies that address all crucial steps (e.g. Kays *et al.*, 2011; Kleyheeg, 2015).

Species range modelling provides an alternative approach to demonstrating upstream dispersal (Table 1, type 1.4). When data are available on changes in the geographic distribution of populations over time, dispersal direction, distance and speed can be quantified. Such information is rarely available for common species but may be available for rare or invasive species (e.g. Wang *et al.*, 2011). This approach is limited by the need for detailed historical records or time-series data. More importantly, this method is biased to lower dispersal distances as it will not take into account colonists that dispersed from source populations further away from the leading edge of the expansion.

Indirect (molecular) methods

The indirect study of dispersal using molecular markers (Table 1, type 2) encompasses a multitude of analytical techniques (e.g. Freeland, 2005; Broquet & Petit, 2009). Quite a large number of studies addressing upstream dispersal have tested whether genetic diversity increases along the river, assuming that the unidirectional dispersal hypothesis would result in loss of genetic diversity upstream (Ritland, 1989; Barrett, Eckert & Husband, 1993; Honnay *et al.*, 2010; Table 1, type 2.1). Alternatively, there were many studies that examined the degree of genetic differentiation among populations (e.g. F_{st}, AMOVA; Table 1, type 2.2). Important drawbacks of these approaches, particularly the required long-term migration-drift equilibria, have been identified elsewhere (Whitlock & McCauley, 1999; Honnay *et al.*, 2010). Moreover, neither of these methods reflects solely contemporary dispersal, and they cannot provide information on dispersal distances (Table 1, type 2.1), directions (Table 1, type 2.2) nor frequencies (both).

Coalescent genealogy analysis (Table 1, type 2.3) is based on simulation of genealogies for sampled individuals based on coalescent theory and a predefined (meta) population model (Kuhner, 2009). Genealogy samplers can estimate the number of migrants per generation among populations in either direction (rates A to B and B to A can be allowed to vary). In principle, this approach can provide the necessary quantitative dispersal data to inform regional population models, however, much prior information is required to properly constrain the analyses. For instance, independent estimates of past population growth rates and the mutation rates of genetic loci are required. Furthermore, all genealogy samplers to date assume random mating within populations (Kuhner, 2009), which is unrealistic in many plant species (c. 60% of species deviate from random mating; Goodwillie, Kalisz & Eckert, 2005). In addition, the models assume stable growth rates over a large number of previous generations (e.g. four times the effective population size; Wilson & Rannala, 2003). As a consequence of these limitations, it will be difficult to apply coalescent genealogy models to species of dynamic habitats, such as streams, rivers and riparian zones.

Assignment methods (AMs; Table 1, type 2.4) are statistical methods that use genetic information to ascertain population membership of individuals (Manel, Gaggiotti & Waples, 2005; Anderson *et al.*, 2010). The logic of AMs is straightforward: knowing the allele frequencies of a number of potential source populations one can calculate the likelihood of the genotype of the focal individual arising from each of those populations. If the focal individual is assigned – based on this likelihood – to a population other than the one it was sampled from it is labelled as a migrant. When this procedure is applied to many propagules or established seedlings it is possible to get insight into the dispersal direction, frequency and distance of the study species in the sampled area. In addition, when propagules or seedlings have been sampled we can be certain that the inferred gene flow is contemporary (Robledo-Arnuncio, 2012). Furthermore, compared to the other molecular analyses, AMs require few prior assumptions about the population biology of the study species (Broquet & Petit, 2009). Consequently, assignment methods can be a powerful tool for detection and quantification of dispersal events.

However, there are three important caveats to consider. First, the reliability of the assignment of seedlings to source populations using AMs depends on the inclusion of the true propagule source in the sample. In many, especially common, species it is unfeasible to sample all potential sources which reduces the probability of correct assignment. Second, long-lived seed banks present a complication as these can harbour genetic diversity that goes unnoticed in surveys on established plants and can make a significant contribution to (meta)population genetic patterns (Honnay *et al.*, 2008). Seed banks are problematic because AMs may be forced to attribute offspring to the wrong parent and thus distort the estimated dispersal kernels. A large number of species in our literature review were reported to form seed banks (41 of 48 species, and 18 with seed banks persisting more than 1 year). Third, AMs, like all other molecular approaches (Table 1, type 2.1–3), infer gene flow and not necessarily the dispersal of propagules. It may be the case that the inferred dispersal events in fact reflect patterns of pollen flow, but none of the reviewed studies using AMs were designed to separate seed from pollen dispersal. In many of the reviewed studies, this possibility is rejected on the argument that the distances between populations are simply too large for pollen flow to play a significant role (e.g. Tero *et al.*, 2003; DeWoody *et al.*, 2004; Liu *et al.*, 2006; Markwith & Scanlon, 2007; Honnay *et al.*, 2009; Jacquemyn *et al.*, 2010). One study (Barrett, Cole & Herrera, 2004) supports this argument with their own (unpublished) data, while others cite work that demonstrates that pollen flow declines with distance (e.g. Honnay *et al.*, 2010). This practise of discounting pollen flow is unfortunate because – as for seed dispersal – little is known about pollen flow curves (Ashley, 2010). However, we do know that (i) large proportions of pollen remain viable after long-distance transport through the air (Lindgren *et al.*, 1995; Williams, 2010); and (ii) that pollen are found at vast distances (up to thousands of kilometres) outside the range of the focal species (Campbell *et al.*, 1999; Rousseau *et al.*, 2005). Furthermore, recent molecular studies have reported cases where wind and insect dispersed pollen to travel up to 30 km (Robledo-Arnuncio & Gil, 2005) and 160 km (Ahmed *et al.*, 2009) respectively. In general, pollen flow studies have been unable to attribute substantial amounts of pollen (generally >20%) to parents within their study areas (Ashley, 2010). This suggests substantial influx of pollen into the area from elsewhere over unknown distances. Therefore, it would be safer to assume, as a null model, that pollen flow can be pervasive and may strongly affect estimates of dispersal derived from gene flow.

Opportunities for future studies to detect dispersal events

Using upstream dispersal by plants in river systems as a case study, we have shown that few studies exist that quantify dispersal events in the field. This is the case even for basic statistics such as direction and frequency. Furthermore, a substantial part of the currently available data on upstream dispersal is based on approaches that are subject to clear methodological limitations. For a large part, this lack of quantitative data has resulted from the fact that in many studies neither the identification nor the quantification of upstream dispersal was the primary aim. Instead, such dispersal was invoked as a *post-hoc* explanation for the observed spatial population genetic structure. Nevertheless, if dispersal events contributing to the spatial population dynamics of riparian and aquatic species (or any other species inhabiting discrete, patchy and dynamic habitats) are to be detected in future studies, there are several methodological advances that in combination would greatly promote success.

Given the clear spatial limitations of direct methods, we propose that molecular methods are best used to *detect* dispersal events. Assignment methods (Manel *et al.*, 2005; Anderson *et al.*, 2010) can in principle provide strong empirical evidence about dispersal directions and distances. In short, AMs have the potential to produce data that: (i) represent contemporary gene flow; (ii) detect the direction of gene flow and (iii) quantify the distance travelled. We suggest the use of AMs also because they require fewer assumptions to infer dispersal than the other methods (e.g. coalescent approaches). Yet, AMs are unable to *identify the mechanism* of dispersal, for this a combination of direct methods and mechanistic modelling is fundamental. As the latter combination is being applied successfully across a range of dispersal syndromes (e.g. Soons *et al.* 2004; Soomers *et al.*, 2013; Viana *et al.* 2013), we here suggest a study design that improves the urgently needed detection and quantification of dispersal by utilising the three important qualities of AMs.

Quantifying directional dispersal using assignment methods

Our AM based study design features four key improvements over current practise. (i) we recommend sampling both juveniles (e.g. seeds, seedlings, eggs, statoblasts and other dormant propagules) and adults and assignment of juveniles only. In cases where the juvenile stage is not the stage when dispersal typically occurs the sampling should be adjusted to account for this (see below); (ii) the use of exploratory network-based model simulations are needed to design spatial sampling protocols; (iii) assignment of potential migrants by combining a Bayesian clustering analysis with an exclusion method to prevent errors due to unsampled populations and (iv) use of both uniparentally inherited DNA (e.g. chloroplast, mitochondrial) and nuclear DNA markers to allow separation of gene flow via gametes (e.g. pollen, sperm) and propagule-mediated dispersal.

Sampling design

We suggest a two-stage sampling approach where both a group of potential migrants and a reference group of known residents are sampled. In plants, residents are established and fertile individuals, while the potential migrant group is formed by seeds and seedlings (see e.g. (Honnay *et al.*, 2009). In freshwater invertebrates, the situation is less clear cut, but separation may be possible by separately sampling benthic individuals and those that are found in the water column or elsewhere. For example, the potential migrant group may be sampled from bird droppings (Van Leeuwen *et al.*, 2012), on the exterior of other animal vectors (Bilton *et al.*, 2001) or using other propagule trapping techniques (e.g. Boedeltje *et al.*, 2004). Using these two groups, migrant detection is then restricted to the potential migrant group, and the known resident genotypes represent a reference set of potential source populations to test against (Pritchard, Stephens & Donnelly, 2000). In cases where such two-stage sampling is unfeasible, assignment is still possible, although the statistical power is reduced (Pritchard *et al.*, 2000). The proposed two-stage sampling approach is advantageous, because (i) one can be sure that the identified dispersal is contemporary; and (ii) it is possible to attach a precise timeframe to the migration rates (Broquet *et al.*, 2009; Robledo-Arnuncio, 2012).

Assignment methods can only assign individuals to sampled populations. Ideally, the goal is therefore to sample all potential source populations. However, in practise, this will often not be possible. Determination of the right spatial extent of the study area, as well as the inter-population distances for sampling, is an underdeveloped area in landscape genetics (Balkenhol *et al.*, 2009; Anderson *et al.*, 2010). Naturally, the largest distance among the set of sampled populations dictates the maximum dispersal distance that can be inferred in a given study (this is a limitation of all the reviewed approaches; Table 1). In general, it is recommended to sample an area that is larger than the expected dispersal distances (Anderson *et al.*, 2010). This is problematic because the dispersal distances are exactly what we wish to quantify. However, previous genetic data (e.g. pairwise F_{st}), demographic data and direct dispersal data on the same or related (taxonomically or functionally) species, can serve as a rough guide. When mechanistic modelling and direct and indirect dispersal detection methods are applied in combination, explorative analyses using realistic and case-specific models (Artzy-Randrup & Stone, 2010; Mari *et al.*, 2014) can inform key links among populations and help focus sampling effort. While dispersal and gene flow data could be used to parameterise spatial population models to optimise their applicability to population management, we recommend that such models are built *first*, and parameterised with estimated dispersal data, to optimise the following sampling effort.

Simulation studies show good performance of AMs when 30–50 individuals (adults) are sampled per population (Cornuet *et al.*, 1999; Manel, Berthier & Luikart, 2002), but more individuals need to be sampled in case few markers are screened (e.g. ≤ 5) or their variability is low (e.g. $H < 0.3$). User-friendly software is now available to simulate complex population genetic histories to estimate statistical power prior to sampling (Hoban, Gaggiotti & Bertorelle, 2013). Such analyses prior to sampling could also be carried out to optimise spatial aspects of the sampling protocol.

Pollen- or sperm mediated gene flow can be distinguished from the dispersal of propagules by the inclusion of uniparentally inherited markers in the study design (e.g. chloroplast or mitochondrial markers; see Avise *et al.*, 1987; Ennos, 1994; Kamm *et al.*, 2009). Since chloroplast and mitochondrial markers are usually transmitted only through maternal tissues and lack recombination in virtually all taxa (Avise *et al.*, 1987; Palmer, 1987), their genotype is inherited effectively as a single (clonal) locus. Each migrant should thus show a match with the assigned source at the chloroplast DNA.

Finally, most molecular marker systems can be used for AMs, but highly variable codominant markers (e.g. microsatellites) are strongly recommended because of their discriminatory power. When the variability in the markers is high ($H > 0.5$) one can achieve a high proportion of correctly assigned individuals with 10–20 loci, even when the level of genetic differentiation is quite low (e.g. $F_{st} = 0.05$–0.10; (Cornuet *et al.*, 1999; Faubet & Gaggiotti, 2008). A larger panel (e.g. 100 loci) of single nucleotide polymorphisms (SNPs) can be used with similar power (Anderson & Garza, 2006).

Data analysis

We propose a three-stage analytical approach for the detection of migrants. First, as Bayesian implementations of AMs can allow for deviations from Hardy–Weinberg Equilibrium (e.g. due to non-random mating) and in simulation studies often outperform maximum likelihood based methods (Manel *et al.*, 2002), we advocate the use of a Bayesian clustering programme (e.g. Wilson & Rannala, 2003; Gao, Williamson & Bustamante, 2007; Faubet & Gaggiotti, 2008) to assign sampled seedlings to source populations. Second, to account for the fact that Bayesian AMs deal poorly with unsampled source populations (Cornuet *et al.*, 1999), we suggest applying an exclusion test (Paetkau *et al.*, 2004; Piry *et al.*, 2004) on the putative migrants to estimate the likelihood that they have indeed originated from the assigned population; if the P-value is high the assignment is accepted. If it is below a certain critical threshold (e.g. $P < 0.05$), the Bayesian assignment is rejected and it is concluded that the individual belongs to an unsampled population. The third step is to distinguish between propagule-based dispersal (e.g. seeds, eggs, statoblasts and other dormant propagules) from gene flow in general (i.e. including pollen, sperm). Given that maternally inherited markers have been included in the dataset, genotypes can easily be matched to potential maternal parents, for instance using maternity analysis (reviewed in Jones *et al.*, 2010). In species where these uniparentally inherited genomes provide insufficient resolution to distinguish between potential source populations, due to low molecular variability, it may be necessary to perform paternity analyses (Robledo-Arnuncio & Gil, 2005; Jones *et al.*, 2010) on propagules with known mother individuals (e.g. seeds on a plant). These data can then be used to separately quantify the male gamete gene-flow kernel and this can be combined with the estimated dispersal kernel based on the AM data to arrive at a propagule-mediated dispersal kernel probabilistically. Finally, following the collection of a set of confirmed dispersal events, the results can be tallied in terms of direction, distance and frequency.

Application

In general, the proposed study design could be applied to almost any plant species of interest, but clearly, data collection will be much more complete for species with a limited distribution and well-defined populations (as most species of conservation interested – or invasive species in their early stages – are likely to be). The same holds true for freshwater invertebrates, although some specific modifications (as indicated in the previous section) are needed depending on life histories.

Furthermore, as study species we recommend using species that are diploid. Although AMs can in principle be applied to plant species irrespective of their ploidy level, it is technically difficult to determine the allele dosages (e.g. AaAA versus AaAa) of polyploid individuals (e.g. Van Puyvelde, Van Geert & Triest, 2010; De Groot *et al.*, 2012). The associated uncertainty in determining exact genotypes will hamper efficient use of AMs, and in fact several AM software packages do not even accommodate polyploid genetic data.

Finally, the results provided by the application of combined AMs, direct methods and mechanistic models would provide a mechanistic dispersal framework quantifying the dispersal of propagules between existing populations and towards potential new habitat patches. This information provides critical input for regional population models, which need to incorporate immigration rates in local populations as part of their recruitment or growth rate (e.g. Hanski, 1998). More mechanistic and spatially explicit population models ideally include dispersal kernels for different vectors, for instance to allow insights in the influence of species traits on patch recolonisation and source/sink dynamics (Dunning *et al.*, 1995; Jongejans, Skarpaas & Shea, 2008). The predicted dispersal distances calculated using recent mechanistic models suggest that waterfowl-mediated dispersal may be of much greater importance than wind-mediated dispersal of riparian plant species (Table S2), although the evaluated species all produce relatively heavy seeds. Such quantitative dispersal information is critical to the conservation practice, where management decisions promoting (or, in the case of invasive species, preventing) dispersal need to be considered when planning restoration activities and in regional nature management plans (e.g. Verhoeven *et al.*, 2008; Brederveld *et al.*, 2011; Sarneel *et al.*, 2011).

Conclusions

Using upstream dispersal of aquatic and riparian plants in riverine systems as a case study, we show that even in such a spatially relatively simple system there exists very little empirical information on dispersal. Even simple quantitative aspects of dispersal such as frequency, direction and range of distances are not well known. Across the few studies that present quantitative data, the general consensus is that upstream dispersal may be less frequent than downstream dispersal, but that it occurs in many species (it was observed in nine of 12 studies) and over vast distances (up to hundreds of km). This applies both to aquatic and riparian plant species, which also share the same main upstream dispersal agents: waterfowl and, to a lesser extent, wind. Analysis of potential reasons for the lack of data resulted in the identification of several important pitfalls in data collection using molecular ('indirect') estimates of dispersal. We used this information to outline a research design based on assignment methods (AMs) to improve this situation. The four key improvements are: (i) assignment of propagules and/or seedlings to a reference set of adults; (ii) combination of 'indirect' molecular methods with 'direct' methods and mechanistic dispersal models, and use of spatially explicit population models to inform sampling designs; (iii) combining both Bayesian and exclusion-based AMs to account for unsampled populations and (iv) combined use of uniparentally inherited (e.g. chloroplast or mitochondrial) and nuclear DNA markers to allow separation of propagule dispersal and gamete (e.g. pollen, sperm) mediated gene flow. In combination, these methods allow efficient quantification of dispersal direction, frequency and distance among regional sets of plant populations in general.

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Supporting Information

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

Table S1. List of references for the selected studies.

Table S2. Predicted dispersal distances using mechanistic models for species with upstream dispersal data.

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