

Modeling seed dispersal of black cherry, an invasive forest tree: how microsatellites may help?

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Abstract: We used empirical models and three dispersal functions (Weibull, lognormal and 2Dt) to model seed distributions derived from the black cherry (*Prunus serotina* Ehrh.) understorey of a pine-dominated stand. Two different approaches were used to disentangle the overlapping seed shadows: the traditional inverse modeling approach and the genetic approach that uses microsatellite markers to assign a dispersed seed to its maternal parent. The distinction was made between the seeds passively dispersed by gravity (fruits with mesocarp) and those dispersed by birds (fruits without mesocarp). Our main objectives were to compare the three dispersal functions and assess the differences between the two approaches used. The functions performed equally well, but the lognormal function often showed a better data correlation. The best dispersal curves obtained by both traditional and genetic approaches were quite similar for the seeds dispersed by gravity, and 95% of these seeds were predicted to fall 5 and 3 m away from the parent tree for the traditional and genetic approaches, respectively. Differences were more important for the seeds dispersed by birds. The traditional approach predicted a lower number of seeds near the parent plant and a higher dispersal distance. Microsatellites provided accurate information on individual dispersal events and led to a better insight into the dispersal process.

Résumé : Nous avons utilisé des modèles empiriques et trois fonctions de dispersion (Weibull, lognormale et 2Dt) pour simuler la dispersion des graines du cerisier tardif en sous étage d'une plantation de pin. Deux approches différentes ont été utilisées pour séparer des pluies de graines provenant de sources non isolées. La première est basée sur la méthode traditionnelle de simulation en sens inverse, tandis que la seconde se sert de marqueurs microsatellites pour retrouver l'origine maternelle d'une graine dispersée. Une distinction a été faite entre les graines dispersées passivement par gravité (fruits avec mésocarpe) et les graines dispersées par les oiseaux (fruits sans mésocarpe). Les objectifs principaux de l'étude étaient tout d'abord de comparer les trois fonctions utilisées et d'estimer ensuite les différences entre les courbes obtenues par les deux approches. Les fonctions étaient comparables mais la fonction lognormale présentait souvent une meilleure corrélation avec les données. Les meilleures courbes de dispersion obtenues par les approches traditionnelles et génétiques étaient globalement semblables pour les graines dispersées par gravité et prédisaient que 95 % de celles-ci tomberaient à des distances de 5 et 3 m respectivement pour les deux approches. Les différences étaient plus marquées pour les graines dispersées par les oiseaux. L'approche traditionnelle prédisait en effet un nombre plus faible de graines tombant près de l'arbre et une plus grande distance de dispersion. Les microsatellites ont fourni des données précises sur les événements individuels de dispersion et ont dès lors mené à une meilleure évaluation du processus de dispersion.

Introduction

Biological invasions are causing significant ecological impacts and economical costs and are thought to be the second greatest threat to biodiversity, after habitat fragmentation (Wilcove et al. 1998). Invasive plants have been shown to alter ecosystem processes, threaten endangered or rare species, and displace native species (Forman 2003). An alien becomes invasive when it can reproduce and increase its range

in its new environment. The invasion process usually involves an increase in population size and a geographical range expansion. Therefore, the success of an invasive species will likely depend on its ability to spread and migrate; an effective dispersal strategy is often considered preeminent for fast and successful spread of invasive species (Cain et al. 2000; Jordano and Godoy 2001).

The American black cherry (*Prunus serotina* Ehrh.) is good example of an invasive plant species in Europe. Intro-

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duced at a large scale and massively planted by foresters in the 19th century, it rapidly filled the gaps between the plantings and established dominant thickets (Starfinger et al. 2003). Belonging to the Rosaceae family, black cherry produces small cherries nearly every year. The bulk of the seed crop falls to the ground in the vicinity of the parent tree but some seeds are dispersed by mammals and birds (Marquis 1990). In forests, the long-distance dispersal of black cherry is more limited than it is in open fields with hedges because of a later and less abundant fruit production. Therefore, there seems to be strong evidence for a very close spatial relationship between the initial planting and subsequent spontaneous spread in forests, and dense shrub layers in pine stands developed almost exclusively in the direct vicinity of the initial plantings (Starfinger et al. 2003). To date, no thorough investigation of the short-distance dispersal of black cherry in forests in its introduced range has been conducted, although this form of dispersal seems to be the most important mechanism driving the spontaneous spread of the species.

Studies of seed dispersal by wind and by animals in numerous plant species consistently reveal a rapid decline in seed density with distance from the parent plants (Nathan and Casagrandi 2004). Mathematical tools have often been used to model this decline. Two types of mathematical models have been described: (i) mechanistic models, which can be parameterized using independent data on the dispersal vector and medium, and (ii) phenomenological (empirical) models, which are functions fitted to observed seed shadow (Levin et al. 2003).

Mechanistic models have more often been applied for wind dispersal because wind directions and speeds are easily described (e.g., Calogeropoulos et al. 2003; Skarpaas et al. 2004). Animal behavior is much more difficult to characterize, because it depends not only on abiotic conditions and vegetation, but also on abundances and characteristics of alternate food sources, competing species, and predators (Herrera et al. 1994; Alcantara et al. 2000; Jordano and Schupp 2000; Nathan and Muller-Landau 2000; Parciak 2002; Deckers et al. 2005). Therefore mechanistic models of animal-dispersed seeds require quantification of detailed behavioral information.

Given this complicated picture, phenomenological models are often used to describe the total seed shadow. A phenomenological model is used to fit data on seed densities as a function of distance from their source (Nathan and Muller-Landau 2000). Different mathematical functions are used, and their parameters are estimated to best fit the observed data. Two major limitations of this modeling approach are usually outlined. The first one is related to the shape of the model curves. It has indeed often been argued that because different mechanisms are involved in the seed dispersal, none of the empirical curves described can provide adequate predictions for both short and long dispersal distances (Levin et al. 2003). Different mathematical kernels have been designed to overcome that problem, and some authors have mixed different kernels to take the different dispersal mechanisms into account (e.g., Higgins and Richardson 1999; Bullock and Clarke 2000). In our study, two main mechanisms are involved: passive dispersal by gravity and dispersal by birds. The seeds dispersed by each mechanism are

easily identified because the mesocarp is in some cases present (dispersal by gravity) and in others absent (eaten by birds). We chose to model the dispersal of these two fruit types separately to overcome the first limitation of the phenomenological models. A second general problem with these models is the identification of the specific source location of dispersed seeds. This has led researchers to choose isolated individuals so that the source of the seed is not ambiguous. However, situations where the seed shadows of different individuals do not overlap are sometimes hard to find, especially in the context of plant invasions, as adult plants tend to form conspecific aggregations. A way to disentangle the overlapping seed shadows in such cases has been described by Ribbens et al. (1994) and further refined by Clark and collaborators (Clark et al. 1998, 1999). The approach is based on the assumption that the seed rain is the summed contributions of potential parent trees. With this traditional approach, it is not possible to check the quality of the model because no data are available on the individual seed shadow. Molecular tools and particularly microsatellite markers, because of their highly variable character (Ouborg et al. 1999), present an interesting solution to the problem of overlapping seed shadows. It is indeed possible to find the origin of the dispersed seed by genotyping both the maternal tissue of the dispersed seeds and the parents in the population. This technique, first developed on *Prunus mahaleb* L. (Godoy and Jordano 2001), has proven its usefulness in other species such as *Prunus avium* L. (Schueler et al. 2003), *Abies alba* Mill., *Quercus robur* L. (Ziegenhagen et al. 2003), *Quercus lobata* Née (Grivet et al. 2005), and *Jacaranda copaia* (Aubl.) Don (Jones et al. 2005). This method provides a direct measure of dispersal, even if it implies an exhaustive sampling.

The combination of empirical modeling approach with the direct parental genetic analysis in studies of dispersal in closed forest canopies could provide bright prospects for further progress in the understanding of dispersal mechanisms of plant species. Genetic analysis can indeed supply more accurate data on individual dispersal events (Nathan and Muller-Landau 2000), bringing insight into the components of the seed rain, even in conditions of overlapping seed shadows, while models can be used to fit density–distance curves using empirical functional relationships. This paper aims to study the advantages provided by microsatellite markers for checking the accuracy of the models obtained by the traditional approach and modeling the short-distance seed dispersal of an invasive tree species in a forest stand.

The specific goals of the study were then to (i) fit three empirical models to the data, using the traditional approach described by Ribbens et al. (1994) for disentangling overlapping seed shadows, (ii) fit the same models using accurate data on individual dispersal events provided by seed genotyping, and (iii) compare the best models of both approaches and assess the accuracy of the traditional approach using the data provided by molecular tools.

Material and methods

Study site and experimental plot

The study site is located in central Belgium (50°79'N, 04°71'E), in a pine stand of 2.29 ha. The overstorey is composed of *Pinus sylvestris* L. and *Pinus nigra* Arn., which

were planted in 1927 and 1940, respectively. The understory is invaded by *Prunus serotina* that has never been planted nor managed. Beech (*Fagus sylvatica* L.) forest surrounds the study site.

The experimental plot had a surface of 0.55 ha, and two of its edges (the northern and the northeastern) were in contact with the surrounding beech forest. The two other edges were continuous with the rest of the pine stand and other black cherry individuals. All reproductive adults were mapped with an electronic compass (Impulse 200) combined with a mapstar compass (Laser Technology Inc., Englewood, Colorado), and data were entered in an ArcGIS database (ESRI 2001) to know the distances between each tree and each trap. The minimum distance between a tree and a trap was 0.2 m, and the mean distance was 37.0 m. Only 2.5% of the distances were smaller than 6.7 m. The circumference at breast height (CBH) was taken on all 305 reproductive trees and ranged from 21 to 86 cm. A subsample of 23 adults was chosen among the trees with different CBH classes to measure the approximate age and mean crown diameter. Trees were 11 to 32 years old, with a mean ($\pm$ SD) crown diameter of 5.64 ± 1.69 m.

Experimental design and fruit collection

Traps of 0.25 m^2 were arranged in the experimental plot following a $10 \text{ m} \times 10 \text{ m}$ grid, and an additional seed trap was placed on nine nodes of the grid (Fig. 1). The total number of traps in the plot was 62. Fruits were collected weekly, from the beginning of September 2004 to early December 2004, and were sorted in three different groups: fruits with mesocarp, fruits without mesocarp, and nonmature fruits. Nonmature fruits were not used for further analyses. Distinction was made between fruits with and without mesocarp because their dispersal mechanisms were different. Fruits that still had a dark fleshy mesocarp presumably fell near the parent plant (dispersal by gravity), whereas fruits that did not have a mesocarp were regurgitated by birds and likely to have been carried further away from the parent plant. Fruits with mesocarp were washed to remove their pulp and were soaked in a fungicide solution (2% Rovral, Bayer Cropscience, Mannheim, Germany) for 5 min. All seeds were kept separately at 4°C until further treatment.

For modeling the dispersal curve by the traditional approach, all fruits with and without mesocarp (4212 and 1000 fruits, respectively) in the 62 traps were used, whereas a subsample of the traps was chosen for genotyping. This subsample was chosen in two main parts of the plots. First, because the northern and northeastern parts were contiguous with beech forest and therefore not in contact with other black cherry adults, the northern part was chosen to limit the probability of finding nonassigned seeds. Second, the parcel surrounding the central trap line in the southwestern direction was chosen to catch longer dispersal events along a transect (Fig. 1). All parents (total number of 192) in these two parts were genotyped as well as the fruits with and without mesocarp from 5 and 15 traps, respectively, representing a total number of 148 and 188 fruits and a mean number of 32.2 ± 35.2 and 16.3 ± 9.9 fruits/trap, respectively.

Identification of maternal parent by genetic methods

Microsatellite markers were used to determine the exact

source of the fruits found in the traps by genotyping the adult trees and the endocarps (maternal tissue) of these fruits. To assess the genotypic relationship between the mother tree and the endocarp of its fruits, seeds from five trees were collected in July 2003, and the genotype matching was checked. Seed endocarp exhibited the same multilocus genotype as the mother tree.

Leaves were collected on the 192 adult trees in July 2004 and kept at -80°C until DNA extraction. DNA was extracted following the cetyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1990). The endocarps of the fruits to be genotyped were grinded in porcelain mortar with the help of liquid nitrogen, and DNA was extracted following a modified procedure of Cheung et al. (1993).

Previous work has shown that six microsatellite markers gave high polymorphism in Belgium (Pairon and Jacquemart 2005). Four markers were chosen to genotype the adult trees of the population (i.e., M4c, pchpgms3, pchgms2, and Ps12a02). PCR reactions and fragment analysis were done using the same equipment and following the same protocol as previously described (Pairon and Jacquemart 2005).

Polymorphism was high and a two-locus genotype (primers M4c and pchpgms3) showed 178 unique genotypes among the parents (one realization each) and seven matched pairs. Primer pchgms2 did not help to resolve those pairs, while adding the genotype at locus Ps12a02 was enough to provide one genotype realization for each tree. Therefore, only primers M4c and pchpgms3 were used as markers for the endocarps, and primer pchgms2 was added when a seed was assigned to one of the seven pairs of parents that shared the same genotype. The source tree for individual dispersed seed was identified by comparing the endocarp multilocus genotype with the complete set of genotypes of reproductive trees. The matching between the genotypes of the parent and the seed was automated with procedure MERGE in SAS version 8.2 for windows (SAS Institute Inc. 1999). Unfortunately, it was not possible to compute the probability of identity that estimates the probability of erroneously assigning the parentage of a seed to a tree on the plot when it has, in fact, been produced by a tree having the same multilocus genotype outside the plot. Black cherry is tetraploid and segregates disomically at meiosis (Pairon and Jacquemart 2005). Therefore, the allelic frequencies in the population as well as the allelic proportions in gametes could not be computed.

Theoretical background for seed dispersal modeling

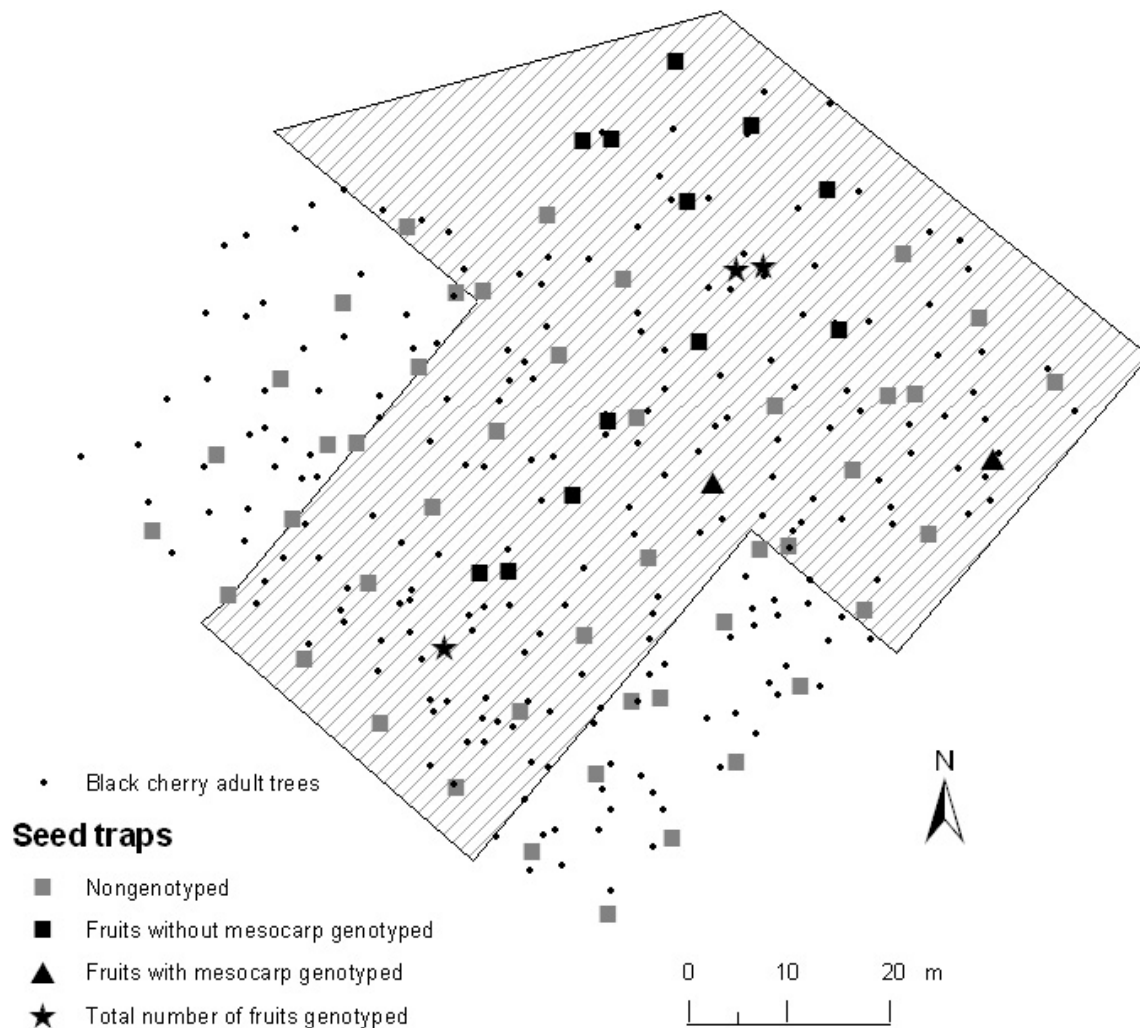
Traditional inverse modeling approach

The density curve for a point source (S_{DPi}) is defined as the density of seeds deposited at distance x from a point source bearing Q seeds:

$$[1] \quad S_{DPi} = Qf(x)$$

where $f(x)$ is the dispersal term. In our case, seed density was evaluated in 62 seed traps, placed at various distances from different point sources (each adult tree in the plot was considered to be a different point source). Therefore, the observed seed density (S_{Di}) was the sum of the contributions of different point sources, placed at various distances x_i from one trap (Clark et al. 1998, 1999; Turchin 1998; Lepage et al. 2000; Hille Ris Lambers and Clark 2003; Greene et al. 2004; Skarpaas et al. 2004).

Fig. 1. Experimental design used to collect and genotype seeds from *Prunus serotina* adult trees in the understorey of a pine plantation in Belgium. Three hundred and five adult trees were mapped, and 62 seeds traps were arranged following a continuous 10 m × 10 m grid to study the seed dispersal of this invasive species in its typically invaded habitat. All seeds from some traps were genotyped to allow the direct assignment to a maternal parent. The fruits with mesocarp were dispersed by gravity, while the fruits without mesocarp were dispersed by birds. Hatched area indicates the zones where adult trees were genotyped.



$$[2] \quad S_{Di} = \sum_{i=1}^n Q_i f(x_i)$$

where Q_i is the number of seeds produced by the tree i , $f(x_i)$ is the dispersal kernel, which depends on the distance (x_i) from the tree i to the considered trap, and n is the total number of trees in the plot.

Different dispersal kernels have already been used (Greene and Calogeropoulos 2002). Among those, three were chosen to fit the data (Greene et al. 2004): the Weibull probability function (Ribbens et al. 1994; Clark et al. 1998, 1999), the 2Dt function (Clark et al. 1999), and the log-normal function (Greene and Johnson 1989) (Table 1). Each kernel is a two-parameter distribution, which depends on a shape parameter (θ) and a scale parameter (B).

The total number of seeds produced by a tree has often been characterized by an allometric relationship (e.g., Greene et al. 2004), which depends on a tree size parameter (here chosen as the circumference at breast height (CBH)) and two parameters to be fitted (A and ϕ):

$$[3] \quad Q_i = A(\text{CBH}_i)^\phi$$

Genetic approach

The approach was similar to the traditional approach and used the data sets obtained from seed genotyping. There were two main differences between the two approaches. First, because a fruit was assigned to one parent and because each tree was therefore considered to be an independent point source, the summation of the contributions of the different trees was no longer necessary in the genetic approach. Second, only on a subsample of the traps was used for genotyping, because each fruit was one observation, whereas each trap represented one observation in the traditional approach.

Fruit production

The mean predicted production ($\bar{Q}_p$) was evaluated on the basis of the allometric equation (eq. 3) of the best models. The fruit production (Q) was calculated for each tree i , and the mean of Q_i was taken.

Table 1. The three dispersal kernels, $f(x)$ used to model the seed dispersal of *Prunus serotina* in the understorey of a pine plantation in Belgium, with x being the distance from the source tree for the three models.

Model	Dispersal kernel $f(x)$
Weibull	$\frac{\theta}{B} \left(\frac{x}{B}\right)^{(\theta-1)} \exp\left[-\left(\frac{x}{B}\right)^\theta\right]$
2Dt	$\frac{\theta}{B^2 \pi \left(1 + \frac{x^2}{B^2}\right)^{\theta+1}}$
Lognormal	$\left(\frac{1}{[(2\pi)^{1.5} \theta x^2]}\right) \exp\left[-\left(\frac{[\ln(x/B)^2]}{2\theta^2}\right)\right]$

Note: These are kernels for density of seeds as a function of distance from a source plant. B is the scale parameter, and θ is the shape parameter.

The mean observed production ($\bar{Q}_o$) is

$$[4] \quad \bar{Q}_o = \frac{S}{n} \left[\frac{1}{t} \left(\sum_{j=1}^t T_j \right) \right]$$

where n is the total number of trees in the plot, t is the total number of seed traps, T_j is the number of seeds in trap j , and S is the surface of the plot multiplied by a constant factor that transforms the number of seeds per trap to a number of seeds per square metre.

Traditional inverse modeling approach

Because the plot was not isolated from conspecifics and because of long dispersal events, fruits from outside the plot fell in the traps. We hypothesized that the number of fruits coming in from different sources outside the plot was equal to the number of fruits leaving the plot. We supposed therefore that the mean observed production ($\bar{Q}_o$) was neither overestimated nor underestimated by the sampling design. However, the model fit for fruits without mesocarp resulted in a dispersal kernel that accounted for dispersal beyond the maximal distances between trees and traps. The integration of this kernel over the maximal distances gave values inferior to one, leading to an overestimation of the production predicted by the models. A correction was therefore applied on the mean predicted production ($\bar{Q}_p$) for fruits without mesocarp using a correction factor corresponding to the area under the curve from 0 to 37 m.

Genetic approach

Because seeds leaving the plot were not taken into account by the sampling design, the mean observed production of fruits without mesocarp was underestimated. In this case, the true production of fruits without mesocarp was estimated by the model that accounted for the seeds leaving the plot.

Statistical methods

Models were fitted to two different data sets, the observed density of fruits with mesocarp and that of fruits without mesocarp. The total number of fruits was also used in a preliminary analysis to assess the necessity of handling both types of fruits separately. Nonlinear minimization (function “nlm” in R, The R foundation for statistical computing 2004) was used to fit the models via minimization of the negative log-likelihood ($-\ln L$). The likelihood function used was the Poisson likelihood (Clark et al. 1999; Lepage et al. 2000; Skarpaas et al. 2004).

Overall goodness of fit of models was estimated with Pearson’s correlation and the value of the negative log-likelihood. Standard deviations for parameters were obtained by inverting the Hessian matrix and taking the square root of the covariance for each parameter.

The distance within which 95% of the produced seeds (Q_i) had fallen was taken as a reference distance to compare the models.

Results

Descriptive analysis

The total number of fruits with and without mesocarp collected in the traps was 4215 and 1000, respectively, representing mean values of 271.9 and 64.5 fruits/m², respectively. A subsample of these fruits were genotyped to determine their exact source locations. As expected, fruits with mesocarp all came from a nearby maternal parent (mean distance traveled 2.5 m), and 95% of them had fallen within 0–5 m from the source (Fig. 2a). The maximum distance recorded was 11.3 m. The pattern of dispersion was more complex for fruits without mesocarp. Of the 188 genotyped fruits of that class, we could only assign 126 of them to a maternal parent in the plot; therefore almost 33% of seeds had unknown parents. The maximum distance traveled by a seed was 44.8 m, and the mean distance was 10.8 m. Indeed, 95% of fruits without mesocarp for which the parents had been identified were found to fall within 0 to 25 m (Fig. 2a).

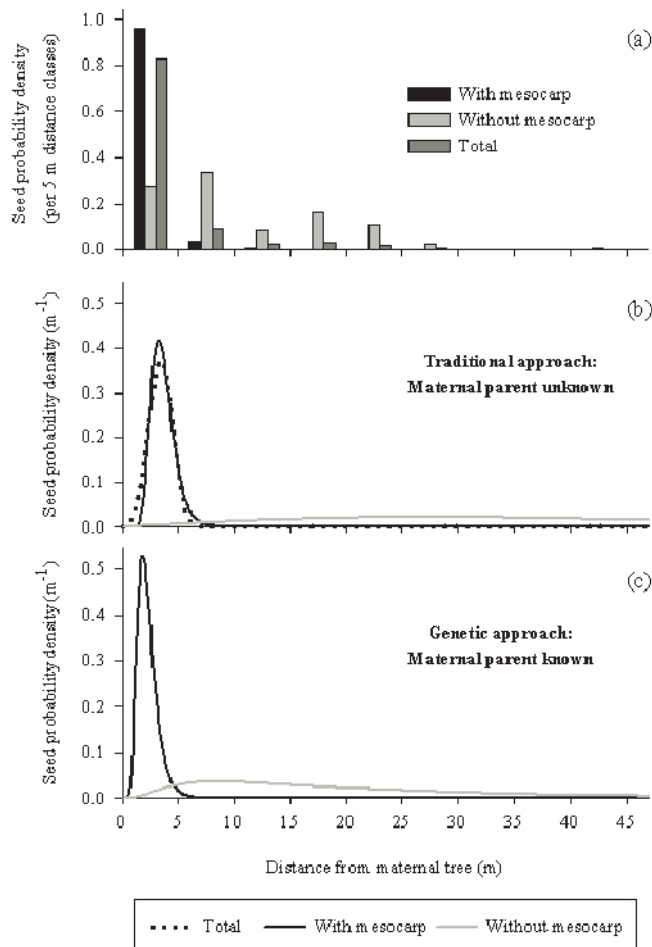
The proportion of bird-dispersed seeds was low, as the vast majority (83%) of seeds had fallen within 5 m from the source tree. This percentage does not take into account the unassigned seeds without mesocarp (33% of seeds).

Production

Parameter ϕ of the allometric equation (eq. 3) had to be fixed to a value of zero for fruits with mesocarp (Table 2). Indeed, when this parameter was evaluated by the traditional four-parameter model, it fell down to values lower than zero. This would suggest that a tree with smaller circumference produces more fruits than one with a larger circumference. By setting the exponent of CBH to zero, we assumed that the production of fruits with mesocarp was best estimated by a constant term, independent from the tree size parameter.

The mean observed total fruit production ($\bar{Q}_o$) of one tree was 6344.7, of which 21% had been eaten by birds. The mean predicted productions ($\bar{Q}_p$) calculated by the allometric equation with the parameters of the best models were comparable with the mean observed production (Table 3). The production of fruits with mesocarp was higher for the model

Fig. 2. (a) Frequency distribution of seed dispersal distances estimated by direct genotyping of seed endocarp of bird dispersed *Prunus serotina* seeds sampled in seed traps in the understorey of a pine plantation in Belgium. (b)–(c) Empirical dispersal models fitted to data with the traditional approach (maternal parent unknown) and with seed genotyping (maternal parent known). The models are represented as a seed probability density per metre. The total number of fruits is the sum of fruits with and without mesocarp. Note that the seed probability density is higher in Fig. 2a because it is evaluated by distance classes, each class being of 5 m.



obtained by the traditional approach (maternal parent unknown) than for the model obtained by the genetic approach (maternal parent known). The five traps chosen to genotype these fruits had a lower number of fruits with mesocarp than the number found in the rest of the traps, showing the high heterogeneity of the production within the plot. The differences in sampling and therefore in predicted production did not affect the comparability of the dispersal curves of both approaches because kernels are expressed in probability densities.

Model quality

Several cases of colinearity between the scale (B) and the shape (θ) parameters occurred, reflected by an algorithm convergence failure, high parameter standard deviations, or a noninvertible Hessian matrix. Whenever an algorithm failed

to converge, the shape parameter was fixed to a given value and the models were then reduced to three-parameters models (Table 2). Standard deviations were high or the Hessian matrix was not invertible for the 2Dt function in all but one case and for the lognormal function fitted to the nongenotyped fruits without mesocarp. Because our aim was to compare, whenever possible, the goodness of fit obtained for the different full models, the shape parameter was not fixed in these cases. An over simplification of the models may indeed have led to a loss of flexibility and to a roughly similar dispersal curve for every model. The estimated parameters of those curves are nevertheless not to be considered different from zero, and therefore, they do not have a biological significance. This is well illustrated when the lognormal function for nongenotyped fruits without mesocarp is considered. In this case, the scale parameter (B) had a high standard deviation, reflected by the abnormally high distance (878 m) within which 95% of the seeds had fallen. When a B value close to 0 was taken instead, the distance within which 95% of the seeds had fallen was reduced to a distance comparable to the distances given for the two other curves (around 50 m).

Comparison among the curves

For fruits with mesocarp, the models that gave the best log-likelihood value were the lognormal, both for the genotyped and nongenotyped seeds. For fruits without mesocarp, the best models were the Weibull for the nongenotyped seeds and the lognormal for the genotyped seeds (Table 2). The overall performances of the three models for each data set were always in the same range, except for the models fit on the data set of genotyped (maternal parent known) fruits with mesocarp. The lognormal was indeed in this case much better, with a correlation of 0.65 (versus 0.41 and 0.42 for the Weibull and the 2Dt, respectively). These findings are congruent with the comparison that can be done among the different curve shapes (Fig. 3). The shapes are indeed always quite similar. Differences are, however, stronger at very short distances and between the curves of the nongenotyped fruits without mesocarp where the lognormal presents a highly different shape.

Comparison between approaches

Some difference can be seen when comparing the curves obtained with the traditional approach and those resulting from the approach using microsatellite tools to know the exact location of the maternal tree (Fig. 3). For fruits with mesocarp, the curves are flatter with the traditional approach, showing a lower probability density (roughly around 0.05 and 0.02 for the genotyped and nongenotyped seeds, respectively) and therefore a further dispersal distance (5 and 4 m, respectively). For fruits without mesocarp, the same trends are observed. A lower probability density near the source and a wider tail characterize the curves obtained by the traditional method.

To test the quality of the best models obtained by the traditional approach, the predicted proportions of seeds as a function of distance from maternal tree were compared with the frequency distribution of seed dispersal distances estimated by genotyping (Fig. 2). Results showed that the frequency distribution of fruits with mesocarp was well estimated

Table 2. Parameters of the three dispersal models used to empirically model dispersed seeds of *Prunus serotina* in the understorey of a pine plantation in Belgium for the data set in which the maternal parent was unknown and the data set in which the maternal parent was known through genotyping.

Fruit type	Model	A	ϕ	B	θ	(-ln L)	cor	d
Maternal parent unknown								
Total	Weibull*	1448.89±142.95	0.44±0.029	3.80±0.027	3.71±0.087	4920.63	0.47	4
	Lognormal	644.51±54.87	0.69±0.025	3.69±0.028	0.40 [†]	4953.12	0.47	5
	2Dt	843.42±108.23	0.61±0.037	51.13±38.21 [‡]	129.071±72.10	5182.40	0.44	5
With mesocarp	Weibull	5276.81±53.44	0	3.81±0.034	3.90±0.12	5806.39	0.42	5
	Lognormal*	5287.36±46.99	0	3.53±0.024	0.28±0.0071	5745.28	0.43	5
	2Dt	5411.45±45.15	0	220.00±nd [§]	2381.67±nd	6116.09	0.39	7
Without mesocarp	Weibull*	213.98±162.67	0.70±0.21	38.79±2.51	2	1152.55	0.30	49
	Lognormal	70.69±48.88	1.32±0.11	198.70±207.079	1.95±0.27	1154.33	0.31	878
	2Dt	288.24±nd	0.64±nd	2.51×10 ³ ±nd	3.53×10 ³ ±nd	1153.84	0.30	54
Maternal parent known by genotyping								
With mesocarp	Weibull	1879.08±78.15	0	2.69±0.075	1.71±0.057	1011.62	0.41	5
	Lognormal*	1974.26±82.52	0	2.12±0.032	0.38±0.0099	826.39	0.65	3
	2Dt	1878.01±77.34	0	4.20±1.75	3.26±0.47	963.89	0.42	5
Without mesocarp	Weibull	0.91±0.7	2.10±0.19	20 063±1054	1.79±0.081	655.10	0.43	27
	Lognormal*	1039±0.78	2.10±0.19	18 032±1.81	0.85±0.050	649.07	0.47	39
	2Dt	0.97±0.74	2.09±0.19	29.53±27.47	2.59±2.16	656.12	0.43	28

Note: The origin of a seed is said to be known when it has been genotyped and when a matching genotype has been found among the parents of the population. The four parameters of models are given (A, ϕ , B, θ) ± their standard deviations. Results for the total number of fruits (with and without mesocarp) are given only for the data set with unknown maternal parents. Fruits with mesocarp were dispersed by gravity, while fruits without mesocarp were dispersed by birds. As estimators of the model fit, the negative Poisson log-likelihood at convergence (-ln L) and the Pearson correlation (cor) are given. The distance, in metres, at which 95% of the seeds had fallen (d) is shown.

*Models that show the lowest negative likelihood.

[†]Parameters that are not followed by ± were those that were fixed to a given value and were not estimated by the model.

[‡]Standard deviations in italic are high and indicate that the confidence interval is higher than the parameter value given.

[§]Standard deviation could not be evaluated either because the Hessian matrix was not invertible or because the covariance was negative and the square root could therefore not be taken.

Table 3. Mean predicted and observed productions of *Prunus serotina* adult trees in the understorey of a pine plantation in Belgium for the data set in which the maternal parent was unknown and the data set in which the maternal parent was known through genotyping.

Fruit type (model)	$\bar{Q}_o$	$\bar{Q}_p$
Maternal parent unknown		
Total (Weibull)	6538.2	6344.7±1365.9
With mesocarp (lognormal)	5422.8	5287.4
Without mesocarp (Weibull)	1202.2	1324.2±887.6
Maternal parent known		
With mesocarp (lognormal)	2149.5	1974.3
Without mesocarp (lognormal)	1178.1	1773.1±1943.5

Note: The mean observed production ($\bar{Q}_o$) is the production calculated by summing all seeds collected in seed traps, multiplying it to obtain an estimate of the number of seeds that fell in the plot, and dividing this number by the total number of adult trees in the plot. The mean predicted production ($\bar{Q}_p$) is the production evaluated with the parameters of the allometric equation (A, ϕ) given by the best models for each fruit type. The best models are those that showed the lowest log-likelihood. Standard deviation is given for the total number of fruits and for fruits without mesocarp. The with mesocarp fruit type does not have standard deviations because the tree size parameter of the allometric equation (ϕ) was set to zero.

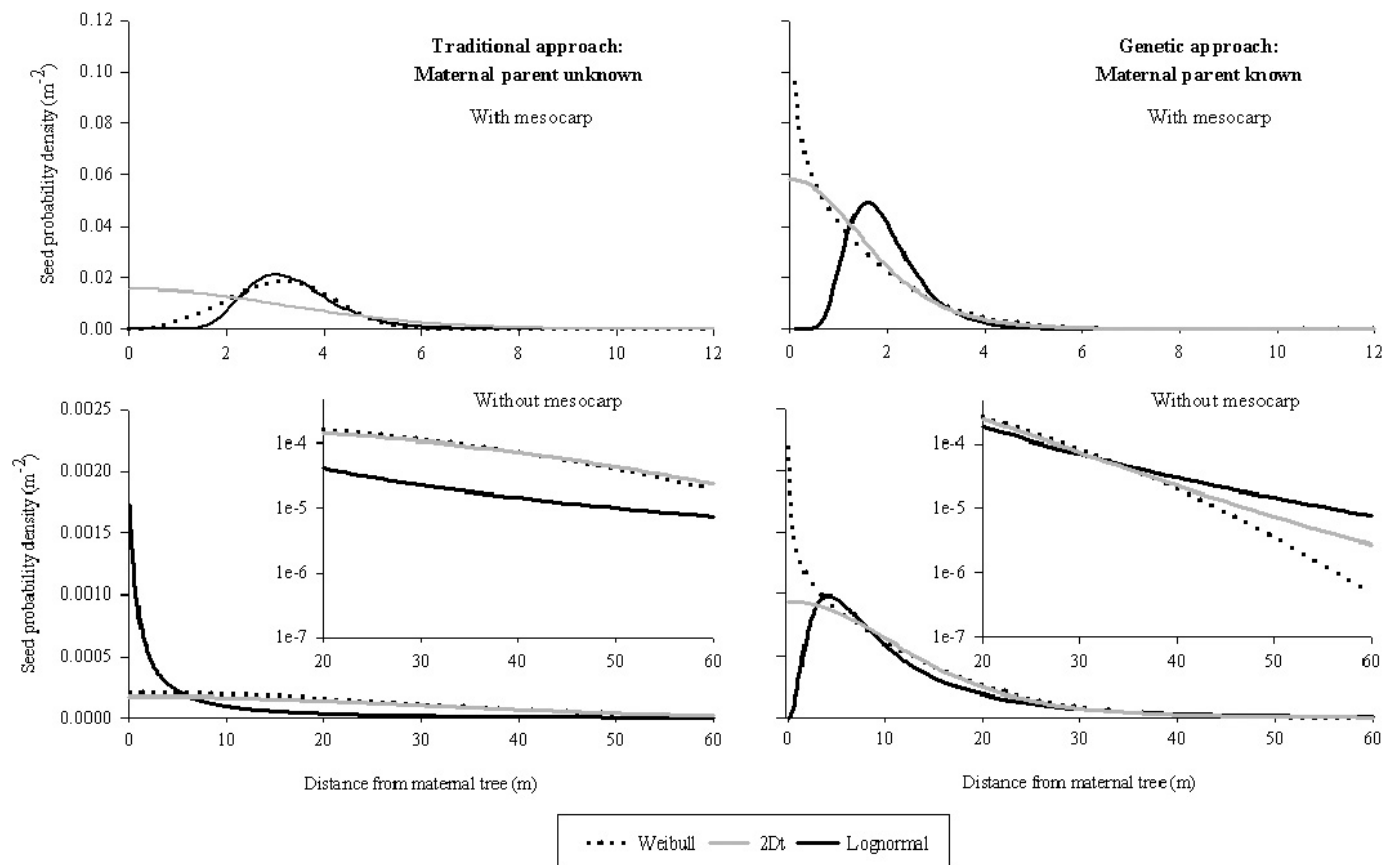
with the traditional approach and was different between the two approaches for the fruits without mesocarp. The curve for the fruits without mesocarp predicted a mode much farther away from the source and a wider tail, showing quite a high number of seeds falling farther than 45 m.

Discussion

Microsatellite markers were successfully used to directly assign a seed to its maternal parent in a black cherry population. Results showed that 83% of the seeds assigned to a parent in the plot had fallen within a 5 m radius of the mother tree (Fig. 2). These results are congruent with those of Hoppes (1988) and Smith (1975), who both concluded that the greatest proportion of the black cherry seeds are dispersed near the source in the species' native range. Indeed, 88% of the total number of seeds that Smith (1975) collected around a single adult were found within 5 m of that tree. Hoppes (1988) studied the seed fall pattern of *Prunus serotina* around three tree-fall gaps of different shapes and radius and found the median of dispersal distance to be 1 m away from the source. Similarly, Godoy and Jordano (2001) studied the dispersal of *Prunus mahaleb* and showed that most dispersal distances were short, within 10 m of the maternal source tree (median dispersal distance of 6.1 m).

If the vast majority of the seeds fall near the parent plant, a nonnegligible proportion of fruits is handled by frugivore birds and potentially dispersed. The pattern of distribution of these fruits was more complex, with a dispersal distance up to 30 m, and 33% of the seeds could not be assigned to a parent in the stand. Unfortunately, it was not possible to conclude whether the unassigned seeds originated from highly distant sources because our stand was embedded within a larger plot that contained other black cherry individuals. The dispersal distances observed can be compared with those

Fig. 3. Empirical dispersal models (probability density per square metre) computed for seeds of *Prunus serotina* collected in seed traps in the understorey of a pine plantation in Belgium. Models were fitted to two types of data, the first type using the traditional approach (maternal parent unknown) and the second type refined with the help of the molecular tools (maternal parent known). The graphs are given for both fruits with and without mesocarp.



found in the species' native range by Smith (1975), who found 0.4% of the seeds in traps at distances from 20 to 25 m. In his study, only 71% of the seed crop was accounted for at distances ranging from 0 to 25 m, and the author invoked longer bird flights and seed dispersion for over 25% of the crop. Similarly, Godoy and Jordano (2001) recorded that around 10% of seeds traveled farther than 100 m away for *Prunus mahaleb* trees, with some seeds traveling more than 3–5 km.

Production

The seed production function estimated well the production observed in the stand. However, the trunk circumference did not seem to be an accurate tree size parameter for predicting the production of fruits with mesocarp. This observation is in contrast with the findings of Jordano and Schupp (2000), who mentioned a significant effect of plant size in determining fruit production in *Prunus mahaleb*. However, flowering intensity depends highly on light availability, which varies between the edge of a stand and the forest interior, especially in shrub cover. Moreover, black cherry individuals that do not give the production that would be expected according to their crown size, age, and position in the stand have been observed in the native range (Marquis 1990), suggesting that other elements may be important in determining production.

The total mean observed seed production for a tree in the

stand was estimated to be around 6538 fruits. This value is in good correspondence with a production value of 6011 fruits found in the invasive region in France (D. Closset-Kopp, personal communication, 2005). However, these values are in contrast with the situation in the native region where Smith (1975) and Hoppes (1988) found a production of 2849 and 4000 fruits, respectively. Such high variations in individual fecundity have been found in *Prunus mahaleb* and can be caused by a variety of factors, including not only individual size differences, but also the intensity of flowering and the consistent interannual variation in fruit set (Jordano and Schupp 2000).

Model quality and comparison among the curves

As in Greene et al. (2004), the lognormal model was generally the best data descriptor of the three models. In all but one case, however, the magnitude of differences in log-likelihoods between the best and the worst models were subtle, indicating that all three kernels were roughly comparable as data descriptors. This is in accordance with Greene and Calogeropoulos (2002), who argued that any of these kernels would achieve a similar level of significance at short distances.

The curves of the three models differed in their shapes at very short distances, presenting a concave curve, a convex curve, or a mode away from the source. The difference was

particularly important at short distances (<2 m for fruits with mesocarp and <5 m for fruits without mesocarp), a finding that has already been mentioned in previous studies (Greene and Calogeropoulos 2002; Greene et al. 2004). In the present study, this finding can be explained by the relatively small amount of data at those distances and the intrinsic differences of the mathematical functions. The concave curves never showed the best correlations with the data, which is in agreement with the findings of Clark et al. (1999), who claimed that a concavity in the near shape is never observed for point sources. The lognormal curve generally differed from the other curves because it presented a maximum at nonzero distances. This shape seemed to be better supported by the data. We think that an increase in the density of fruits falling 1 or 2 m away from the source is possible but that a production close to zero near the trunk seems unlikely.

Because short- and long-distance dispersal can be associated with different dispersal mechanisms, we chose to model the dispersion of fruits with mesocarp differently from that of fruits without mesocarp. This approach had proven beneficial for understanding the two dispersal mechanisms involved. None of the dispersal kernels was indeed flexible enough to take into account the small proportion of seeds dispersed by birds, and the shape of the curves obtained for the total number of fruits were almost similar to those obtained for fruits with mesocarp (Fig. 2b).

Molecular markers and models

Molecular markers are known to represent a solution to the problem of deconstructing overlapping curves (Cain et al. 2000; Wang and Smith 2002; Nathan et al. 2003) and to provide a greater accuracy for interpreting patterns of seed dispersal (Shimatani 2004; Jones et al. 2005). In our study, they greatly helped us acquire a better understanding of the seed rain, mainly for fruits dispersed by birds. The traditional approach was indeed quite accurate in predicting the fate of the seed passively dispersed by gravity that mainly fell near the parent tree but less reliable for seeds dispersed by birds. The direct assignment of these seeds has indeed shown that a quite high number of them fall near the parent tree, suggesting that birds may stay perched in branches and eat and regurgitate the seeds almost where they had picked them up. The traditional approach did not reflect this tendency. However, because the experimental plot was not isolated from conspecifics, the study area was artificially reduced to 50 m for the genetic approach. Seeds originating from nearby trees situated outside the plot could not be taken into account, and 33% of unassigned seeds could not be included in the model. The differences in seed probability density observed between the two approaches at 40 m from the parents could therefore only be due to the sampling scheme and to the noninclusion of the real percentage of seeds originating from distant sources in the genetic model. None of the two approaches was therefore accurate for predicting seed dispersal at long distances, suggesting once again that caution should be taken before extrapolating dispersal kernels beyond the observed distances in any study. However, genetic approaches have more potential than nongenetic approaches for gaining insights into the dispersal shape at long distances, as new methods for estimating dispersal curves with the help of microsatellite markers in situ-

ation of nonisolated populations are being described. These methods, up to now mainly used for pollen dispersal studies (e.g., Sork et al. 1999; Smouse and Sork 2004), are not comparable anymore to the traditional inverse modeling approach. They are therefore beyond the scope of this study but show the high potential of microsatellites for characterizing dispersal curves.

Conclusion

Seed dispersal and its consequences in different systems is a complex process. Using highly variable microsatellite markers to assign seeds to their maternal parents greatly helped to check the accuracy of the models obtained by the traditional approach and improved the predictions. These molecular tools indeed supplied accurate data on individual dispersal events, bringing information on individual seed shadows, even in situation of high conspecific densities. Therefore, even if microsatellite markers may seem to be labor intensive, time consuming, and expensive, the insights they could bring into the true dispersal events and the potential information they hold are incomparable to any other nongenetic approach. The development of methods for modeling seed dispersal curves in nonisolated populations with microsatellites is certainly going to add important benefits to the technique, especially for studying both local and long-distance scales in complex and potentially large nonisolated populations.

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