Methods for studying earthworm dispersal

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Abstract

Dispersal is a key driver of species composition and functional traits in earthworm communities. However, it has been largely overlooked in ecological literature on earthworms because it is particularly difficult to study. In this publication, we review recent developments that have been made in this field of research. We present methods to assess dispersal distance, such as Capture-Mark-Recapture and molecular tools, and methods using dispersal corridors or X-ray imagery aiming at identifying the mechanisms triggering dispersal in earthworms communities.

Keywords

Movements, landscape structure, connectivity, behavior, capture mark recapture methods

Introduction

Dispersal plays a major role in shaping biodiversity, evolution, and ecosystem functioning. It connects localities together through fluxes of individuals and alleles. The direct consequence is that species abundance and genetic composition in different places of a landscape are not independent. In other words, local population abundance, genetic structure and community structure not only depend on local factors and processes such as habitat features, demography, genetic drift or species sorting and competition, but are also dependent on the properties of neighboring populations and communities (Leibold et al., 2004). In this perspective, we need to study local community and genetic structure at both local and regional scales in order to understand the structure of local populations or communities, as well as their functional role.

The magnitude of the dependence between local and regional scales directly results from dispersal rate. Theoretically, when dispersal is very high, local sites are well interconnected and tend to behave like a unique population or community (Economo and Keitt, 2008; Mouquet and Loreau, 2003). Local interactions are then a major driver of species composition and a low genetic differentiation among populations is expected. When dispersal is very low, local sites are isolated and behave like islands. Local populations and communities are well differentiated and severe genetic drift occurs. Extinction risk is then high for small populations. In nature, dispersal is generally between these two extremes, and complex behaviors such as source – sink dynamics may occur. In such case, certain sites can act as sources of dispersers (source), while others behave like sinks. Such dynamics can prevent extinction or speciation in certain sites. Local populations connected by dispersal are called "metapopulation" (Hanski and Gilpin, 1997), local communities connected by dispersal are called "metacommunity"(Leibold et al., 2004). In both cases, we need to understand the

45 drivers and the magnitude of dispersal in order to understand the behavior and the properties
46 of the system, either at local and regional scales.

47 Dispersal is usually defined as the movement of individuals away from their natal habitat, or
48 from their usual home range, to a new habitat (Clobert et al., 2012). It is usually decomposed
49 in three successive steps. First step is departure from the usual home range. Second is the
50 transfer between the departure site and the arrival site. Third is the establishment in a new
51 habitat (Fig. 1).

52

53 Figure 1

54

55 All these steps can originate from two very distinct processes: it can come from individuals'
56 own willing, a process called "active dispersal", or from movements driven by an external
57 force such as wind, water runoff, displacement by another animal, or by human activities
58 (Matthysen, 2012). Dispersal direction is generally controlled in active dispersal but not in
59 passive dispersal.

60 Studying dispersal is challenging for all organisms (Nathan, 2001), because it is hard to track
61 individuals. This is particularly true for earthworms, because they are subterranean and cannot
62 be seen from surface. Several approaches have been developed to address these difficulties,
63 and can be classified in two groups. The first ones focus on dispersal patterns. They intend to
64 measure typical dispersal distances of organisms during life span, or during a precise period
65 of time. This approach aims at producing a histogram of distances travelled over a period of
66 time, the so called "dispersal kernel" (Nathan et al., 2012). It is thus centered on the spatio-
67 temporal aspects of the dispersal. The second group of methods focuses on the factors that

drive dispersal, such as habitat quality or conspecific density. The products of this approach are dispersal rules, like positive density dependent dispersal. This approach is often framed in game theory and evolutionary ecology. It aims at predicting dispersal behavior and understanding the reasons why different dispersal behaviors evolved.

In this work, we present techniques that are currently available to study earthworms' dispersal from these two angles: 1) dispersal distances and 2) the factors that drive dispersal.

1. Methods for studying earthworm dispersal distance

One of the most basic question regarding dispersal of organisms is to determine how far they can move. In order to address this point, we need to estimate the distribution of the distances travelled for a given period of time. For this, two kinds of movements are often defined : the usual and most frequent movements, related to foraging, and the rare long distances movements (Nathan et al., 2008). In this framework, true dispersal usually refers to the rare and long distances dispersal events (LDD), located in the tail of the dispersal distribution(Nathan et al., 2012). These rare events play a critical role in colonization processes and invasion processes (Trakhtenbrot et al., 2005).

The estimation of traveling distances is challenging for earthworms, but can be done in two broad ways: by tagging and by studying the genetic profiles of individuals or populations.

1.1 Tags

Methods based on tags usually share the same principle: a group of individuals is captured, marked with a tag, then released at a known location in the field or in an experimental device. After a period of time, the marked individuals are searched at proximity from the release point. The distribution of the recapture distances from the release point (Fig. 3A) gives the

91 “dispersal kernel" of the individuals over the respective period of time. These methods are
92 generally referred to as Capture Mark Recapture (CMR) methods (Seber, 1982). They are
93 better suited for short distances estimation than for LDD. In theory, individuals can be
94 released and recaptured several times, but this is difficult to achieve in the field on
95 earthworms. Variations in CMR methods include the type of tags, the capacity to use tags
96 specific to individuals, and the number of recapture events. In earthworms, several types of
97 tags have been used so far. Early attempts used food colorants such as E102 + E132 (green) or
98 E124 (red) and red biological stains such as Safranin and Phloxine (Mazeaud, 1979). More
99 recently, $^{15}NH4+$ and U-^{13}C glucose (Dyckmans et al., 2005), Rubidium (Ben Hamou et al.,
100 2007), or ^{60}Co (Capowiez et al., 2001) have been used with success. However, all these
101 methods are difficult to apply because they require a significant equipment or lab work to
102 detect the tags, because they are not visible with naked eyes. We now present in detail two
103 recent methods that circumvent these caveats.

104 **1.1.1 Visual Tags for earthworm: VIE**

105 Visible Implant Elastomers (VIE, Fig. 2A&B and 3A) are colored tags that are injected below
106 the skin (Butt and Lowe, 2007; Gonzalez et al., 2006). They are injected in a liquid state but
107 soon become solid through a polymerization process. They are available from Northwest
108 Marine Technology, Inc (http://www.nmt.us/) and are relatively affordable.

109

110 Figure 2

111

112 This kind of tags has been used with success on a variety of organisms such as fish, frogs,
113 turtles and seeds, in order to assess population size or dispersal distance. They are visible

114 through the skin of the animals, particularly with a UV light. The tags are well supported by
115 earthworms (Butt et al., 2009). They can stay up to 27 months in their body, which is much
116 longer than the previously mentioned tags (Butt et al., 2009).

117

118 Figure 3

119

120 The main drawback of this method is the impossibility of monitoring individuals in a
121 continuous manner. It is not possible to track individuals between two capture events.
122 Marking and recapturing require a lot of time, and tags are hardly specific to individuals, even
123 though combining tags of different colors is possible for large individuals (>1.5g, Fig. 2B).
124 This method requires releasing a high number of marked animals for statistical reasons, which
125 can trigger density dependence dispersal (McCrea and Morgan, 2014). Indeed, in many
126 species, high level of conspecific density leads to active dispersal (Caro et al., 2013; Mathieu
127 et al., 2010). Finally, statistical methods for capture recapture data are complex (Amstrup et
128 al., 2005; Lee and Chao, 1994). Despite these difficulties, this approach is very useful to get
129 an estimation of the dispersal kernel of individuals.

130 **1.1.2 Electronic tags: RFID tags**

131 A new promising technique is the use of miniaturized RFID tags (Radio Frequency
132 IDentification. These tags (Fig.4) offer the possibility to mark each individual specifically,
133 with a unique barcode ID. A scanner (Fig. 4B) retrieves the ID of an individual when the tag
134 is close enough from the receptor, typically less than 0.5 cm.

135

136 Figure 4

137

138 Detecting individuals in a continuous fashion can be achieved by installing antennas at the
139 surface of the ground, which detect any individual with a tag that comes close to the antenna.
140 This offers the possibility to track individuals continuously and at different sites.

141 This method suffers from several limitations that still impede its use on a regular base. First,
142 RIFD tags are more harmful than VIE tags. They are bigger (1.4 x 8mm) and are frequently
143 ejected out of the body by earthworms. During preliminary tests, only larger individuals,
144 typically >1g, supported them. Second, detection can only be done at very short distance from
145 the antenna (<0.5cm), and cannot be done through the soil. Even if the antenna can be buried,
146 it is much more efficient to detect individuals on the surface. Thus, this technique is better
147 suited for large epigeic and anecic species, which crawl on surface, but not for endogeic ones.
148 Third, the tags are completely invisible once injected, which makes it difficult to find their
149 location in the body during manual scanning, or to determine if an individual is tagged or not.
150 A VIE can be injected in addition, close to the RIFD tag, however this increases the risk of
151 mortality. At the moment, miniaturized RFID can only be detected by the scanners provided
152 by the RFID manufacturers, which are expensive and have a limited efficiency. However, in
153 the future it should be possible to build its own system of scanners or antenna and data logger.

154 **1.2 Molecular approach**

155 Recent progresses in molecular biology offer new opportunities for the estimation of dispersal
156 distance of earthworms from genetic data (Dupont, 2009; Torres-Leguizamon et al., 2012;
157 Zeller et al., 2012). As dispersal leads to gene flow, genetic information can be used to infer
158 dispersal patterns. They can be assessed in two ways: first by comparing observed

159 populations' genetic structure to theoretical ones under no dispersal, and second by statistical
160 assignment methods that allow identifying the parents or the population of origin of
161 individuals, based on their genetic profile (Broquet and Petit, 2009). In addition, the effect of
162 landscape structure on dispersal patterns can be assessed with the tools developed within the
163 framework of landscape genetics (Manel et al., 2003). The principle is to determine if there is
164 any relationship between the spatial patterns of populations' genetic structure, or individuals'
165 genetic profile, and landscape features. This approach was applied at the plot scale (100x50m)
166 on the earthworm *Allolobophora chlorotica* (Dupont et al., 2015). It suggested that soil
167 properties influence dispersal in the field, and estimated that gene flow occurs at
168 approximately 7 m.year-1. At the landscape level, it showed the role of landscape features on
169 the dispersal of two earthworm species, *A. chlorotica* and *Aporrectodea icterica* (Dupont et
170 al., 2017).

171

172 Figure 5

173

174 The genetic approach is based on the variation of highly variable DNA sequences (i.e.
175 molecular markers) which are selectively neutral (i.e. the variation is not selected by the
176 environment). Microsatellites, Amplified fragment length polymorphisms (AFLPs), and
177 single nucleotide polymorphisms (SNPs) are typical markers used for this kind of study
178 (Dupont et al., 2011; Torres-Leguizamon et al., 2012). Because population genetic models are
179 dedicated to diploid (2N) species, this kind of approach can hardly be used for studying
180 dispersal of earthworm species with a ploidy level different to 2N. Once the molecular data
181 are acquired, they can be matched with landscape data in two ways. First, they can be
182 compared to landscape structure in the neighborhood of the sampling sites (Fig. 5). For this,

183 an area (a "buffer") is defined around each sampling site, and the relevant features of
184 landscape structure are described with the help of a GIS. For instance, the distance to the
185 nearest forest, the percentage of crops or the length of roads can be computed (McGarigal and
186 Marks, 1995). The result of this process is a table describing different landscape features for
187 each sampling site. These features can then be matched with genetic descriptors of
188 populations, such as allelic richness and heterozygote frequency, using, for instance,
189 traditional regression techniques. The second way to proceed is through connectivity analysis.
190 In this case, the landscape is considered in its totality, not only around a certain radius of each
191 sampling site. In this approach, all sites are compared by pairs, both in terms of genetic
192 difference and geographical distance (Fig. 5). This is done by correlating the geographical
193 distance between pairs of sites to their genetic distance. If there is any significant relationship
194 between the two matrixes, it suggests that landscape feature, and thus dispersal, has an
195 influence on spatial genetic structure. Geographical distance is calculated by estimating the
196 cumulated costs of movements between each pairs of sites. For this, specific costs are
197 attributed to the different elements of the landscape. The product of this step is a "resistance
198 map". This map is used to calculate the cumulative traveling costs between each pair of sites,
199 using different algorithms of movements. Finally, either the cost of the shortest path, or the
200 weighted mean of the different paths, is used to estimate the distance between all pairs of sites
201 (McRae et al., 2008). The final product of this step is a pairwise squared distance matrices
202 giving the traveling costs between pairs of sites. This process can be realized with different
203 scenario of costs. By comparing different scenarios of costs, this technique allows identifying
204 which elements are most likely to play a role on dispersal, either by facilitating it (corridors)
205 or by impeding it (barriers) (Dupont et al., 2017). In order to match the distance matrix with
206 genetic features, the genetic differentiation between all sampling sites must also be estimated.
207 This can be done through different indices, but the most widespread is the fixation index Fst

208 (Wright, 1951). The result of this step is a pairwise squared dissimilarity matrix between sites.
209 In order to correlate the matrix of genetic differentiation to the matrixes of traveling costs,
210 specific statistical techniques must be used, because the same sites are involved in several
211 comparisons, and there is non independency in the residuals. The historical technique used for
212 this was Mantel test, but it has been criticized and the recommend test is now MLPE
213 (Maximum Likelihood Population Effect). This technique identifies the scenario of costs that
214 match with differences in genetic structure (Van Strien et al., 2012)

215

216

217 **2. Methods for studying mechanisms of earthworm dispersal**

218 Another important aspect in the study of dispersal is to determine the conditions that drive
219 individuals to disperse. These drivers can be internal, such as body size, hormonal status and
220 age, or external, such as habitat quality or biotic interactions (Clobert et al., 2012). In
221 earthworms, only external drivers of dispersal have been studied so far. Two techniques are
222 now well established: the dispersal corridors (Mathieu et al., 2010), and the X ray imagery
223 (Caro et al., 2012).

224 **2.1 Dispersal corridors**

225 The principle of dispersal corridors is to expose individuals to a stimuli, to wait a certain
226 amount of time, and then to compare the movements of individuals in the stimuli treatment to
227 the control (Clobert et al., 2012). In this kind of experiment, it is necessary to be able to make
228 the difference between active dispersal and random diffusion. Active dispersal results from a
229 decision to leave a patch, without knowing in advance where to go, and what type of
230 environment will be available after leaving the initial patch. Random diffusion results from

usual movements, such as foraging, that do not imply a decision to leave. To be able to make the distinction between active dispersal and random diffusion, a specific experimental setup must be used (Fig. 6).

Figure 6

The experimental device is elongated and separated in three parts: 1) the “departure section”; 2) the “crossing section”; 3) the “settlement section”. The “departure section” is the section where the stimuli or the control treatment is implemented, and where the earthworms are inoculated. The treatment can consist for example in soil quality, litter quality, conspecific density, or interspecific density. The crossing section must be an unhospitable soil, which individuals would cross only if the conditions in the departure section are really unhospitable. This is the key point to make the distinction between random diffusion and active dispersal. The “settlement section” must contain a very hospitable soil, which individuals will not leave once they reached it. In top of that, it is required to put litter on the surface of the departure and of the settlement zones, even for endogeic species. After a fixed period of time after inoculation, the soil and the litter of the tree sections are separated and the number of earthworms in each section is retrieved. The percentage of dispersal is compared between control and stimuli treatment with a logistic model (Caro et al., 2013).

1.3 X ray imagery

Another promising tool is X ray imagery. This method takes advantage of recent progress in imagery machines. It consists in using X ray imaging device such as Philips Diagnostic C

254 generator to scan the soil (Fig. 7). This kind of device is capable of differentiating earthworms
255 and their gallery in the soil, if the soil is less than 6 cm thick. The soil must not be too mineral
256 otherwise X ray cannot penetrate. Adding soft material, such as peat, greatly increase image
257 quality. Introducing by incision a small metal tag (1mm) below the skin of individuals can
258 also help to localize them on the X ray shots. With this kind of setup, it is possible to
259 determine in real time the location of individuals in the ground (Caro et al., 2012), to tell if it
260 is located inside or outside a gallery, and to measure its movement speed [VIDEO].

261

262 Figure 7

263

264 **Conclusion**

265 The several techniques that now exist to study earthworm dispersal (Table 1) can give us
266 insights about dispersal distances and mechanisms leading to active dispersal, either in the
267 field or in experimental devices. They offer new opportunities to quantify this process, and to
268 estimate its contribution to population dynamics, community assembly, and finally ecosystem
269 functioning.

270

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362 **Figure captions**

363 Fig. 1 - Dispersal is usually decomposed in three steps: Emigration from the source, transfer,
364 and immigration to a new site.

365 Fig. 2 - A/ Tagging of a small *Allolobophora chlorotica* B/ An individual of *Aporrectodea*
366 *longa* tagged with two yellow bands.

367 Fig. 3 - Summary of the steps required to study earthworm dispersal with tags.

368 Fig. 4 - A/ Picture of a RFID tag ready to be injected. B/ The scanner recognizes a tagged
369 *Allolobophora chlorotica* individual.

370 Fig. 5 - Overview of the workflow to perform landscape genetics on earthworm.

371 Fig. 6 - Example of a dispersal corridors with its three sections. The triangle indicates the
372 inoculation point of earthworms.

373 Fig. 7 - Experimental setup to take X ray shots of earthworms in the soil.

374

375

376 Table 1 Comparison of the methods currently available for studying earthworm dispersal.

377

Method	Focus on dispersal	Type of method	Estimation of dispersal	Type of dispersal	Spatial extent †	Place	Cost	Computing complexity ‡	Time required ‡	References §
Landscape genetics	Distance	Molecular	indirect	passive	++ to +++	Field	$$$$	+++	+++	1)
VIE Tags	Distance	Capture Mark Recapture	direct	active	+ to ++	Field & Lab	$$	++	++	2)
RFID Tags	Distance	Capture Mark Recapture	direct	active	+ to ++	Field & Lab	$$$	++	++	3)
Dispersal corridors	Mechanisms	Experimental	direct	active	+	Lab	$	+	+	4)
X rays	Mechanisms	Experimental	direct	active	+	Lab	$$	+	+	5)

378

379 †: + = small extent, +++ = large extent, ‡: + = least, +++ = most, § References : 1) Dupont, L. and al ., 2017. Mol Ecol 26, 3128-3140. 2) Butt, K.R., Lowe, C.N.,
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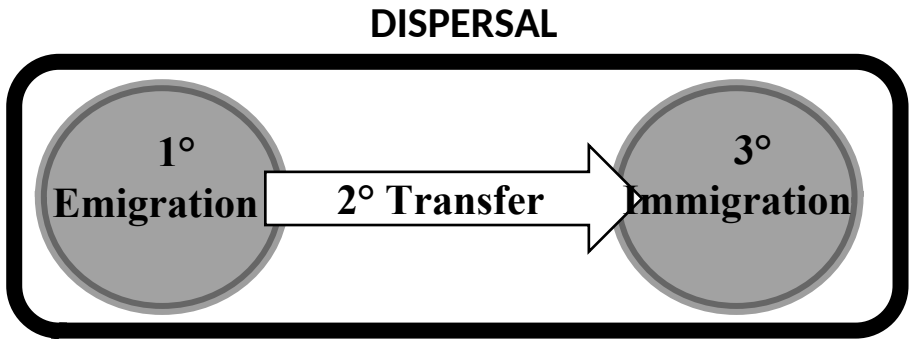
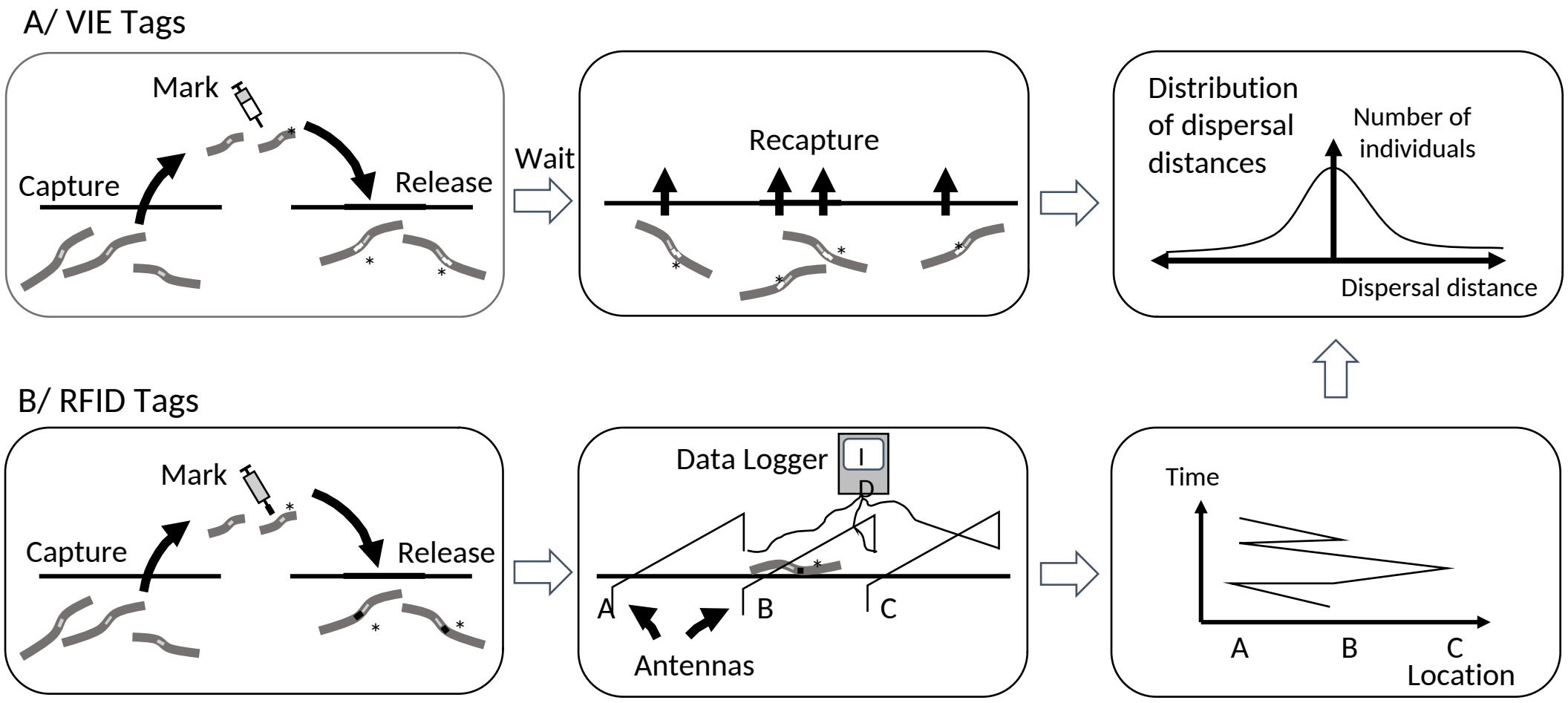
382


DISPERSAL
1°
Emigration
2° Transfer
3°
Immigration


A


B


A/ VIE Tags
Mark
Capture
Release
Wait
Recapture
Distribution of dispersal distances
Number of individuals
Dispersal distance
B/ RFID Tags
Mark
Capture
Release
Data Logger
A
B
C
Antennas
Time
A
B
C
Location


A


Bluetooth
ID:
1159369542009473
B
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Earthworm sampling

Molecular analysis

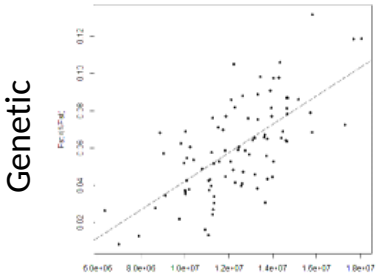
Genetic structure of populations
Or
Genetic profile of individuals

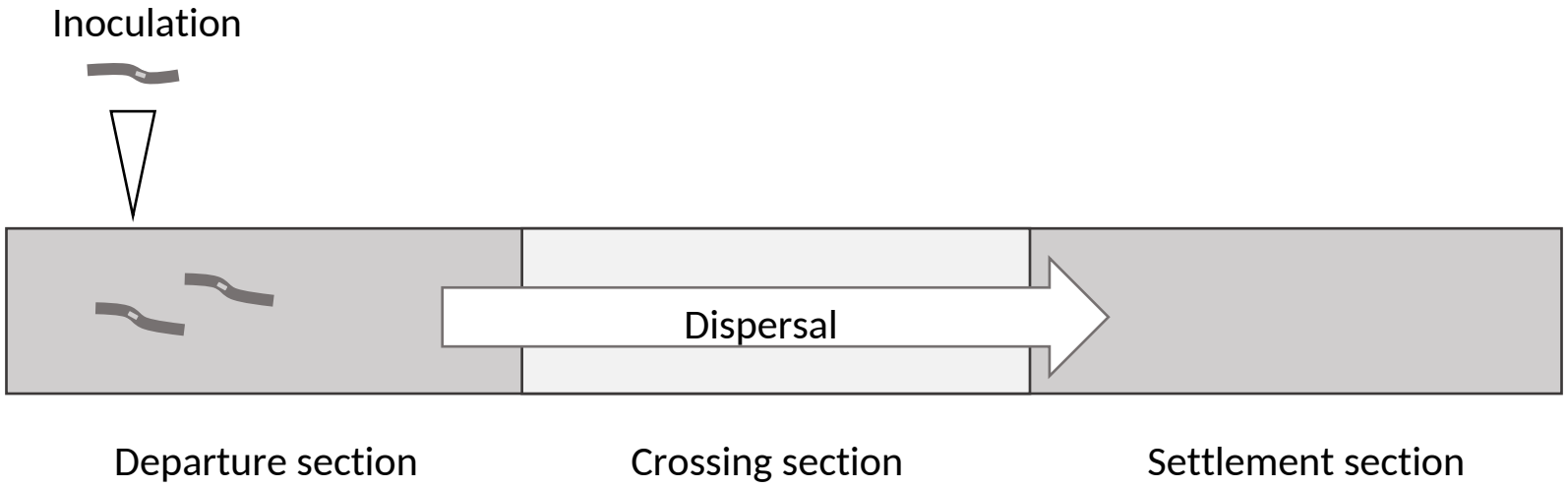
Landscape Analysis

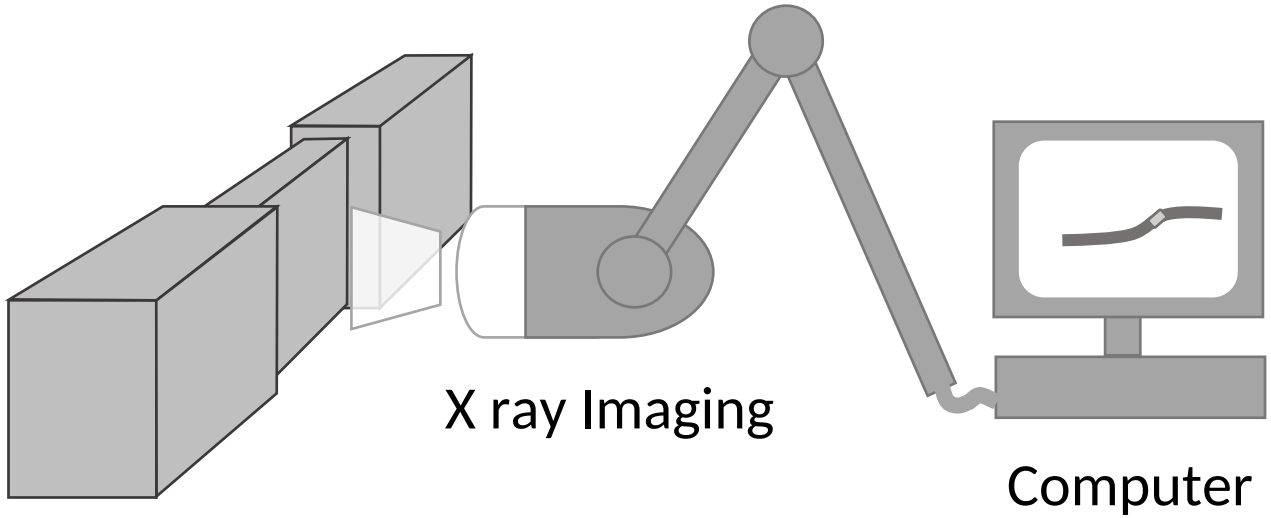
Landscape structure

Landscape connectivity

Statistical analysis




Inoculation
Dispersal
Departure section
Crossing section
Settlement section


X ray Imaging
Computer
Experimental device