

1 **Apparent negative density-dependent dispersal in tsetse (*Glossina* spp)**
2 **is an artefact of inappropriate analysis**

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23 **Abstract**

24 Analysis of genetic material from field-collected tsetse (*Glossina* spp) in ten study areas has
25 been used to predict that the distance (δ) dispersed per generation increases as effective population
26 densities (D_e) decrease, displaying negative density dependent dispersal (NDDD). This result is an
27 artefact arising primarily from errors in estimates of S , the area occupied by a subpopulation, and
28 thereby in D_e , the effective subpopulation density. The fundamental, dangerously misleading, error
29 lies in the assumption that S can be estimated as the area (\hat{S}) regarded as being covered by traps.
30 Errors in the estimates of δ are magnified because variation in estimates of S is greater than for all
31 other variables measured, and accounts for the greatest proportion of variation in δ . The errors
32 result in anomalously high correlations between δ and S , and the appearance of NDDD, with a slope
33 of -0.5 for the regressions of $\log(\hat{\delta})$ on $\log(\hat{D}_e)$, even in simulations where dispersal has been set as
34 density independent. A complementary mathematical analysis confirms these findings. Improved
35 error estimates for the crucial parameter b , the rate of increase in genetic distance with increasing
36 geographic separation, suggest that three of the study areas should have been excluded because b is
37 not significantly greater than zero. Errors in census population estimates result from a fundamental
38 misunderstanding of the relationship between trap placement and expected tsetse catch. These
39 errors are exacerbated through failure to adjust for variations in trapping intensity, trap
40 performance, and in capture probabilities between geographical situations and between tsetse
41 species. Claims of support in the literature for NDDD are spurious. There is no suggested
42 explanation for how NDDD might have evolved. We reject the NDDD hypothesis and caution that
43 the idea should not be allowed to influence policy on tsetse and trypanosomiasis control.

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47 **Author summary**

48 Genetic analysis of field-sampled tsetse (*Glossina* spp) has been used to suggest that, as
49 tsetse population densities decrease, rates of dispersal increase – displaying negative density
50 dependent dispersal (NDDD). It is further suggested that NDDD might apply to all tsetse species
51 and that, consequently, tsetse control operations might unleash enhanced invasion of areas cleared
52 of tsetse, prejudicing the long-term success of control campaigns. We demonstrate that NDDD in
53 tsetse is an artefact consequent on multiple errors of analysis and interpretation. The most serious of
54 these errors stems from a fundamental misunderstanding of the way in which traps sample tsetse,
55 resulting in huge errors in estimates of the areas sampled by the traps, and occupied by the
56 subpopulations being sampled. Errors in census population estimates are made worse through
57 failure to adjust for variations in trapping intensity, trap performance, and in capture probabilities
58 between geographical situations, and between tsetse species. The errors result in the appearance of
59 NDDD, even in modelling situations where rates of dispersal are expressly assumed independent of
60 population density. We reject the NDDD hypothesis and caution that the idea should not be allowed
61 to influence policy on tsetse and trypanosomiasis control.

62 **Introduction**

63 Using critical assumptions about gene flow, and a model developed by Rousset (1), de
64 Meeûs et al. (2) (henceforth DM) claimed that dispersal distance per generation, in tsetse flies
65 (*Glossina* spp), increases as a power function of decreasing population density. The study was
66 based on genetic analysis of material from five different species of tsetse, captured in ten studies in
67 six different countries in West and East Africa (3–11). The authors concluded that negative density-
68 dependent dispersal (NDDD) probably applied to all tsetse species. They predicted that mean
69 dispersal distance (δ) would increase by 200-fold when the effective population density (D_e) of
70 adult tsetse decreased from about 24,000 to 1 per square kilometre, the order of density decline
71 commonly associated with tsetse control operations (12). This led DM to warn that such control
72 measures could unleash enhanced invasion of areas cleared of tsetse, so prejudicing the long-term
73 success of tsetse control campaigns. That in turn prompted them to suggest the necessity of using
74 "area-wide and/or sequential treatments of neighboring sites" to counter the added invasion, and
75 they implied that small control operations risk doing more harm than good. The term "area-wide"
76 applied to campaigns is jargon for the eradication of whole infestations up to natural barriers to
77 reinvasion and is commonly associated with recommendations for the costly and complex use of the
78 sterile insect technique (13). It would be dismaying if area-wide control were indeed necessary,
79 since small-scale operations run by local communities offer an economically viable way forward
80 (14–16).

81 Much therefore rests on the DM notion of NDDD and the idea thus merits prompt and
82 thorough scrutiny. Modelling studies have already shown that, even if the NDDD hypothesis were
83 correct, the threats to tsetse control are greatly exaggerated (17). It remains to show, however,
84 whether there is any valid evidence that NDDD in tsetse actually exists at all. For that purpose, we
85 now dissect the evidence adduced by DM. We show that the methods that they used to estimate
86 their parameters are subject to massive errors, and that such errors create the illusion of NDDD,
87 even in simulated populations where NDDD has been specifically proscribed.

88 **Methods**

89 First, we identify the DM procedure and the variables that it involves. Then, employing the
90 values that DM actually use for these variables, kindly provided by Dr Thierry de Meeûs
91 (Supplementary File S1, Table S1), we expose anomalous relationships between them. We show
92 that these are due to the cardinal and erroneous importance that the DM procedure gives to trap
93 placement in the study areas, aggravated by subjective aspects of the way that such placements are
94 viewed. By using simulations in Excel spreadsheets, we show that the errors in the DM procedure
95 lead to the illusion of NDDD, even in simulations which specify that dispersal rates are independent
96 of density.

97 The above considerations are all that need be offered to the generality of entomologists and
98 tsetse control officers in order to invalidate completely the DM evidence for NDDD in tsetse, and
99 thus to dispel the anxieties that DM have created. However, in the hope of interesting the specialist
100 students of ecology, genetics and statistics, we expose many other problems in the DM arguments.
101 We stress that we make no explicit or implicit criticism of the model of Rousset (1997), nor of the
102 genetic analyses in any of the studies, which generated the data used by DM. We simply enumerate
103 the errors, inconsistencies and illusions that arise from the way in which DM have used and
104 interpreted the Rousset model and the genetic data.

105

106 **Results**

107 **The DM procedure and its variables**

108 DM build the idea of NDDD around their Equation (1):

$$109 \quad \delta \approx \frac{1}{\sqrt{\pi b D_e}} = \frac{\sqrt{S}}{\sqrt{\pi b N_e}} \quad (1)$$

110 where δ is the predicted dispersal distance per generation of a tsetse population, N_e is the effective
111 population size, roughly defined as the number of adults in a population that will leave a genetic
112 signature to the next generation, S is the surface area occupied by the effective population, and b is

113 the slope of the linear regression of the genetic distance between subpopulations plotted on the log-
114 transformed geographic distance between those subpopulations. D_e is the effective population
115 density, equal to N_e/S . We will show that using Equation (1) in this way is inappropriate and prone
116 to finding spurious negative correlations between the estimated values of \hat{D}_e and $\hat{\delta}$. We represent
117 estimated, as opposed to true, values of the variables by \hat{S} , \hat{N}_e , \hat{D}_e , \hat{b} and $\hat{\delta}$.

118 DM did not measure $\hat{\delta}$: they simply predicted it from Equation (1), using estimates \hat{b} and \hat{N}_e
119 obtained from genetic analyses, and estimates \hat{S} of the area occupied by the population, calculated
120 from the disposition of the traps that sampled the population. We investigated the contributions of
121 various factors to the value of $\log(\hat{\delta})$ by taking base-10 logarithms of both sides of Equation (1) to
122 get:

$$123 \quad \log(\hat{\delta}) \approx -0.5 \log(\pi) + 0.5 \log(\hat{S}) - 0.5 \log(\hat{N}_e) - 0.5 \log(\hat{b}) \quad (2)$$

124 This allowed us to plot the relationships between various constituents of $\log(\hat{\delta})$ in DM's data (Fig 1,
125 Row A). The primary result found by DM was a strong linear correlation ($R^2 = 0.85$; $P < 0.01$)
126 between $\log(\hat{\delta})$ and $\log(\hat{D}_e)$ for the 10 populations used in the DM study (Fig 1, A1). DM also
127 found a strong negative correlation ($R^2 = 0.86$; $P < 0.02$) between their predicted values of $\log(\hat{\delta})$ and
128 the estimated census population densities ($\hat{D}_c = \hat{N}_e/\hat{S}$), with \hat{N}_e estimated from trap catches. These
129 relationships form the central structure of the DM argument for NDDD. Notice that $\log(\hat{b})$ is not
130 significantly correlated with $\log(\hat{D}_e)$ (Fig 1, A3; $P > 0.05$), while $\log(\hat{N}_e)$ is weakly correlated with
131 $\log(\hat{D}_e)$ (Fig 1, A5), and not significantly correlated with $\log(\hat{S})$ (Fig 1, A4). Surprisingly, $\log(\hat{\delta})$ is
132 strongly correlated with $\log(\hat{S})$ (Fig 1, A2), and $\log(\hat{S})$ is strongly negatively correlated with $\log(\hat{D}_e)$
133 (Fig 1, A6).

134

135 **Fig 1. Relationships between various parameters in DM Equation (1) in real and simulated**
136 **experiments.** Row A: Relationships using DM estimates. Row B: Simulation using DM estimates
137 for \hat{N}_e and \hat{b} from the Opiro et al. (2017) study (11) and various values of \hat{S} spanning the range for
138 the 10 studies used by DM. Random error added to \hat{N}_e and \hat{b} . Row C: As for B but \hat{N}_e and \hat{b}

139 measured without error. Data kindly supplied by Dr Thierry de Meeûs and available here in
140 Supplementary File S1, Table S1.

141

142 **Errors in the estimation of S , the true area occupied by a subpopulation**

143 There is no biological reason to expect the high correlation between $\log(\hat{D}_e)$ and $\log(\hat{S})$ nor
144 between $\log(\hat{\delta})$ and $\log(\hat{S})$. The fact that strong correlations were found, suggests that variation in \hat{D}
145 $_e$ in DM's data is mostly driven by changes in the estimated area (\hat{S}) covered by traps, rather than
146 by true changes in D_e . We investigated the importance of S in determining the δ value predicted by
147 Equation (2), relative to contributions from other terms. Since the equation has the form of a
148 multiple linear regression, we borrowed a tool from regression to estimate, for the DM data, the
149 relative importance of each of the predictor variables, $\log(N_e)$, $\log(\hat{S})$, and $\log(b)$, in determining the
150 predicted value of $\log(\hat{\delta})$.

151 We measure “relative importance” by the percentage of the total variance in $\log(\hat{\delta})$ that is
152 explained by variation in each of the three predictor variables. Since these three variables are inter-
153 correlated across the ten DM studies, we used hierarchical partitioning (18–20) to estimate their
154 relative importance. In this approach, one of the variables, say $\log(\hat{S})$, is added to each of the four
155 possible regression models that contain neither, either, or both of the other two predictors. In each
156 case, the increase in multiple R^2 due to the addition of $\log(\hat{S})$ is recorded, and the average of the
157 four increases is the estimated proportion of total variance in $\log(\hat{\delta})$ that is explained by $\log(\hat{S})$. The
158 procedure is then repeated for the other two predictors.

159 Because $\log(N_e)$, $\log(\hat{S})$, and $\log(b)$ were used to predict $\log(\hat{\delta})$ in the first place, via
160 Equation (2), they jointly explain 100% of its total variance. Application of hierarchical partitioning
161 to the DM data, using either the *hier.part* or *relaimpo* package of the R language, yielded the
162 following percentages for the three predictors: $\log(N_e) = 25\%$, $\log(\hat{S}) = 65\%$, and $\log(b) = 10\%$.
163 Thus, the variation in $\log(\hat{S})$ explained almost twice as much of the variation in $\log(\hat{\delta})$ as did the
164 other two variables combined.

165 The central importance of S in accounting for variation in the dispersal rate (δ) leads us
166 immediately to the main problem with the DM development, embodied in their statement (2): “The
167 average surface (S) occupied by a subpopulation can be computed as the surface area occupied by
168 the different traps used in a given survey site. When only one trap was available per site or when the
169 GPS coordinates of corresponding traps (one subsample) were not available, we computed $S =$
170 $\pi(D_{min}/2)^2$ where D_{min} is the distance between the two closest sites taken as the distance between the
171 centers of two neighboring subpopulations”.

172 This statement is untrue and dangerously misleading. The true area (S) covered by a
173 biological subpopulation bears no relation to the area (\hat{S}) estimated to be covered by a set of traps.
174 Assuming a subpopulation is well-mixed with random mating and no intra-population genetic
175 structure (as is assumed by all methods for estimating N_e), then N_e can be estimated by catching
176 flies from any area smaller than the geographic range of that subpopulation.

177 Fig 2 shows three different possibilities for the trap-sampling areas ($\hat{S}_1 - \hat{S}_3$) in relation to the
178 true area (S_t) occupied by the actual, well-mixed subpopulation. Sampling flies from within either \hat{S}
179 $_1$ or \hat{S}_2 will produce the same expected value of the estimate \hat{N}_e , of the true value of N_e , but will use
180 different values of \hat{S} , which is the denominator for calculating \hat{D}_e . Thus, the estimated population
181 density will vary according to the area covered by the traps, introducing error into the \hat{D}_e estimates.

182
183 **Fig 2. Sampled and true subpopulation areas.** Illustration of three different possible sampling
184 areas ($\hat{S}_1 - \hat{S}_3$) relative to the true area (S_t) covered by a subpopulation.

185
186 If \hat{S}_3 is used (covering an area larger than the size of what can be considered a well-mixed
187 subpopulation), then the assumptions underlying the estimates of \hat{N}_e will be violated, and the
188 estimates of \hat{N}_e will be flawed, so introducing yet more error to \hat{D}_e . The extent to which these
189 erroneous estimates of \hat{N}_e will scale with \hat{S} – as it grows above the size of S – is not entirely clear,
190 but previous work suggests that it will not scale linearly (21) and will thus continue to produce

191 incorrect values of \hat{D}_e that are a function of trap placement. Correct values of \hat{D}_e can be obtained
192 only in the special case where $S = S_t$. Thus, in DM's data, variation in \hat{D}_e is due largely to the choice
193 of trap placement rather than true biological variation in N_e .

194

195 **How errors in \hat{S} invalidate estimates of the dispersal distance $\hat{\delta}$**

196 Some simple matters emerging from the DM paper allow us to check the validity of its
197 estimates of population densities and dispersal distances. The most important of these is that, for
198 each of the 10 populations used by DM, it is implicitly assumed that there is a single true value of δ
199 that is roughly applicable throughout the entire area occupied by the population. Thus it should be
200 true that, if investigators deployed traps in different patterns within the same population, but still
201 followed the DM rules for estimating S in each case, the resulting estimates of δ should be the same,
202 regardless of the trap distribution adopted – subject only to experimental errors in estimating b and
203 N_e . We now show that this expectation is grossly violated, even in situations where b and N_e are
204 measured without error.

205

206 **In-depth analyses of the studies in Tanzania and Uganda.** To see how such problems arise,
207 consider the DM analysis of the *G. pallidipes* data for Tanzania (7). The original report states that
208 sampling of tsetse was carried out using two traps at each of seven sites. Because GPS coordinates
209 were apparently not available for these traps, DM analysed the data as if only one trap was used at
210 each site. Given the definition provided above, and $D_{min} \approx 6.7$ km, they computed $\hat{S} = \pi(D_{min}/2)^2 =$
211 34.87 km². Using their estimated values of $\hat{b} = 0.0168$ and $\hat{N}_e = 44$, DM calculated a value of $\hat{\delta} =$
212 3875 m per generation from Equation (1), the highest estimate in any of the studies they cited.

213 As noted above, however, there were actually two traps at each site, not one. Suppose that
214 GPS coordinates were available for the traps. Then it would be logical to use DM's alternative
215 definition for estimating S : "For all analyses, when more than one trap was available in a site, the
216 surface area of the site was computed as $S = \pi(D_{max})^2$ where D_{max} is the distance between the two

217 most distant traps in a given site, taken as the radius of the corresponding subpopulation.” If they
218 had done that, they would have found that $D_{max} = 0.1$ km, resulting in a value of $\hat{S} = \pi(D_{max})^2 \approx$
219 0.031 km², differing from the DM estimate of \hat{S} by a factor of more than 1000. Notice that,
220 whichever definition of trap spacing was used, the same flies would have provided the genetic and
221 geographic information employed in the DM estimates of \hat{b} and \hat{N}_e , which would thus have been
222 identical in each case. Accordingly, the revised estimates of effective population density is $\hat{D}_e \approx$
223 $44/0.031 = 1401$ tsetse per km², and dispersal distance is $\delta \approx \sqrt{0.031}/\sqrt{\pi \times 0.0168 \times 44} = 116$ m,
224 differing from the DM estimate of δ by a factor of 33.4.

225 This analysis suggests that the estimates DM obtained for δ , in all of their studies, were
226 strongly dependent on the spacings of the traps used to sample the population, and also on the
227 decision about how to interpret those spacings. Alternatively, if it is objected that – by regarding the
228 trap deployment as either one, or two, traps per site – one is measuring the movement rates in two
229 different subpopulations, we would be forced to conclude that values of δ could differ by orders of
230 magnitude between subpopulations of the same population. Either scenario is sufficient to
231 undermine completely the basis of the DM analysis.

232 The foregoing objections to the DM analysis do not, of themselves, explicitly negate the
233 NDDD hypothesis as it applies to tsetse. This hypothesis is, however, seen to be similarly
234 compromised by analysis of data from another of the studies used in the DM analysis. In a study of
235 *G. fuscipes fuscipes* in Uganda, six traps were deployed at each of 42 sites, spread across an area of
236 about 4000 km² (11). Traps at each site were separated by a distance of at least 100 m. For this trap
237 spacing, DM calculated $\hat{S} = 0.02$ km², using $\hat{S} = \pi(D_{max})^2$ (see above). Using finite estimates of \hat{N}_e ,
238 from 30 of the 42 sites, DM calculate an arithmetic mean of $\hat{N}_e = 425$ flies for the effective
239 population (Fig 3). The other 12 sites did not provide finite estimates of \hat{N}_e . Similarly, they
240 estimated $\hat{b} = 0.0202$ using information on genetic and geographic distances between all available
241 sites. Using the above estimates for \hat{S} , \hat{b} and \hat{N}_e , they calculated $\hat{\delta} = 27$ metres per generation, the
242 lowest among all of the 10 studies cited by DM, and the one where the estimated effective

243 population density, \hat{D}_e , was the second highest. We now show that these estimates are also artefacts
244 of the way in which the traps were deployed, and the way that DM chose to analyse the data.

245

246 **Fig 3. Distribution of estimates of \hat{N}_e from the Opiro et al. (2017) study (11).** Of the 42 study
247 sites, 30 provided finite estimates of \hat{N}_e , using the LD method.

248

249 To demonstrate the problem, suppose we make 10 separate estimates of the DM parameters,
250 distributing traps throughout the roughly 4000 km² in the study area (11) – with different trap
251 deployment patterns for each of the 10 estimating procedures. For five of these procedures, suppose
252 that – as in the original design – six traps are deployed at each site but that r , and thus D_{max} , is
253 varied such that $\hat{S} = \pi D_{max}^2$ takes values of 0.02, 0.08, 0.32, 0.64 and 2.56 km², respectively. For
254 the other five procedures, larger values of \hat{S} are generated by using only one trap per site, and
255 making the shortest distance (D_{min}) between any two sites take values of 2, 6, 10, 14 and 20 km,
256 respectively, giving values of $\hat{S} = \pi (D_{min}/2)^2$ ranging from 3 to 314 km². Taken together, the above
257 design provides 10 different sampling systems – all of which are capturing flies throughout the
258 4000 km² study area, and all of which should thus produce estimates of \hat{b} and \hat{N}_e with the same
259 expected values.

260 We consider two different scenarios, where b and N_e are measured either with, or without,
261 added stochastic error. The procedure for analysing these situations is described in Supplementary
262 File S2W, and the calculations are carried out as in Supplementary File S2E. The results for a single
263 realisation of the stochastic process are shown in Fig 1, Rows B and C. For each of the 10 different
264 trap deployments, and values of \hat{S} , all with the same expected values \hat{b} and \hat{N}_e , and with stochastic
265 error added to $\log(\hat{b})$ and $\log(\hat{N}_e)$, we calculate the corresponding values of \hat{D}_e and $\hat{\delta}$.

266 If we assume, as DM clearly do, that the expected value of δ is constant across the whole
267 study area (11), then the DM approach, used to analyse the data from all 10 experiments, should
268 provide the same value of δ in each case – allowing for errors in the measurement of b and N_e . That

269 is to say, the measured value of δ should not depend on how the traps are deployed. In particular, if
270 $\log(\hat{\delta})$ is plotted against $\log(\hat{D}_e)$ or against $\log(\hat{S})$, the results should approximate horizontal lines.
271 The reality is markedly different. The results of a single realisation of the simulation procedure are
272 shown in Fig 1, Row B, from which it is seen that the simulation essentially reflects all of the
273 properties of the NDDD picture provided in Fig 1, Row A. In particular we see that: $\log(\hat{\delta})$ is
274 strongly correlated with $\log(\hat{D}_e)$, with a slope around -0.5 (Fig 1, A1, B1); $\log(\hat{\delta})$ is strongly
275 correlated with $\log(\hat{S})$, with a slope around 0.5 (Fig 1, A2, B2); $\log(\hat{b})$ and $\log(\hat{D}_e)$ are poorly
276 correlated (Fig 1, A3, B3); $\log(\hat{b})$ and $\log(\hat{D}_e)$ are poorly correlated (Fig 1, A4, B4); $\log(\hat{N}_e)$ and
277 $\log(\hat{D}_e)$ are positively, but rather weakly, correlated (Fig 1, A5, B5); $\log(\hat{S})$ declines linearly with
278 increasing $\log(\hat{D}_e)$ (Fig 1, A6, B6). The reader is invited to make serial iterations of the stochastic
279 procedure – with each iteration using a different randomly generated error for $\log(\hat{b})$ and $\log(\hat{N}_e)$.

280 Notice that we make no assumption about the true underlying nature of dispersal: it could be
281 NDDD, DID (density independent dispersal) or PDDD (positive density-dependent dispersal).
282 Regardless of this, however, the output always strongly resembles NDDD. Moreover, while we
283 have used only one of the DM studies as an example (11), the result is entirely general and the same
284 problem will arise in the analysis of all of the studies cited by DM. Notice also that, if b and N_e are
285 measured without error, the outcome still gives the appearance of NDDD (Fig 1, C1-C6). That is to
286 say, the illusion of NDDD is entirely due to the fundamentally erroneous assumption that \hat{S} , as
287 measured by the distribution of traps used in the sampling procedure, provides a good estimate of
288 the true area (S) occupied by the tsetse population under study.

289 The foregoing analyses show that, depending on trap placement and the choice of how \hat{S} is
290 calculated from such placement, the Opiro and Manangwa studies (7,11) can both be made to
291 reflect either an extremely high effective population density and low dispersal rate, or completely
292 the reverse. Clearly, in each study, these scenarios cannot both be correct. Indeed, both are almost
293 certainly incorrect because, as explained above, \hat{S} is virtually never equal to the true area (S)
294 occupied by a subpopulation.

295

296 **The illusion of NDDD in a simulated population with assumed DID.** We reinforce the above
297 results by considering the effects of errors in estimates of S on a simulated population where we
298 specifically require that the true value of the dispersal distance (δ) is independent of effective
299 population density (D_e), i.e., a population exhibiting DID. Since $\hat{D}_e = \hat{N}_e / \hat{S}$, errors in the measures of
300 S are reflected in the \hat{D}_e estimates and that is problematic for the DM approach. This is illustrated in
301 Supplementary File S3, where we simulate a group of populations where δ is constant, D_e varies
302 within some arbitrary range, and b is calculated from δ and D_e according to Equation (1). Given the
303 assumption of DID, the calculated values of b decline as a power function of D_e (Fig 4A). We use
304 these true values of D_e in this simulated population to generate “estimates” of \hat{D}_e with large error.
305 For this, \hat{D}_e is calculated as true D_e multiplied by some random factor between 0.2 and 5; these
306 errors can be made additive instead, without consequence to the conclusion. For simplicity, we
307 assume that \hat{b} is estimated without error ($\hat{b} = b$). When there is error in D_e , however, $\log(b)$ appears
308 uncorrelated with $\log(D_e)$ (Fig 4B). We then calculate $\hat{\delta}$ using Equation (1), replicating the method
309 of DM. As the error input increases from 1 (no error) to 3 (three-fold error), there emerges a
310 negative correlation between $\log(\hat{D}_e)$ and $\log(\hat{\delta})$, with a slope tending towards -0.5 (Fig 4C). This
311 approximates the slope apparent in the DM analysis of their own real data.

312

313 **Fig 4. Simulation of a group of populations where the true dispersal rate (δ) is independent of**
314 **the true population density (D_e).** A. Relationship between $\log(b)$ and $\log(D_e)$ when D_e is measured
315 without error. B. Single realisation of the same relationship when D_e is measured with error.
316 C. Single realisation of the relationship between $\log(\hat{\delta})$ and $\log(\hat{D}_e)$ when D_e is measured with error.

317

318 The reader can vary the input values of Supplementary File S3 to observe the consequences
319 for the slope of $\log(\hat{\delta})$ against $\log(\hat{D}_e)$. Since the simulation is stochastic, the slope changes with
320 each realisation of the process but, for fold-error greater than around 1.2, the slope is invariably less

321 than zero. That is to say, the population appears to exhibit NDDD, despite the fact that it has been
322 set up such that dispersal is actually independent of population density. Furthermore, the
323 dominating influence of large errors in \hat{D}_e caused by highly variable and arbitrary values of \hat{S} , also
324 explain the otherwise perplexing correlation we have noted between \hat{S} and $\hat{\delta}$ in DM's data.

325

326 **Why DM's method results in apparent NDDD with a slope of -0.5.** Equation (1) is a
327 rearrangement of the original derived by Rousset (1) to describe the value of b that would arise in a
328 population as a result of the values of D_e and δ , where the latter is a distance measure somewhat
329 different from that used by DM. As δ increases, the resulting genetic mixing leads to subpopulations
330 becoming less genetically differentiated, i.e., b decreases, and the equality defined in Equation (1) is
331 maintained. In the absence of density-dependent dispersal, an increase in the effective population
332 density (D_e) slows the rate of genetic drift, so that subpopulations differentiate genetically from
333 each other more slowly, again leading to decreased genetic differentiation between subpopulations
334 and hence a reduction in b . The equality defined in Equation (1) is thus maintained again, with no
335 change in δ . If, however, dispersal is density dependent, then a change in D_e will lead to a change in
336 δ , and the resulting change in b will be the outcome of the changes that both D_e and δ have on b ,
337 still maintaining the equality defined by Equation (1).

338 The properties above relate to the *true* values of D_e , δ and b – and, thus, to the true values of
339 the constituents of D_e (N_e , the effective population size and S , the area occupied by that effective
340 population). But that is not the case for errors in the estimate \hat{D}_e . Error in \hat{D}_e will not be
341 accompanied by a corresponding change in \hat{b} , and thus, when Equation (1) is used to calculate $\hat{\delta}$
342 the same error will propagate to $\hat{\delta}$, so that an overestimate of \hat{D}_e will lead to an underestimate of $\hat{\delta}$
343 and vice-versa. When \hat{D}_e and $\hat{\delta}$ are then plotted against each-other, the result is the error in \hat{D}_e being
344 plotted against itself. If the error in \hat{D}_e is large enough – and we have shown that errors can be
345 >1000-fold – this autocorrelation will overwhelm any true relationship between D_e and δ . As the

346 error in \hat{D}_e increases, \hat{b} approaches independence from \hat{D}_e , and $d(\ln(\delta))/d(\ln(D_e))$ can then be
347 calculated from Equation (1) with b as a fixed parameter.

348 In Supplementary File S4 we present a complementary mathematical analysis of the effects
349 of errors in \hat{D}_e on observed results. The analysis confirms that, as long as estimation errors strongly
350 dominate the values of \hat{D}_e , the relationship between $\log(\hat{D}_e)$ and $\log(\hat{\delta})$ will appear to suggest the
351 presence of NDDD, even in circumstances where the true situation is DID.

352

353 **Errors in the estimation of D_c , the true census density**

354 DM found a strong linear correlation ($R^2 = 0.86$; $P < 0.02$) between the logs of estimated
355 dispersal distance ($\hat{\delta}$) and census population density ($\hat{D}_c = \hat{N}_c/\hat{S}$), where the \hat{N}_c values were derived
356 from trap catches. As we shall now demonstrate, the errors in \hat{D}_c are even more serious than those in
357 \hat{D}_e . We consider a model world consisting of a continuous population of tsetse covering a large
358 area, say $100 \text{ km} \times 100 \text{ km} = 10,000 \text{ km}^2$. The population is uniform, across its entire extent, in
359 terms of its true population density, which for the sake of illustration, we take as 1000 flies per km^2 .
360 This assumption of fixed true density implies that the true effective density, D_e , is also fixed, and
361 hence δ is fixed, assuming fixed b . Finally, we stipulate that a single trap, used in isolation,
362 produces a daily catch of flies equal to 1% of the population in the surrounding 1 km^2 , i.e., 10 flies
363 per trap per day in our model world.

364

365 **Sampling the model population using more than one trap at a site.** Using the DM definition for
366 this case, $S = \pi(D_{max})^2$, where D_{max} is the distance between the two most distant traps in a given site.
367 Note that this immediately assumes that the true area (S) of the subpopulation being sampled, and
368 the surface area of the trapping site (\hat{S}), are identical. This assumption leads to problems: suppose,
369 for example, a subpopulation is sampled using six traps deployed as shown in Fig 5A, with a single
370 trap placed at the centre a circle of radius r , and a further five traps placed at equally spaced

371 distances along the circumference of the circle. This trap placement provides the most compact
372 disposition conforming with the trap spacing used, for example, in (11).

373

374 **Fig 5. Surface area occupied by a subpopulation, as estimated by DM.** A. More than one trap
375 deployed at a site. In the example shown (*cf*(11)) five traps are spaced at equal intervals on the
376 circumference of a circle of radius r units, with a further trap at the centre of the circle. DM
377 calculate the area of the site as $\hat{S} = \pi(D_{max})^2$, where D_{max} is the distance between the two most
378 distant traps in a given site, taken as the radius of the corresponding subpopulation. B. Two trapping
379 sites with one trap deployed at each site. For this scenario, the surface area occupied by the sub-
380 population sampled is calculated from $S = \pi(D_{min}/2)^2$, where D_{min} is the distance the distance
381 between the centres of two neighbouring subpopulations and thus as the average diameter of a
382 subpopulation.

383

384 Now consider the effect of changing the value of r , and thus D_{max} , on the measured values of
385 the census population (\hat{N}_c) and the census population density (\hat{D}_c) associated with the subpopulation
386 being sampled. Initially, assume that the traps are packed very closely together, such that \hat{S} ($r \approx 1$
387 m) = $\pi(D_{max})^2 = 10 \text{ m}^2$. Clearly, these closely packed traps will interfere with each other and the
388 catch per unit time would probably differ little whether one used all six traps, or just the one at the
389 centre – which would provide an expected catch of 10 flies per day if used in isolation anywhere in
390 our population space (see above). The expected total catch per day from all six traps would thus be
391 $N_c \approx 10$ flies. If r is increased to 3 m, such that $\hat{S} \approx 100 \text{ m}^2 = 10^{-4} \text{ km}^2$, there will be less interference
392 between the traps, but we expect that there will still be *some* interference and we thus expect $\hat{N}_c <$
393 10 flies per trap per day, and the expected catch from all six traps will thus be $10 < \hat{N}_c < 60$ flies.

394 If r is increased to 100 m, as in (11), \hat{S} ($r = 100 \text{ m}$) $\approx 113,500 \text{ m}^2 = 0.114 \text{ km}^2$, the traps are
395 now sufficiently far apart that they are effectively acting independently (22). Each trap is, therefore,
396 expected to catch 10 flies per day, and the expected total daily catch will be $\hat{N}_c = 60$ flies. For all

397 further increases in trap spacing, and increases thereby in \hat{S} , there will be no further increase in \hat{N}_c
398 (22). Thus, the expected total daily catch is $\hat{N}_c = 60$ flies – regardless of the value of r , as long as all
399 traps used are separated from each other by distances of the order of at least 100 m. Since, however,
400 the census population density is given by $\hat{D}_c = \hat{N}_c/\hat{S}$ it follows that, as r increases above 100 m, the
401 measured value of \hat{D}_c decreases as the square of the change in r . Thus, if we take $\hat{S}(r=100\text{ m}) \approx$
402 0.114 km^2 then $\hat{D}_c(r=100\text{ m}) = \hat{N}_c(r=100\text{ m})/\hat{S}(r=100\text{ m}) \approx 60/0.114 \approx 529$ flies per km^2 . If,
403 however, we double r such that $\hat{S}(r=200\text{ m}) \approx 0.454\text{ km}^2$ then $\hat{D}_c(r=200\text{ m}) = \hat{N}_c(r=200\text{ m})/\hat{S}(r$
404 $=200\text{ m}) = 60/0.454 \approx 132$ flies per km^2 , which is one quarter of the density for the situation where
405 $r = 100$ m.

406

407 **Sampling subpopulations using one trap at each site.** Using the same model world as defined
408 above, consider a situation where we sample two sites, using one trap at each site (Fig 5B). The
409 arguments follow the same course as for the situation where multiple traps are used at a single site.
410 As before, the true value of δ is unaffected by the distance (D_{min}) separating the centres of the
411 neighbouring sub-populations. And the estimate of the census population number (\hat{N}_c) is also
412 independent of D_{min} , and thus of \hat{S} . Since, however, S increases in proportion to the square of D_{min} ,
413 it follows that the census population density ($D_c = N_c/\hat{S}$) decreases in proportion to the square of
414 D_{min} .

415 That is to say, for our model world, while the catch per trap per unit time is independent of r
416 ≥ 100 m, the estimated population density declines with increasing r – whereas the true population
417 density is constant, being independent of the pattern of trap deployment. At the same time,
418 however, since the catch per trap is constant, the total catch increases in direct proportion to the
419 number of traps deployed and the duration of deployment. As evidenced by the use made of data
420 from (11), DM took no account of these problems in their calculations of N_c and D_c for the various
421 studies they used to derive the data for their Fig 1B. If this is the case, we must expect that their
422 estimated values \hat{N}_c and \hat{D}_c , for any given study, bear little or no relation to the true values of

423 population size and density for that study. Since, also, the various studies used to estimate \hat{N}_c and \hat{D}_c
424 c used markedly different trap numbers and spacings, it follows that the ratio of these estimated
425 values to the true values will be different in every study. These considerations cast considerable
426 doubt on the validity of the values of \hat{D}_c used by DM on the abscissa in their Fig 1B.

427 The implications of the above problems with \hat{D}_c are again exemplified by contrasting the
428 results from (11) and (7). For the former, DM use $\hat{N}_c = 108$, in accord with the fact that six traps,
429 placed at least 100 m from each other, caught an average of 18 flies per trap over a period of 3-4
430 days, or about 5 flies per trap per day (11). Using the DM estimate of $\hat{S} = 0.02 \text{ km}^2$ for this study, \hat{D}_c
431 $c = \hat{N}_c/\hat{S} = 108/0.02 = 5400$ tsetse per km^2 . DM do not quote a value of \hat{D}_c for (7) – but we know
432 that two traps were used at each site and that each trap caught about 20 flies per day. For
433 comparison with (11), suppose the total catch from the two traps over a 3-day period was then
434 $2 \times 3 \times 20 = 120$ flies. Using the DM estimate of $\hat{S} \approx 34.87 \text{ km}^2$ for this study gives $\hat{D}_c = \hat{N}_c/\hat{S} =$
435 $120/34.87 = 3.44$ flies per km^2 . We thus have the ludicrous situation where the calculated census
436 density is $5400/3.44 \approx 1569$ times higher in (11) than in (7), despite the catch in (11) being a
437 quarter, i.e., $5/20$, of that in (7). The apparent implication is that the availability to traps varies by at
438 least 6000-fold between study areas, thereby suggesting that trap catches are hopeless indices of
439 tsetse abundance. That alone is sufficient to render Fig 1B of DM entirely meaningless, although
440 few field entomologists would credit that trap performances differ quite so markedly. The main
441 problem, which is equally damaging, is probably erroneous estimates of \hat{D}_c stemming from gross
442 errors in \hat{S} .

443

444 **Absence of correlation between \hat{N}_c and \hat{N}_e**

445 DM state that they expect a strong correlation between the true values of N_e and N_c .
446 Otherwise – as they say – all population genetics studies of tsetse would need to be called into
447 question. Their data do not, however, support their expectation. The linear correlation between their
448 estimates of \hat{N}_c and \hat{N}_e for the tsetse populations used in their own study is only 0.39 and is not

449 statistically significant (Fig 6A). Moreover, whereas DM claim that N_e values should be less than
450 N_c , in reality $N_c < N_e$ in five out of six situations where DM provide data for the two variables
451 (Supplementary File S1, Table S1). Notice, however, that if we divide each \hat{N}_c and \hat{N}_e value by \hat{S} , to
452 create the population densities, \hat{D}_c and \hat{D}_e , there is a very high correlation between the log
453 transformed versions of these two variables (Fig 6B). This is consistent with our earlier suggestion
454 that many of the DM results are artefacts resulting from inappropriate estimates of S , which varies
455 over a range large enough to swamp all other sources of variation. Nonetheless, the following
456 section suggests that there are additional good reasons to expect serious errors in the estimates of
457 N_c .

458

459 **Fig 6. Effective and census population numbers and densities using DM data.** Plots of: A.
460 census population size (\hat{N}_c) against effective population size (\hat{N}_e); B. census population density (\hat{D}_c)
461 against effective population density (\hat{D}_e). All data transformed by taking logs to the base 10.

462

463 **Failure to allow for the intensity and duration of trapping.** Scrutiny of (11) exposes further
464 problems with the DM estimates of census numbers \hat{N}_c and density \hat{D}_c . As detailed above, the DM
465 value of $\hat{N}_c = 108$ for this study reflects the number of flies captured per site – regardless of the
466 number of traps used, and without regard to the trapping period. Failure to adjust for trapping
467 intensity in different studies presents a serious problem. For example, with reference to Fig 5B,
468 suppose the three traps at the white-filled squares were removed. Clearly, the expected catch – and
469 thus \hat{N}_c – would be halved. For the three traps remaining at the grey-filled square, however, D_{max} –
470 and thus \hat{S} – would be unchanged, so that \hat{D}_c would be halved.

471 Moreover, there appears to have been no attempt to adjust for the trapping duration, which is
472 approximated as an ill-defined 3-4 days. If the traps had only been run for one day, the expected
473 catch, and \hat{N}_c , would have been reduced by around two-thirds or three-quarters. The DM paper and

474 its Supplementary Materials provide no suggestion, for any of the study sites considered, that the
475 catches were standardised relative to the number of traps employed and the duration of trapping.

476

477 **Failure to allow for differences in trap performance.** DM cite (23) as support for their claim that
478 the census of flies captured in the studies they used were correlated with the real census of the
479 corresponding tsetse populations. This is inappropriate, for two reasons. First, the paper cited
480 provides no tsetse census data – real or otherwise. Second, it presents data only for *G. m. morsitans*
481 and *G. pallidipes*: DM do not provide census population estimates for either species, and neither
482 species is represented in their Fig 1B. For both of these reasons the DM census results cannot
483 justifiably be correlated with tsetse census data from the paper cited. What the paper did suggest
484 (23), was that there was an order of magnitude difference between the probability of capturing *G.*
485 *m. morsitans* and *G. pallidipes* in an odour-baited trap (23). This underlines the danger of assuming,
486 as DM have done implicitly in their Fig. 1B, that differences in trap catches of different species are
487 correlated with differences between the true population densities for those species.

488 In offering these estimates of capture probability (23), the authors were careful to stipulate
489 that all catches referred to a single device, run for a one-day sampling period. They also expressed
490 the probabilities as a percentage of the population of flies occurring in a 1-km² neighbourhood of
491 the sampling device. In their own study, as detailed above, DM failed to correct their trap catches
492 for trap type, trapping duration, numbers of traps, or the number of flies in a well-defined
493 neighbourhood of the trap.

494 Even if DM had corrected the catch for the intensity and duration of trapping, there is still
495 the problem that the studies considered by DM employed a variety of trapping techniques,
496 involving different sorts of trap used to catch different species of tsetse. This is important because
497 the design of traps and the species against which they are deployed can affect, by at least one order
498 of magnitude, the numbers caught (23–27). The problem is aggravated further by the fact the some
499 of the traps in the studies adopted by DM were employed with highly effective odour attractants (7),

500 and others were not (11). In view of all of the above problems, it appears that the trap catches, as
501 employed by DM, are meaningless indices of population numbers and density.

502

503 **Unsupported claims of reinvasion dynamics**

504 When discussing possible causes of NDDD and asserting that dispersal is strongly density
505 dependent in tsetse, DM pay no attention to the dynamics of altered dispersal that are predicted by
506 their Equation (1). Similarly, in their abstract and on p.5, DM assert that "... control campaigns
507 might unleash dispersal from untreated areas." This could occur only if tsetse were reacting to the
508 population density in the destination of their movement, i.e., the treated area, rather than in their
509 current, untreated, location. However, this model of movement contravenes the assumptions of the
510 underlying model (1), which clearly requires that the dispersal and density parameters must describe
511 the same population. Thus, given that an untreated area would have a much higher population
512 density than a treated area, flies in the former would have a lower dispersal rate than flies in the
513 treated area. Control would not, therefore, be expected to "unleash dispersal", that is, rapid
514 reinvasion. DM also suggest that "... the bigger the decrease in the (treated) population, the higher
515 potential for reinvasion...", and this is also due to density dependence. However, this dynamic,
516 even if correct, is not implied by their model (Equation (1)). That model says nothing about a
517 population's potential for reinvasion.

518

519 **Other problems**

520 **Errors in the estimation of b .** Equation (1) makes sense only if $b > 0$. Thus, DM tested the null
521 hypothesis of $b = 0$ for each of their 10 studies, and claim that b is significantly greater than zero in
522 every case. We show, in Supplementary File S5, that DM under-estimated the errors in b and at
523 least three of seven estimates tested were not significantly different from zero. If all sources of
524 uncertainty could be accounted for, it is likely that some of the other b -values estimated by DM
525 would also lose their statistical significance.

526

527 **Contradictory evidence from trap catches.** The DM results often contradict common sense. An
528 extreme example is provided by the DM estimates derived from (11), for *G. fuscipes fuscipes* in
529 Uganda. The very low value of $\hat{S} = 0.02 \text{ km}^2$, coupled with almost the highest value of $\hat{D}_e > 21,000$
530 tsetse per km^2 among all 10 studies, led to an absurdly low estimate of $\hat{\delta} = 27 \text{ m}$ for the dispersal
531 per generation. With a 60-day generation time assumed by DM, this is equivalent to a daily flight
532 distance of about 4 m (17). This makes no sense: female tsetse must locate and feed on a host at
533 least three times in a 9-day inter-larval period if they are to produce a healthy pupa (28,29). This
534 seems virtually impossible if a fly moves only 4 m each day. The very low rate of movement is also
535 in stark contrast to a mark-recapture estimate of 338 m/day for *G. f. fuscipes* in Uganda (30), >80
536 times higher than the DM estimate, and equivalent to a dispersal of $\delta = 1.9$ to 2.6 km per
537 generation.

538 Moreover, if DM are correct in claiming that census population density is at least as large as
539 effective population density, then we might also expect that the true absolute density of tsetse in
540 (11) could be of the order of 21,000 per km^2 . However, this makes little sense given that the results
541 in (11) show that tsetse catches averaged only 5 per trap per day. By contrast, in (30), a single team
542 of stationary men caught tsetse at the rate of 120 *per hour*, suggesting that the actual population
543 density could have been 2-3 orders of magnitude higher than in (11). The problem then is that if, as
544 suggested by the NDDD hypothesis, dispersal rates decrease markedly with increasing population
545 density, expected dispersal rates should have been far lower in (30) than in (11), rather than the
546 reverse.

547 Similar concerns are raised by the estimates derived from the study on *G. pallidipes* (7).
548 Here the effective population density is quoted as $\hat{D}_e = 1.3$ tsetse per km^2 , four orders of magnitude
549 lower than the value estimated by DM from the study on *G. f. fuscipes* (11). It then makes no sense
550 that tsetse catches were about four times higher in (7) than in (11). These matters all make sense,

551 however, when one realizes that the \hat{D}_e and \hat{D}_c estimates in the DM study are essentially artefacts of
552 the gross variation in trap placements and the huge resultant errors in \hat{S} .

553

554 **PDDD more likely than NDDD.** There is no credible evidence from field work with tsetse that
555 control operations, which often cause huge reductions in population densities, result in increased
556 dispersal rates through NDDD. In a critique of DM, an evidence-based suggestion was advanced
557 that control operations might actually have the very opposite effect, causing what is effectively
558 PDDD (31). Tsetse control campaigns, which raise the death rate among adult tsetse, are associated
559 with a marked increase in the proportion of young adult females in the tsetse population (32), and
560 such flies are unlikely to disperse far since their flight capacity is poor (33). This argument is
561 certainly no evidence that a reduction in density will itself cause a decreased dispersal, but it does
562 weaken further the claim that control measures will cause problems by enhancing dispersal. No
563 effort was made to refute the above suggestion (31,34).

564

565 **No field evidence for larviposition pheromone in tsetse.** In apparent justification of the idea that
566 females might return to previous larviposition sites, DM claim that *G. morsitans* in the field secrete
567 a larviposition pheromone that attracts other females to the same site, leading to a strong
568 aggregation of pupae (35). Inspection of the work cited shows, however, that it makes no mention
569 of pheromones of any sort. Laboratory evidence for such a pheromone has indeed been published
570 (36–38) but no such chemical has ever been shown to produce aggregation of pupae in the field.
571 Nonetheless, the suggestion by DM that tsetse dispersal is in some way linked to the existence of a
572 larval deposition pheromone has been reiterated by some of the DM co-authors (38). The arguments
573 adduced in support of this idea are, however, confused or unconvincing in several ways. First,
574 allowing that the predation of tsetse pupae increases with the local density of the pupae in the wild
575 (39), deliberate aggregation of pupae would be expected to reduce, rather than increase, survival
576 probability. Second, there is the potential problem that predators could benefit from a signal that

577 pupae occur at a particular site – pupae are best hidden, not advertised. Finally, and most
578 confusingly, it is claimed that the pheromone would have its greatest benefit at low population
579 densities, because sparse populations become extinct if they disperse rapidly (38). This contradicts
580 completely the DM idea that sparse populations evolve to disperse widely: in short, the DM co-
581 authors are themselves arguing against their own notion of NDDD.

582

583 **Inappropriate pooling of data for different situations.** The DM study involved tsetse populations
584 in ten locations, in six countries. We question the validity of performing a pooled analysis on such
585 an *ad hoc* collection of results that refer to a variety of tsetse species in different circumstances. The
586 pertinent question is, rather, how dispersal rates might change with the variation in population
587 density for a given species in a given place from time to time. Hence, when DM interpret their
588 Figure 1 as they do, they must have made the assumption that the relationship between density and
589 dispersal rate applies to all species in all circumstances at all times. Indeed, somewhat strangely,
590 after making this assumption to interpret their results, DM then announce that their interpretations
591 support the assumption. However, that assumption is invalidated by our consideration (see above)
592 of the way that the estimates of D_e and D_c vary due to sampling procedures and species behaviour at
593 any given place and time. Furthermore, there are many variables other than population density that
594 are likely to affect mobility substantially, including vegetation type and fly size (40), and perhaps
595 also climate, and the type and abundance of hosts and sympatric species of tsetse and other biting
596 flies.

597 Even if we did give credence to the procedures of DM, we would have to accept that their
598 Figure 1 would make sense only if the habitat and population density were each homogeneous
599 within each of the ten situations considered. Most situations in tsetse belts are not like that. For
600 example, in the study area for *G. f. fuscipes* in Northern Uganda the terrain is stated to be very
601 heterogeneous (11). In such conditions it is usual to place traps where experience has suggested that
602 catches will be the greatest, ignoring the much larger areas where catches per trap are lower, but

603 where a substantial part of the population is likely to be present at low density. Hence, to what part
604 of the study area, if any, do the outputs for dispersal rate and density refer?

605

606 **Absence of any suggestion for a mechanism by which NDDD might have evolved.** Whether or
607 not we allow that the population density within each study area is varied or uniform, there is a
608 problem in seeing a credible biological mechanism for the occurrence of the NDDD apparent in
609 Figure 1 of DM. When asked for their understanding of this, all that was offered in response was
610 that evolution was involved, with no explanation of how such evolution was driven (31,34).

611 However, the idea of a particular dispersal rate evolving in response to a specific density in a given
612 situation, as conceived in DM Figure 1, creates a dilemma. On one hand, the plots of density vs
613 dispersal in their Figure 1 would be nonsensical if the density were variable at any one time within
614 the study area covered by each plot. On the other hand, if density were uniform within each study
615 area at a given time, then density could not exert any direct selection pressure, since however much
616 the flies moved, they would experience the same population density – they would not find densities
617 more favourable to survival.

618 As an alternative, a combination of three phenomena might be proposed: (i) that tsetse have
619 some optimal density, (ii) natural selection has resulted in tsetse behaving in such a way as to tend
620 to stabilise the density at that optimum value, and (iii) the optimal density is achieved via changes
621 in the dispersal rate, in direct response to the population density experienced by the flies. Any such
622 change in dispersal rate would have to operate by increasing dispersal when local densities are
623 above the optimum, and causing aggregation when local densities are sub-optimal. However, the
624 dispersal mechanism just described is clearly PDDD, not NDDD. It is thus most confusing that, as
625 indicated above, at least some of the DM co-authors appear eager to promote this idea (38).

626

627 **Errors in claimed support for NDDD.** DM cite various works in arguing that NDDD in tsetse
628 populations has been known for some time (35,39,41–43). None of the papers cited, however, make

629 any explicit or even implicit mention of NDDD; nor do they provide any support for the idea. As an
630 example, DM cite (39) in support of the idea that the efficacy of population suppression may be
631 reduced at low densities if normal density-dependent constraints are removed. This idea has long
632 been accepted by the generality of tsetse workers, but it does not imply any recognition of NDDD.

633

634 **Confusion between correlation and causation; possible reverse causality and confounding.**

635 Even if we disregard all of the many problems detailed above, and allow that the correlations
636 apparent in Fig. 1 of DM are valid, correlation does not necessarily imply causation. Note that the
637 gene flow theory behind Equation (1) treats dispersal and density as independent population
638 parameters, with no suggestion of a mechanistic relationship between them (1). Moreover, even if
639 we accepted the idea of a dependent relationship between these parameters, we could not be sure
640 whether that meant that declining density caused increasing dispersal, or that increased dispersal
641 caused decreased density, or that the levels of density and dispersal were each caused independently
642 by one or more other factors. Given all of these doubts about any causal background to their Fig. 1,
643 we conclude that DM have failed to produce evidence that tsetse control in any one place will
644 induce any increase in the dispersal rate – let alone a gross increase. It is also presumptuous to
645 predict that the claimed causal relationship will apply to all species of tsetse everywhere.

646 **Discussion**

647 We have exposed a wide range of errors in the DM paper. Those of core importance relate to
648 errors of measurement, which create the illusion of NDDD and ensure that the slope of $\log(\hat{\delta})$
649 against $\log(\hat{D}_e)$ in DM's Fig 1 approximates -0.5. DM's method of using their Equation (1) to
650 calculate an estimate of δ from measured estimates of D_e and b is inherently biased towards finding
651 NDDD (a negative correlation between D_e and δ) even when none exists. This bias occurs
652 whenever there is any error, even random, in the estimate of D_e . In DM's data, by far the most
653 serious errors in measurement appear to be in the estimate \hat{S} , and these stem from the DM notion
654 that the true areas inhabited by the various subpopulations under study are determined by the

655 distance between traps. That is the root error, since inter-trap distances are not features of tsetse
656 populations. Instead, they are strongly constrained by logistical issues, such as the numbers of
657 available paths and traps, the mode of transport employed, the purpose of the trapping study and the
658 whims of the researcher.

659 It is surprising and discouraging that no worker in the field of tsetse genetics appears to have
660 objected to the glaring errors in the DM analysis. In particular, none of the nearly 50 co-authors on
661 the papers cited by DM seems to have raised any concerns. We suggest that this is due to the fact
662 that molecular genetics is a highly specialised topic, often involving abstruse theoretical
663 considerations, arcane terminology and complex mathematical statistics, impenetrable to all but a
664 narrow group of specialists. The consequence is that very few people have any real understanding
665 of what is being said and implied in most mathematically-based papers on genetics. Instead, they
666 look mainly at the implications of the results, taking on trust that the arguments and procedures
667 which produced the results have been thoroughly checked before publication. The need for such
668 trust is especially evident in the DM case since the procedure is particularly difficult to follow – so
669 much so that even the editors and reviewers seem to have been overly trusting and, accordingly,
670 misled entirely.

671 In consequence of all this, our willingness to credit genetic arguments in general has been
672 weakened. We suggest that the genetics field should become more transparent, providing much
673 clearer descriptions of analytical processes, and easy access to all of the data required to check
674 those processes. In short, where geneticists wish to address and sway non-specialists, as DM clearly
675 intended to do, they should be obliged to make it abundantly clear, in simple manner, exactly what
676 they have assumed and done.

677 It is particularly worrying that although NDDD was initially offered by DM as a strongly
678 supported hypothesis in need of testing, some of the co-authors are already treating the hypothesis
679 as established fact, stating for example that: “through genetic studies, De Meeûs et al. (2019) have
680 shown that a strong negative density-dependent dispersal occurs after control operations” (38) –

681 clearly implying, quite falsely, that DM measured dispersal before and after control. Such
682 statements, taken with the DM warning that NDDD will unleash massive invasion from
683 neighbouring untreated areas, must be seen as having potentially serious impacts on disease control
684 policy. It is for that reason that we have felt it essential to expose so fully the errors involved in the
685 NDDD hypothesis.

686 Nonetheless, it would clearly be beneficial if genetic analysis could be used to provide
687 useful indications of the dispersal rate of tsetse, or indeed of any other creature. As we have seen, a
688 central problem is that of providing accurate and meaningful estimates of population numbers and
689 density. That problem has taxed tsetse workers for nearly a century – since C.H.N. Jackson
690 pioneered the use of mark-recapture in a remarkable series of population studies with tsetse (44–47)
691 – and we still do not have good solutions. The best estimates of population numbers have involved
692 mark-recapture exercises applied to island populations, closed to immigration and migration
693 (48,49). When such exercises are carried out on open populations, the results are much more
694 difficult to interpret, because of the confusing effect of fly dispersal.

695 Modern field studies of tsetse generally rely, as in the studies quoted by DM, on trap
696 samples to estimate population numbers and densities, but this approach is fraught with difficulties.
697 For example, random movement implies that a few of the tsetse caught in a trap, in a given
698 sampling period, will have come from extreme distance, with progressively greater numbers coming
699 from shorter distances. The area sampled in any given time is therefore a complex concept. Even if
700 we decide to define S as the area from which originated some arbitrary proportion of the catch, we
701 can assess that area only by knowing the dispersal rate of the species under study – that is, by
702 having established in advance the very thing that procedures like those of DM, are developed to
703 assess. It appears, therefore, that such procedures will always be difficult. Nonetheless, we are
704 interested in collaborating with geneticists trying to solve such problems and in moving, thereby,
705 towards a sensible use of genetic analysis in the illumination of the population dynamics of tsetse in
706 the field.

707

708 **Acknowledgements**

709 The contents of this publication are the sole responsibility of the authors and do not
710 necessarily reflect the views of any funding agency. The authors declare that they have no
711 competing interests.

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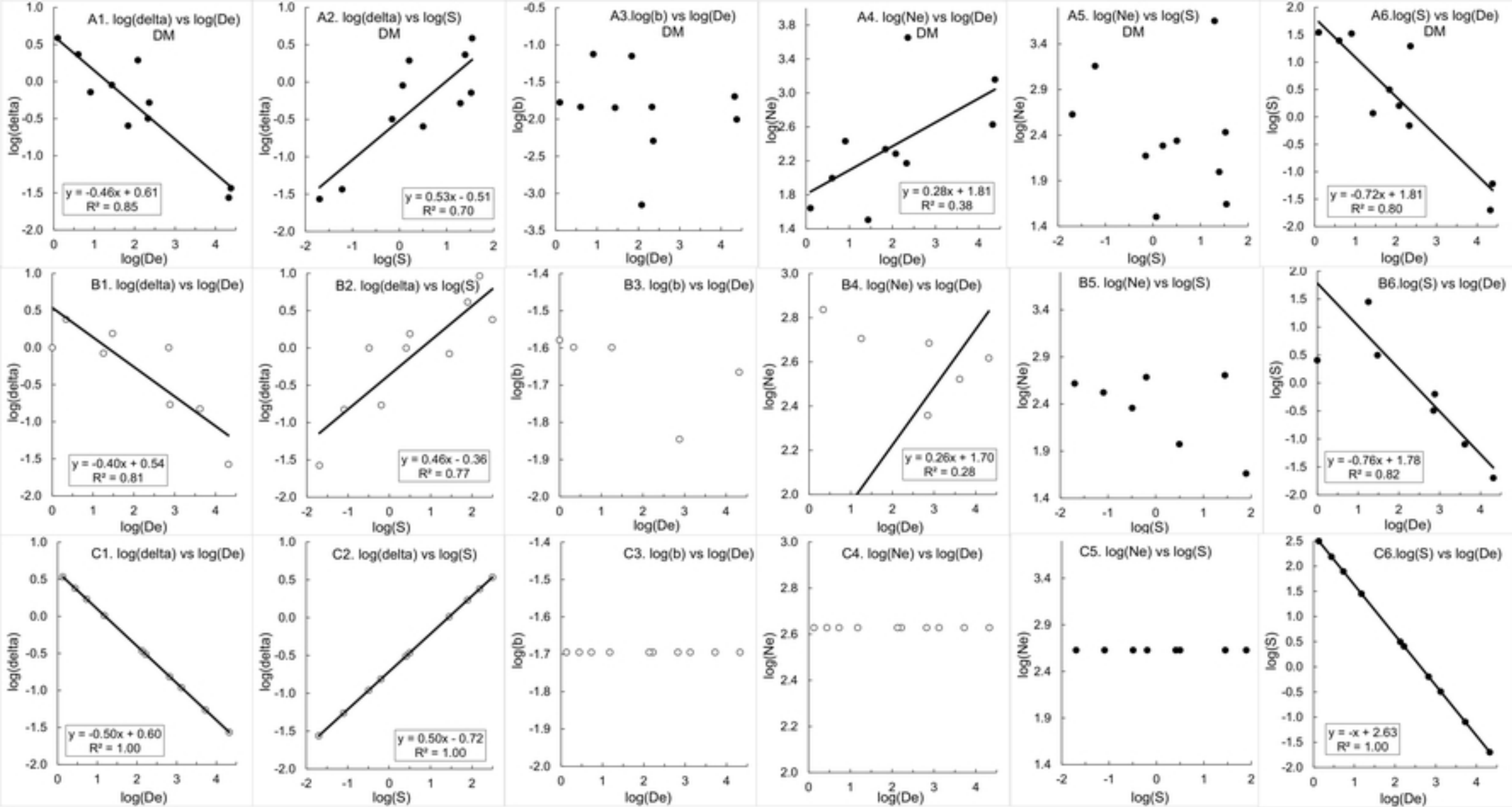


Figure 1

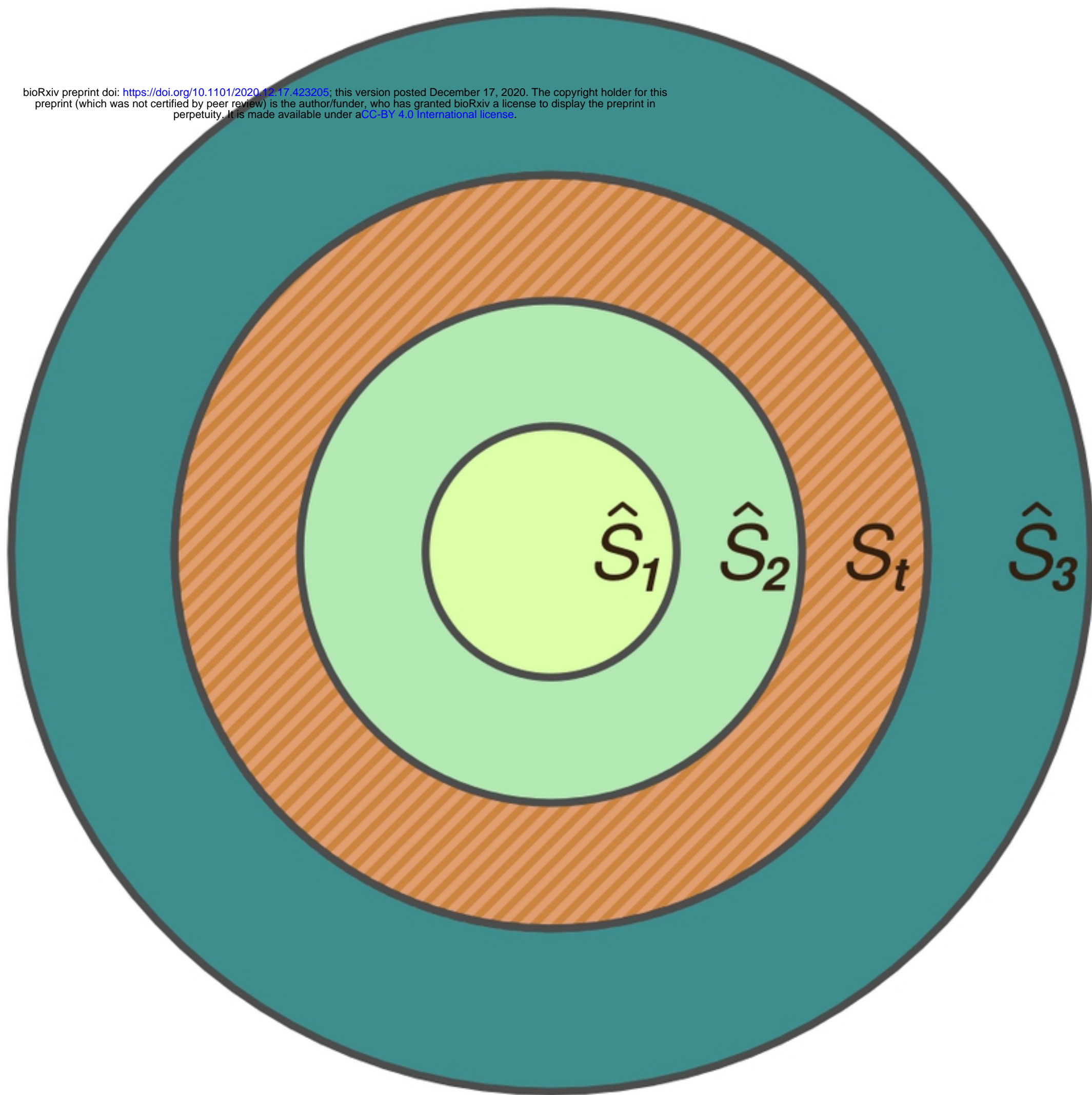


Figure 2

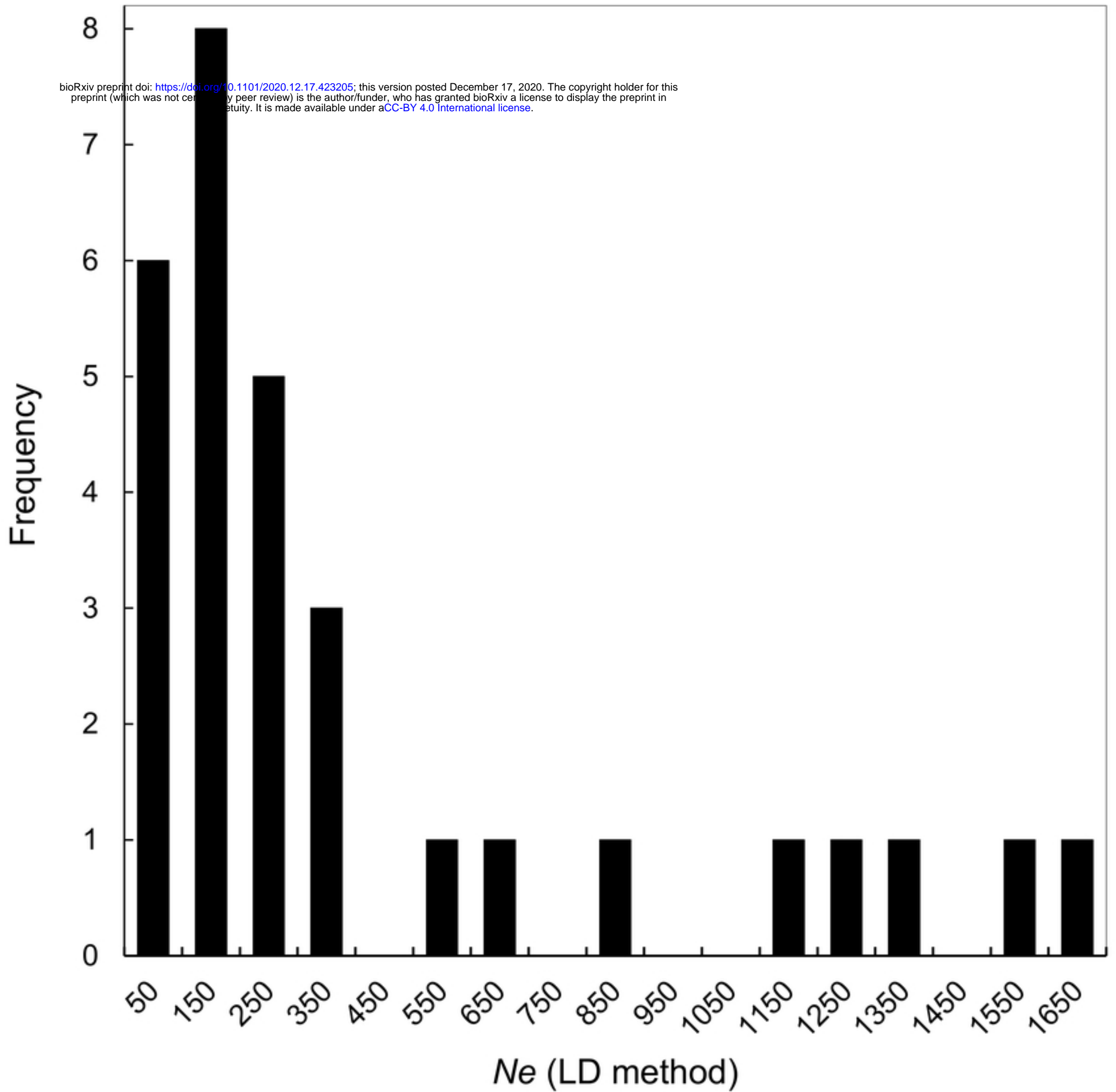


Figure 3

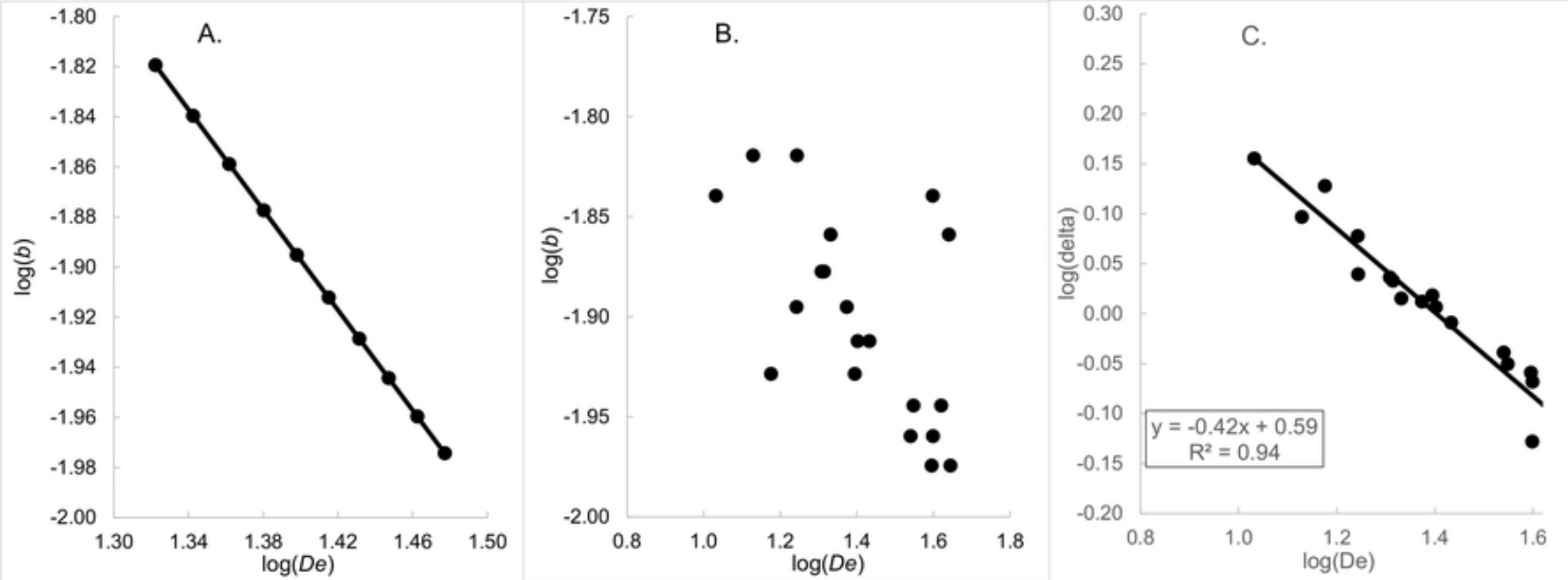


Figure 4

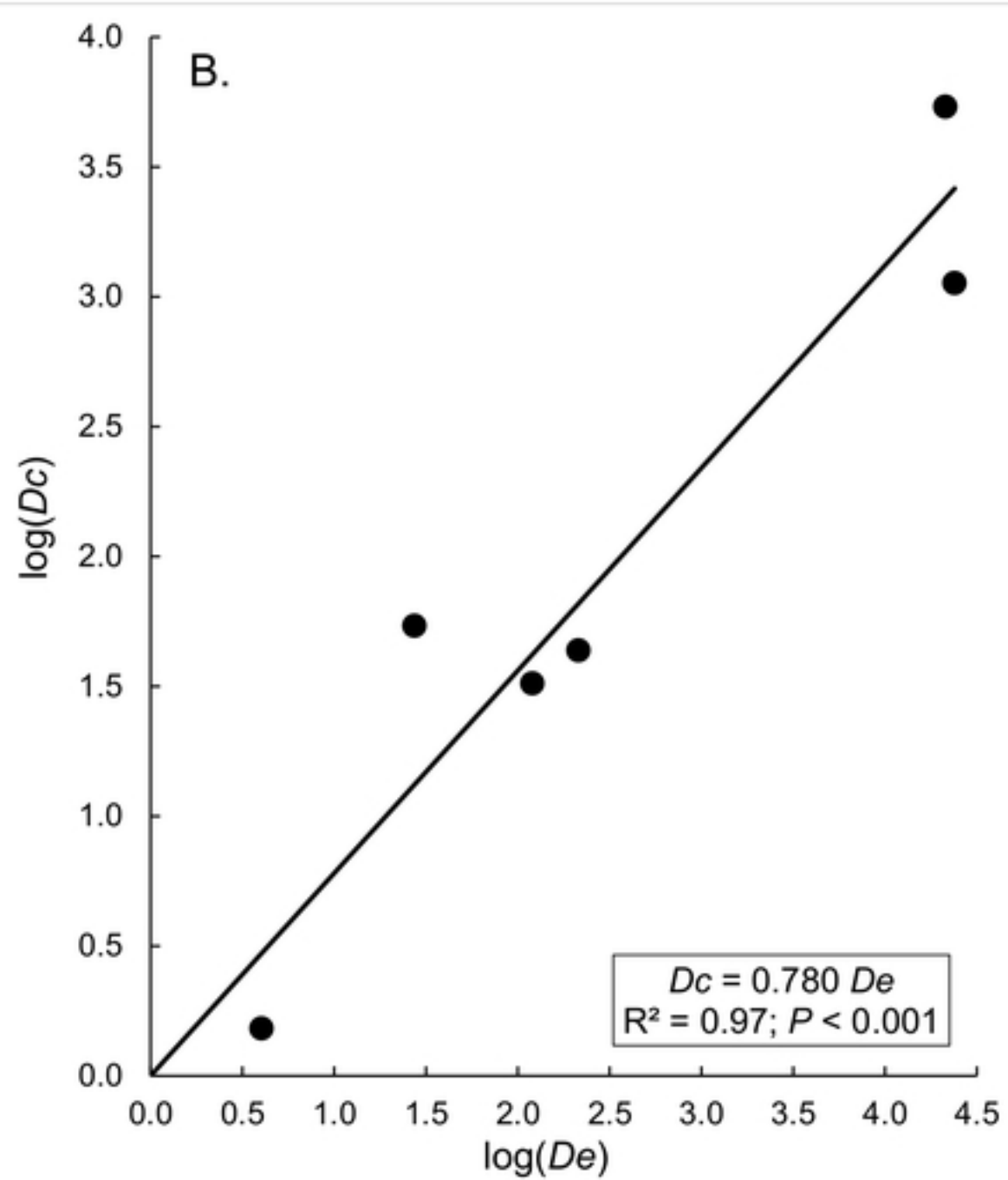
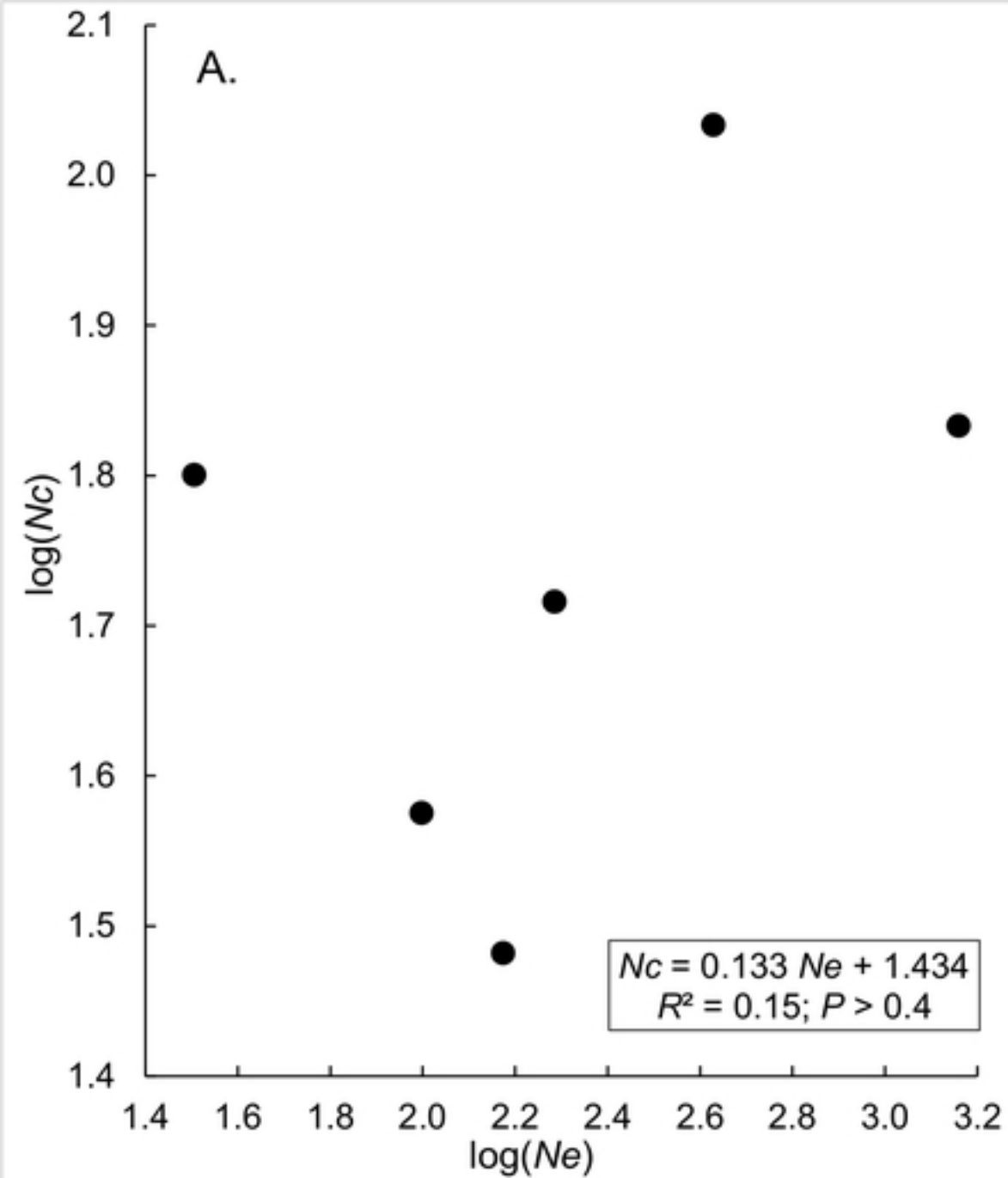


Figure 5

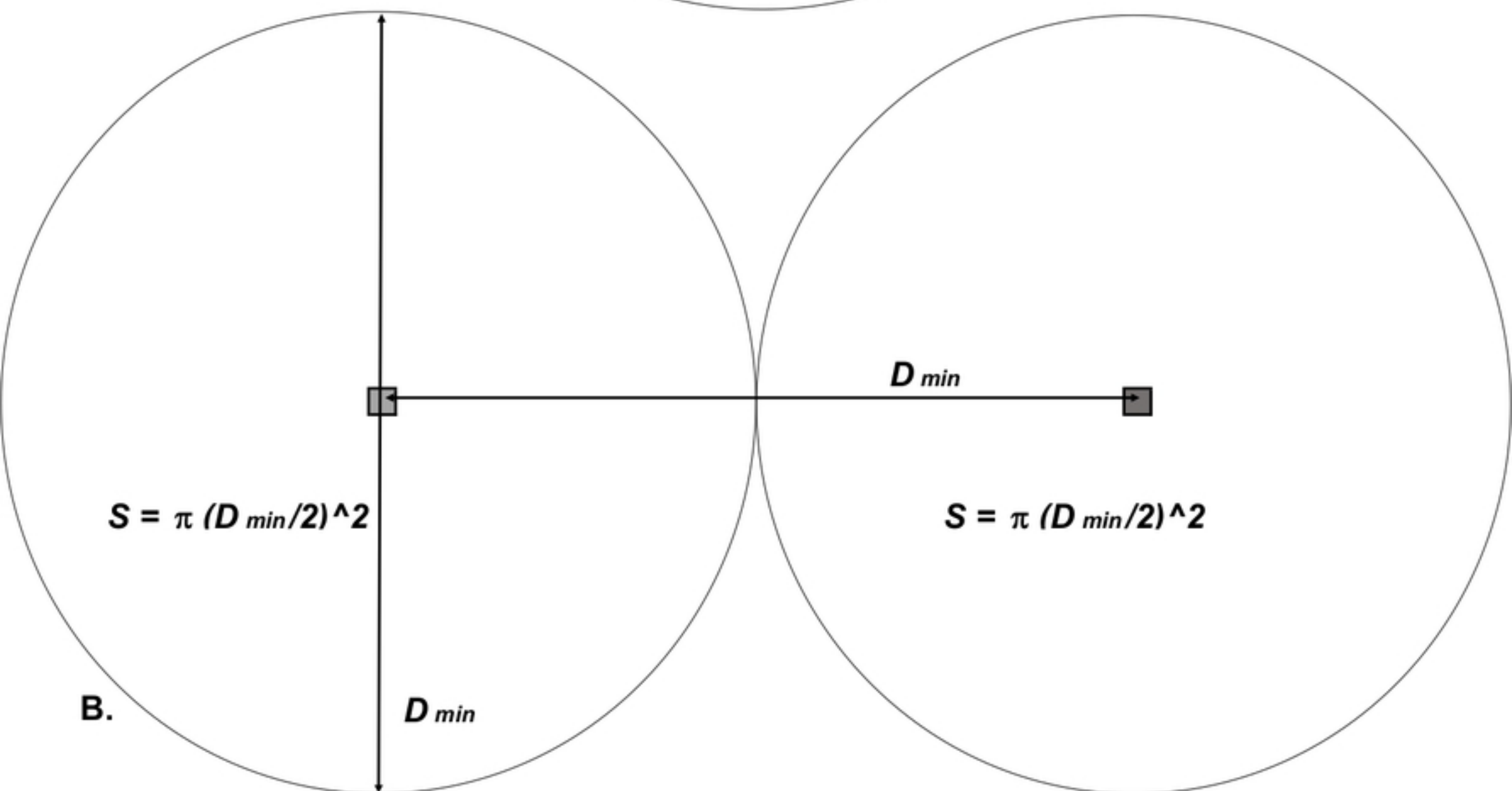
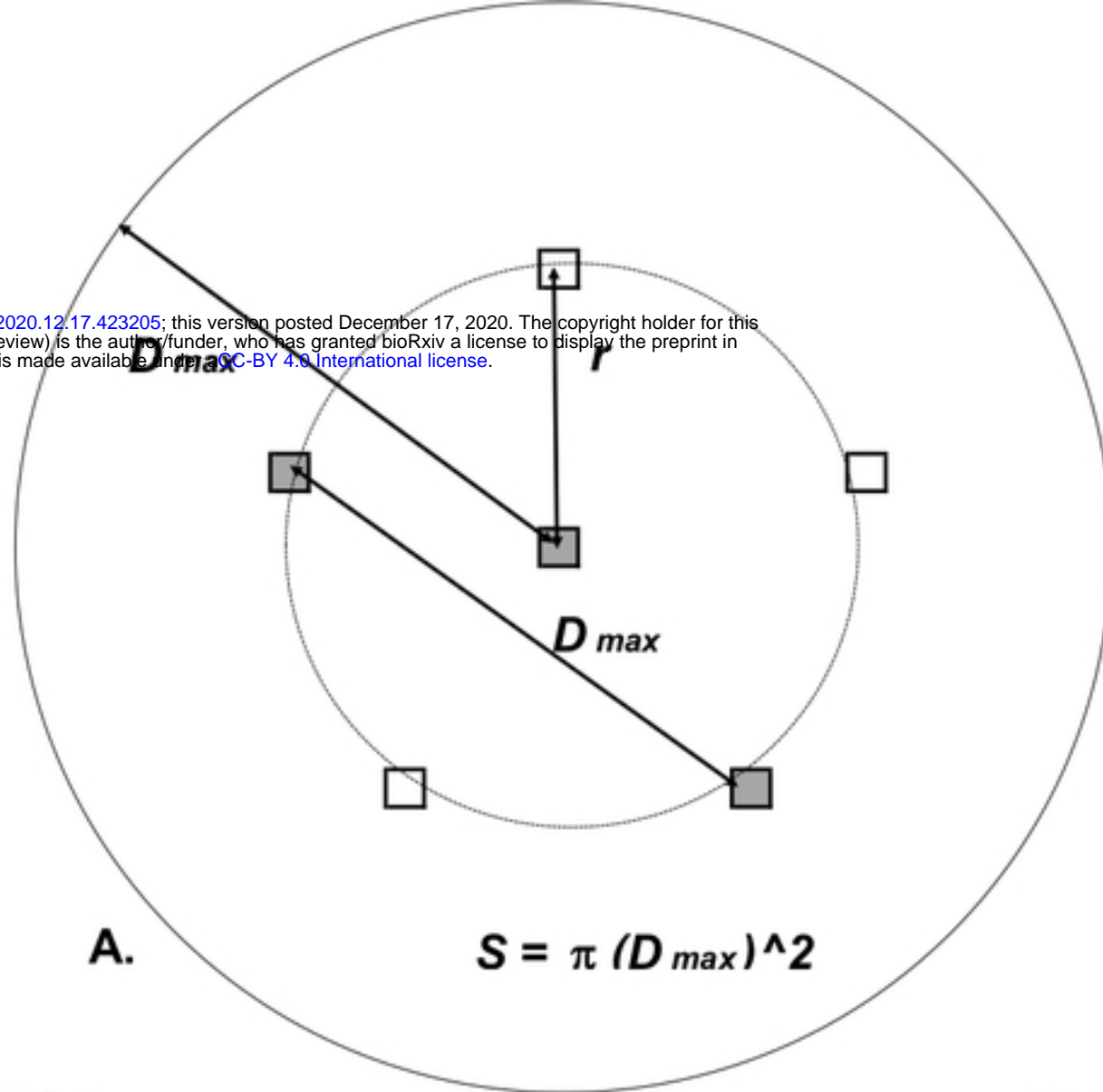


Figure 6