

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/333533834>

# Ecological scaffolding and the evolution of individuality: the transition from cells to multicellular life

Preprint · May 2019

DOI: 10.1101/656660

---

CITATIONS

2

READS

339

3 authors, including:



Andrew James Black  
University of Adelaide

28 PUBLICATIONS 653 CITATIONS

[SEE PROFILE](#)



Pierrick Bourrat  
Macquarie University

57 PUBLICATIONS 655 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Causal specificity [View project](#)



New Historical and Philosophical Perspectives on Quantitative Genetics - Special Issue of Studies in History and Philosophy of Science [View project](#)

1 Ecological scaffolding and the evolution of individuality: the transition from cells to  
2 multicellular life

3

4

5 Andrew J Black<sup>1\*</sup>, Pierrick Bourrat<sup>2,3</sup> and Paul B Rainey<sup>4,5\*</sup>

6

7

8 <sup>1</sup>School of Mathematical Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

9 <sup>2</sup>Philosophy Department, Macquarie University, New South Wales 2109, Australia.

10 <sup>3</sup>Department of Philosophy & Charles Perkins Centre, The University of Sydney, New South Wales  
11 2006, Australia.

12 <sup>4</sup>Department of Microbial Population Biology, Max Planck Institute for Evolutionary Biology, Plön  
13 24306, Germany.

14 <sup>5</sup>Laboratoire de Génétique de l'Evolution, Ecole Supérieure de Physique et de Chimie Industrielles de la  
15 Ville de Paris (ESPCI Paris), CNRS UMR 8231, PSL Research University, 75231 Paris, France.

16

17

18 \*Corresponding authors:

19 AB, School of Mathematical Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

20 Email: andrew.black@adelaide.edu.au

21 PBR, Department of Microbial Population Biology, Max Planck Institute for Evolutionary Biology,  
22 Plön 24306, Germany.

23 Email: rainey@evolbio.mpg.de

24

25 ABSTRACT

26 Evolutionary transitions in individuality are central to the emergence of biological complexity. Recent  
27 experiments provide glimpses of processes underpinning the transition from single cells to multicellular  
28 life and draw attention to the critical role of ecology. Here we emphasise this ecological dimension and  
29 argue that its current absence from theoretical frameworks hampers development of general explanatory  
30 solutions. Using mechanistic mathematical models, we show how a minimal ecological structure  
31 comprised of patchily distributed resources and between patch dispersal can scaffold Darwinian-like  
32 properties on collectives of cells. This scaffolding causes cells to participate directly in the process of  
33 evolution by natural selection as if they were members of multicellular collectives, with collectives  
34 participating in a death-birth process arising from the interplay between the timing of dispersal events  
35 and the rate of resource utilisation by cells. When this timescale is sufficiently long and new collectives  
36 are founded by single cells, collectives experience conditions that favour evolution of a reproductive  
37 division of labour. Together our simple model makes explicit key events in the major evolutionary  
38 transition to multicellularity. It also makes predictions concerning the life history of certain pathogens  
39 and serves as an ecological recipe for experimental realisation of evolutionary transitions.

40

## 41 INTRODUCTION

42 Evolutionary transitions in individuality (ETIs) are central to the emergence of biological complexity<sup>1-3</sup>.  
43 Each ETI involved the formation of collective-level entities from the interaction of particles<sup>4,5</sup>. For  
44 example, chromosomes evolved from the joining of once independently replicating genes. Sexually  
45 reproducing types evolved from asexual organisms. Multicellular life evolved from independently  
46 replicating cells .

47 Central to each of these transitions was the emergence of properties at the newly formed level that  
48 allowed individuals — at this level — to participate directly in the process of evolution by natural  
49 selection<sup>5-9</sup>. This required newly formed collectives to be discrete and vary one to another, to reproduce  
50 and to leave offspring that resemble parental types<sup>10</sup>. These essential and intertwined Darwinian  
51 properties of variation, differential reproduction and heredity are such fundamental features of living  
52 systems that it is easy to overlook the fact that individuality is a derived state and in need of  
53 evolutionary explanation<sup>3,7-9,11-13</sup>.

54 With focus on multicellular life, it is evident that reproduction, in even simple multicellular forms, is a  
55 complex process<sup>9,11,12,14</sup>. It is therefore tempting to invoke selection as its cause. But this is problematic  
56 because the earliest collectives lacked capacity for collective-level reproduction and thus to invoke  
57 selection at the collective level as the cause of collective-level reproduction is to invoke the trait  
58 requiring explanation as the cause of its own evolution. Clearly such an explanation is unsatisfactory.

59 One way to avoid this dilemma is to recognise opportunities for co-option of pre-existing cellular traits.  
60 For example, in the colonial volvocine algae, group formation evolved by co-option and expansion of  
61 cell cycle regulation evident in the unicellular ancestor *Chlamydomonas*<sup>15</sup>. In experimentally evolved  
62 snowflake yeast, collective-level reproduction emerged via co-option of apoptotic capacity already  
63 apparent in single cell precursors<sup>16</sup>.

64 We do not wish to downplay the importance of co-option, but there is conceivable value in asking  
65 whether Darwinian properties might emerge in the absence of co-option. Such a take-nothing-for-  
66 granted line of inquiry presents a challenge as it requires conceiving possibilities for the emergence of  
67 properties essential for collectives to participate in the process of evolution by natural selection from  
68 starting states that lack any manifestation of collective-level Darwinian properties. In essence it begs  
69 explanations for how Darwinian properties might emerge from non-Darwinian entities and therefore by  
70 non-Darwinian means. Solutions stand to inform not only how multicellular states arise from single  
71 cells, but how Darwinian properties might emerge during each of the major evolutionary transitions,  
72 including that from non-living matter.

73 A solution that we advance draws heavily on ecology, the significance of which we suggest has been  
74 overlooked — even though the importance of population structure has been emphasised by literature  
75 on the levels of selection<sup>17</sup>. It recognises that Darwinian properties can be “scaffolded” by the  
76 environment — that these properties can be exogenously imposed in such a way as to cause lower level  
77 entities (e.g., cells) to become unwitting participants in a selective process that occurs over a longer  
78 timescale than the timescale over which cell-level selection occurs, and as part of a larger entity (a  
79 collective). In time, such exogenously imposed — Darwinian-like — properties stand to become  
80 endogenous features of evolving systems (for development of general views on scaffolding processes  
81 see<sup>18</sup>).

82 Ecological scaffolding underpinned a recent (and on-going) experimental exploration on the evolution  
83 of multicellularity<sup>19,20</sup>. Discrete lineages established from the bacterium *Pseudomonas fluorescens* were  
84 propagated under conditions that required, for long-term persistence, repeated completion a two-phase  
85 life cycle involving soma and germ-like states. In the experiment, variation was discretised using glass  
86 microcosms, but the design is synonymous with an environment such as a pond in which reeds extend  
87 from the water<sup>19,21</sup>. Each reed allows establishment of a single microbial mat (the soma-like phase),  
88 with the spacing of reeds ensuring variation at the level of mats. Mats that collapse, for example,  
89 through physical disturbance, are extinguished, allowing the possibility that an extant mat might, via  
90 production of a dispersing (germ-like) phase, increase its representation among the population of mats.  
91 Thus the possibility of a selective process unfolds at the level of mats. After ten lifecycle generations,  
92 the fitness of derived mats significantly improved, with the most successful lineage having even evolved  
93 a simple genetic switch that ensured reliable developmental change between soma and germ-line  
94 phases<sup>19</sup>. Not only does this study demonstrate that scaffolding works, but it also showed that  
95 externally imposed Darwinian properties can begin the shift toward endogenisation. Or to quote Van  
96 Valen: “*evolution is the control of development by ecology*”<sup>22</sup>.

97 Our goal here is to move from the experimental *Pseudomonas* study to a more general formulation. Our  
98 focus is the transition from single cells to multicellular life, but argue that scaffolding has broad  
99 implications. Our goal is to show that a minimal set of ecological conditions (and ensuing evolutionary  
100 responses) are sufficient to effect evolutionary transitions in individuality. We develop our thesis using  
101 mechanistic models.

102

103

104 RESULTS

105 Scaffolding Darwinian properties

106 Before moving to mathematical models, we describe the simplest conceivable example of a population  
107 structure that confers Darwinian-like properties on collectives of particles (cells). Consider an  
108 environment in which resources are distributed across patches. A single cell founds a patch. Available  
109 resources allow exponential growth of the founding type, however, because resources within patches are  
110 finite, they are rapidly depleted causing the population to decline (equations describing the birth /  
111 death process and relationship with resources are shown in Box 1 and the Supplementary Information  
112 file). Long-term persistence of cells requires dispersal to a new patch. Dispersal occurs at a fixed regular  
113 time interval via, for example, some external factor such as wind, water splash or tidal flow.

114 Cell fate within the environment of patches depends on performance over two timescales. The first  
115 timescale is defined by the doubling time of cells. The second is defined by the timing of dispersal  
116 events. To make apparent the impact of the second timescale on the ensuing evolutionary dynamics,  
117 consider a second variant cell. This type grows faster than the former (cells consume resources more  
118 rapidly), which means that in a patch founded by both types, faster growing cells rapidly exclude slower  
119 growing cells. In the following we therefore limit the number of colonisers to a single founding cell  
120 type, thus limiting within patch competition.

121 Consider a single slow (depicted in Figure 1 as green) and fast (blue) cell that colonise separate patches  
122 (Figure 1). Cells of both types grow and divide, but different growth rates mean that blue cells deplete  
123 resources more rapidly than green cells. If dispersal occurs early (Figure 1A) when cells are in  
124 exponential growth, then the number of extant blue cells exceeds the number of green cells and thus  
125 future recursions of patches are dominated by blue cells (Figure 1B). If, however, dispersal occurs at a  
126 later time point, for example, once resources are depleted and population size is in decline, as in Figure  
127 1C, then future patch recursions are dominated by green types despite the fact that within a patch,  
128 green types lose in competition with blue types (Figure 1D). Under this scenario parallels are evident  
129 with models of virulence evolution in pathogens, where patches equate with hosts, cells are pathogens  
130 and dispersal is synonymous with transmission<sup>23-26</sup>. Virulent pathogens that kill their host before  
131 transmission face extinction.

132 Thus far, our focus has been the consequences of this population structure on the long-term fate of cells  
133 with different growth rates, but it is possible to switch perspective: there is a coupled evolutionary  
134 dynamic occurring at the level of patches (Figures 1C and 1D). Patches manifest Darwinian-like  
135 properties of variation (spatially distributed resources ensure that variation is discretised and that

136 patches vary one to another), differential reproduction (successful patches give rise to patches via  
137 dispersal) and heredity (offspring patches resemble parental patches because new patches are founded by  
138 single cells) that are also features of the founding cells. These properties are externally imposed  
139 (scaffolded) on patches by virtue of the structure of the environment.

140 Note that we refer to the properties of patches as “Darwinian-like”. Indeed, it makes no sense to think  
141 of patches as multicellular organisms (they are not) — if the ecological scaffold was to be removed  
142 (patchily distributed resources and a means of dispersal) — the Darwinian-like properties of the patches  
143 would instantly disappear. Yet, under the scenario outlined, cell fate is determined by selective  
144 conditions operating over the second (longer) timescale, just as if the cells themselves were members of  
145 multicellular collectives. Such a scaffolded framework of patch-level selection, based on nothing other  
146 than patchily distributed resources and a means of dispersal between patches, establishes conditions  
147 sufficient for the evolution of traits that are adaptive at the level of patches. We elaborate the  
148 mechanistic bases using models developed in the following section.

149 **Evolution in nested Darwinian populations**

150 To explore the evolutionary dynamics of the above ecological model we allow mutation to affect the  
151 growth rate of individual cells (the within-patch model is described in Box 1). With such a model it  
152 becomes possible to determine the effect of the timing of dispersal — the second timescale — on the  
153 dynamics of within- and between-patch competition. Mathematical details are provided in the  
154 Supplementary Information file, but see also Box 1.

155 The full evolutionary model consists of  $M$  patches that are each founded by a single cell of a single  
156 phenotype (growth rate  $\beta$ ). Cells within patches replicate and consume resources with mutation giving  
157 rise to types that vary in growth rate. Once resources are depleted the population size within patches  
158 declines. After a fixed time interval,  $T$ , which defines the second timescale, dispersal takes place.  
159 Dispersal is effected by randomly selecting  $M$  patches (with replacement) in proportion to the number  
160 of cells within each patch, and then randomly selecting a cell, within chosen patches, in proportion to  
161 numbers within the patch. In effect, the procedure is equivalent to pooling all viable cells from all  
162 patches at the time of dispersal and picking  $M$  cells at random. The dispersal regime thus rewards  
163 patches containing the greatest number of cells.

164 The bottleneck wrought at the moment of dispersal means that types founding new patches are freed  
165 from competition with faster growing types. Figure 2 shows the number of cells within a patch for a  
166 single realisation with initial (arbitrarily chosen) growth rate  $\beta = 1.8$ . The bottleneck imposes a strong  
167 homogeneity on the composition of the patch as the original population has to grow significantly before

168 mutants start to arise. The peak number of cells within the patch is reached at time  $T = 16.5$ , thus for  
169 cells with this initial growth rate, setting a dispersal time of  $T = 10$  is fast (i.e., still within the  
170 exponentially growing phase), and  $T = 30$  is slow (cells have significantly declined since their peak  
171 numbers).

172 From the patch perspective, the bottleneck reduces within patch variation and ensures high fidelity of  
173 transmission of patch phenotype (the size of the patch at the time of dispersal). Cells chosen for  
174 dispersal are individually transferred to new patches thus marking the founding of a new generation of  
175 patches. The mechanistic nature of the model allows the average growth rate of cells within a  
176 generation, number of cells in patches at the time of dispersal, and genealogy to be determined.

177 Figure 3 shows the time resolved dynamics of 50 independent realisations of the full evolutionary  
178 model, where patches experience 200 recursions, under slow ( $T = 30$ ) and fast ( $T = 10$ ) dispersal  
179 regimes. In these simulations the maximum cell growth is set to rate of  $\beta = 2$ , which in a real system  
180 would arise from chemical and physical constraints to the rate of cell replication. The state of  
181 populations at the time of dispersal are shown in Figures 4A and 4B. Single realisations of the model  
182 under slow and fast dispersal regimes are also shown in Supplementary Movie Files 1 and 2.

183 Under both fast and slow dispersal regimes patch fitness (the number of cells within patches at time of  
184 dispersal) increases rapidly before reaching a plateau (Figure 3B and 3D). This is consistent with  
185 predictions arising from the logic of Darwinism: imposition (by ecological scaffolding) of Darwinian-  
186 like properties on patches ensures patches participate in a selection process akin to evolution by natural  
187 selection, one that could be the starting point for patches to be units in their own right, provided they  
188 eventually acquire features classically associated with evolutionary individuals<sup>5,27</sup>. The plateau arises  
189 because under the slow dispersal regime growth rate evolves to maximise the number of particles  
190 available at the time of dispersal. Under the fast dispersal regime, the plateau is a consequence of  
191 reaching the maximum limit imposed by the growth rate. As this maximum rate is arbitrarily set,  
192 allowing it to increase would result in the evolution of patches of larger final size.

193 The cause of enhanced evolutionary success of patches resides in properties of individual cells. Under  
194 both fast and slow dispersal regimes, selection favours patches that harbour the greatest number of cells  
195 at the time of dispersal. Under both regimes fast growing cells outcompete slow growing types within  
196 patches, however, under the slow dispersal regime, selection rewards patches containing slow growing  
197 mutants and selects against patches dominated by fast growing cells. The opposite is true of patches  
198 evolving under the fast dispersal regime.

199 Under the slow dispersal regime this results in the seemingly counter intuitive finding that patch fitness  
200 increases at the expense of cell fitness (Figures 3A and 3B). Yet within our model, this is readily  
201 explained: fitness of a cell is measured over the short timescale while patch fitness is measured over the  
202 long timescale<sup>28,29</sup>. This captures precisely — and explains mechanistically — the notion of “fitness  
203 decoupling” thought to occur during the earliest stages of the evolution of multicellular life, but which  
204 has often been difficult to intuit<sup>3,30</sup>.

205 Under the fast dispersal regime, fast growing cells are favoured both within patches and over the second  
206 timescale. From the perspective of the evolution of multicellular life, the selection regime imposes the  
207 same directionality at both timescales leading to the view that fitness at both timescales (levels) are  
208 “coupled”.

209 It is interesting to note the difference in speed of the selective response under the two dispersal regimes  
210 and also the magnitude of difference in patch population size at equilibrium. The slower response  
211 under the slow dispersal regime is a consequence of the time taken for slow growing mutants to invade  
212 from rare in the face of within-patch competition for fast growth. The maximum population size under  
213 the fast dispersal regime (for these parameters) is a consequence of the imposed maximum limit on the  
214 growth rate. If faster rates were allowed, then larger population sizes would evolve (up to a limit where  
215 rate maximises patch size at the dispersal time).

216 Figures 5A and 5B show the evolutionary fate (genealogy) of 10 independent lineages under the slow  
217 and fast dispersal regimes, respectively. Mapped on the phylogenies are changes in cell growth rate and  
218 patch size at time of dispersal. That a genealogical representation is possible derives from both the  
219 mechanistic nature of the model and the fact that patches are founded from single cell types. Movie  
220 versions of the Figures 5A and 5B are shown in Supplementary Movie Files 3 and 4.

221 It is important to emphasise that the parameters and timescales chosen in the above simulations are  
222 arbitrary. For any initial growth rate  $> 1$ , it is always possible to choose fast and slow dispersal times  
223 relative to the time of the peak patch population that will result in selection over the timescale of  
224 dispersal feeding back to affect the growth rate of cells. In contrast, if the dispersal time is equal to the  
225 initial peak time, then no evolutionary change in cell growth rate will be observed.

## 226 Evolution of patch traits

227 The above model shows how a second timescale, defined by dispersal events necessary for establishment  
228 of new patches, affects the evolution of cell growth rate, and how changes in cell growth rate affect the  
229 evolutionary dynamics of patches. From the patch perspective, derived patches are more fit than

230 ancestral patches, but this is not a consequence of traits adaptive at the patch level. Under both slow  
231 and fast dispersal regimes, selection favours cells whose growth rate maximises the number of cells  
232 available for dispersal. Changes in cell growth rate thus fully explain the evolutionary dynamics of  
233 patches. This cell-level perspective further emphasises the previous comment that patches are not to be  
234 confused with even the most basic manifestations of multicellular life forms.

235 Nonetheless, our prediction is that an ecological scaffold that couples short and long-term timescale  
236 dynamics establishes conditions conducive to the evolution of traits adaptive at the level of patches. By  
237 this we mean traits that would be difficult to explain from the view point of cells. This prediction  
238 becomes intuitive upon switching perspectives: from a cell-level to a patch-level perspective. Although  
239 patches are endowed with Darwinian-like properties, there is scope for patches to evolve genuine  
240 Darwinian properties — in a ratchet-like manner<sup>31</sup> — so that patches participate in the process of  
241 evolution by natural selection and thus become bearers of adaptations at the patch level.

242 What might such patch-level adaptations entail and what might constitute their mechanistic (cell-level)  
243 basis? A fundamental requirement given the need for patches to pass through single bottlenecks at each  
244 recursion, is evolution of a stochastic epigenetic switch (a simple developmental programme), such as  
245 observed previously in numerous experiments<sup>32,33</sup> including those arising from experimental  
246 explorations of the evolution of multicellular life<sup>19</sup>.

247 To investigate this possibility we extend the basic model to include two types of cell. The first type,  
248 which we denote  $G$ , is essentially the same as in our first model, with the exception that at each  
249 reproduction event there is some probability,  $q$ , that instead of giving rise to another  $G$  cell, a different  
250 cell type, denoted  $S$ , is produced instead. The  $S$  cells also consume resources, but unlike  $G$  cells,  $S$  cells  
251 cannot replicate or be dispersed. The production of  $S$  cells is thus costly: they deplete resources and  
252 reduce the number of cells available for dispersal. Full mathematical details of the model are given in  
253 the Supplementary Information file, but see also Box 2. The phenotype of  $G$  cells is quantified by their  
254 growth rate,  $\beta$ , and the probability of production of  $S$  cells,  $q$ . All other parameters are fixed.

255 In this formulation,  $S$  cells are a rough approximation for soma. Like soma,  $S$  cells are an evolutionary  
256 dead end. In this switch to considering  $S$  cells as proxy for soma, it follows that  $G$  cells approximate  
257 germ cells: like germ cells, these dispersing cells found the next collective generation.

258 To connect with our previous results, simulations of the model were first performed with dispersal  
259 depending solely on the number of  $G$  cells within the patch at the time of dispersal, thus patches that  
260 optimise the number of  $G$  cells maximise the number of descendent patches. As to be expected given  
261 the cost of maintaining  $S$  cells, in repeated simulations of the model in which mutation affects both

262 growth rate and the probability of production of  $S$  cells, the rate of  $S$  cell production under both slow  
263 and fast dispersal regimes declines to zero (Figure 6A-C). The equilibrium fitness of both cells and  
264 patches tend to the same values as in the previous model.

265 To determine whether the ecological scaffold established by patchily distributed resources and dispersal  
266 between patches establishes conditions favouring evolutionary emergence of a division of labour, the  
267 model was re-run, but with  $S$  cells now endowed with ability to aid dispersal of  $G$  types. Mathematically  
268 this was achieved by defining the probability that a cell within a patch is chosen for dispersal be a  
269 function of both the number of  $G$  and  $S$  cells in the patch (see Box 2 for details).

270 As shown in Figures 6D-F (and especially 6E)  $S$  cells are favoured under the slow dispersal regime (the  
271 probability of  $S$  cell production rapidly evolves away from zero and plateaus at 0.08). Under this  
272 scenario the equilibrium cell growth rate is higher than when dispersal depends solely on the number of  
273  $G$  cells (contrast this with the solid lines in Figures 6A and D). This is because increased production of  
274  $S$  cells slows the rate of production of  $G$  cells allowing the population to peak at a comparatively higher  
275 growth rate. Mean patch fitness depends on the contribution that  $S$  cells make toward dispersal of  $G$   
276 types.

277 Under the fast regime  $S$  cells are not favoured and the growth rate of  $G$  simply increases to its  
278 maximum limit. However, if the maximum allowable growth rate is increased beyond the limit of  
279  $\beta = 2$ , production of  $S$  cells under the fast dispersal regime can be favoured. The key point is that  
280 under the slow dispersal regime, production of  $S$  is always favoured. When dispersal time is fast,  
281 production of  $S$  cells is favoured only if cell growth rate can increase to the point at which peak  
282 population size is reduced (through early and rapid depletion of resources). In real systems it is likely  
283 that cells would already be close to their maximum growth rate and thus further increases would  
284 depend on rare beneficial mutations. In contrast, decreases in growth rate are readily achieved via  
285 deleterious mutations.

286

## 287 DISCUSSION

288 The major evolutionary transitions in individuality pose some of the most intriguing and complex  
289 problems in biology. Numerous perspectives have been offered, ranging from theoretical multi-level  
290 selection frameworks<sup>3,34-39</sup>, to views that give prominence to explanations for the evolution of  
291 cooperation<sup>40-42</sup>; from perspectives that emphasise the importance of specific mechanisms<sup>4,43-45</sup> through  
292 those, like us, that emphasise the pivotal importance of the origins of group-level Darwinian  
293 properties<sup>5,7,9,11,13,46,47</sup>.

294 Encompassed within these diverse views are central concepts that are often ambiguous. This is  
295 particularly true of scenarios in which ETIs are described in terms of “shifts in levels of selection”, or  
296 more specifically, shifts between multi-level selection (MLS) frameworks MLS1 (where individual cells  
297 are the focus of attention) and MLS2 (where groups are replete with Darwinian properties). A  
298 thorough analysis lead Okasha<sup>3</sup> to conclude the existence of a “grey area” between early and later stages  
299 of ETIs where both a MLS1 and MLS2 perspective can be taken. Closely allied is the notion of “fitness  
300 decoupling”<sup>30</sup> — a sense that as selection shifts from a lower to a higher level the fitness of the higher  
301 level decouples from that of the lower — and the related idea of “de-Darwinisation” of lower level  
302 components<sup>5</sup>. While to the initiated all these terms convey meaning, they remain metaphorical and  
303 descriptive: discussion of issues surrounding ETIs needs to become mechanistic. The challenge is to  
304 know where to start.

305 Our mechanistic approach places emphasis on simplicity, causality and gives prominence to ecological  
306 factors. The ability of natural selection to act on collectives of cells depends on emergence of some  
307 manifestation of heritable variance in fitness at the collective level. In our take-nothing-for-granted  
308 approach the possibility that this arises from co-option of pre-existing cell-level traits was recognised,  
309 but put aside. While resulting in a high bar, it gives emphasis to the fact that reproduction, heredity  
310 and variation are derived traits and their existence should not be presumed<sup>7,11,14</sup>. It has also made  
311 transparent a genuine dilemma, namely, the need to explain how Darwinian properties emerge from  
312 non-Darwinian entities and thus by non-Darwinian means. If Darwinian properties do not pre-exist,  
313 or cannot arise by co-option of pre-existing lower-level traits, then their earliest manifestation  
314 necessarily lies in some exogenous factor(s). The solution we advocate involves recognising the  
315 continuity between organisms and their environments; the idea that Darwinian-like properties can be  
316 scaffolded by the environment in much the same way that reproduction in viruses is scaffolded by the  
317 host cell<sup>5</sup>, or that development can be scaffolded by overlap of parts between parents and offspring<sup>48</sup>.

318 The mechanistic models outlined here show that certain ecological structures can scaffold Darwinian-like properties on collectives, causing the constituent cells to experience selective conditions as if they  
319 were members of nascent multicellular organisms — even to the point where traits emerge that are  
320 defining features of multicellular life. The circumstances are minimal: nothing more than patchily  
321 distributed resources and a means of dispersal between patches. The existence of patches ensures that  
322 variation among collectives is discreet, while establishment of future recurrences of patches via single  
323 founding cells not only reinforces discreteness but is akin to reproduction. At the same time passage  
324 through a single cell bottleneck establishes high fidelity between parent and offspring patches.  
  
325  
326 The second timescale is of critical importance in that it underpins a death-birth process at the level of  
327 patches<sup>49,50</sup>. Without this feature there would be no, or minimal, evolutionary impact of the second  
328 timescale on the fate of cells. Patches fail or succeed based on properties of the cells. The fact that slow  
329 growing cells are favoured when dispersal time is long is a direct consequence of the feedback between  
330 the patch-level birth-death process and the evolutionary dynamics of cells. Although within-patch  
331 selection favours fast growing cells, patches dominated by fast growing cells contain few viable cells for  
332 dispersal. Long-term success of cells thus comes from alignment of cell and patch fitness. The model  
333 thus explicates the concept of fitness decoupling. If the growth rate of cells is fast relative to the longer  
334 timescale, such that there is suboptimal patch occupancy at the time of dispersal, then patch-level  
335 selection will drive the evolution of reduced cell growth rate, leading to enhanced patch fitness. As  
336 noted above there are numerous parallels with certain models of disease evolution.  
  
337 An at first unexpected, albeit important, subtlety surrounding the second timescale arises from its  
338 frequency of occurrence relative to the initial growth rate of cells. Beginning from a position where the  
339 growth rate of cells leads to suboptimal patch occupancy at the time of dispersal, as in Figure 3, a  
340 dispersal time that coincides with the exponential growth phase of cells drives an increase in cell growth  
341 rate (Figure 3D), while also marginally increasing patch fitness. More significant though is the fact that  
342 the fast dispersal regime is not conducive to the evolution of a reproductive division of labour. Under  
343 the fast dispersal regime growth rate of cells is the sole factor governing patch success: any reduction in  
344 total yield of  $G$  cells due to production of  $S$  cells is not offset by contributions that  $S$  cells make to  
345 dispersal.  
  
346 For the evolutionary emergence of a reproductive division of labour the second timescale needs to occur  
347 when cells are not in exponential growth phase. When evolutionary success depends on being the  
348 fastest, limited opportunities exist for exploration of phenotypic novelty<sup>51-53</sup>. This stems from the  
349 simple fact that manifestations of phenotypic novelty typically come at some cost to growth rate. In

350 other words, opportunities for evolution to explore phenotypic space require the possibility that slow  
351 growing types not be excluded by selection. Selection comes to reward persistence and not simply types  
352 that grow fastest.

353 There are additional aspects of the longer timescale that are illuminating. It is usual when discussing the  
354 transition from cells to multicellular life to consider the cell the “lower level” and the group the “higher  
355 level”. Accordingly, the transition from cells to multicellular life is often referred to as a levels of  
356 selection problem<sup>3,5,34,37</sup>. The same is true for any of the major ETIs. It is clear from our patch model  
357 that the evolution of multicellular life is better articulated as a problem to be solved by understanding  
358 conditions leading to the emergence of a second timescale over which a birth-death process operates on  
359 discretely packaged variation. This shift from levels to timescales does much to clarify the kinds of  
360 conditions necessary to effect ETIs<sup>22,28,29</sup>.

361 The occurrence of ecological conditions in nature that generate birth-death dynamics at a timescale  
362 longer than the doubling time of particles are likely rare, perhaps explaining why transitions to  
363 multicellular life are rare<sup>13</sup>. Elsewhere we have articulated spatially structured environments afforded by  
364 reeds in ponds about which surface-colonising microbial mats form, allowing cells to access oxygen that  
365 is otherwise limiting. Periodic collapse of mats marks death events that allow the possibility that new  
366 mats arise by dispersal from extant types<sup>7,21</sup>. Precisely this scenario underpinned design of on-going  
367 lineage selection experiments exploring the evolution of multicellular life<sup>19,20</sup>.

368 Once attention focusses on dispersal events, along with recognition that such events may effect  
369 collective reproduction, then attention turns to emergence of simple primordial lifecycles. Those  
370 involving more than a single phase, for example those involving soma- and germ-like phases, establish  
371 by virtue of the life cycle, a second timescale over which selection acts<sup>19</sup>. A significant aspect of such life  
372 cycles is the fact that birth-death events depend on the efficacy of developmental processes that become  
373 the focus of selection. Indeed, the evolution of lifecycles is intimately connected to the transition of  
374 multicellular life. Even in our simple conceptual model outlined above, the moment that  $S$  cells have a  
375 selected advantage, then a rudimentary life cycle manifests, with selection able to set the developmental  
376 programme via effects on  $q$ , the rate at which  $S$  cells are produced. Arguably this marks an early step in  
377 the process of endogenisation: the process by which externally imposed Darwinian-like properties  
378 become integral features of the new entity<sup>3,14</sup>. Another possibility would be for  $S$  cells to provision new  
379 environments with resources as does the cotyledon in a plant seed, thus freeing evolving collectives from  
380 dependence on patchily distributed resources. In the *Pseudomonas* experiment, as generations of  
381 selection have passed, dependence of the evolving lineages on the scaffold has lessened. This is

382 especially noticeable via evolution of a simple developmental programme controlling the switch  
383 between soma-like and germ-like phases<sup>19</sup>.

384 Thus far we have been silent on cooperation and the causes of cooperation that typically feature so  
385 prominently in discussions on the evolution of multicellular life<sup>41,54</sup>. There is no doubt that cooperation  
386 is a basic feature of multicellular organisms, but the scaffolding perspective does not presuppose  
387 cooperation among cells as a necessary first step. Nonetheless behaviours that might reasonably be  
388 labelled as cooperation stand to evolve given appropriate ecological scaffolds. For example, the slow  
389 growing cells favoured when dispersal time is slow (Fig 3A, 4A and 5A) could be labelled cooperating  
390 types because they show restraint in the face of plentiful resources and the fast growing mutants arising  
391 within patches might be termed “cheats”, but there is no need to use such labels. Indeed, applying  
392 these labels brings focus to the individual cell, and detracts from the ecological context and importance  
393 of timescales that is critical to scaffolding, and to where causal processes lie.

394 One behaviour that is readily labelled as an extreme form of cooperation — suicidal altruism — is  
395 evident in the *S* cells. Such behaviours can be seen from the perspective of single cells with the  
396 temptation to invoke inclusive fitness<sup>55</sup>, but to do so, would be to ignore the importance of ecology<sup>56</sup>  
397 and population structure<sup>17</sup>. It is the meta population structure determined by ecological circumstances  
398 that ensures patches are founded by single cells and this both limits within patch conflict, while also  
399 being necessary for the emergence and maintenance of a reproductive division of labour. In our model  
400 within-patch close kinship is thus a consequence of environmental structure.

401 The models developed in this paper are the simplest possible constructs that illustrate the process of  
402 ecological scaffolding. In simplifying to its most basic formulation a strict ecology has been assumed.  
403 For example, a single cell is only ever dispersed into a new patch, there is no migration between patches,  
404 and dispersal occurs for all patches at the same moment on a fixed, deterministic, timescale. On-going  
405 work explores the effects of relaxing these assumptions and in a forthcoming paper we show that the  
406 overall effects of scaffolding are robust, up to a point, to increases in bottleneck size and to  
407 asynchronous dispersal.

408 The concept of ecological scaffolding has a number of applications and implications. In concluding we  
409 mention briefly three areas. The first concerns the emergence of the first self-replicating chemistries at  
410 the moment life emerges from non-living material. Recognition that Darwinian-like properties might  
411 emerge from the interplay between chemistry and environment opens the door to conceptual and  
412 experimental scenarios whereby chemistries that lack capacity for autonomous replication might begin  
413 to transition, through a process of templated production of bioactive compounds, toward a replicative

414 process. Alkaline thermal vents appear to offer as much: these highly compartmentalised structures sit  
415 at the interface between hydrogen-rich fluids arising from the flow of heated water across serpentine,  
416 and acidic ocean waters with the possibility that carbon-dioxide is reduced to biologically active  
417 species<sup>57,58</sup>. Ensuing “growth” of products within the porous compartments sets in place the possibility  
418 of a replicative process. Incorporation of such ecological structures in future experimental designs may  
419 provide Darwinian ingredients that are typically absent from explorations of the chemical origins of life.  
  
420 The second area of relevance is infectious disease biology. To a pathogen, the eukaryotic host offers a  
421 discrete patch of resource. Pathogens that rely on transmission for long-term persistence experience  
422 selection over two timescales. Our model leads to the prediction that pathogens that passage through  
423 restrictive bottlenecks, such as HIV, are likely to have evolved more complex life histories than  
424 currently appreciated, involving, for example, a division of labour. This seems to be true of *Salmonella*  
425 *typhimurium* that switches stochastically between virulent and avirulent cell types, that invade the  
426 lumen, or colonise the gut, respectively. Cells that colonise the lumen, trigger the inflammatory  
427 response, which benefits the faster growing avirulent cells in the gut. However, unlike cells in the gut,  
428 lumen colonising cells are killed by the intestinal innate immune defences and are thus, like soma, an  
429 evolutionary dead-end<sup>59</sup>. A division of labour is also hinted at in the case of HIV and other chronic  
430 RNA viruses in humans that appear to escape the deleterious effects of short-sighted within-host  
431 evolution over prolonged time intervals. Growing evidence suggests that this is attributable to  
432 establishment of germ-line-like lineages<sup>60</sup>.  
  
433 The third example concerns application of ecological scaffolding for in vitro engineering of evolutionary  
434 transitions and particularly for top-down engineering of microbial communities in which communities  
435 eventually become a single symbiotic entity. In the laboratory environment, and aided particularly by  
436 advances in micro / millifluidic technologies, it is a relatively trivial matter to confine populations and /  
437 or communities to thousands of discretised droplets that can then be subject to a death-birth process<sup>21</sup>.  
438 In a forthcoming paper we detail this process and its outcome for the evolution of interactions that  
439 build integrated communities.

## 440 ACKNOWLEDGEMENTS

441 We are grateful to Silvia De Monte, Guilhem Doulcier and members of our respective teams for  
442 stimulating discussion. AJB acknowledges an ARC DECRA fellowship (DE160100690) and support  
443 from both the ARC Centre of Excellence for Mathematical and Statistical Frontiers (CoE ACEMS),  
444 and the Australian Government NHMRC Centre for Research Excellence in Policy Relevant Infectious  
445 diseases Simulation and Mathematical Modelling (CRE PRISM<sup>2</sup>). PB acknowledges a Macquarie  
446 University Research Fellowship and a Large Grant from the John Templeton Foundation (Grant ID  
447 60811). PBR acknowledges generous financial support from MPG core funding and previously from  
448 the Marsden Fund Council from New Zealand Government funding, administered by the Royal  
449 Society of New Zealand.

450 **SUPPLEMENTARY MATERIAL**

451 Supplementary Information

452 Supplementary Movie File 1

453 Supplementary Movie File 2

454 Supplementary Movie File 3

455 Supplementary Movie File 4

456 **BOX 1. Mathematical model**

457 We construct the simplest possible model to demonstrate the dynamics of two nested Darwinian  
458 populations. We assume a fixed population of  $M$  patches each provisioned with a fixed amount of  
459 resource that is consumed by the cells in order to divide. The dynamics within each patch are  
460 independent. Within each patch, cells undergo a birth/ death process for a fixed length of time,  $T$ , after  
461 which a dispersal event occurs leading to a new generation of  $M$  patches (with replenished resources).

462 We first describe the basic birth / death dynamics within a patch (with no mutations), we then describe  
463 how this is modified by allowing for mutation. Cells have a mean life time that, without loss of  
464 generality, is set to 1 and individual cells reproduce at a rate  $\beta$  in proportion to the density of resource  
465 in the patch. Under these dynamics, the density of cells within the patch follows modified Lotka–  
466 Volterra equations, where there is no replenishment of the resource, i.e.,

$$\begin{aligned} \frac{dy}{dt} &= -\beta xy, \\ \frac{dx}{dt} &= (\beta y - 1)x, \end{aligned} \tag{1}$$

467 where  $y(t)$  and  $x(t)$  are the proportions of resource and cells within the patch respectively. The scaling  
468 for these proportions is taken to be the initial amount of resource within the patch,  $N$  61. Initially,  
469  $y(0) \approx 1$ , so cells grow exponentially with the resource being depleted at the same rate. At some point,  
470 the resource becomes significantly depleted leading to a reduction in growth rate. The population peaks  
471 once the rate of growth matches the rate that cells die. After this point the population of cells begins to  
472 decline (the trajectories shown in Figure 1 are examples of these dynamics). The amount of growth and  
473 hence the peak population size is limited by the initial amount of resource in the patch, so high growth  
474 rates lead to large populations that peak early, but which then decrease quickly.  
475

476 We now outline how mutations and hence different competing cell types are included in the model.  
477 We assume that mutations only affect the growth rate of cells and, for simplicity, possible growth rates  
478 are discretised (with step size  $\mu$ ) so that cells can be grouped according to their growth rate. We define  
479 the growth rate of the  $i$ 'th type as  $\beta_i$ . At each reproduction event there is a probability,  $p$ , of creating a  
480 child cell with a different growth rate, and hence with probability  $1 - p$  a cell of the same type is  
481 produced. If a mutant is produced there is a probability  $\nu$  that it has a growth rate larger than the  
482 parent, and therefore  $1 - \nu$  it is smaller than the parent cell. As before, each cell type has the same  
483 mean life span which is set to 1. Unless otherwise indicated these parameters are set as  $p = 0.01$ ,  
484  $\mu = 0.05$  and  $\nu = 0.5$ .

485 Simulating such a model is more difficult than just solving a system of ordinary differential equations  
486 (ODEs) as the introduction of new types through mutations is a stochastic process. Simulation of a full  
487 stochastic model of the process defined above is possible, but undesirable as it is computationally  
488 expensive. Instead we approximate the dynamics with a piecewise deterministic process, where the times  
489 of the introduction of new types via mutations are modelled stochastically, but the growth dynamics  
490 between these times are modelled deterministically<sup>62</sup>. Hence between the introduction of new types,  
491 the proportions of each type already in the patch evolves via the set of  $m + 1$  ODEs,

492

493

$$\begin{aligned} \frac{dy}{dt} &= -y \sum_{i=1}^m \beta_i x_i, \\ \frac{dx_i}{dt} &= (\beta_i y - 1)x_i, \quad i = 1, \dots, m, \end{aligned} \tag{2}$$

494 Where  $m$  is the number of types currently in the patch. This is equivalent to Lotka–Volterra dynamics  
495 with competition, but where resources are not replenished.

496 The use of the piecewise deterministic models, where times of new mutants arising are stochastic but  
497 the birth-approach is a pragmatic comprise between computational efficiency and realism, but ignores  
498 other stochastic effects, such as the time for the cells to grow to an appreciable number before  
499 exponential growth is fully underway. Full stochastic models can account for this and display a  
500 distribution of patch sizes with a much larger variance. Forthcoming work shows that the evolutionary  
501 dynamics are similar.

502 The particular dynamics of the system allow further approximation to improve the computational  
503 efficiency of the model. The bottleneck imposes a strong homogeneity to the patch, which means that  
504 new mutants cannot reach appreciable frequencies within a single patch generation. This means it is  
505 possible to limit the number of mutants in the system, typically to just the first two types, which means  
506 that it is not necessary to allow mutant types to create further mutants. An example trajectory is shown  
507 in Figure 2 with full details provided in the Supplementary Information.

508 Simulation of the full model over multiple generations proceeds as follows. Each of the  $M$  patches are  
509 seeded with a single cell (at the same time). For each patch the model described above is simulated up  
510 to the dispersal time  $T$ . For the first model, the dispersal dynamics only depend on the proportion of  
511 cells within each patch. A new generation of patches is founded by randomly selecting a patch in  
512 proportion to the number of cells within it and then randomly selecting a cell, within the chosen patch,  
513 again in proportion to its number within the patch. This procedure is equivalent to simply picking

514 particles randomly from the whole population of patches. Hence the larger the number of a given type  
515 within the population, the more likely it is to be dispersed. This two-step procedure is simulated for a  
516 given number of generations and quantities, such as the average growth rate within a generation, can be  
517 calculated. Because the model is mechanistic, it is possible to track the genealogies of both the cells and  
518 patches, as shown in Figure 5.

519

520 **Box 2: Mathematical model with two cell types**

521 We take a similar approach to simulating this version of the model, employing a piecewise-deterministic  
522 approximation where the times of the introduction of new types due to mutations are stochastic and the  
523 birth / death dynamics are deterministic. Hence between mutations the dynamics evolve as

$$\begin{aligned}\frac{dy}{dt} &= -y \sum_{i=1}^m \beta_i x_i - dyz, \\ \frac{dx_i}{dt} &= \beta_i(1 - q_i)yx_i - x_i, \quad i = 1, \dots, m(t), \\ \frac{dz}{dt} &= y \sum_{i=1}^m q_i \beta_i x_i - z,\end{aligned}$$

524  
525 where  $z(t)$  is the proportion of  $S$  cells in the patch. This model introduces two new parameters:  $q_i$ ,  
526 which is the per event probability of producing an  $S$  cell and  $d$ , which is the rate at which  $S$  cells  
527 consume the resource. The parameter  $d$  remains fixed, but  $q_i$  is subject to mutation. As the phenotype  
528 space is now two-dimensional the scheme for generating mutants is different to the first model, but the  
529 number of new mutants remains limited to the first two. We assume that only  $G$  cells can be dispersed,  
530 hence the bottleneck enforced by the dispersal mechanism means a patch is always seeded from a single  
531  $G$  type cell. More details of the model and mutational process are given in the Supplementary  
532 Information.

533 For dispersal we assume the system to be composed of  $k = 1, \dots, K$  patches. Then let  $x_i^{(k)}$  be the  
534 proportion of type  $G_i$  and  $y^{(k)}$  be the proportion of type  $S$  in patch  $k$ . Each patch is founded by a  
535 single  $G$  cell with phenotype  $(\beta, q)^{(k)}$ . These cells reproduce, mutate, and create  $S$  cells until the  
536 dispersal time,  $T$ , at which point a sample is taken from the resulting populations to create the next  
537 generation. This occurs in two steps:

- 538 1. Randomly select a patch  $k$ , in proportion to its weight,  $w_k$ , which is function of the proportion of  
539 its constituents,  $x_i^{(k)}$   $i = 1, 2, 3$  and  $y^{(k)}$ .
- 540 2. From the patch selected in step 1, randomly select a  $G$  cell from the total patch population.

541 We consider two ways to assign a weight to patches for the first step. Most simply, if we take  
542  $w_k = \sum_{i=1}^m x_k$ , then the dispersal process is as in the first model, i.e., the probability of choosing a patch is  
543 proportional only to the number of  $G$  cells, so patches with more  $G$  cells at the time of dispersal are  
544 more likely to be sampled from. The other function we consider is

545

$$w_k = (1 + 200y^{(k)}) \sum_{i=1}^m x_i^{(k)}.$$

546 This can be interpreted as the  $S$  cells aiding dispersal from the patch, for example by attracting the  
547 dispersal agent. The constant multiplying  $y^{(k)}$  term represents the strength of this effect.

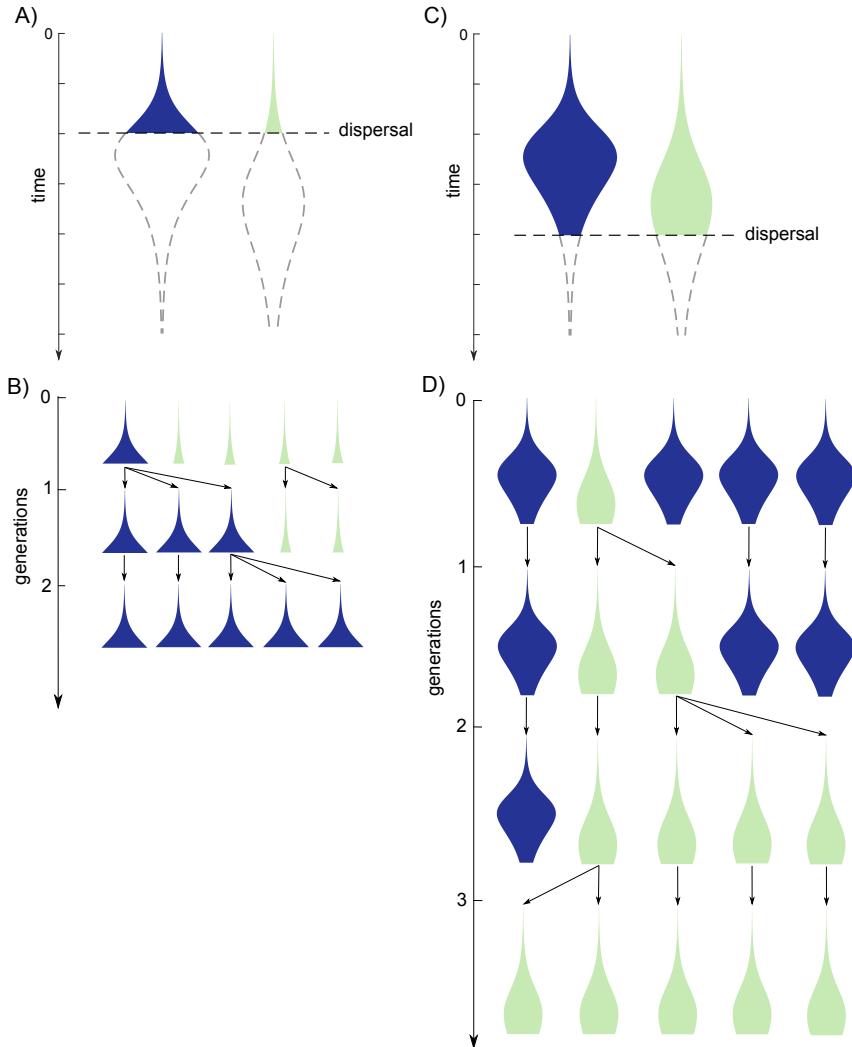
548 REFERENCES

- 549  
550  
551 1 Buss, L. W. *The Evolution of Individuality*. (Princeton University Press, 1987).  
552 2 Maynard Smith, J. & Szathmáry, E. *The Major Transitions in Evolution*. (Freeman, 1995).  
553 3 Okasha, S. *Evolution and the Levels of Selection*. (Oxford University Press, 2006).  
554 4 Michod, R. E. *Darwinian Dynamics: Evolutionary Transitions in Fitness and Individuality*.  
555 (Princeton University Press, 1999).  
556 5 Godfrey-Smith, P. *Darwinian Populations and Natural Selection*. (Oxford University Press,  
557 2009).  
558 6 Hull, D. L. Individuality and selection. *Ann. Rev. Ecol. Syst.* **11**, 311-332, (1980).  
559 7 Rainey, P. B. & Kerr, B. Cheats as first propagules: a new hypothesis for the evolution of  
560 individuality during the transition from single cells to multicellularity. *Bioessays* **32**, 872-880,  
561 (2010).  
562 8 Libby, E. & Rainey, P. B. A conceptual framework for the evolutionary origins of  
563 multicellularity. *Phys. Biol.* **10**, 035001, (2013).  
564 9 De Monte, S. & Rainey, P. B. Nascent multicellular life and the emergence of individuality. *J.  
565 Biosci.* **39**, 237-248, (2014).  
566 10 Lewontin, R. C. The units of selection. *Ann. Rev. Ecol. Syst.* **1**, 1-18, (1970).  
567 11 Griesemer, J. The units of evolutionary transition. *Selection* **1**, 67-80, (2000).  
568 12 Bourrat, P. From survivors to replicators: Evolution by natural selection revisited. *Biol. Phil.* **29**,  
569 517-538, (2014).  
570 13 Rainey, P. B. & De Monte, S. Resolving conflicts during the evolutionary transition to  
571 multicellular life. *Annu. Rev. Ecol. Evol. Syst.* **45**, 599-620, (2014).  
572 14 Okasha, S. The strategy of endogenization in evolutionary biology. *Synthese*,  
573 doi.org/10.1007/s11229-11018-11832-11226, (2018).  
574 15 Hanschen, E. R. *et al.* The Gonium pectorale genome demonstrates co-option of cell cycle  
575 regulation during the evolution of multicellularity. *Nat. Commun.* **7**, 11370, (2016).  
576 16 Ratcliff, W. C., Denison, R. F., Borrello, M. & Travisano, M. Experimental evolution of  
577 multicellularity. *Proc. Natl. Acad. Sci. USA* **109**, 1595–1600, (2012).  
578 17 Wade, M. J. *Adaptation in Metapopulations: How Interactions Change Evolution*. (University  
579 of Chicago Press, 2016).  
580 18 Capra, L. R., Griesemer, J. R. & Wimsatt, W. C. in *Vienna Series in Theoretical Biology* (eds  
581 G. B. Müller, G. P. Wagner, & W. Callebaut) 426 (The MIT Press, Cambridge, MA, 2014).  
582 19 Hammerschmidt, K., Rose, C., Kerr, B. & Rainey, P. B. Life cycles, fitness decoupling and the  
583 evolution of multicellularity. *Nature* **515**, 75-79, (2014).  
584 20 Rose, C. J., Hammerschmidt, K. & Rainey, P. B. Meta-population structure and the evolutionary  
585 transition to multicellularity. *bioRxiv*, doi.org/10.1101/407163, (2019).  
586 21 Rainey, P. B., Remigi, P., Farr, A. D. & Lind, P. A. Darwin was right: where now for  
587 experimental evolution? *Curr Opin Genet Dev* **47**, 102-109, (2017).  
588 22 Van Valen, L. Energy and evolution. *Evolutionary Theory* **1**, 179-229, (1976).  
589 23 Ewald, P. W. *Evolution of Infectious Disease*. (Oxford University Press, 1994).  
590 24 Levin, B. R. & Bull, J. J. Short-sighted evolution and the virulence of pathogenic  
591 microorganisms. *Trends Microbiol.* **2**, 76-81, (1994).  
592 25 Frank, S. A. Natural selection. III. Selection versus transmission and the levels of selection. *J Evol  
593 Biol* **25**, 227-243, (2012).  
594 26 Lythgoe, K. A., Pellis, L. & Fraser, C. Is HIV short-sighted? insights from a multistrain nested  
595 model. *Evolution* **67**, 2769-2782, (2013).  
596 27 Clarke, E. The problem of biological individuality. *Biol. Theor.* **5**, 312-325, (2010).

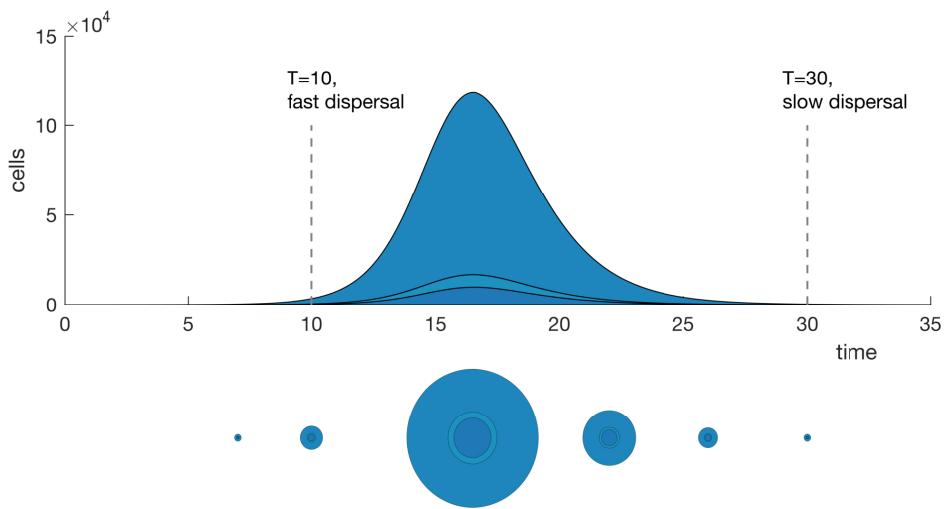
- 597 28 Bourrat, P. Levels of selection are artefacts of different temporal fitness measures. *Ratio* **28**, 40-  
598 50, (2015).
- 599 29 Bourrat, P. Levels, time and fitness in evolutionary transitions in individuality. *Philos Theor Biol*  
600 7, e601, (2015).
- 601 30 Michod, R. E. & Roze, D. in Mathematical and Computational Biology: Computational  
602 Morphogenesis, Hierarchical Complexity, and Digital Evolution (ed C. L. Nehaniv) 47-92  
603 (American Mathematical Society, 1999).
- 604 31 Libby, E. & Ratcliff, W. C. Ratcheting the evolution of multicellularity. *Science* **346**, 426-427,  
605 (2014).
- 606 32 Gallie, J. *et al.* Bistability in a metabolic network underpins the de novo evolution of colony  
607 switching in *Pseudomonas fluorescens*. *PLoS Biol.* **13**, e1002109, (2015).
- 608 33 Remigi, P. *et al.* Ribosome provisioning activates a bistable switch coupled to fast exit from  
609 stationary phase. *Mol Biol Evol*, 10.1093/molbev/msz1041, (2019).
- 610 34 Calcott, B. & Sterelny, K. in *The Vienna Series in Theoretical Biology* (eds G. B. Müller, G. P.  
611 Wagner, & W. Callebaut) 319 (The MIT Press, Cambridge MA, 2011).
- 612 35 Godfrey-Smith, P. & Kerr, B. Gestalt-switching and the evolutionary transitions. *Brit J Philos Sci*  
613 **64**, 205-222, (2013).
- 614 36 Clarke, E. Origins of evolutionary transitions. *J. Biosci.* **39**, 1-14, (2014).
- 615 37 Shelton, D. E. & Michod, R. E. Group selection and group adaptation during a major  
616 evolutionary transition: insights from the evolution of multicellularity in the volvocine algae. *Biol.  
617 Theor.* **9**, 452-469, (2014).
- 618 38 Clarke, E. A levels-of-selection approach to evolutionary individuality. *Biol Philos* **31**, 893-911,  
619 (2016).
- 620 39 Bourrat, P. Evolutionary transitions in individuality: A formal analysis. *Synthese*, in press, (2019).
- 621 40 Queller, D. C. & Strassmann, J. E. Beyond society: the evolution of organismality. *Phil. Trans. R.  
622 Soc. Lond. B* **364**, 3143-3155, (2009).
- 623 41 Bourke, A. F. *Principles of Social Evolution*. 267 (Oxford University Press, 2011).
- 624 42 West, S. A., Fisher, R. M., Gardner, A. & Kiers, E. T. Major evolutionary transitions in  
625 individuality. *Proc. Natl. Acad. Sci. USA* **112**, 10112-10119, (2015).
- 626 43 Boraas, M. E., Seale, D. B. & Boxhorn, J. E. Phagotrophy by a flagellate selects for colonial prey:  
627 A possible origin of multicellularity. *Evol Ecol* **12**, 153-164, (1998).
- 628 44 van Gestel, J. & Tarnita, C. E. On the origin of biological construction, with a focus on  
629 multicellularity. *Proc. Natl. Acad. Sci. USA* **114**, 11018-11026, (2017).
- 630 45 Herron, M. D. *et al.* De novo origins of multicellularity in response to predation. *Sci Rep* **9**,  
631 2328, (2019).
- 632 46 Rainey, P. B. Unity from conflict. *Nature* **446**, 616, (2007).
- 633 47 Bourrat, P. Evolutionary transitions in heritability and individuality. *Theor Biosci*, in press,  
634 (2019).
- 635 48 Griesemer, J. in *Towards a Theory of Development* eds A. Minelli & T. Pradeu) 183-202  
636 (Oxford University Press, 2014).
- 637 49 Simon, B., Fletcher, J. A. & Doebeli, M. Towards a general theory of group selection. *Evolution*  
638 **67**, 1561-1572, (2012).
- 639 50 Doebeli, M., Ispolatov, Y. & Simon, B. Towards a mechanistic foundation of evolutionary  
640 theory. *eLife* **6**, (2017).
- 641 51 Muller, G. B. & Wagner, G. P. Novelty in Evolution - Restructuring the Concept. *Ann. Rev.  
642 Ecol. Syst.* **22**, 229-256, (1991).
- 643 52 Pfeiffer, T., Schuster, S. & Bonhoeffer, S. Cooperation and competition in the evolution of ATP-  
644 producing pathways. *Science* **292**, 504-507, (2001).
- 645 53 Pfeiffer, T. & Bonhoeffer, S. An evolutionary scenario for the transition to undifferentiated  
646 multicellularity. *Proc. Natl. Acad. Sci. USA* **100**, 1095-1098, (2003).

- 647 54 Wilson, D. S. & Sober, E. Reviving the superorganism. *J. Theor. Biol.* **136**, 337-356, (1989).  
648 55 Hamilton, W. D. The genetical evolution of social behavior, I & II. *J. Theor. Biol.* **7**, 1-52,  
649 (1964).  
650 56 Wilson, E. O. & Holldobler, B. Eusociality: origin and consequences. *Proc. Natl. Acad. Sci. USA*  
651 **102**, 13367-13371, (2005).  
652 57 Martin, W. & Russell, M. J. On the origin of biochemistry at an alkaline hydrothermal vent.  
653 *Phil. Trans. R. Soc. Lond. B* **362**, 1887-1925, (2007).  
654 58 Lane, N. The Vital Question: Why is Life the Way it is? , 352 (Profile Books Ltd, 2015).  
655 59 Diard, M. *et al.* Stabilization of cooperative virulence by the expression of an avirulent  
656 phenotype. *Nature* **494**, 353-356, (2013).  
657 60 Lythgoe, K. A., Gardner, A., Pybus, O. G. & Grove, J. Short-Sighted Virus Evolution and a  
658 Germline Hypothesis for Chronic Viral Infections. *Trends Microbiol.* **25**, 336-348, (2017).  
659 61 Black, A. J. & McKane, A. J. Stochastic formulation of ecological models and their applications.  
660 *Trends Ecol. Evol.* **27**, 337-345, (2012).  
661 62 Davis, M. H. A. Piecewise-deterministic Markov processes: A general class of non-diffusion  
662 stochastic models. *J. R. Statist. Soc. B* **46**, 353-388, (1984).  
663

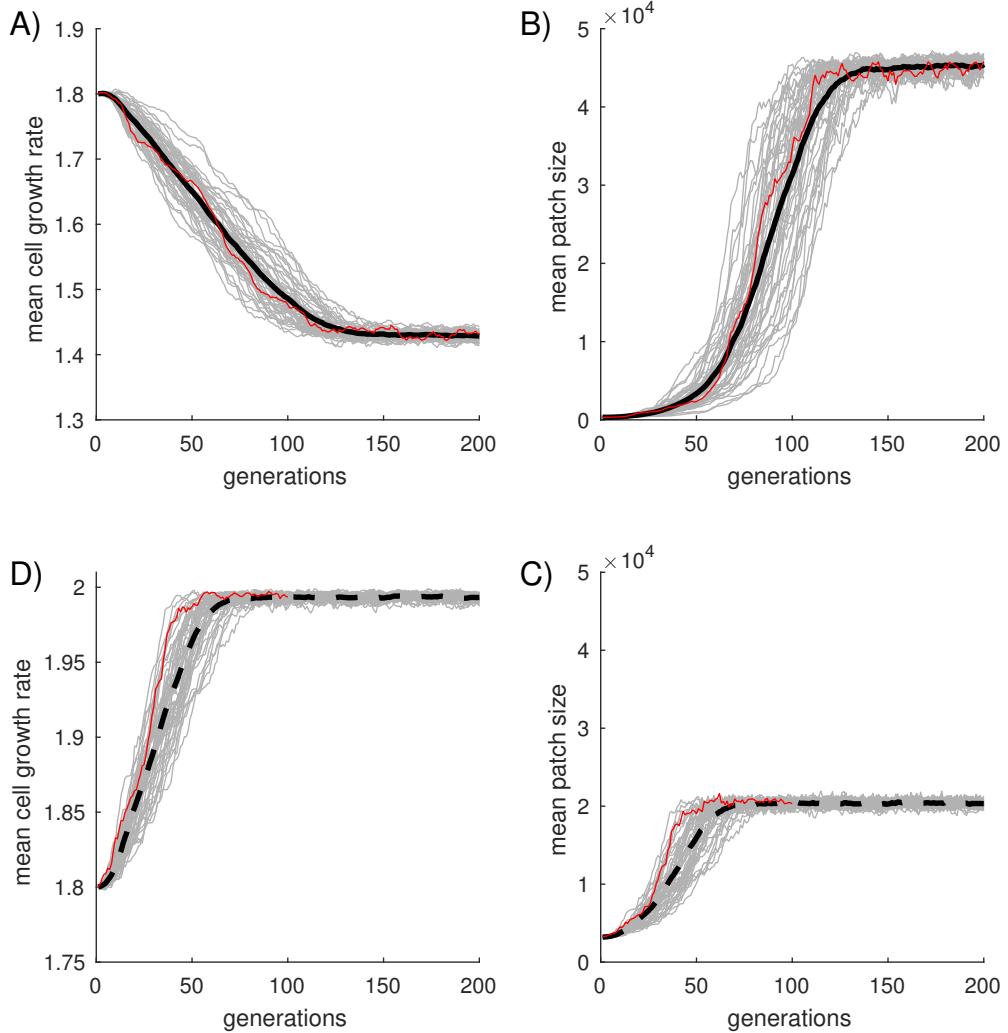
## FIGURES



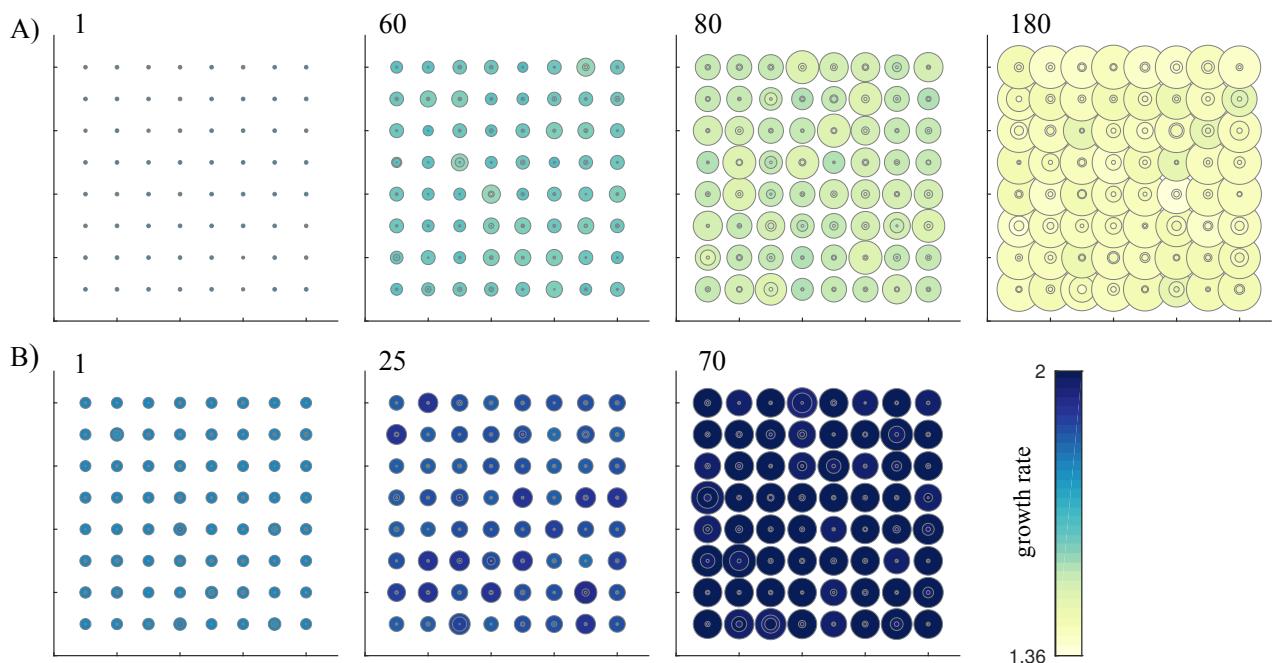
**Figure 1. Scaffolding Darwinian properties.** Patchily distributed resources provide opportunity for two cell types (blue and green) to replicate (blue cells grow faster than green according to equation 1 (Box)). Single cells of each type colonise discrete patches at time  $t = 0$  and consume resources. Difference in growth rate means the relationship between cell density and time differs for blue versus green populations. Should a dispersal event occur during exponential growth (dashed line) then more blue cells will be dispersed relative to green (A) and thus the blue population will be more successful over the long term (B). Conversely, should dispersal occur at a later stage and after resources are depleted (C), then the population of green cells will out compete green over the long term (D).



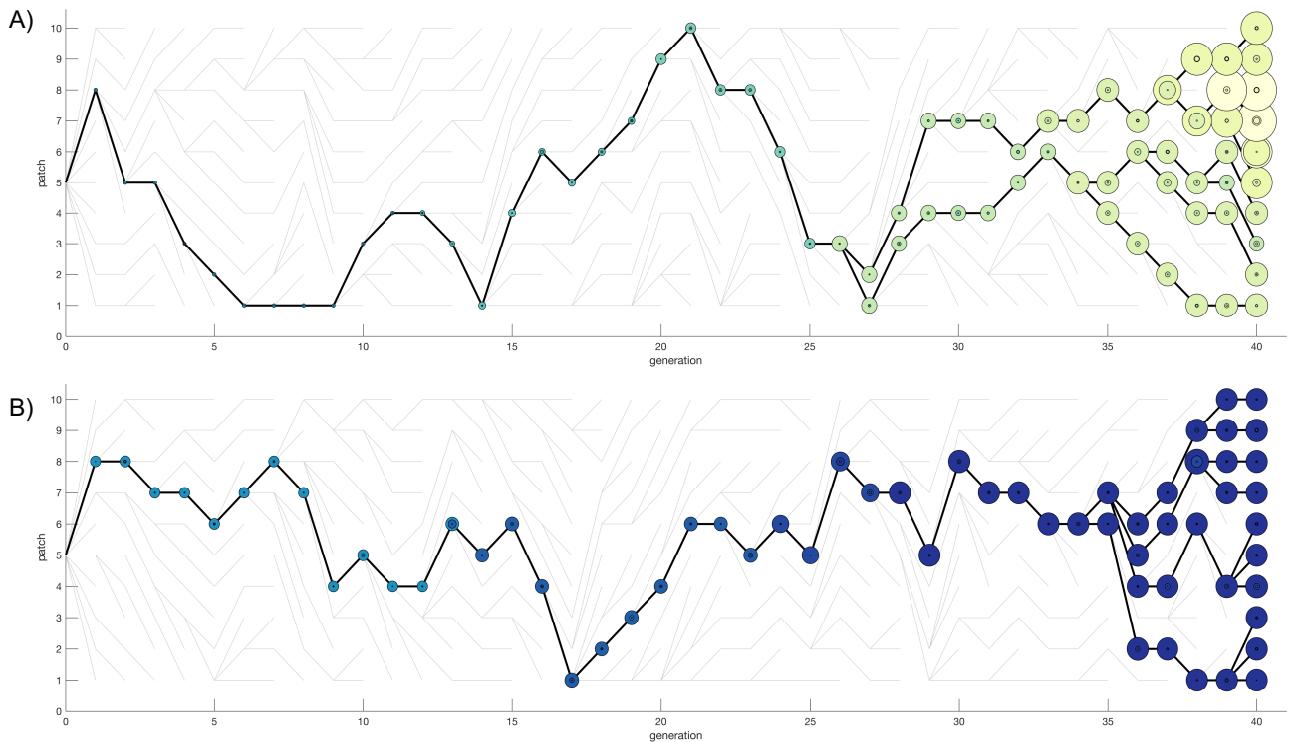
**Figure 2. Single realisation of the within patch model with mutation.** The number of cells is plotted as a function of time starting from a single cell with growth rate  $\beta = 1.8$  and the amount of resource  $N=10^6$ . The darker shaded regions show the numbers of mutant cells. Darker and lighter colours correspond to faster and slower growing cells, respectively. The circles below the main plot are representations of cell numbers (used in the video and Figures 3 and 4) at times 7, 10, 16.5, 22, 26 and 30. In this representation, the *area* of each region is proportional to the number of cells of each type within the patch (with the same colour scheme). The peak number of cells within the patch is reached at time  $\sim 16.5$ , thus for the initial growth rate of  $\beta = 1.8$ , setting a dispersal time of  $T=10$  is considered fast, and  $T=30$  slow (shown by the dashed lines).



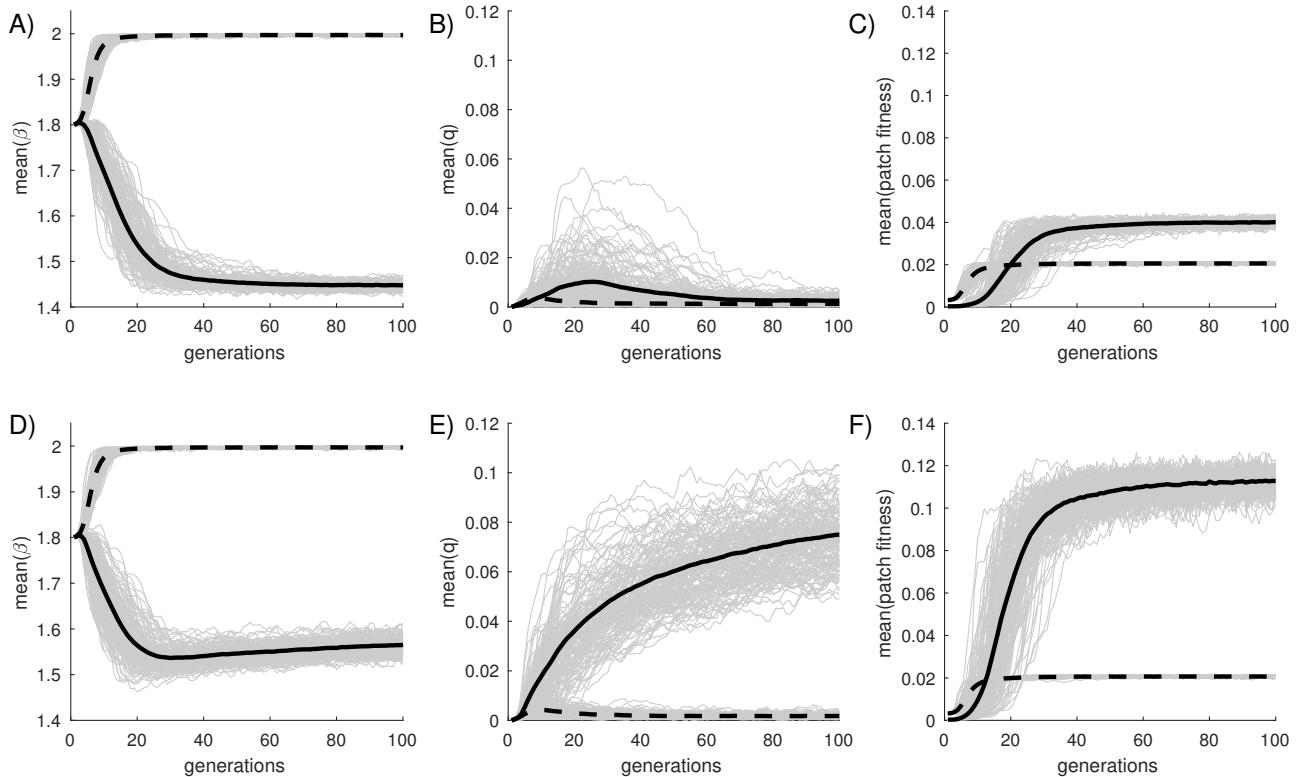
**Figure 3. Effect of dispersal timescale on properties of cells and patches.** Each grey line is from an independent realisation of the stochastic model. The solid black lines are averages over 50 realisations. A and B show the evolution of the average growth rate over all patches in the generation with slow dispersal ( $T=30$ ). C and D are the same but with fast dispersal ( $T=10$ ). Both regimes start with a homogenous population of cells with  $\beta = 1.8$  and in both cases the average patch size increases, but for slow dispersal this is achieved by cells decreasing their average growth rate. Figure 4 shows the 64 patches at the moment of dispersal for two single realisations (shown in red above) after a number of generations.



**Figure 4. Evolution of patch size under slow and fast regimes.** The dynamic of patch-size evolution and corresponding effects on cell growth rate under slow (A) and fast (B) dispersal regimes. Movies of simulations are shown in Supplementary Movie Files 1 and 2. Colour corresponds to cell growth rate and patch size is proportional to the number of cells within patches at the moment immediately prior to dispersal. Generation number is indicated above each panel and corresponds to the realisations highlighted in red in Figure 3.



**Figure 5. Genealogy of patches under slow (A) and fast (B) dispersal regimes.** The simulations to produce these have only 10 patches and modified mutational parameters compared with those in Figures 2 and 3. This is to allow a clearer visualisation of the process, which otherwise requires many more generations to see change. Movie versions of these are included in the Supplementary Movie Files 3 and 4. As in the previous figures, the cell numbers in each patch are proportional to the area of the circles and the growth rates are indicated by the colours, as shown by the colour bar in Figure 4. The mutational parameters are larger for these simulations ( $\mu = 0.05, p = 0.05$ ) so evolution occurs on a quicker timescale as compared with the results shown in Figures 3 and 4.



**Figure 6. Simulations of the two-type model.** A, B and C show the simulations the the model where the probability of dispersal only depends on the number of G cells in the patch at T. A shows the mean growth rate (averaged over generations), B is the mean probability of a reproduction event creating a B cell, and C shows the mean patch fitness. Each grey line is a single stochastic realisation and the solid and dashed lined are averages over 100 independent realisations for slow (T=30) and fast dispersal (T=10) respectively. Panels D, E, and F, show the similar simulations, but where the probability of dispersal from a patch is a function of both the number of G and S cells (see Box 2). In all scenarios, the maximum cell growth rate is limited to 2. The difference in the total number of generations between these simulations (100) and previous results (200) are due to different mutational schemes between the models.