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Structured growth and genetic drift raise relatedness in the social amoeba *Dictyostelium discoideum*

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1 **Structured growth and genetic drift raise relatedness in the social amoeba *Dictyostelium discoideum*.**

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11

12 **One condition for the evolution of altruism is genetic relatedness between altruist and beneficiary,**
13 **often achieved through active kin recognition. Here we investigate the power of a passive process**
14 **resulting from genetic drift during population growth in the social amoeba *Dictyostelium discoideum*.**
15 **We put labelled and unlabelled cells of the same clone in the centre of a plate, and allowed them to**
16 **proliferate outward. Zones formed by genetic drift, due to the small population of actively growing**
17 **cells at the colony edge. We also found that single cells could form zones of high relatedness.**
18 **Relatedness increased at a significantly higher rate when food was in short supply. This study shows**
19 **that relatedness can be significantly elevated before the social stage without a small founding**
20 **population size or recognition mechanism.**

21

22 1. Introduction

23 The fitness and success of a group of cooperators is under constant threat of collapse by the
24 infiltration and spread of selfish individuals, known as cheaters, that reap the benefits of cooperation
25 without paying the costs (1, 2). Kin selection theory predicts that cooperative behaviours will be selected
26 if relatives preferentially interact with one another by kin recognition or high population viscosity (3-5).
27 High relatedness is key to kin selection and has been shown in the social insects (6, 7), birds (8, 9),
28 mammals (10, 11) and microbes (12-14).

29 When a microbial colony grows from a single point, zones of highly-related individuals can form
30 when genotypes, identical except for their fluorescent labels, are mixed in moderate numbers
31 (~1000)(15). Models (16) suggest that the mechanism behind this is genetic drift; only a few cells are
32 actively dividing in any region of the growing colony edge and there are no phenotypic differences
33 between cells (**Fig 1A**). Selection could also produce zones, but it is not required. Simulations suggest
34 that segregation rate is dependent upon availability of nutrients; diffusible nutrients cannot penetrate
35 far into the colony, lowering the effective population and promoting segregation by drift. This outward
36 growth results in the formation of growing zones of clonemates (**Fig 1A**).

37 Further simulations show that when genotypes have clear altruist or cheater phenotypes,
38 altruists obtain the group benefit of their behaviour because of increased relatedness through
39 structured growth (16). However, the effect structured growth has on relatedness has not been
40 quantitatively assessed for any social microbe, and so to address this issue, we use the social amoeba
41 *Dictyostelium discoideum*, now a model organism for social evolution studies (17-19). When food is
42 scarce, starving *Dictyostelium* cells aggregate to form a fruiting body where around one-fifth die to form
43 a stalk, lifting the remaining cells aloft as viable spores (20). Such a costly act of altruism must have

44 evolved in groups of highly-related individuals (3, 4, 21). However, during the aggregation stage, several
45 genotypes mix together forming chimeras (22). Cheater genotypes avoid contributing their fair share of
46 cells to the stalk and are overrepresented in the sporehead (18, 22).

47 We investigate whether genetic drift during the non-social growth stage can act as a mechanism
48 for the formation of highly-related groups prior to the social stage. Whether it will form clear zones like
49 bacteria is not obvious given the high mobility of *Dictyostelium* amoebae. Natural formation of zones of
50 high relatedness during growth should increase the opportunity for altruism to evolve and be
51 maintained, even in initially mixed populations (**Fig 1**).

52 Relatedness within fruiting bodies should increase with distance from the origin because the
53 zones widen with distance whilst the area of an aggregation territory for a given density of amoebae
54 remains constant, increasing the likelihood that cells of only one genotype will fall within an aggregation
55 territory (23) (**Fig 1B**).

56 Here, we demonstrate that even with large initial populations and mobile cells, founder effects
57 and genetic drift at the growing edge act as a mechanism to increase and maintain relatedness in *D.*
58 *discoideum* and that this effect is greater in low food conditions. We also show that clear zones can form
59 from a single cell.

60 **2. Material and Methods**

61 Wild *D. discoideum* clones were grown in the presence of *Klebsiella aerogenes* (Ka) bacteria on
62 SM agar (10g peptone, 1g yeast extract, 10g glucose, 1.9g KH_2PO_4 , 1.3g K_2HPO_4 , 0.49g MgSO_4
63 (anhydrous), 17g agar per litre) plates and incubated 22°C. We obtained amoebae by spreading spores
64 evenly over SM plates in the presence of Ka. Log-phase cells were harvested and washed by repeated
65 centrifugation in KK2 buffer (16.1mM KH_2PO_4 , 3.7mM K_2HPO_4).

66 Clones were transformed with actin15-RFP following the procedure in Pang et al. (24). Clones
67 expressing the markers were then further selected on G418-SM agar plates (30µg G418/ml). Mixes
68 were performed with two clones that were isogenic except for the RFP marker, to eliminate any effects
69 of kin recognition.

70 *Genetic drift assay*

71 Log-phase *NC28.1* and *NC28.1*-RFP cells were mixed in three proportions (wt:RFP; 50:50, 95:5
72 and 99:1) and suspended at a density of 4×10^8 cells/ml in KK2 with 1×10^7 total cells pipetted per plate in
73 the centre of a 150mm wide SM plate spread with Ka. The low-nutrient treatment diluted nutrients 50-
74 fold. Images of whole plates were taken using an SLR digital camera with a 660 nm filter and a 514nm
75 LED as a light source (25).

76 Ten fruiting bodies were taken at each of four distances (at <1, 3, 5 and 7 cm) from the centre
77 and suspended in spore buffer (20mM EDTA and 0.1% NP-40). The proportion of the two genotypes in
78 each fruiting body was then determined by direct counting of spores using a fluorescent microscope.
79 The relatedness (r) within individual sporeheads was calculated as:

$$r = \frac{p(p - \bar{p})}{(1 - \bar{p})} + \frac{q(q - \bar{q})}{(1 - \bar{q})}$$

80 where p and q are the proportion of the two genotypes in the sporehead and are the proportions of p
81 and q for the whole population (26).

82

83 **3. Results**

84 For all three mixtures of *NC28.1* and *NC.28*-RFP, clear zones formed readily (**Fig 2A**), and as a
85 consequence of structured growth, relatedness within fruiting bodies increased with distance from the

86 origin, where relatedness was zero ($R^2 = 0.5944$; $P = 0.0033$; **Fig 2B**). Clones did mix; there was low
87 relatedness in fruiting bodies at the 'borders' between zones.

88 A fortuitous mutation showed that a clonal zone can form from a single mutant cell. On a clonal
89 plate of the genotype *NC34.1* a spontaneous mutation occurred during growth producing a zone of cells
90 that could not form normal fruiting bodies (**Fig 2C**).

91 When the availability of food was decreased, relatedness almost doubled, reaching 0.68 at the
92 plate edge, compared to 0.36 with the regular SM plate ($R^2 = 0.7576$, $P < 0.001$; 2-way ANOVA: nutrient
93 level, $F_{1,24} = 12.7$, $P = 0.003$; distance from origin, $F_{3,24} = 18.1$, $P < 0.001$; **Fig 2B**).

94 For data see the supplemental material.

95

96 **4. Discussion**

97 In any given population, the fitness of an altruistic gene is dependent upon the structure of the
98 population (3, 4, 27, 28). Our results show that high relatedness can be obtained by structured growth
99 and genetic drift without a small initial group size (**Fig 2A**). This result is important for two reasons. First,
100 high relatedness means there will be distinct local groups of cooperators and cheaters, so that the
101 cooperators are protected from exploitation. Second, these results provide one explanation for the
102 disparity between ready chimera formation *in vitro* (22, 29), and high relatedness in wild fruiting bodies
103 (19).

104 This, however, provides another problem; if local dispersal produces areas of high relatedness
105 to neighbours, then it also makes neighbours closest competitors, cancelling out the relatedness effects
106 (5). However, combining structured growth with regular spore dispersal can maintain altruism, as spore
107 dispersal relaxes the effects of competing with highly-related neighbours (5).

108 During the evolution of multicellularity a degree of unrelatedness – and therefore conflict – had
109 to be overcome, perhaps by recognition or via some other mechanism e.g. (30-33). We show that a
110 sufficient degree of structured growth may be sufficient to achieve this, despite the mobility of amoebae
111 that tends to mix cells. Relatedness also increased at a higher rate when there were fewer nutrients,
112 creating more distinct zones (**Fig 2B**). This suggests that in the wild where there are likely to be even
113 fewer nutrients, high relatedness may be generated even more readily. Thus, combined with the added
114 friction of a rough natural substrate this effect upon relatedness may be further enhanced. Other
115 mechanisms such as recognition (30) may have evolved to inflate relatedness further, an important
116 adaptation if motile vegetative *Dictyostelium* cells cause mixing of genotypes slowing the rate of
117 increase.

118 Our study shows that structured growth is a potentially important cause of high relatedness.
119 Once altruism evolves, groups of altruists can form whilst excluding cheaters, allowing more complex
120 mechanisms to evolve.

121

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124 work supported by NERC and NSF grant Nos. DEB 0918931 and DEB 1011513.

125

126 **Figures**

127 **Figure 1: Zones of high relatedness form by genetic drift.** (A) A thin layer of red and green cells,
128 identical except for their colour actively grow at the colony edge, simple sampling error creates small
129 groups of the same genotype. Cells growing in the direction of the arrows form zones of coloured cells.

130 (B) Relatedness increases within fruiting bodies with distance from centre of origin (O). Each aggregation
131 territory (blue-grey lines) is of a similar size, so the probability a territory is contained within a single
132 zone increases with distance from O.

133 **Figure 2: Zones of high relatedness form readily without recognition.** (A) *NC28.1* mixed in three
134 different proportions with *NC28.1*-RFP. The following mixes were (wt:RFP): (i) 50:50, (ii) 95:5 and (iii)
135 99:1. In each case distinct zones of *28.1*-RFP formed. (B) Relatedness (r) within sporeheads increases
136 from the point of origin in a 50:50 mix of *NC28.1* and *NC28.1*-RFP. When food was plentiful (dashed line)
137 r increased significantly with distance from origin (O) ($R^2 = 0.5944$, $P = 0.003$). When food was reduced
138 (solid line), r also increased ($R^2 = 0.7576$, $P < 0.001$) and to a significantly higher level than when grown
139 on plentiful food (2-way ANOVA: nutrient level $F_{1,24} = 12.7$, $P = 0.003$; distance from origin: $F_{3,24} = 18.1$, P
140 < 0.001). (C) A non-fruiting mutant forms a zone from a population of originally clonal cells of *NC34.1*.
141 The mutation occurred at some point between O and the apex of the zone (dashed line). The wide
142 angles suggest the mutant had an accelerated growth rate.

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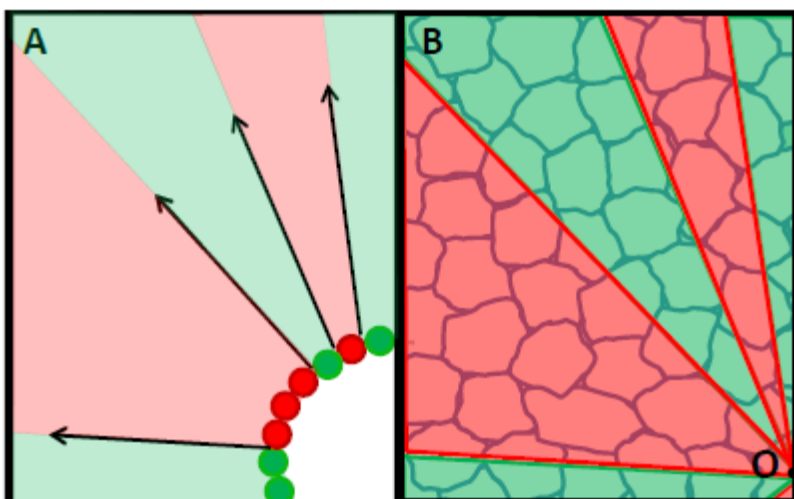
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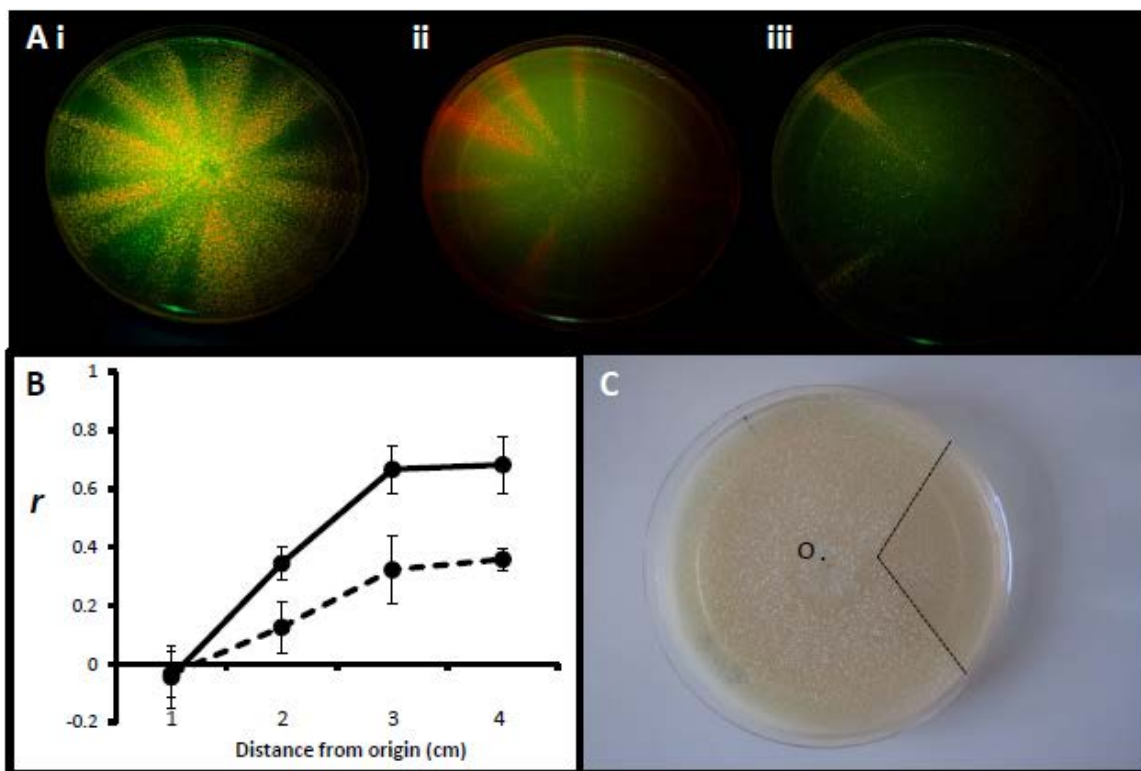
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Fig 1



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Fig 2



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