biology
letters
Evolutionary biology

Biol. Lett. (2012) **8**, 794–797
doi:10.1098/rsbl.2012.0421

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

Neil J. Buttery[1,*], **Chandra N. Jack**[2], **Boahemaa Adu-Oppong**[1], **Kate T. Snyder**[3], **Christopher R. L. Thompson**[4], **David C. Queller**[1] **and Joan E. Strassmann**[1]

[1]*Department of Biology, Washington University in St Louis, One Brookings Drive, Campus Box 1137, St Louis, MO 63130-4899, USA*
[2]*Department of Bioengineering, and* [3]*Department of Ecology and Evolution, Rice University, 6100 Main St., Houston, TX 77005, USA*
[4]*Faculty of Life Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, UK*
**Author for correspondence* (*buttery@wustl.edu*).

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

Keywords: social evolution; genetic drift; social microbes; *Dictyostelium*

1. INTRODUCTION

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

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

Further simulations show that when genotypes have clear altruist or cheater phenotypes, altruists obtain the group benefit of their behaviour because of increased relatedness through structured growth [16]. However, the effect structured growth has on relatedness has not been quantitatively assessed for any social microbe, and so to address this issue, we use the social amoeba *Dictyostelium discoideum*, now a model organism for social evolution studies [17–19]. When food is scarce, starving *Dictyostelium* cells aggregate to form a fruiting body where around one-fifth die to form a stalk, lifting the remaining cells aloft as viable spores [20]. Such a costly act of altruism must have evolved in groups of highly related individuals [3,4,21]. However, during the aggregation stage, several genotypes mix forming chimaeras [22]. Cheater genotypes avoid contributing their fair share of cells to the stalk and are over-represented in the sporehead [18,22].

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

Relatedness within fruiting bodies should increase with distance from the origin because the zones widen with distance while the area of an aggregation territory for a given density of amoebae remains constant, increasing the likelihood that cells of only one genotype will fall within an aggregation territory [23] (figure 1*b*).

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

2. MATERIAL AND METHODS

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

Clones were transformed with actin15-red fluorescent protein (RFP) following the procedure in Pang *et al.* [24]. Clones expressing the markers were then further selected on G418-SM agar plates (30 μg G418 ml^{-1}). Mixes were performed with two clones that were isogenic except for the RFP marker, to eliminate any effects of kin recognition.

(a) *Genetic drift assay*

Log-phase *NC28.1* and *NC28.1*-RFP cells were mixed in three proportions (wt : RFP; 50 : 50, 95 : 5 and 99 : 1) and suspended at a density of 4×10^8 cells ml^{-1} in KK2 with 1×10^7 total cells pipetted per plate in the centre of a 150 mm wide SM plate spread with Ka.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2012.0421 or via http://rsbl.royalsocietypublishing.org.

Received 3 May 2012
Accepted 11 June 2012

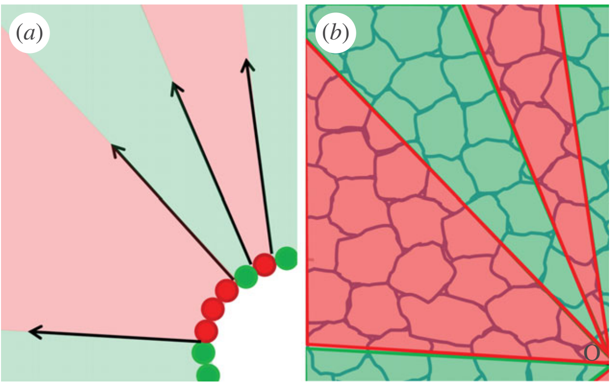

Figure 1. Zones of high relatedness form by genetic drift. (*a*) A thin layer of red and green cells, identical except for their colour actively grow at the colony edge, simple sampling error creates small groups of the same genotype. Cells growing in the direction of the arrows form zones of coloured cells. (*b*) Relatedness increases within fruiting bodies with distance from centre of origin (O). Each aggregation territory (blue–grey lines) is of a similar size, so the probability a territory is contained within a single zone increases with distance from O.

The low-nutrient treatment diluted nutrients 50-fold. Images of whole plates were taken using an SLR digital camera with a 660 nm filter and a 514 nm LED as a light source [25].

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

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

where p and q are the proportion of the two genotypes in the sporehead and are the proportions of p and q for the whole population [26].

3. RESULTS

For all three mixtures of *NC28.1* and *NC28.1*-RFP, clear zones formed readily (figure 2*a*), and as a consequence of structured growth, relatedness within fruiting bodies increased with distance from the origin, where relatedness was zero ($R^2 = 0.5944$; $p = 0.0033$; figure 2*b*). Clones did mix; there was low relatedness in fruiting bodies at the ‘borders’ between zones.

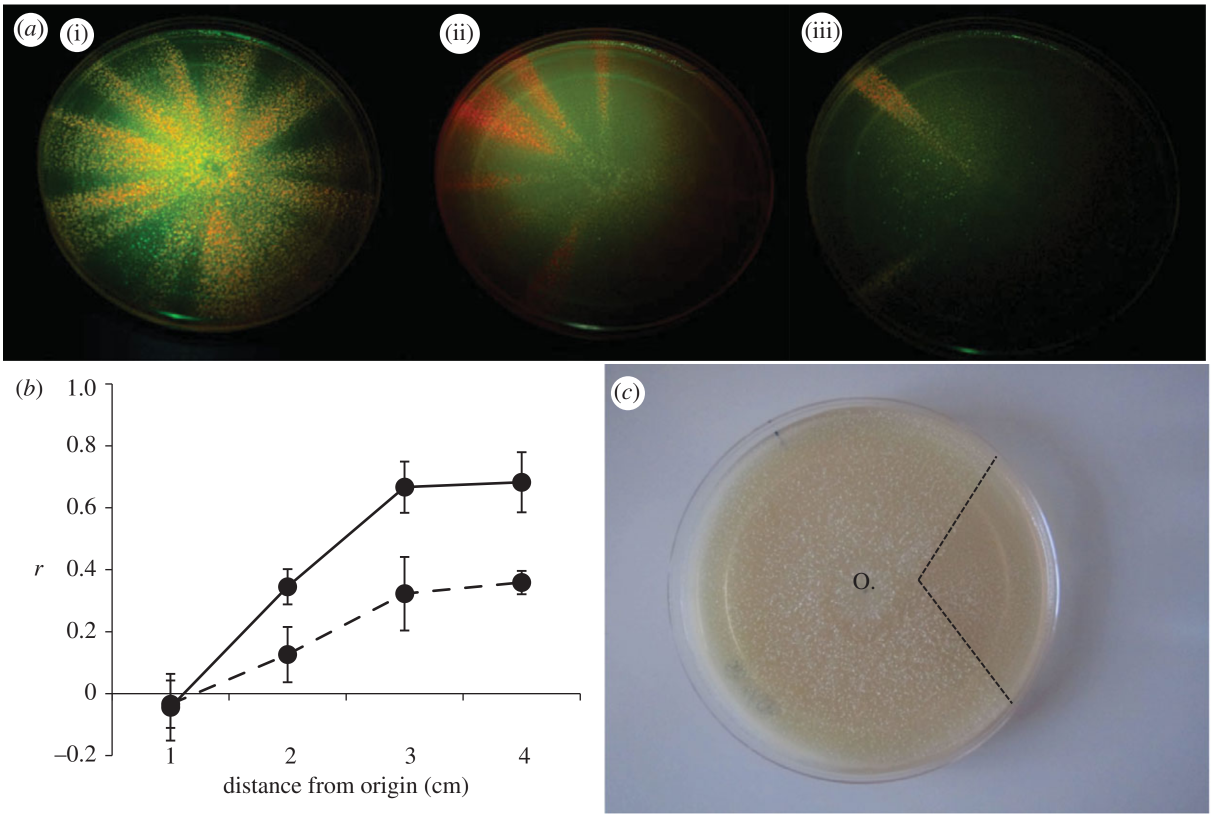

Figure 2. Zones of high relatedness form readily without recognition. (*a*) *NC28.1* mixed in three different proportions with *NC28.1*-RFP. The following mixes were (wt : RFP): (i) 50 : 50, (ii) 95 : 5 and (iii) 99 : 1. In each case, distinct zones of *28.1*-RFP formed. (*b*) Relatedness (r) within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

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

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

For data, see the electronic supplemental material.

4. DISCUSSION

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

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

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

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

Many thanks to the Queller–Strassmann Laboratory for their helpful comments. This material is based on work supported by NERC and NSF grant nos. DEB 0918931 and DEB 1011513.

1 West, S. A., Diggle, S. P., Buckling, A., Gardner, A. & Griffins, A. S. 2007 The social lives of microbes. *Annu. Rev. Ecol. Evol.* **38**, 53–77. (doi:10.1146/annurev.ecolsys.38.091206.095740)

2 West, S. A., Griffin, A. S., Gardner, A. & Diggle, S. P. 2006 Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* **4**, 597–607. (doi:10.1038/nrmicro1461)

3 Hamilton, W. D. 1964 Genetical evolution of social behaviour I. *J. Theor. Biol.* **7**, 1–16. (doi:10.1016/0022-5193(64)90038-4)

4 Hamilton, W. D. 1964 Genetical evolution of social behaviour 2. *J. Theor. Biol.* **7**, 17–52. (doi:10.1016/0022-5193(64)90039-6)

5 Queller, D. C. 1992 Does population viscosity promote kin selection. *Trends Ecol. Evol.* **7**, 322–324. (doi:10.1016/0169-5347(92)90120-Z)

6 Boomsma, J. J. & Grafen, A. 1991 Colony-level sex-ratio selection in the eusocial hymenoptera. *J. Evol. Biol.* **4**, 383–407. (doi:10.1046/j.1420-9101.1991.4030383.x)

7 Silk, J. B. 2009 Nepotistic cooperation in non-human primate groups. *Phil. Trans. R. Soc. B* **364**, 3243–3254. (doi:10.1098/rstb.2009.0118)

8 Griffin, A. S. & West, S. A. 2003 Kin discrimination and the benefit of helping in cooperatively breeding vertebrates. *Science* **302**, 634–636. (doi:10.1126/science.1089402)

9 Sharp, S. P., McGowan, A., Wood, M. J. & Hatchwell, B. J. 2005 Learned kin recognition cues in a social bird. *Nature* **434**, 1127–1130. (doi:10.1038/nature03522)

10 Charpentier, M. J. E., Peignot, P., Hossaert-Mckey, M. & Wickings, E. J. 2007 Kin discrimination in juvenile mandrills, *Mandrillus sphinx*. *Anim. Behav.* **73**, 37–45. (doi:10.1016/j.anbehav.2006.02.026)

11 Oriain, M. J. & Jarvis, J. U. M. 1997 Colony member recognition and xenophobia in the naked mole-rat. *Anim. Behav.* **53**, 487–498. (doi:10.1006/anbe.1996.0299)

12 Chaine, A. S., Schtickzelle, N., Polard, T., Huet, M. & Clobert, J. 2010 Kin-based recognition and social aggregation in a ciliate. *Evolution* **64**, 1290–1300.

13 Kuzdzal-Fick, J. J., Fox, S. A., Strassmann, J. E. & Queller, D. C. 2011 High relatedness is necessary and sufficient to maintain multicellularity in *Dictyostelium*. *Science* **334**, 1548–1551. (doi:10.1126/science.1213272)

14 Vos, M. & Velicer, G. J. 2009 Social conflict in centimeter and global-scale populations of the bacterium *Myxococcus xanthus*. *Curr. Biol.* **19**, 1763–1767. (doi:10.1016/j.cub.2009.08.061)

15 Hallatschek, O., Hersen, P., Ramanathan, S. & Nelson, D. R. 2007 Genetic drift at expanding frontiers promotes gene segregation. *Proc. Natl Acad. Sci. USA* **104**, 19 926–19 930. (doi:10.1073/pnas.0710150104)

16 Nadell, C. D., Foster, K. R. & Xavier, J. B. 2010 Emergence of spatial structure in cell groups and the evolution of cooperation. *PLoS Comput. Biol.* **6**, e1000716. (doi:10.1371/journal.pcbi.1000716)

17 Buttery, N. J., Thompson, C. R. L. & Wolf, J. B. 2010 Complex genotype interactions influence social fitness during the developmental phase of the social amoeba *Dictyostelium discoideum*. *J. Evol. Biol.* **23**, 1664–1671. (doi:10.1111/j.1420-9101.2010.02032.x)

18 Ennis, H. L., Dao, D. N., Pukatzki, S. U. & Kessin, R. H. 2000 *Dictyostelium* amoebae lacking an F-box protein form spares rather than stalk in chimeras with wild type. *Proc. Natl Acad. Sci. USA* **97**, 3292–3297. (doi:10.1073/pnas.050005097)

19 Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E. & Queller, D. C. 2007 High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl Acad. Sci. USA* **104**, 8913–8917. (doi:10.1073/pnas.0702723104)

20 Raper, K. B. 1984 *The dictyostelids*. Princeton, NJ: Princeton University Press.

21 Bourke, A. F. G. 2011 *Principles of social evolution*. New York, NY: Oxford University Press.

22 Strassmann, J. E., Zhu, Y. & Queller, D. C. 2000 Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408**, 965–967. (doi:10.1038/35050087)

23 Bonner, J. T. & Dodd, M. R. 1962 Aggregation territories in the cellular slime molds. *Biol. Bull.* **122**, 13–24. (doi:10.2307/1539317)

24 Pang, K. M., Lynes, M. A. & Knecht, D. A. 1999 Variables controlling the expression level of exogenous genes in *Dictyostelium*. *Plasmid* **41**, 187–197. (doi:10.1006/plas.1999.1391)

25 Chin-Sang, I. & Zhong, W. 2009 Using LEDs as a low-cost source to detect GFP and DsRED. *The Worm Breeder's Gazette* **18**, 6.

26 Queller, D. C. & Goodnight, K. F. 1989 Estimating relatedness using genetic-markers. *Evolution* **43**, 258–275. (doi:10.2307/2409206)

27 Griffin, A. S., West, S. A. & Buckling, A. 2004 Cooperation and competition in pathogenic bacteria. *Nature* **430**, 1024–1027. (doi:10.1038/nature02744)

28 West, S. A., Pen, I. & Griffin, A. S. 2002 Conflict and cooperation: cooperation and competition between relatives. *Science* **296**, 72–75. (doi:10.1126/science.1065507)

29 Buttery, N. J., Rozen, D. E., Wolf, J. B. & Thompson, C. R. L. 2009 Quantification of social behavior in *D. discoideum* reveals complex fixed and facultative strategies. *Curr. Biol.* **19**, 1373–1377. (doi:10.1016/j.cub.2009.06.058)

30 Benabentos, R. *et al.* 2009 Polymorphic members of the lag gene family mediate kin discrimination in *Dictyostelium*. *Curr. Biol.* **19**, 567–572. (doi:10.1016/j.cub.2009.02.037)

31 Foster, K. R., Shaulsky, G., Strassmann, J. E., Queller, D. C. & Thompson, C. R. L. 2004 Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431**, 693–696. (doi:10.1038/nature02894)

32 Ostrowski, E. A., Katoh, M., Shaulsky, G., Queller, D. C. & Strassmann, J. E. 2008 Kin discrimination increases with genetic distance in a social amoeba. *PLoS Biol.* **6**, 2376–2382. (doi:10.1371/journal.pbio.0060287)

33 Queller, D. C., Ponte, E., Bozzaro, S. & Strassmann, J. E. 2003 Single-gene greenbeard effects in the social amoeba *Dictyostelium discoideum*. *Science* **299**, 105–106. (doi:10.1126/science.1077742)