The costs and benefits of being a chimera

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Most multicellular organisms are uniclonal. This is hypothesized to be because uniclonal organisms function better than chimeras (non-clonal organisms), owing to reduced levels of internal genetic conflict. We tested this idea using the social amoeba or slime mold *Dictyostelium discoideum*. When starving, the normally solitary amoebae aggregate to form a differentiated multicellular slug that migrates towards light and forms a fruiting body, facilitating the dispersal of spores. We added 10^7 amoebae to Petri plates containing 1, 2, 5 or 10 clones mixed together. We found an intrinsic cost to chimerism: chimeric slugs moved significantly less far than uniclonal slugs of the same size. However, in nature, joining with other clones to form a chimera should increase slug size, and larger slugs travel further. We incorporated this size effect into a second experiment by giving chimeras more cells than single clones (single clones had 10^6 cells, two-clone chimeras had 2×10^6 cells and so on). The uniclonal treatments then simulated a clone in a mixture that refuses to form chimeras. In this experiment, chimeras moved significantly further than the uniclonal slugs, in spite of the intrinsic cost. Thus, chimerism is costly, which may be why it evolves so seldom, but in *D. discoideum* the benefits of large size appear to compensate.

Keywords: chimera; *Dictyostelium discoideum*; evolution of multicellularity; levels of selection; reproductive conflict

1. INTRODUCTION

The cells of most multicellular organisms are genetically identical, typically developing mitotically from a single cell (Bonner 1974; Dawkins 1982; Maynard Smith 1988; Maynard Smith & Szathmáry 1995; Grosberg & Strathmann 1998; Michod 1999). Furthermore, in exceptional species where mixing with non-self cells sometimes occurs, cellular self-recognition mechanisms normally prevent fusion with all except self and close kin (Buss 1982; Grosberg & Strathmann 1998). Uniclonality may be favoured because it prevents disruptive internal conflict within the organism that compromises its function (Dawkins 1982; Maynard Smith 1988; Maynard Smith & Szathmáry 1995; Grosberg & Strathmann 1998; Michod 1999; Pál & Papp 2000). Reproductive competition among cells within chimeras has been shown in several species (Buss 1982; Grosberg & Strathmann 1998), including mutant strains of the bacterium *Myxococcus xanthus* (Velicer *et al.* 2000), the fungi *Didymium iridis* (Clark & Collins 1973) and *Neurospora crassa* (Ryan & Lederberg 1946), natural clones in the ascidian *Botryllus schlosseri* (Sabbadin & Zaniolo 1979; Stoner *et al.* 1999) and both mutant (Ennis *et al.* 2000) and natural clones of *Dictyostelium discoideum* (Strassmann *et al.* 2000). In some species, mutant cheater strains do not function as well as wild types (Ennis *et al.* 2000; Velicer *et al.* 2000). However, it is not known whether chimerism among natural clones disrupts organismal function.

Rinkevich & Weissman (1992) compared the size and reproductive patterns in chimeric pairs and isolated clones of the compound tunicate *B. schlosseri*. They found no significant difference in size (comparison of chimeric pairs with two single clones) or reproductive patterns. However, inter-individual variance was high, making it difficult to identify patterns (Rinkevich & Weissman 1992), and *B. schlosseri* clones typically form chimeras only with close kin (Scofield *et al.* 1982; Grosberg & Quinn 1986), which is predicted to reduce competition and any resulting costs. In this study, we compared chimeras and clones of the social amoeba *D. discoideum*, which is unusual among chimeric species because clones mix indiscriminately to form chimeras (Strassmann *et al.* 2000).

Dictyostelium discoideum is a predator of bacteria and is common in the soil (Bonner 1967; Raper 1984; Francis & Eisenberg 1993). When starving, the usually solitary single-celled amoebae aggregate and form a differentiated multi-cellular organism, which contains 10^4–10^6 cells when cultured in the laboratory (Bonner 2001). They generally first form a pseudoplasmodium or slug that migrates away from ammonia and towards light and heat (Bonner 1967; Bonner *et al.* 1950; figure 1). This takes the cells to a more suitable microenvironment and towards the soil surface, where the slug metamorphoses into a fruiting body composed of a spherical sorus of spores and a stalk that holds the sorus aloft. Fruiting at the soil surface increases the chance of spore dispersal by passing invertebrates (Bonner 1982; Huss 1989; Kessin 2001). The stalk cells of the fruiting body die; therefore, competition to become a spore cell is expected among clones in chimeras (Armstrong 1984; Strassmann *et al.* 2000; Kessin 2001; Crespi 2001). *Dictyostelium discoideum* clones readily mix to form chimeras (Strassmann *et al.* 2000), and multiple clones are often found in small volumes of soil (0.2 g), suggesting that chimerism occurs naturally (A. Fortunato, J. E. Strassmann, L. Santorelli and D. C. Queller, unpublished data). Furthermore, there is evidence for internal conflict within chimeras, with one clone

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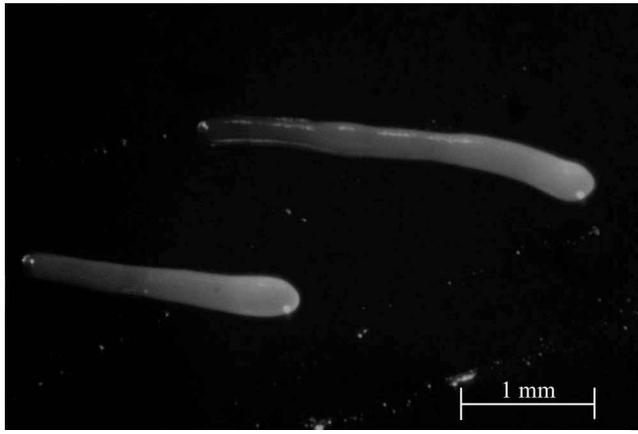DOI 10.1098/rspb.2002.2163

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Figure 1. Photo of *Dictyostelium discoideum* slugs migrating towards light (from left to right). Chimeric slugs are less mobile than uniclonal slugs of the same size.

often over-represented in the living spores relative to the dead stalk cells (Strassmann *et al.* 2000).

We examined the effect of chimerism on an important group function in *D. discoideum*: their ability to migrate to a more favourable location for sporulation and dispersal (Bonner 1967, 1982; Raper 1984; Kessin 2001). Chimerism was intrinsically costly: chimeric slugs moved less far than clonal slugs of the same size. However, in the wild, chimerism will increase the size of slugs by increasing the number of cells that aggregate together. In a second experiment, we incorporated this size factor so that cell number was proportional to the number of clones present, i.e. chimeras of two clones had twice as many cells as a single clone. Now chimeric slugs moved further than clones, showing that the size benefit outweighs the intrinsic cost of chimerism.

2. MATERIAL AND METHODS

(a) *Experiment 1: the cost of chimerism*

In our first experiment, we compared the distance moved by migrating uniclonal slugs with that moved by chimeric slugs containing 2, 5 or 10 clones, at a constant total cell number (figure 2a). We assume that slug mobility is linked to fitness. Slug movement is a complex trait (Kessin 2001) that demands adaptive explanation because it cannot be explained by chance or as a side-effect of another function (Williams 1966; Michod 1999). It makes sense adaptively in getting amoebae away from the starving environment and towards open spaces suitable for spore dispersal (Bonner 1982; Kessin 2001). We used 10 natural clones isolated from the type locality, Little Butts Gap, NC, USA (Francis & Eisenberg 1993; Strassmann *et al.* 2000) (34.2, 63.2, 69.1, 75.2, 85.1, 85.2, 98.1, 99.1, 101.1 and 105.1). Ten pairs of clones were studied: 34.2–85.2, 34.2–105.1, 63.2–75.2, 63.2–85.1, 69.1–98.1, 69.1–75.2, 85.1–101.1, 85.2–101.1, 98.1–99.1 and 99.1–105.1. The mixture of five clones was 85.2, 98.1, 99.1, 101.1 and 105.1, and the 10 category used all 10. We raised clones from spores stored on silica gel using SM/5 plates and the bacteria *Klebsiella aerogenes*. We added a total of 10^7 amoebae from 1, 2, 5 or 10 clones (in equal proportions) to separate starving plates in 200 µl of water. One plate was prepared for each clone, one plate for each pair and five plates each for the 5 clone and 10 clone mixtures (30 plates in total). Starving plate agar contained activated charcoal to minimize light

(a) experiment 1: constant total cell number
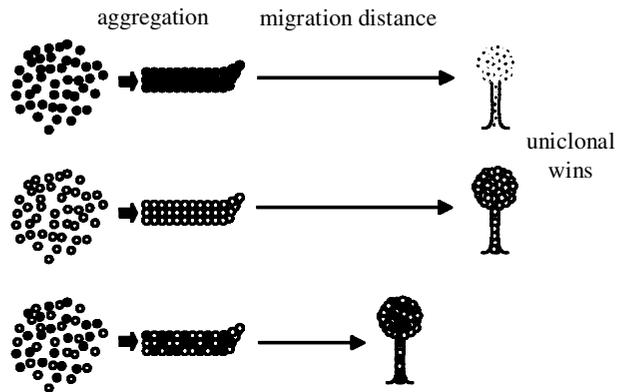
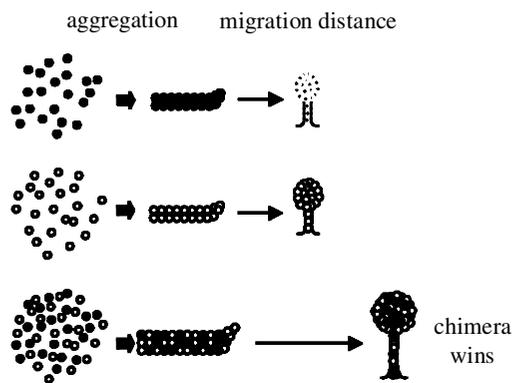### (b) experiment 2: constant cell number per clone

Figure 2. Diagram of results. (*a*) Experiment 1: chimera and uniclonal treatments have equal total number of cells and slugs are of equal size. Uniclonal slugs move further showing an intrinsic cost to chimerism (figure 3). (*b*) Experiment 2: the number of cells of each clone is constant whether in a chimera or uniclonal treatment. The uniclonal treatment now mimics the case of a clone that does not form chimeras. Not mixing with other clones reduces the number of cells available for aggregation and reduces slug size. In this experiment, chimeric slugs move further, showing that the size benefit of chimerism outweighs the intrinsic cost (figure 4).

reflectance. Activated charcoal can accelerate development in *Dictyostelium* (Bonner 1967; Raper 1984). However, in our experiments it was contained in the agar and would affect all treatments equally.

(i) *Slug mobility*

We used the phototactic behaviour of *D. discoideum* slugs (Bonner *et al.* 1950; Bonner 1967) to assess their mobility. Amoebae were placed behind a start line 8 mm from the edge of the plate. The plates were then stacked between discs of black opaque card, and enclosed in a black opaque card cylinder. A hole (diameter 1 mm) in the cylinder aligned with each plate on the other side of the start line provided a directional light source to attract the slugs. After 8 days, we removed the plates from their casing. By this time, all slugs had stopped moving and formed fruiting bodies. We assessed slug mobility by dividing the plates into 10 equal sections perpendicular to the direction of movement and counting the number of fruiting bodies in each section. All fruiting bodies were counted (mean ± s.d. fruiting

bodies per plate $= 291 \pm 200$ across experiment 1 and experiment 2). The distance travelled was calculated as the mean number of sections crossed by slugs for each plate converted to centimetres. Petri-plates are 8.9 cm in diameter, so the maximum distance travelled by a slug is less than 9 cm. Note that we underestimate overall slug mobility because slugs that reached the edge of the plate could move no further. The effect of this is to reduce the true difference between treatments, making our results conservative.

(ii) Slug size and total spore production

We estimated slug size from the diameter of the resulting fruiting body's sorus. Up to 10 haphazardly chosen fruiting bodies were measured in each section of the plate, except for sections containing more than 50 fruiting bodies, where 25 were measured, and section 10 (the furthest from the start line on the plate), where all sori were measured because size variance was particularly high. This high variance occurred because section 10 contained all of the slugs that were stopped by the edge of the plate. We made haphazard choices by nudging the plate and measuring the fruiting body closest to the microscope crosshairs. Diameter measurements were converted to volumes using $4/3\pi r^3$, where r is the radius of a sphere. The mean sorus volume for a plate was calculated as the average of the section volumes, weighting the value for each section by the proportion of fruiting bodies in that section.

The number of spores produced on each plate was estimated by combining the sorus volume for each section with the number of fruiting bodies $S = \sum_{i=1}^{10} V_i N_i$ where V_i is the mean sorus volume and N_i is the number of fruiting bodies for section i of the plate.

(iii) Spore-to-stalk ratio

The relative allocation to spore and stalk was assessed from the ratio of sorus diameter to stalk length for 30 haphazardly chosen fruiting bodies per plate. To gain a representative sample from each section, the number measured in each section was in proportion to the actual number of fruiting bodies in that section.

(iv) Statistics

In analysis, the data from the mixtures of 5 and 10 clones, which were replicates of identical mixtures, were averaged and treated as a single datum each, so that in total $n = 22$. All Spearman's rank correlations were corrected for ties. One-tailed tests were used when there was a clear *a priori* prediction of the trend direction. In experiment 1, we had *a priori* predictions of the effect of chimerism on slug movement (the cost of chimerism should reduce slug movement) and spore-to-stalk ratio (clones behaving more selfishly should increase spore-to-stalk ratio; DeAngelo *et al.* 1990). In experiment 2, we had an *a priori* prediction that, because *D. discoideum* forms chimeras, the benefit of chimerism would outweigh the cost, making chimeric slugs move further (see § 2c).

(b) Repeat of experiment 1

We repeated experiment 1 comparing 15 clones and 10 chimeric mixtures of these clones for which genetic data have shown mixing (always) and cheating (sometimes) in chimeras (Strassmann *et al.* 2000). The fifteen clones were 28.1, 28.2, 34.1, 34.2, 39.1, 63.2, 69.1, 85.1, 85.2, 70.1, 75.2, 98.1, 99.1, 101.1 and 105.1, and the chimeric pairs were 28.1–28.2, 34.1–105.1, 63.2–69.1, 98.1–70.1, 85.1–85.2, 75.2–28.1, 34.1–34.2, 85.2–39.1, 105.1–98.1 and 101.1–99.1.

(c) Experiment 2: the cost and benefit of chimerism combined

In experiment 1, the total number of cells added to each plate was held constant across all uniclonal and chimeric treatments (figure 2a). This means that the more clones added to any one plate, the fewer cells of each clone were present (the mix of 10 clones had one-tenth as many of any one clone as the uniclonal plates). While holding total cell number constant is important in identifying any intrinsic costs of chimerism, it is somewhat artificial. In the wild, mixing with other clones to form a chimera should increase the number of cells in a slug. For example, a clone in a mixture of two clones that will join to form chimeras can double the number of cells available for aggregation over a clone that refuses to mix. This is likely to be important in *D. discoideum*, where it has long been known that larger slugs move further than small slugs (Bonner *et al.* 1953). Clones that form chimeras should end up in larger slugs, which move further.

We designed a second experiment to test the prediction that a size benefit of chimerism outweighs any intrinsic costs found in the first experiment. Although we do not have a clone that will not form chimeras, we simulated this behaviour by repeating experiment 1, this time keeping *the number of cells per clone* constant (thus, total cell numbers in solitary-clone treatments were one-half that of pairs of clones, one-fifth that of mixes of 5 clones and one-tenth that of mixes of 10 clones) (figure 2b). The single-clone treatments therefore had the same number of joinable cells as a clone in a mixture that refuses to form chimeras. Using the same clones as experiment 1, we plated out a total of 10^6 amoebae in the uniclones, 2×10^6 in the pairs of clones, 5×10^6 in the mixtures of five clones and 10^7 in the 10-clone chimeras. We repeated the entire experiment with tenfold fewer cells (single clones had 10^5 amoebae). Slug mobility was assessed as in experiment 1. Slugs moved further for a given cell number than in experiment 1. This is probably because experiment 2 was performed later in the year when there was more natural light. Unlike in experiment 1, fruiting body (and slug) size was assessed indirectly, by dividing the number of cells added to the plate by the number of fruiting bodies produced. Use of this measure was possible because experiment 1 revealed that chimerism does not affect the number of amoebae becoming spores (see § 3a(i)).

3. RESULTS

(a) Experiment 1: the cost of chimerism

We found the predicted cost of chimerism. Chimeric slugs moved less far than uniclonal slugs (one-tailed Spearman's, corrected for ties: rho $= -0.5$, $n = 22$, $p = 0.01$, figure 3). This result was confirmed in the repeat experiment using pairs of clones for which chimerism and cheating within chimeras has been previously demonstrated using microsatellite markers (Strassmann *et al.* 2000): uniclonal slugs ($n = 15$) moved significantly further than chimeras containing two clones ($n = 10$) (one-tailed Mann–Whitney U-test: $n = 25$, $p = 0.03$). Consistent with previous work (Bonner *et al.* 1953), larger slugs moved further than small slugs across all treatments, whether chimeric or uniclonal (one-tailed Spearman's for distance moved and mean fruiting body size: rho $= 1.0$, $n = 10$, $p = 0.001$).
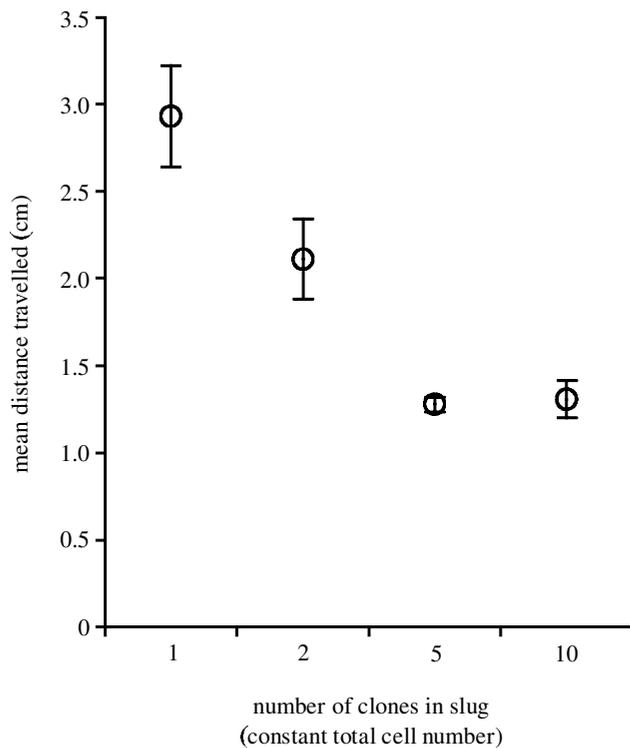
Figure 3. The cost of chimerism. Mean migration distance decreases with number of clones in slugs of *Dictyostelium discoideum* for constant total cell number (one-tailed Spearman's, corrected for ties, rho = −0.5, $n = 22$, $p = 0.01$). Standard errors are shown. We studied 10 clones. Pairs data are ten different combinations of the ten clones. Fives and ten data are five replicates of one mixture for each, so each is treated as a single datum in the statistical test.
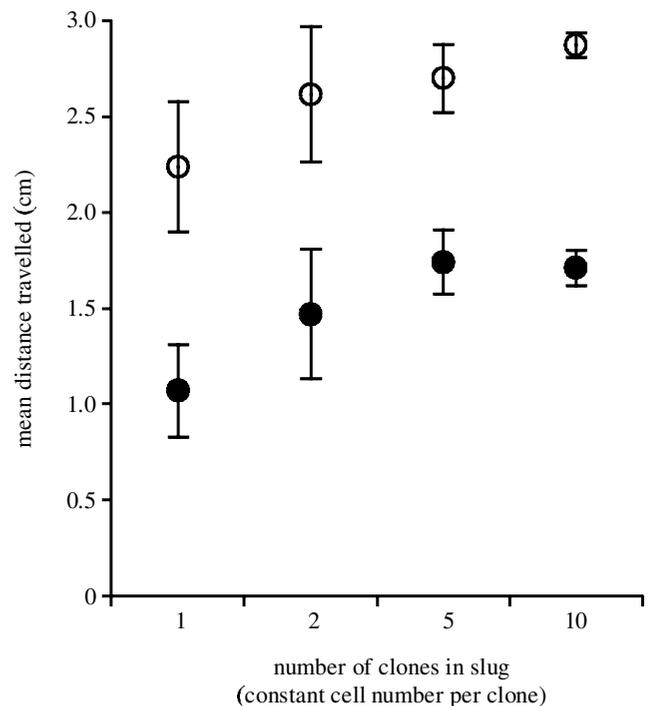

Figure 4. The cost and benefit of chimerism combined. The benefit outweighs the cost: when cell number is proportional to the number of clones in slugs (figure 2), migration distance increases with clone number in *Dictyostelium discoideum*. Filled circles are for cell densities ten times less than the open circles (one-tailed Spearman's of clone number and average distance moved across the two densities, rho = 0.38, $n = 22$, $p = 0.04$). Standard errors are shown.

(i) *Slug size and total spore production*

Chimerism did not significantly affect the number of fruiting bodies produced (two-tailed Spearman's: rho = 0.24, $n = 22$, $p = 0.28$; mean ± s.d. across plates = 489 ± 203), slug size (two-tailed Spearman's on mean sorus volume: rho = −0.19, $n = 22$, $p = 0.39$; mean ± s.d. = 1.97 ± 0.92 mm³) or total spore production (two-tailed Spearman's: rho = 0.10, $n = 22$, $p = 0.64$; mean ± s.d. = 1090 ± 576 mm³).

(ii) *Spore-to-stalk ratio*

Chimerism also had no significant effect on the ratio of sorus diameter to stalk length (one-tailed Spearman's: mean ± s.d. = 0.08 ± 0.01, rho = −0.01, $n = 22$, $p = 0.43$). Reduced cooperation in chimeras relative to single clones of *D. discoideum* might result in a reduced stalk in chimeras as clones behave more selfishly (DeAngelo *et al.* 1990). DeAngelo *et al.* studied a single pair of clones and found that chimeras had shorter stalks than uniclonal fruiting bodies, but the trend reversed with richer medium (Hilson *et al.* 1994). Our data demonstrate that the reduced allocation to stalk is not a general feature of chimerism in *D. discoideum*.

(iii) *Macrocysts*

A small minority of cells formed macrocysts (Bonner 1967; Raper 1984; Kessin 2001) in 5 out of 10 clones, 8 out of 10 pairs of clones, none of the 5 clone mixtures and all five of the 10 clone mixtures. Macrocysts are giant cells that form by amoeba fusion and represent the sexual stage of social amoebae (Raper 1984). Total spore production by each class (see § 3a(i)), however, was very similar, showing that macrocyst formation had little effect on multicellular development. There was no correlation between number of macrocysts and mean distance migrated by slugs (two-tailed Spearman's: rho = 0.3, $n = 22$, $p = 0.17$). In experiment 2, macrocysts occurred only in the mixes of 10 clones.

(b) *Experiment 2: the cost and benefit of chimerism combined*

Slug size was strongly positively correlated with the number of clones added to each plate in both replicates of experiment 2 (one-tailed Spearman's: high density: rho = 0.64, $n = 22$, $p = 0.002$; low density: rho = 0.46, $n = 22$, $p = 0.02$), so that chimeric slugs were significantly larger than uniclonal slugs. Furthermore, chimeras moved significantly further than uniclonal slugs (one-tailed Spearman's of number of clones and average distance moved across the two densities: rho = 0.38, $n = 22$, $p = 0.04$; figure 4). This demonstrates, as predicted, that the intrinsic cost of chimerism is outweighed by the size advantage (figure 2*b*).

4. DISCUSSION

In experiment 1, chimeric slugs moved less far than uniclonal slugs of the same size, demonstrating an intrinsic

cost of chimerism (figures 2*a* and 3). What causes this cost? It could be a purely mechanical effect of the clones behaving differently in the slug and not working well together. However, since clones are known to differ in their abilities to become spores in chimeras (Strassmann *et al.* 2000), the cost may well result from evolved competitive strategies (Kessin 2001). Pre-spore cells are located towards the posterior of the slug, so competition to get into this region may slow down the forward movement of the slug. Furthermore, clones have the potential to poison or physically harm each other in their bid to become spores (Atzmony *et al.* 1997), which may consume resources and slow the slug.

There is also a benefit to chimerism in *D. discoideum*. By being chimeric, *D. discoideum* clones can join with more cells, thereby making larger slugs and fruiting bodies than could a clone that mixes only with itself. Raising the effective cell density through chimerism will only benefit a clone when cell number is limiting. This means that chimerism may lose its size benefit when cell densities are so high that a single clone can produce the maximum-size slugs. However, we believe that cell density in nature is lower than in our experiments because wild fruiting bodies found on deer dung, which is one of the richest natural growth substrates for *D. discoideum*, were extremely small (mean sorocarp volume on dung = 0.016 mm³, $n = 29$; T. Platt, unpublished data). Therefore, the size benefit of chimerism shown in experiment 2 will also occur under natural conditions. Increased size is probably a general feature of chimeras that form by fusion. In the tunicate *B. schlosseri*, mature chimeras of two fused clones are roughly twice the size of single clones (Rinkevich & Weissman 1992). Buss (1982) suggested that the increased size of chimeras over clones would benefit chimeras when size-specific ecological processes, such as competitive ability, predation risk and fecundity, existed. In support of this, larger size increases growth rate and survival in *B. schlosseri* and in corals that are known to form chimeras with close kin (Hughes & Jackson 1980; Jokiel & Morrissey 1986; Grosberg & Quinn 1986). In addition, a recent study has shown that grouping by sperm in wood mice increases swimming velocity (Moore *et al.* 2002). Comparably, in *D. discoideum*, large slugs move further than small slugs, increasing dispersal distance (Bonner *et al.* 1953; see § 3a(i)). When the benefit and cost are taken together, chimerism increases slug movement (experiment 2, figures 2*b* and 4), which may be why *D. discoideum* adopts this unusual mode of life.

There are at least two possible additional benefits to the larger size of chimeras, which we did not study. Increased cell number results in larger fruiting bodies, which should increase the chance of dispersal by a passing invertebrate (Huss 1989). Larger fruiting bodies also have proportionally smaller stalks, which means that more amoebae disperse as spores (Ràfols *et al.* 2002). In combination with our data, this suggests that the benefits of size strongly outweigh the costs of chimerism in *D. discoideum*. There are further clone-level costs and benefits that may occur if one clone cheats another of reproduction in a chimera (Strassmann *et al.* 2000). These costs and benefits of cheating should not affect the average payoffs of chimerism, because one chimeric clone gains at the expense of another chimeric clone. However, if there are some clones that always lose in chimeras, these particular clones might be selected to exclude other clones.

Overall, chimerism seems to benefit *D. discoideum* clones. However, it is significant that, for slugs of equal size, clones out-perform chimeras (figure 3). This provides, to our knowledge, the first experimental evidence for an intrinsic functional cost of chimerism that would select for uniclonality in organisms that lack the strong group size advantage of *D. discoideum*. Such costs of chimerism have been invoked to explain several patterns in the development of multicellular organisms. First is the ubiquity of a single-cell bottleneck in multicellular development (Bonner 1974; Dawkins 1982; Maynard Smith 1988; Maynard Smith & Szathmáry 1995; Grosberg & Strathmann 1998; Michod 1999). Developing from a single cell promotes uniclonality and may be selected because it minimizes the functional costs of chimerism. However, a single-cell bottleneck may also be selected because it helps to purge deleterious mutations from populations (Kondrashov 1994), or it may simply be a pleiotropic effect of sex. The cost of chimerism probably has more significance in other developmental patterns after the single-cell bottleneck. Significantly, it can explain the rarity of fusion between multicellular organisms, and the existence of kin-recognition mechanisms that maximize within-organism relatedness in those that do fuse (Buss 1982; Grosberg & Strathmann 1998).

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