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Interspecies and intraspecies interactions in social amoebae

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Abstract

The stable co-existence of individuals of different genotypes and reproductive division of labour within heterogeneous groups are issues of fundamental interest from the viewpoint of evolution. Cellular slime moulds are convenient organisms in which to address both issues. Strains of a species co-occur, as do different species; social groups are often genetically heterogeneous. Intra- and interspecies 1 : 1 mixes of wild isolates of *Dictyostelium giganteum* and *D. purpureum* form chimaeric aggregates, following which they segregate to varying extents. Intraspecies aggregates develop in concert and give rise to chimaeric fruiting bodies that usually contain more spores (reproductives) of one component than the other. Reproductive skew and variance in the proportion of reproductives are positively correlated. Interspecies aggregates exhibit almost complete sorting; most spores in a fruiting body come from a single species. Between strains, somatic compatibility correlates weakly with sexual compatibility. It is highest within clones, lower between strains of a species and lowest between strains of different species. Trade-offs among fitness-related traits (between compatible strains), sorting out (between incompatible strains) and avoidance (between species) appear to lie behind coexistence.

Introduction

The basis of co-existence in a species whose members differ in fitness-related traits is a long-standing issue in evolutionary biology (Futuyma, 1998). The situation is especially baffling when it comes to species in whose life cycles the sexual phase is rare, intermittent or absent (Rainey *et al.*, 2000). The cellular slime mould or social amoebae present us with a glaring example. Groups can consist of distinct strains of a species (in nature; Sathe *et al.*, 2010) or even different species (in the laboratory and by inference also in nature; Raper & Thom, 1941; Bonner & Adams, 1958; Jack *et al.*, 2008; this study). Crucially, members of a group differ in the efficiencies with which they form reproductives (spores). This raises the evolutionary question: 'What makes it possible for different strains of a species, or different species, to co-exist in the long run?'

Many factors can lie behind the co-occurrence of different species in a shared environment. The species can occupy different niches or the niches can overlap. In addition, the species can exhibit commensalism, symbiosis or exploitation (Tokeshi, 1999). If two species compete for the same resources, it is believed that they cannot co-exist stably ('Gause's principle'; Gause, 1934). In practice, the application of this principle requires several caveats (Hardin, 1960). Hutchinson (1961) claimed that the high diversity of phytoplankton and the limited range of resources on which they survive refuted it. The existence of stable polymorphisms within an asexual sympatric species whose members differ in fitness-related traits (Rainey *et al.*, 2000) is also a possible refutation. Bonner (2009, 2013) has put forward a radical hypothesis to account for the co-existence of diverse forms in microorganisms: they could be neutral phenotypes, that is, their morphologies could be more the result of drift than selection. Among the organisms used by Bonner as illustrations are

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diatoms, foraminifera and CSMs. The present study is concerned with the last of these.

The CSMs or social amoebae are the simplest eukaryotes known to exhibit social life with reproductive division of labour (Bonner, 1967, 2009; Kessin, 2001). Their characteristic is that when starved, large numbers of amoebae aggregate by chemotaxis; thereafter, some cells differentiate into stress-resistant spores, whereas others die and form a columnar stalk that supports the spore mass. CSMs have been found in soils all over the world and in animal dung (Raper, 1984; Suthers, 1985; Swanson *et al.*, 1999; Sathe *et al.*, 2010). The pandemic nature of CSM species makes them exemplars of the 'everything is everywhere' concept in microbial ecology (O'Malley, 2008).

Cellular slime moulds (CSMs) feed on soil (or dung) bacteria and yeasts (Raper, 1984). They may do so without appreciable discrimination between bacterial species (Anscombe & Singh, 1948; our unpublished observations) or exhibit relative, although not absolute, preferences (Horn, 1971). Nevertheless, CSM amoebae that are found together must necessarily interact – both indirectly because they compete for the same bacteria and actively because they may share chemical attractants (Raper, 1984; Schaap *et al.*, 2006) and adhesion factors (Nicol & Garrod, 1978).

Heterogeneous CSM groups occur in nature (Sathe *et al.*, 2010). They are readily generated in the laboratory by mixing spores or amoebae. Previous studies have reported several outcomes (summarized in Table S1). For mixes between different species, their gist is that (a) there are barriers to free mixing – for example, on account of different chemoattractants (Raper & Thom, 1941); (b) they function at more than one developmental stage – for example, aggregated cells sort out because of different adhesion systems (Raper & Thom, 1941; Nicol & Garrod, 1978); (c) a barrier may be present at a particular stage and can be overcome if the stage is bypassed (Raper & Thom, 1941; Bonner & Adams, 1958); (d) the barrier varies in strength from one pair to another (Raper & Thom, 1941; Bonner & Adams, 1958); and (e) species contribute disproportionately to the spore population (Jack *et al.*, 2008; this study).

Intraspecies mixes display many of the features reported in interspecies mixes (Table S1). Usually, strains of a species form chimaeric fruiting bodies; some do with lesser efficiency than others, and the outcome may depend on whether they are mixed as spores or as amoebae (Bonner & Adams, 1958; Kaushik *et al.*, 2006; this study). One strain may even inhibit the development of the other (Kaushik *et al.*, 2006); and within a chimaeric fruiting body, the strains can make disproportionate contributions to the reproductive tissue (Strassmann *et al.*, 2000; Fortunato *et al.*, 2003; Kaushik *et al.*, 2006; Khare *et al.*, 2009; this study). In short, the range of outcomes seen in pairwise interspecies mixes overlaps with that seen in intraspecies mixes. However, it has been unclear whether the behaviour exhibited by one strain of a species is typical of all strains of that species or whether there are significant strain-to-strain differences. We have explored the extent and implications of chimaera formation in intra- and interspecies mixes by working with several wild-type strains of two sympatric species, *Dictyostelium giganteum* and *D. purpureum*.

We have monitored the ability of pairs of strains to form chimaeric social groups under two different conditions. The first is by mixing spores, spreading them on an agar substrate with bacterial food and following development all the way until fruiting body formation. The second is by mixing starved amoebae and allowing them to develop on a non-nutrient medium. We recorded the occurrence of chimaeric groups, their morphology, relative efficiencies of sporulation within and among chimaeras and whether the likelihood of chimaerism depended on the geographical proximity of the original isolates.

In sum, developmental interactions between the members of a chimaera can impinge on the long-term maintenance of genetic polymorphisms. The main aim of this study is to quantify reproductive asymmetry, which is an especially important consequence of such interactions. A subsidiary aim is to look for patterns in reproductive skew between intra- and interspecies groups. As we will see, reproductive asymmetry is ubiquitous. Not only that, some pairs of strains (of the same species) are more prone to display it than others, and members of different species display it to the strongest extent. Further, there is a positive correlation between reproductive asymmetry and somatic incompatibility. These observations lead to two inferences: first, the members of a species appear to be divided into guilds of mutually compatible strains, and second, somatic incompatibility between species is at one extreme of the range of compatibilities within species.

Materials and methods

Media, chemicals, growth and development conditions were as described previously (Kaushik *et al.*, 2006; Sathe *et al.*, 2010).

Species and strains

The strains come from the Mudumalai wildlife sanctuary, a dry deciduous forest in Tamil Nadu, South India (Sathe *et al.*, 2010). We used five strains of *D. giganteum* (Dg8, DgF5, DgF16, DgE1 and Dg5B1; 'Dg' = *Dictyostelium giganteum*) and three of *D. purpureum* (Dp3, Dp5.2 and Dp14, 'Dp' = for *Dictyostelium purpureum*). Dg8, Dp3 and Dp5.2 were isolated from three different fruiting bodies which had developed on the same pellet of spotted deer dung (Fig. S1). DgF5 and DgF16 were spores in the same fruiting body formed on elephant dung

ca. 10 km away from the spotted deer pellet; DgE1 is from a different fruiting body on the same elephant dung sample. Dg5B1 and Dp14 are from soil samples collected from the 5th and 14th hectares, respectively, of a study plot. Published criteria were used for identifying species (see Supporting Information for details). Strains were genetically distinct based on RAPD and microsatellite polymorphisms (Sathe *et al.*, 2010, 2011).

Spore germination

Spores of each strain were collected separately by harvesting 3- to 4-day-old spore masses with sterile tips. Spores were transferred to 2 mL buffer ('KK_2', KH_2PO_4 2.25 g, K_2HPO_4 0.67 g, H_20 1000 mL, pH 6.4) and mixed thoroughly by vortexing for 2–3 min. The suspension was centrifuged at 300 *g* for 3 min at room temperature, the supernatant was discarded, and spores were suspended in 2 mL KK_2. The spore suspension was once again centrifuged and the pellet was resuspended in 2 mL KK_2. Spores were appropriately diluted in KK_2, counted using a haemocytometer and inoculated at a density of 5×10^4 spores mL^{-1} in a 250-mL sterile flask containing 50 mL 1% peptone; the spore count was repeated. The flask was shaken in a reciprocal shaker at 150 strokes min^{-1} at 22 °C for 12 h. Samples were collected from the flask at various times, and the number of emerged amoebae (as a proxy for germinated spores) and ungerminated spores was counted using a haemocytometer. The results of spore germination were computed as: spore germination = total number of amoebae counted/(total number of amoebae + ungerminated spores).

Growth

In order to obtain spores or amoebae for an experiment, cells were routinely cultured on slime mould medium (SM) agar plates along with *Klebsiella aerogenes* bacteria (SM agar: dextrose 10 g, peptone 10 g, yeast extract 1 g, magnesium sulphate 1 g, K_2HPO_4 0.66 g, KH_2PO_4 2.25 g, agar 20 g, H_2O 1000 mL; all the components were purchased from Hi-media, Mumbai, India).

Doubling times and developmental rates

Doubling times of exponentially growing amoebae and developmental time sequences of freshly starved amoebae were estimated on solid agar plates using standard techniques (Kaushik *et al.*, 2006 and Supporting Information).

Sexual compatibility

The ability of strains to recombine sexually was studied by looking for macrocysts, which are large (~100 μm) encysted structures formed after amoebae belonging to opposite mating types co-aggregate, fuse and form a diploid premeiotic cell that proceeds to engulf the other cells in the aggregate (Blaskovics & Raper, 1957; Saga & Yanagisawa, 1982; Kaushik *et al.*, 2006; http://dictybase.org/techniques/media/mating_types.html; detailed protocol described in Supporting Information).

Interspecies chimaerism

By chimaerism, we mean the presence of cells of two genotypes in the same social group. Most often, the group in question is a fruiting body, in which case chimaerism could apply to the spore population, the stalk population or both. In some situations, amoebae of two species aggregate together and subsequently bifurcate and form distinct fruiting bodies, each containing cells of only one of the two species. In other situations, cells belonging to two strains (or species) form strain-specific (or species-specific) clusters. Both outcomes are referred to as 'sorting out'. Interspecies chimaerism was studied under two different conditions: (i) spores of the two CSM species along with bacteria were inoculated together and (ii) freshly starved amoebae were mixed and allowed to co-develop. The reason for mixing cells in two ways is the observation that spores of one strain of *D. giganteum* could inhibit the development of spores of the other, but amoebae of the same two strains developed freely as chimaeras (Kaushik, 2002). Spores of *D. giganteum* and *D. purpureum* germinated, and amoebae grew, at different rates (this study). Because the differences would be relevant under natural conditions, we took into account their effect on chimaera formation. Details of how spores or amoebae were mixed are provided in the Supporting Information (Data S1).

Intraspecies chimaerism

Freshly starved amoebae or spores belonging to different strains of the same species were mixed pairwise in the same way. All three combinations of *D. purpureum* from three strains (Dp3, Dp5.2 and Dp14) and all ten combinations of *D. giganteum* from five strains (Dg8, DgF5, DgF16, DgE1 and Dg5B1) were tested.

Chimaerism and sorting out were monitored in individual fruiting bodies. Dg-type and Dp-type fruiting bodies formed by each mix were picked randomly with a needle and transferred to a water drop on a glass slide. Coverslips were fixed over these preparations and observed using a 60× objective lens in a Leica DM-IRB fluorescent microscope with appropriate filters. Stained and unstained spores were counted manually. An estimate of the extent of chimaerism in fruiting bodies was calculated separately for Dg-type and Dp-type fruiting bodies using the formula: index of chimaerism (CI) in Dg-type fruiting bodies = number of fruiting bodies containing both Dg and Dp cells/total number of Dg-type fruiting bodies analysed, and similarly for Dp-type

fruiting bodies. Chimaerism in spore masses could be detected easily and reliably, but identifying a chimaeric stalk was difficult on account of autofluorescence from stalk cells; relying on cell morphology alone was questionable. We took a conservative approach and classified a stalk as chimaeric when both morphology and staining concurred. For this reason, it is possible that we have missed some chimaeric stalks, and therefore underestimated the intensity of interspecies antagonism. (We mention later that fruiting bodies in which cells of one species are restricted to the stalk are extremely rare.) There were 4 of 548 cases, all from Dp3 + Dg8 mixes, in which fluorescence and morphology did not match. They were not included in the tally. When two strains of the same species were stained and mixed as spores, development could be followed but the formation of a chimaera had to be inferred indirectly, because once spores germinated, fluorescence was no longer clearly visible in the resulting amoebae (there was no such problem when the amoebae themselves were stained). In intra- and interspecies mixes, we estimated the asymmetry or skew in chimaeric spore masses as follows. Suppose the proportions of the two strains in the spore mass are X and Y (with $X + Y = 1$). S, defined as $S = 1-(X/Y)$, was used as a measure of the asymmetry in contribution to the spore population. S indicates the reproductive skew between the two genotypes in a chimaeric fruiting body. While calculating the skew, the minor component was always used as the numerator (i.e. $X \leq 0.5$). Thus, when $X = Y$, $S = 0$ (there is no skew), and when $X = 0$, $S = 1$ (the skew is maximal).

Data analysis

Statistical tests are referenced below as needed and were carried out using Excel or other online software. The *t*-tests for means used Welch's modification (Welch, 1947) that takes into account unequal variances (heteroscedasticity). Heteroscedasticity per se was estimated using the Brown–Forsythe test, which is a modification of the *F*-test that is based on medians and does not require the assumption of normality. The correlation between variance in spore proportions and absolute reproductive skew was assessed by means of Spearman's rank-order correlation coefficient, which also does not require normal distributions.

Results

Intraspecies and interspecies chimaera formation was monitored in 3818 fruiting bodies (1156 intraspecies and 2662 interspecies).

Intraspecies mixes

These experiments address the issue of somatic incompatibility within a species. We had assumed that strains of the same species would invariably form chimaeric fruiting bodies and that social groups would display reproductive asymmetries of various extents. The second assumption was borne out but the first was not. Members of a species indeed co-aggregate freely. However, whereas some strain combinations go on to form chimaeric fruiting bodies, others do not. The participating amoebae show different degrees of somatic incompatibility. After forming a single aggregate, they sort out, on occasion completely (Table S3 contains a comprehensive summary). The main implication of this section is that it raises the question of long-term co-existence in the face of significant reproductive asymmetries.

Dictyostelium purpureum

(a) Differences in somatic compatibility. All three pairs of *D. purpureum* strains aggregated freely when mixed as amoebae; the speed of aggregation and further development was comparable between the strains and their combinations (Figs 1 and S5). As evident from roughly equal numbers of stained and unstained cells in mixes (not shown), early aggregations were invariably chimaeras. However, the component amoebae sorted out later to a greater or lesser extent. In the case of good mixers, most fruiting bodies were chimaeric; in the case of poor mixers, most contained spores of one or the other strain, but not both. Strong autofluorescence of stalk cells made it impossible to monitor chimaerism within the stalk, and stalk cells from different strains could not be distinguished morphologically. The index of chimaerism (CI), defined as the ratio of chimaeric fruiting bodies to all fruiting bodies, ranged from very low (17.0%) in the Dp3 + Dp14 mix to very high (80.0%) in the Dp3 + Dp5.2 mix. The Dp5.2 + Dp14 mix also resulted in a low CI of 22.4%. The reason behind the two low values is that in Dp3 + Dp14 and Dp5.2 + Dp14 mixes, ~ 90% of the aggregates developed two tips that went on to initiate separate slugs, one of each strain (this was confirmed later by monitoring spores in spores masses; Fig. 1, Tables S3 and S4). We infer that Dp3 and Dp5.2 are somatically compatible, but neither is compatible with Dp14. As explained earlier, comparable quantitative data could not be obtained when spores, rather than amoebae, were mixed. However, going by the proportion of chimaeric aggregates that formed two slugs and two fruiting bodies, when cells were mixed as spores too the incidence of chimaerism was once again high with Dp3 and Dp5.2 and comparatively low with Dp3 and Dp14 or with Dp5.2 and Dp14 (Fig. S6).

(b) Reproductive skew. All mixes resulted in fruiting bodies with significant reproductive skews. In the case of Dp3 + Dp5.2, where mixing was very good, 32.7 $\pm$ 7.9% of the spores belonged to Dp3 (mean $\pm$ SD), with a reproductive skew of $S = 0.49 \pm 0.17$. The other two pairs, both poor mixers as we have seen, displayed

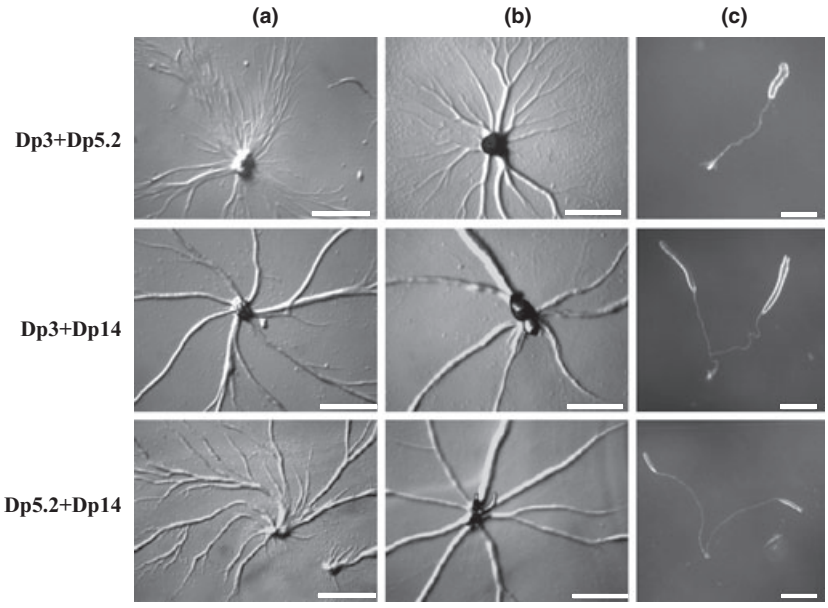

Fig. 1 Development in 1 : 1 mixes of *Dictyostelium purpureum* strains. Amoebae were harvested from nutrient (SM) plates and transferred to non-nutrient (PBA) plates at a density of 10^5 cells cm^{-2}. Plates were incubated at 22 °C and observed every few hours. Amoebae of different Dp strains aggregated freely (loose aggregates; a) and formed tight aggregates from which slugs emerged (b). These structures went on to form migrating slugs (c) which later formed fruiting bodies. In the case of the Dp3 + Dp5.2 mix, the amoebae co-aggregated and remained together in slugs and fruiting bodies, whereas in the case of Dp3 + Dp14 and Dp5.2 + Dp14, the amoebae co-aggregated but aggregates tended to form two different tips (one of each strain) that led to two slugs and fruiting bodies (note bifurcation in last two panels). Scale bar = 250 μm.

higher reproductive skews: 60.0 ± 35.9% of the spores belonged to Dp3 in the Dp3 + Dp14 mix (S = 0.75 ± 0.27) and 65.8 ± 31.9% of the spores belonged to Dp5.2 in the Dp5.2 + Dp14 mix (S = 0.71 ± 0.31). Coefficients of variation in the skew are high in the last two cases because of significant sorting out and because even among chimaeras most spores came from one of the two strains.

(c) Comparable productivity of pure and mixed groups. At the level of the population as a whole too, strains contribute unequally to the next generation. Dp3, which mixed well with Dp5.2, contributed just 29.9 ± 4.0% of the spores. Among the poor mixers, 57.0 ± 8.6% of the spores belonged to Dp3 (Dp3 + Dp14 mix) and 54.5 ± 7.5% of the spores to Dp5.2, respectively (Dp5.2 + Dp14 mix). Note that the ~50% mean fraction of spores contributed by either strain in the last two cases (the naive expectation based on a 1 : 1 mixing ratio) masks much intergroup variation (Table S3). The overall productivity of a pair of strains (i.e. the mean number of spores relative to the initial number of amoebae) was similar to that of a single strain, and this was reflected in the fact that all fruiting bodies appeared similarly proportioned (not shown).

Dictyostelium giganteum

(a) Differences in somatic compatibility (Table S3). All ten pairwise mixes of starved *D. giganteum* amoebae aggregated in concert and developed at the same rate as any of the five strains individually (Figs 2 and S7). Five pairs were good mixers; their cells tended to stay together after aggregation (indices of chimaerism CI from 78.1% to 100%). The remaining five were poor mixers; chimaeric aggregates formed two tips that gave rise to separate slugs and fruiting bodies, each containing spores of a single strain (CI = 7.3–66%).

(b) Reproductive skew. As with *D. purpureum*, the contribution of a strain to the spore population and the reproductive skew was estimated by examining chimaeric fruiting bodies (Table S3). On the whole, the reproductive skew is lower with good mixers than with poor mixers, but there are exceptions. Except for the DgF16 + DgE1 mix, in all the good mixers, the relative contributions are significantly different from 1 : 1 (t-test assuming unequal variances, $P < 0.05$; see Table S3). The corresponding figures in the case of poor mixers are not very different as far as the means go, but the variances are much higher: in several chimaeric fruiting bodies formed by poor mixers, the vast majority of spores belong to one strain (Table S3).

(c) Comparable productivity of pure and mixed groups. We estimated the overall productivity of a pair of strains, meaning the total number of spores formed as a percentage of the number of amoebae spread initially. This includes spore counts from all fruiting bodies, whether pure or chimaeric, and ranges

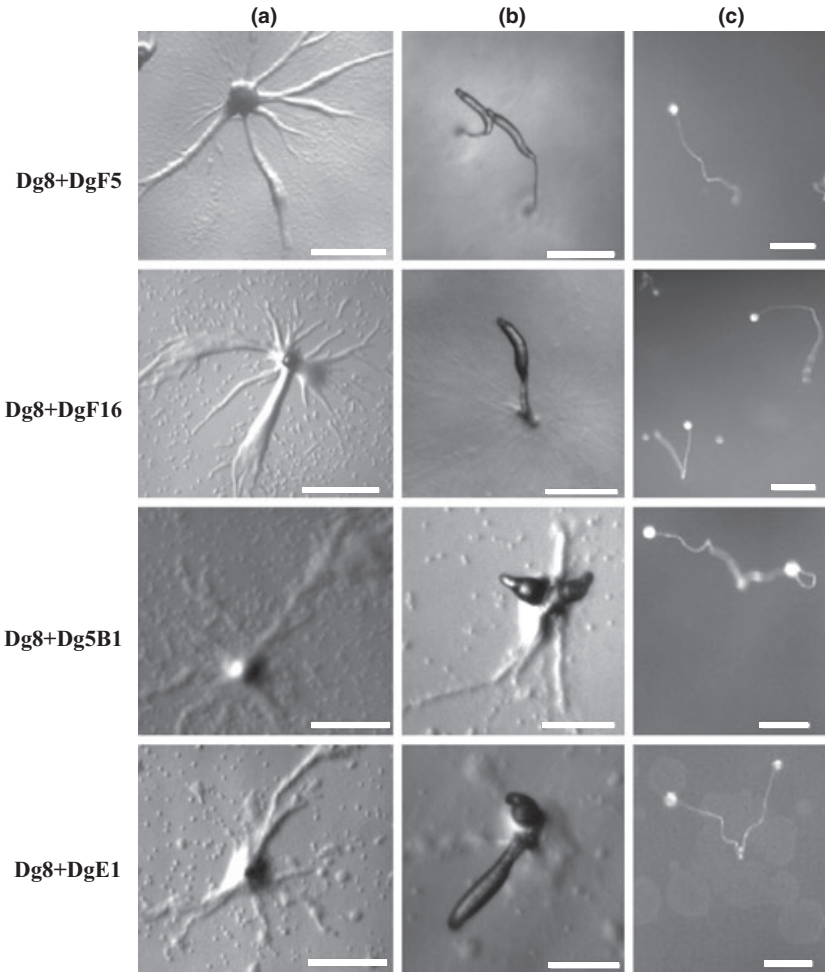

Fig. 2 Development in 1 : 1 mixes of *Dictyostelium giganteum* strains. Amoebae of different *Dictyostelium giganteum* strains were mixed in a 1 : 1 ratio and developed together. Good mixers (e.g. Dg8 + DgF5 and Dg8 + DgF16) formed chimaeric aggregates that remained as chimaeras (slugs and fruiting bodies). Poor mixers formed chimaeric aggregates that frequently split into two slugs and fruiting bodies (e.g. Dg8 + Dg5B1 and Dg8 + DgE1). Aggregates (a), slugs (b) and fruiting bodies (c) are shown in first, second and third columns, respectively. Scale bar = 250 μm.

from 37.5 ± 2.3% (DgF16 + Dg5B1) to 64.3 ± 10.3% (Dg8 + DgF5) (Table S3). As in the case of *D. purpureum*, these figures are comparable to the productivity of a single strain by itself (not shown).

Interspecies mixes

The question raised here is, 'Are interspecies and intraspecies groups qualitatively different in respect of the indices of social behaviour monitored by us?' The short answer is no; interspecies groups lie at the end of a range that is visible in intraspecies groups. Clear morphological differences made it possible to monitor the outcomes of interspecies mixes irrespective of whether spores or amoebae were mixed (Table S2; Figs S2, S4). The rule was that chimaeric fruiting bodies resembled one or the other species type unambiguously (see below for the single exception). This was so even when as many as one-fourth of their spores belonged to the minority species (compare Fig. S2 with Figs 3 and S8–S10; Table S5), and confirms to the observation of Jack *et al.* (2008) on chimaeric fruiting bodies formed by *D. purpureum* and *D. discoideum*.

(a) Two patterns of development. The outcome of mixing was similar until the end of aggregation, but after that, there were two distinct features (Tables S4 and S5). Aggregation was initiated by the species that develops faster, *D. giganteum*, and proceeded to completion at the pace set by it. The upshot was that *D. purpureum* cells formed chimaeric aggregations significantly earlier than when developing on their own. Subsequently, the tempo of development varied. In some cases, cells of the two species sorted out and each component went on to develop at its own species-specific pace. In other cases, *D. giganteum* cells developed at their normal pace, but *D. purpureum* cells were arrested for a considerable time.

(i) Independent and parallel development. The majority of mixes displayed the first pattern. The DgF5 + Dp3 case is illustrative (Fig. 3). Loose aggregates, visible after 5–6 h, went on to form tight aggregates from which one or two tips emerged (Fig. 3). If it was a single tip, the result was a single fruiting body of either the Dg or Dp type. When two tips emerged, they initiated two separate fruiting bodies by 14–16 h, one each of the Dp

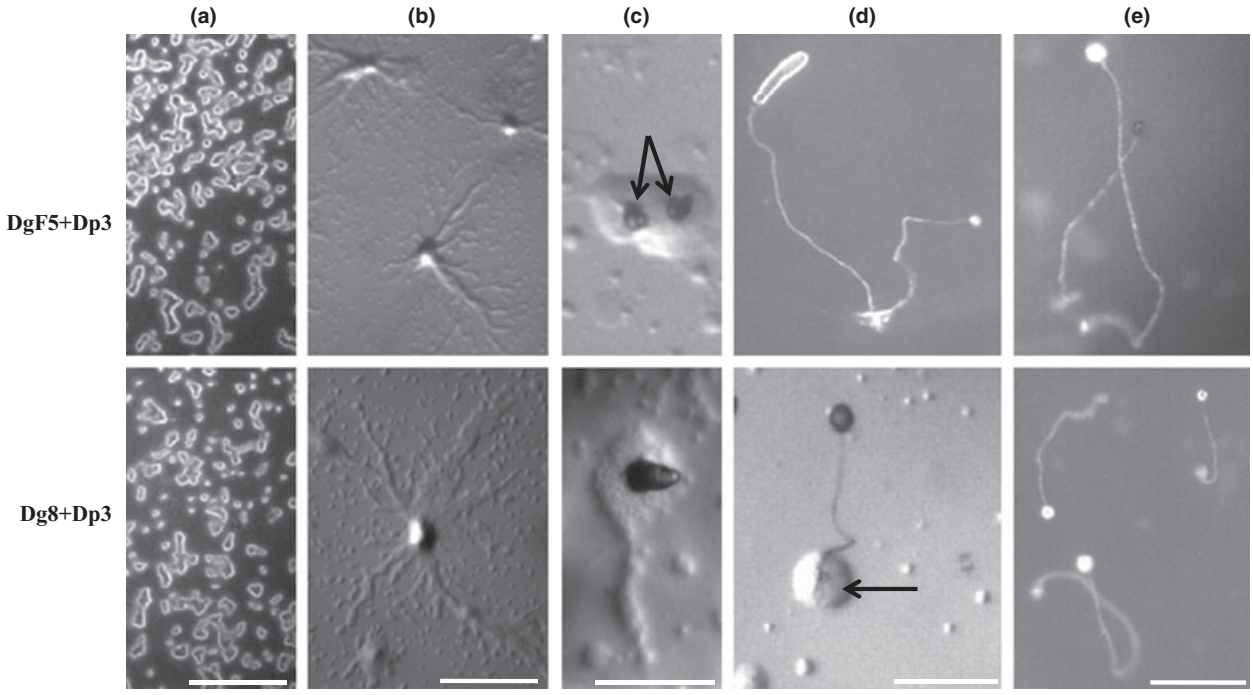

Fig. 3 Development in interspecies mixes. Freshly starved amoebae of *Dictyostelium giganteum* and *D. purpureum* were mixed in a 1 : 1 ratio and allowed to develop on PBA plates. Mixes of DgF5 + Dp3 and Dg8 + Dp3 are shown in the top and the bottom panels, respectively. In the case of DgF5 + Dp3, amoebae co-aggregated and formed chimaeric aggregates; (a) shows the amoebae and (b), the aggregations. Soon two tips emerged from the same aggregate (c); one tip initiated a Dg-type fruiting body and the other tip, a Dp-type slug (d) and Dp-type fruiting body (e). Distinct Dg-type (white spore mass) and Dp-type (purple spore mass) fruiting bodies were seen finally (e). Overall, the rate of development of Dp3 was speeded up (by 6–8 h) after it was mixed with DgF5 and that of DgF5 was slowed down (by 3–4 h). In the Dg8 + Dp3 mix (lower panel), amoebae co-aggregated, the aggregate formed one tip (c) and the single tip initiated a Dg-type slug which later formed a Dg-type fruiting body (d). A clump of Dp amoebae remained at the base of the fruiting body (see arrow in d). After 2–3 days, some of these developmentally arrested clumps formed Dp-type fruiting bodies. The reasons why only some of the clumps developed into fruiting bodies are not known; may be by then many Dp amoebae lose their developmental potential (starved amoebae of *Dictyostelium mucoroides* lose their potential to form fruiting bodies with the passage of time after starvation; Gregg, 1971).

and Dg types. The early boost to developmental rate was maintained: on its own, Dp3 took ~24 h to reach the fruiting body stage, but after it was mixed with DgF5, did so in 16–18 h. At the same time, the presence of Dp3 slowed down the development of DgF5 by 3–4 h (this happened in all the cases where Dg and Dp co-aggregated and bifurcated only post-aggregation). With minor variations (see legends to Figs S8 and S9, Table S4), the outcome was similar in mixes of DgF5 + Dp5.2, DgF5 + Dp14, DgF16 + Dp3, DgF16 + Dp5.2 and DgF16 + Dp14. In mixes of DgE1 or Dg5B1 with any Dp strain, aggregation almost invariably was followed by the emergence of two separate tips and fruiting bodies from a chimaeric aggregate.

(ii) Suppression of development in one species by the other. The outcomes of the Dg8 + Dp3, Dg8 + Dp5.2 and Dg8 + Dp14 mixes confirmed to the second pattern. Amoebae began to aggregate in concert after 4–5 h of plating (Figs 3 and S10). Small slugs (as compared to the slugs formed by Dg alone) emerged from these aggregates by 7–8 h. They were of the Dg type; by 14–16 h, they had become Dg-type fruiting bodies (Fig. 3). Clumps of amoebae could be seen left behind at the base of these tiny fruiting bodies (Fig. 3). After 2–3 days of incubation, some of the clumps became migrating slugs that went on to differentiate into Dp-type fruiting bodies (Fig. 3, Table S4). Eventually, the plates contained two distinct types of fruiting bodies (Dp type and Dg type) with those of the Dp type being in a significant minority (Table S4).

(b) Interspecies chimaerism. Interspecies chimaerism was rare. Just 13% (345/2662 fruiting bodies) of the fruiting bodies were chimaeric. In the remaining (87%) of fruiting bodies, both spore and stalk cells belonged to the same species (all experiments clubbed, Table S5); the mean index of interspecies chimaerism was comparable when cells were mixed as amoebae or as spores (CI = 14.8 ± 8.4 when mixed as amoebae; CI = 10.5 ± 10 when mixed as spores; *t*-test, $n = 30$, $t = 1.95$, d.f. = 58, $p = 0.0549$; all 15 combinations). As with intraspecies mixes, in interspecies mixes too, the extent of mutual discrimination shown by amoebae of two

species varied significantly between fruiting bodies (Figs S11 and S12). Of 1387 Dg-type fruiting bodies, 14.9% (207) were chimaeric, and of the 1275 Dp-type fruiting bodies, 12.6% (161) were chimaeric; the difference is not significant ($\chi^2 = 1.93$, d.f. = 1, $P = 0.15$). When compared with indices of chimaerism for intraspecies mixes, these figures fall at the lower end of the range (Fig. 4; compare Tables S3 and S5).

25/30 (83%) of amoebal mixes and 20/30 (66%) of spore mixes yielded chimaeric fruiting bodies; the difference in proportions is significant (binomial test, $P = 0.02$ or 0.04; http://vassarstats.net/binomialX.html). Chimaeric fruiting bodies formed from Dg8 + Dp5.2, Dg8 + Dp14, DgF5 + Dp5.2, DgE1 + Dp5.2, Dg5B1 + Dp5.2 mixes were all Dg type, and chimaeric fruiting bodies formed from DgE1 + Dp3, Dg5B1 + Dp3, Dg5B1 + Dp14 mixes were all Dp type. Both were seen only when the species were mixed as amoebae but not as spores (Table S5). On the other hand, the Dg5B1 + Dp14 and DgF16 + Dp14 combinations all yielded Dg-type chimaeric fruiting bodies after cells were mixed as spores, but not as amoebae. Irrespective of the mode of mixing, some combinations of strains did not give rise to a single chimaeric fruiting body (CI = 0): this was true of Dg-type fruiting bodies formed from the Dg5B1 + Dp3 mix and Dp-type fruiting bodies formed from the DgE1 + Dp14 mix (Table S5).

(c) Reproductive skew. Most spores in interspecies chimaeric fruiting bodies belonged to one of the two species. 8.41 ± 6.33% of the spores in Dg-type chimaeric fruiting bodies belonged to *D. purpureum* and 10.58 ± 7.16% of the spores in Dp-type chimaeric fruiting bodies were contributed by *D. giganteum*. In seven cases (involving the Dg8 + Dp3 mix), all the spores came from one strain (Dg8) and stalk from both. In three cases (two involving Dg8 + Dp3 and one Dp5.2 + Dg8), the spore mass came from one strain (Dg8 or Dp5.2) and stalk from the other (Dp3 or Dg8). The last instance resulted in a purple spore mass and *D. giganteum*-type stalk and constituted the sole example of a fruiting body whose appearance was ambiguous. To reiterate, these are exceptional cases – even for the mixes referred to.

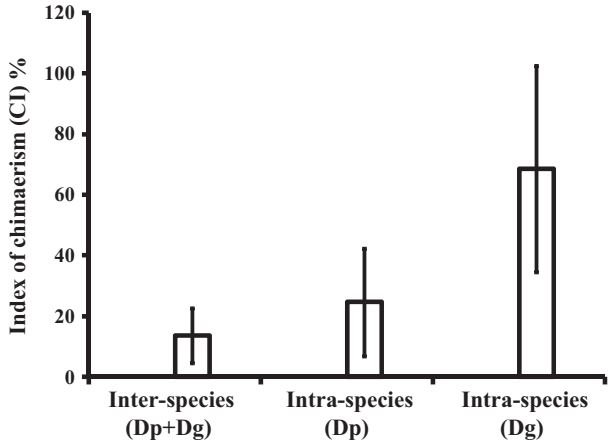

Fig. 4 Extent of chimaerism in intraspecies and interspecies mixes. The index of chimaerism (CI), defined as the ratio of chimaeric fruiting bodies to all fruiting bodies, was calculated for intra- and interspecies mixes. Results are shown as mean ± SD ($n = 10$ intraspecies Dg amoebae mixes; $n = 3$ intraspecies Dp amoebae mixes and $n = 15$ each of interspecies amoebae and spore mixes). The CI is low in interspecies mixes (13.7 ± 9.0; range: 0–31.4%) than in intraspecies mixes (Dp: 24.7 ± 17.7, range: 17–80%; Dg: 68.6 ± 34, range: 7.3–100%).

Sexual compatibility

Pairs of *D. giganteum* and *D. purpureum* strains were mixed as amoebae under conditions that favoured the sexual cycle. None of the *D. purpureum* strains formed macrocysts; all five *D. giganteum* strains did (Table 1; Fig. S13).

Discussion

The results may be summed up as follows: (i) when confronted with each other, members of different species co-aggregate but largely avoid each other thereafter, that is, are somatically very weakly compatible; (ii) members of the same species display somatic compatibilities ranging from avoidance to complete mixing; (iii) there is a weak overlap between somatic and sexual compatibility; and (iv) the reproductive skew is low in clonal groups (where 'skew' is only a formal concept – see below), ranges between low and high in mixed groups of the same species and is very high in mixed groups of different species. The main inference of significance for evolution that we draw is that the individuals that constitute a species exist in nature in the form of 'guilds' of mutually compatible strains. The stable long-term co-existence of a guild must depend on trade-offs between fitness-related traits among its members. Between guilds, whatever interaction there is furthers segregation, not integration. We go on to speculate that separation between guilds is the first step towards speciation.

These findings confirm and extend studies on other species. Chimaeric fruiting bodies are rare: Raper & Thom (1941) found this with four species (*D. discoideum*, *D. mucoroides*, *D. purpureum* and *Polysphondylium violaceum*) and Bonner & Adams (1958) with six (*D. discoideum*, *D. mucoroides*, *D. lacteum*, *D. purpureum*, *Polysphondylium pallidum* and *P. violaceum*). Jack *et al.* (2008) found high chimaerism between *D. discoideum* and *D. purpureum* (50% of fruiting bodies that resembled *D. discoideum* and 22% of those that resembled *D. purpureum* were chimaeras); but as we find too (Table S5), the majority of spores in a fruiting body belonged to one species (~85% and ~94%, respectively). It is noteworthy that the cells

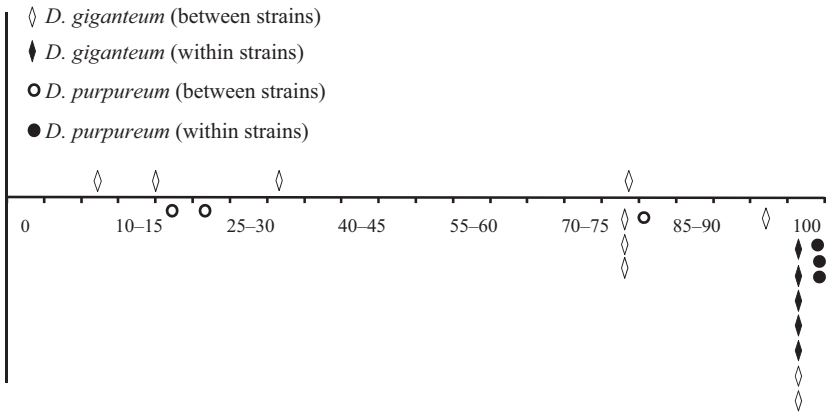

Fig. 5 Somatic and sexual compatibility in *Dictyostelium*. Strains that show a wide range of somatic compatibilities can mate and form macrocysts. The index of chimaerism between a pair of strains is shown on the horizontal line. Symbols above the line indicate sexual compatibility, and those below the line, incompatibility. None of the interspecies mixes formed macrocysts (not shown).

Table 1 Sexual compatibility (macrocyst formation) in *Dictyostelium* strains.

	Dp3	Dp5.2	Dp14	Dg8	DgF5	DgF16	DgE1	Dg5B1
Dp3	M−/C+	M−/C+	M−/C−	M−/C+	M−/C−	M−/C+	M−/C−	M−/C−
Dp5.2		M−/C+	M−/C−	M−/C+	M−/C+	M−/C−	M−/C−	M−/C−
Dp14			M−/C+	M−/C−	M−/C−	M−/C−	M−/C+	M−/C−
Dg8				M−/C+	M−/C+	**M+/C+**	M−/C+	M−/C+
DgF5					M−/C+	M−/C+	M−/C+	**M+/C−**
DgF16						M−/C+	M−/C+	**M+/C+**
DgE1							M−/C+	**M+/C−**
Dg5B1								M−/C+

M+, macrocysts formed; M−, no macrocysts.
M+ indicates that the strain pair in the relevant row and column belonged to different mating types, M− that they did not. The Cs stand for mean indices of chimaerism.
C+ = high frequency of chimaerism (CI ≥ 25% either when mixed as spores or as amoebae), C− = low frequency of chimaerism (CI < 25% when mixed as spores and when mixed as amoebae).
Bold letters differentiate the pair of strains who can successfully enter the sexual life cycle (macrocyst formation) from those who cannot. Grey shades have been used to differentiate *D. purpureum* mixes from *D. giganteum* mixes.

that are in chimaeras interact with one another and go through development in the same manner as cells of the same species or the same clone: the signalling systems that underlie morphogenesis and differentiation must overlap more and more as development proceeds. Now we will sum up the important findings under a small number of heads.

(i) Mutual antagonism between species. On the whole, the interaction between *D. purpureum* and *D. giganteum* is strongly antagonistic (Table S5). Cells of the two species tend not to aggregate together; and when they do, they sort out almost totally. The mean CI across fruiting bodies is less than 15% overall and less than 5% in as many as 40.67% (24/59) of the cases (Table 2, Table S5). Also, interspecies chimaeras show a marked skew in spore distribution. In 44/59 (74.57%) of chimaeric fruiting bodies, fewer than 10% of spores belong to the minority type (Table 2). Chimaerism can have positive or negative consequences for either species. An extreme example is the ability of Dg8 to inhibit further development of any Dp strain after co-aggregating. Some Dp cells develop further with Dg8 but most enter a period of quiescence, indeed so long that they must suffer a loss in fitness relative to conspecifics developing on their own (Figs 3 and S10). On the other hand, in the DgF5 + Dp3 mix, *D. purpureum* benefits because its cells complete development more quickly than when on their own (Fig. 3). Strains of a species vary significantly in their ability to form a chimaeric fruiting body with a given strain of another species (as is true also of *D. discoideum–D. purpureum* chimaeras; Jack *et al.* (2008)). A common history of co-occurrence of Dg8 with the same *D. purpureum* strains (Dp3 and Dp5.2) may account for the suppression of development in mixed groups. Chimaeric fruiting bodies occur more frequently in the Dg8 + Dp5.2 and Dg8 + Dp3 mixes than in Dg8 + Dp14 (Table S5). Dg8 was isolated from the same spotted deer dung pellet as Dp5.2 and Dp3; Dp14 originated from soil ~6 km away (Fig. S1).

All things considered, we conclude that mixed species groups are likely to be uncommon among *D. giganteum* and *D. purpureum*, which implies that they are unlikely

Incidence of chimaerism and skew in relative proportion of spores ('f.b.' = fruiting body)

Category	Number of cases						
(A) Interspecies combinations							
Class interval (%)	0–5	5–10	10–15	15–20	20–25	25–30	> 30
(a) Chimaeric f.bs (mean nos.)							
(i) Dg type	11	6	5	4	1	3	–
(ii) Dp type	13	3	5	3	1	3	1
(b)% of minority spores in f.bs							
(i) Dg type	16	8	4	1	1	–	–
(ii) Dp type	11	9	5	1	3	1	–

(B) Intraspecies combinations					
Class interval (%)	0–20	20–40	40–60	60–80	80–100
Chimaeric f.bs					
(i) Dg mixes	2	1	1	2	4
(ii) Dp mixes	1	1	–	1	–
Class interval (%)	0–10	10–20	20–30	30–40	40–50
% of minority spores in f.bs					
(i) Dg mixes	–	–	2	5	3
(ii) Dp mixes	–	–	–	3	–

Table 2 Chimaera formation in interspecies mixes of *Dictyostelium purpureum* (Dp) and *D. giganteum* (Dg). 'Number of cases' means the number of distinct strain-specific mixes (30 between species, 10 among *D. giganteum* strains and 3 among *D. purpureum* strains). The number of fruiting bodies monitored was much larger. In one case, namely when spores of Dg8. Dp14 were mixed, no Dp-type fruiting bodies were seen (see Table S5). See fig.6. and text for further details.

to have played a role in the evolution of social behaviour within either species. This contrasts with the inference drawn by Foster *et al.* (2002) that chimaeric slugs formed by *D. discoideum* and *D. purpureum* amoebae would be favoured as a means of increasing the size of the slug and (therefore) the efficiency with which cells can move away from an unfavourable environment. Because chimaera formation depends on mutual compatibility, it would be interesting to see whether, as with strains within a species, pairs of species exhibit different degrees of asexual compatibility too.

(ii) Similarities between inter- and intraspecies mixes. Development in inter- and intraspecies chimaeras (and, as we will argue, between them and clonal development) exhibits quantitative differences but is qualitatively similar. In both cases, an aggregate can break up and give rise to two slugs and two fruiting bodies (evidently, the phenomenon is not restricted to *D. purpureum*; Mehdiabadi *et al.*, 2006). The CI ranges from 17.0% to 80% in *D. purpureum* mixes (median 22.4%), 7.3% to 100% in *D. giganteum* mixes (median 72.05%) and 0% to 31.4% in interspecies mixes (median 6.98%). Reproductive skews (mean ± SD) are 0.11 ± 0.06 in within-clone mixtures, 0.30 ± 0.20 in *D. giganteum* mixes, 0.64 ± 0.28 in *D. purpureum* mixes and 0.91 ± 0.12 in interspecies mixes: the ranges overlap (we reiterate that for a clone 'reproductive skew' is a notional concept that refers to the proportions of amoebae belonging to two arbitrary subgroups – e.g. labelled and not labelled – that form spores). With regard to the incidence of chimaerism, the overlap is marked; in fact, some strains of the same species form chimaeras less readily than strains of different species (e.g. Dp3 + Dp14, mean CI = 17.0%, DgE1 + Dg5B1, 7.3%; Dg8 + Dp3, 31.4%; see Tables S3 and S5). Bonner & Adams (1958) found this in *D. mucoroides* and *P. violaceum*: 'differences between strains of one species [can be] as great, or even greater, than those between different species'.

(iii).Positive correlation between different indices of compatibility (chimaerism, reproductive skew and variances in spore proportions). In going from groups of a single clone (appropriately defined), to groups comprising two different clones of one species, to groups of two species, CI displays a declining trend (Fig. 4). In parallel, despite significant differences from case to case, the reproductive skew shows an increasing trend (Tables S3 and S5). Not only that the spread in the relative allocation of the two components of the mix to the reproductive pathway, that is, the variance in spore proportions, also shows an increasing trend (Fig. 6). Across all mixes, there is a significant correlation between the variance in spore proportions and the absolute difference in spore proportions from 0.5 (Spearman's rank-correlation coefficient $r_s = 0.82$, $n = 14$, $P < 0.001$; http://vassarstats.net) (see Supplementary analysis A). Thus, a low CI means not only that chimaeric fruiting bodies are unlikely, but also that when they do occur, they result in a (relatively) high reproductive skew and, concomitantly, a large variance in spore proportions.

With regard to the variances, the Brown–Forsythe test (Brown & Forsythe, 1974) reveals a striking trend (Supplementary analysis B). Within-clone ('self') mixes (with labelled and unlabelled cells constituting the two classes), together with the DgF16 + DgF5 mix [see comment under (*iv*) below], form a homogeneous group and show the least variance. Good mixers belonging to the same species form a second homogeneous group and show a higher variance; poor mixers form a third

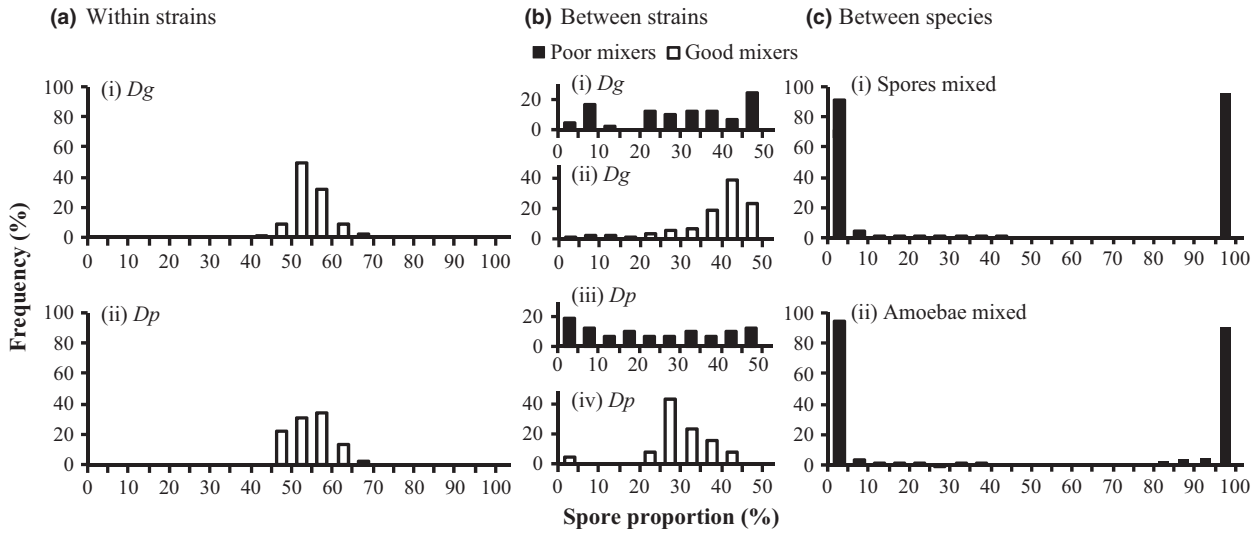

Fig. 6 Differing means and variances in reproductive division of labour. The histograms show relative frequencies of spores in fruiting bodies formed following the mixing cells of cells belonging to the same genotype (panel a), different genotypes of the same species (panel b) or different species (panel c). Cells were mixed as freshly starved amoebae and, in the between-species case, also as spores. Abscissa, spore proportions (class intervals from 0–5 to 95–100); ordinate, relative frequency (%) of fruiting bodies containing spores within that class interval. (a) 'Within strains' refers to experiments in which stained amoebae were mixed in a 1 : 1 ratio with unstained amoebae of the same clone. The numbers of stained and unstained spores were counted later in fruiting bodies (fbs). The data shown are from a typical case. When averaged over all within-clone mixes, the mean ± SD of stained spores was 49.5 ± 3.8% for *Dictyostelium giganteum* strains (n = 134) and 49.7 ± 4.8% for *Dictyostelium purpureum* strains (n = 56). (b) As explained in the text, pairs of strains can be classified as good mixers (CI = 100) or poor mixers (CI < 100). The numbers on the abscissa do not refer to spores of a particular strain, but only to the minority component; this is done here solely in order to make it possible to club data from different mixes. When averaged over all mixes, the proportion of minority spores in *D. purpureum* chimaeric fruiting bodies was 17.2 ± 16.2% (n = 59) for poor mixers and 32.7 ± 7.9% (n = 34) for good mixers. The corresponding figures in *D. giganteum* were 31.8 ± 16.3% (n = 78) for poor mixers and 40.6 ± 8.1% (n = 208) for good mixers. Note that the histograms in this panel do not include pure fruiting bodies (i.e. the 0% and 100% classes); see Table S3 for the full record. (c) When spores or amoebae of different species were mixed, aggregation was followed by almost complete segregation; in most cases, fruiting bodies contained cells of just one of the two species. The histograms show the distribution of *D. giganteum* spores in *D. giganteum*-type fruiting bodies (bars on right side) and *D. purpureum*-type fruiting bodies (bars on left side). The contribution of Dg spores to Dp-type fruiting bodies was 11.3 ± 10.9% (spores mixed, n = 39) and 6. 1 ± 7.4% (amoebae mixed, n = 73). The corresponding contribution of Dp spores to Dg-type fruiting bodies was 7.9 ± 7.3% (spores mixed, n = 23) and 7.6 ± 7.7% (amoebae mixed, n = 115 fbs). A comparison of the panels makes it evident that the variance in outcomes is smallest for mixes between cells belonging to the same strain, somewhat higher for mixes involving different strains and highest of all for mixes involving different species.

homogeneous group with a still higher variance; and interspecies mixes form a group by themselves, with the highest variance of all. In short, both in terms of chimaera formation and in terms of differentiation into reproductives, interspecies mixes represent an extreme case of intraspecies (=interstrain) mixes and intraspecies mixes represent an extreme case of intrastrain (=intraclone) mixes. The reasons behind the trends and correlations remain to be explored.

(iv) Compatibility and environmental effects. Within a species, what distinguishes good and poor mixers? In *D. purpureum*, Dp3 and Dp5.2 show the highest propensity to form a chimaeric fruiting body (mean CI = 80% as against 17% and 22.4% for the two pairs, which are with Dp14). Unlike the other mixes, their aggregates do not give rise to two slugs. Dp3 and Dp5.2 derive from the same pellet of spotted deer dung, whereas Dp14 is a soil isolate.

In *D. giganteum*, DgF5, DgF16 and DgE1 stand out similarly (CI = 100% or 96.6%). DgF5 and DgF16 derive from spores on the same fruiting body. As mentioned above, in terms of variances in spore proportions, they are no more different than labelled and unlabelled cells of the same clone. DgE1 comes from the same dung sample as DgF5 and DgF16, but a different fruiting body. The next highest incidences of chimaerism are for mixes between Dg8 and DgF16 (81.8%) or Dg8 and DgF5 (78.1%). The similarity here is that like DgF16 and DgF5, Dg8 also comes from a sample of animal dung (though deer, not elephant). But mixes between the soil isolate Dg5B1 and the three elephant dung isolates DgE1 (7.3%), DgF5 (15%) and DgF16 (31.2%) result in markedly lower incidences of chimaerism. As in the *D. purpureum* case, only mixes that involve a soil isolate Dg5B1 and four dung isolates DgF5, DgF16, DgE1 (elephant) or Dg8 (deer) break up

and give rise to a pair of slugs. None of the six mixes among the dung isolates do.

(v) Guilds. The question that motivated the present study was: how does the outcome of behaviour in mixed groups throw light on the co-existence of different CSMs – and by extension, the co-existence of individuals of asexual species that differ in fitness-related traits? CSMs belonging to the same species seem to be fragmented into partially overlapping guilds (as we may call them) of mutually compatible strains. The completion of a fruiting body, if not its initiation, appears to involve the members of a guild. From this study, we can identify the following guilds: (i) Dp3 and Dp5.2; (ii) Dp14; (iii) DgF5, DgF16 and DgE1; (iv) Dg8; (v) Dg5B1. The list is bound to get larger, and a strain can belong to more than one guild. Dg5B1 is on its own (so far) because it is incompatible with every other Dg strain (also markedly so with strains of the other species) (Tables S3 and S5).

Are the members of a guild also sexually compatible? The strains we worked with were all heterothallic. No combination of *D. purpureum* forms macrocysts, whereas with *D. giganteum*, Dg5B1 does so with DgF5, DgF16 or DgE1; Dg8 forms macrocysts with DgF16, but not with DgF5, DgE1 or Dg5B1 (Table 1). This indicates that we are dealing with more than one pair of mating types and more than one gene specifying the mating-type system as seen in *D. discoideum* (Bloomfield *et al.*, 2010). The association between sexual and somatic compatibility can be represented as ++ (2), +− (2), −+ (11) and − − (0) where the signs stand for sexual and somatic compatibility, respectively (+ if macrocysts are formed, - - if not; + if the mean CI > 25%, − if not). If the two traits were independent, the expected frequencies would be ++ (3.47), +− (0.53), −+ (9.53) and − − (1.47), and the difference between observation and expectation is just short of significance (Fisher's exact test for a 2 × 2 contingency table, $P = 0.0571$; Fisher, 1954). If we score somatic compatibility by the absence of aggregate bifurcation, the numbers are ++ (1), +− (3), −+ (9) and − − (2), which is significant at the 8% level. A more stringent criterion would be to accept a pair as somatically compatible only if CI > 50%. Then, the associations become ++ (1), +− (3), −+ (11) and − − (0); the expectations on the null hypothesis are ++ (3.2), +− (0.8), −+ (8.8) and − − (2.2) (note that the numbers according to the null hypothesis differ in the three situations). The difference, and by implication the discordance between sexual and somatic compatibility, is highly significant ($P = 0.009$).

Thus, somatic compatibility within a species is highest between members of a clone, marginally lower between strains coming from the same fruiting body, lower between isolates from the same dung pellet, still lower between different dung isolates and lowest level of all between dung and soil isolates (Table S5). This suggests that it can be influenced by both genetic similarity and shared environment. But that cannot be the whole story. The Dg5B1 (soil) +Dg8 (dung) mix displays a higher CI (mean 66.0%) than the Dg8 + DgE1 mix (both dung; mean 52.3%).

(vi) Why are there so many CSM species? Bonner (2009, 2013) has proposed that CSM species display neutral phenotypes (especially as regards morphology), that is, species-specific morphological traits may have evolved by drift. But in mixed groups, amoebae of different strains tend to differ in their spore-forming efficiency. In effect, for the same amoeba, the effect of sporulation on relative fitness depends on the mix (this study, Table S3; Strassmann *et al.*, 2000; Kaushik *et al.*, 2006; Khare *et al.*, 2009). This, along with the observation that interspecies chimaeric social groups lie at one extreme of a continuum in which clonal groups form the other end and intraspecies chimaeras fall in the middle, motivates the following conjecture. Numerous studies on *D. discoideum* have shown that preaggregation amoebae differ in 'quality', that is, in phenotypic traits that influence the probability that an amoeba differentiates into a spore or stalk cell (discussed in Atzmony *et al.*, 1997). The differences exist even between the members of a clone raised in the same environment and are probably stochastic in origin (reviewed in Nanjundiah & Saran, 1992; Nanjundiah & Sathe, 2011; Nanjundiah & Sathe, 2013). Genetic variation among the cells in an aggregate would be an additional source of heterogeneity and would contribute to differences in reproductive fitness between amoebae of different genotypes. Given that, for diverse genotypes to co-exist in the long run, there must be a trade-off between sporulation efficiency and other fitness-related traits. Trade-offs can lead to the formation of guilds whose members are thrown together frequently. If trade-offs do not exist, one of two consequences can follow. When two amoebae of different genotypes are in the same group, suppose that on the average one of them differentiates into a spore with a lower efficiency than the other. Selection will reduce the prevalence of the first genotype and eventually lead to its elimination. Or, selection can act in favour of a trait that reduces the likelihood of its association with the second genotype (note the asymmetry: the second has no problems in associating with the first). In an extreme case, there would be practically no association between the two, leading to the appearance of what we would recognize as two distinct species, each comprising several guilds. The steps leading to speciation would resemble selection for hybrid incompatibility (Maynard Smith, 1993) except that in our case the 'hybrid' would be disadvantageous to one partner, not both. Drift may be a factor behind the continued co-existence of several guilds of a species or of several species.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Older observations on inter- and intra-species mixes along with findings reported here.

Table S2 Morphological characters of *Dictyostelium purpureum* 3 and *D. giganteum* 8.

Table S3 Indices of chimaerism and contribution to spores in intra-species mixes.

Table S4 Fruiting bodies in interspecies mixes.

Table S5 Chimaera formation in inter-species mixes.

Figure S1 Dictyostelium strain sources and isolation.

Figure S2 Development of *Dictyostelium purpureum and D. giganteum.*

Figure S3 Spore dimensions in *Dictyostelium purpureum and D. giganteum* strains.

Figure S4 Spore germination (A) and growth rates (B) of *Dictyostelium purpureum* and *D. giganteum* strains.

Figure S5 Development of *Dictyostelium purpureum* strains.

Figure S6 Slugs formed by *Dictyostelium purpureum* strains individually and in pair wise mixes.

Figure S7 Development of *Dictyostelium giganteum* strains.

Figure S8 Development of a *Dictyostelium giganteum* F5 + *D. purpureum* 5.2 mix.

Figure S9 Development of a DgF5 + Dp14 mix.

Figure S10 Development of a Dg8 + Dp5.2 mix.

Figure S11 Self/non self recognition as seen in inter-species mix: *Dictyostelium giganteum* F16 + *D. purpureum* 3.

Figure S12 Self/non self recognition in intra-species mix: *Dictyostelium giganteum* 8 + *D. giganteum* 5.

Figure S13 Macrocysts formed by sexually compatible *Dictyostelium giganteum* strains.

Data S1 Methods.

Data S2 Data analysis.

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