The Chromosomes of *Dictyostelium Giganteum*

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Abstract

As a first step towards clarifying the basis of the cooperation and conflict seen in chimeric binary mixes of Dictyostelium giganteum, we examined the karyotype of six natural isolates. All six had 5 haploid chromosomes. No meiotic figures were seen. Fluorescence in-situ hybridization was carried out using conserved D. discoideum centromeric DNA sequences as probes. From it, we infer that two chromosomes are sub-metacentric, one is metacentric and two are telocentric.

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Introduction

We report on the chromosomal constitution of six naturally-occurring strains of *Dictyostelium giganteum. D. giganteum* was chosen for two reasons. First, the cytology of this species of Dictyostelid is unkown. Second, our study is motivated by the fact that we have data on social behaviour in pairwise chimaeras made up of different strains of the species [1,2]. The strains in a chimeras differ in their reproductive fitness as measured by the ability to sporulate. That opens up the possibility of identifying correlations between chromosomal or DNA-level variations between strains of a species and relative fitness.

Sorocarpic amoebae, which are found in several major groups, display a remarkable transition from a predatory free-living phase to a cooperative multicellular phase [3]. The best-studied among them belong to the Amoebozoa and are known as the Dictyostelid or cellular slime moulds (CSMs)[4]. Their life cycle makes the CSMs ideally suited to address questions related to the evolution of sociality with reproductive division of labour[5]. There are many studies dealing with the evolutionary basis of social behaviour, in particular of so-called altruistic behaviour and cheating, in the CSMs, and they involve both models and experiments [6–8]. However, while one can speculate on what is responsible for the maintenance of sociality in the CSMs, the absence of information on heritable variation within any CSM species limits the extent to which one can think usefully about how it originated. We lack information on intra-species differences in chromosomal makeup, and know very little about finer differences at the level of nucleotide sequences though a beginning is being made with the latter [9,10]. The present study is the first step towards remedying the situation. We proceed to report on a karyotype for *D. giganteum.* Also, we have carried out fluorescence *in*

situ hybridization of *D. giganteum* chromosomes using probes derived from conserved centromeric sequences of *D. discoideum*, and using them, have attempted to classify *D. giganteum* chromosomes as metacentric or submetacentric and telocentric. A comparison of genomic sequences of the same strains is under way and will be reported elsewhere.

Materials and Methods

Strains

The following six previously described strains of *Dictyostelium giganteum* were used to make chromosome preparations: 46a3, 46c6, F4, F5, F15 and F16. 46a3 and 46c6 are soil isolates from a 50-ha plot of undisturbed forest in the Mudumalai nature reserve [1,2]. F4, F5, F15 and F16 are derived from different spores in a single fruiting body isolated from elephant dung, also from the same reserve [2,11]. Following their isolation from the wild, the strains were sub-cultured and maintained either in the form of fruiting bodies on non-nutrient agar plates or stored as spores in glycerol at -80^{o}C[12]. Dictyostelium can be grown in suspension or in culture dishes and either axenically or in the presence of bacteria. Media and buffers required for culturing were prepared according to published guidelines and available in the Dictyostelium Web resource [13]. *Dictyostelium giganteum* was grown with *Klebsiella aerogenes* on SM agar plates using a modified protocol. A lawn of bacteria was grown first on an SM agar plate by overnight incubation at 37°C. A single spore head taken from a *D. giganteum* fruiting body was picked with a sterile wire loop and transferred to 10µl of autoclaved MilliQ water, and the suspension was dropped in the centre of an agar plate that had a previously grown lawn of *K. aerogenes* on it. The plate was incubated at 22°C for 40 hrs. Amoebae that emerged from the deposited spores grew outwards from the centre of the plate. The method ensures that the cells in the centre enter starvation and begin the phase of multicellular development, while cells in the periphery are always in a vegetative stage, therefore are mitotically active and readily available for making chromosome preparations.

Chromosome preparations

Chromosome preparations were carried out for all the six stains by doing independent sampling of each of them (same stock revived from -80°C) at least 3 times to avoid misinterpretation of results caused due to possible cross contamination of the strains.

Treatment with colchicine

The plate with *D. giganteum* cells was observed under microscope and the central part of growth area which contained cells in developmental phase was cut and removed. The remaining peripheral area with growth which contained mitotically active cells in vegetative phase was treated with 5ml of 400µg/ml colcemid solution (stock solution of 10mg/ml) prepared in 1xKK_2 buffer. The culture plate was incubated for 2 hours with colcemid at 22°C and at very low rpm so that growth of the cells on the SM agar plate would not be disturbed or minimally disturbed while simultaneously getting treated with the colchicine.

Cell collection and washing

The cells were collected by purging the cells with 1ml pipetman in a 10ml falcon tube. The washing was done for four times in 1xKK_2 buffer for 10 minutes at 1000rpm. Washing with 1xKK_2 buffer ensured the removal of the bacterial cells from the suspension. This was followed by incubation of the cells with water at 22°C for 10 minutes (this worked better than the standard 0.56% KCl hypotonic treatment).

Fixation

Amoebae were fixed at least 4 times with a slight modification of the standard protocol (Brody and Williams, 1974) by using a 6:1 ratio of methanol and glacial acetic acid. After fixative washes the pellet was re-suspended in 1 ml of fresh fixative.

Slide preparation and Giemsa staining

Slides for the karyotype study of six strains of *D. giganteum* were prepared by dropping 120 µl of cell suspension on the slide and then warming the slide on a 37°C warmer for 30min. Giemsa staining (using a stock solution of 2%) was carried out for 4min using Sørensen's phosphate buffer and rinsing the slides 15-20 times in autoclaved MilliQ water.

Microscopy, karyotyping and quantitating chromosome features

Microscopy: Metaphase chromosomes from the six strains of *Dictyostelium giganteum* were analysed and imaged using OLYMPUS BX51 microscope at 100x magnification and software from Applied Spectral Imaging (ASI)

Karyotyping: An attempt was made to arrange the chromosomes in the form of a karyotype based on the size of the chromosomes.

Estimation of area of individual chromosomes in a metaphase

Twenty well spread metaphases for each of the six strains were selected. Areas of the individual chromosomes in a metaphase were calculated by using the software Image J [14,15]. Sum of area of all chromosomes in a metaphase was calculated. The proportion of the area of the individual chromosome with the sum of area of all the chromosomes in the metaphase was considered as genome proportion of the individual chromosome. This was done for all the 20 metaphases each of the six strains.

Localization of the chromosome centromeres by FISH

Isolation of genomic DNA of Ax-2

For all the molecular studies, Ax-2 strain was used in order to avoid contamination. Genomic DNA was isolated from vegetative stage of Ax-2 strain of *D. discoideum* with the help of Qiagen genomic DNA isolation Kit. This was used as template DNA for synthesizing centromere specific FISH probes by PCR.

Selection of centromere sequences

The centromere sequences for *Dictyostelium discoideum* are known in the case of chromosomes 2 and 3, while for chromosome 1, the first 100,000 base pairs include the centromere region [16]. To get the most likely centromere sequence in *D. giganteum,* we carried out FISH with four centromeric sequences of three centromeres from *Dictyostelium discoideum* which were Chromosome 3 centromere having accession number FJ387222; Chromosome 2 centromere: FJ387223; Chromosome 2 inner centromere: FJ387224[15] and Chromosome1 centromere as 1st 100000 bp of chromosome1. These sequences were obtained from Dictybase. They showed conservation of very high level for >800bp in four regions whose positions are mentiond in the table above. The sequence alignment was done by ClustalW at http://www.ebi.ac.uk/. ClustalW analysis of the four centromere sequences of *D. discoideum* and found four conserved regions of >800bp (Table 1).

Probe preparation

Primers were designed with the help of Gene Runner Software [17], for the four selected conserved regions of centromere sequences of *D. discoideum* (Table 2). These primers were used to synthesize

Table 1. Table shows ClustalW analysis of 4 centromere sequences of D. *discoideum*.

4 Centromere ClustalW		
Sl. No.	Position	Size
1	29794-32216	2422bp
2	57279-59137	1858bp
3	52002-53557	1555bp
4	25202-26005	0803bp

Table 2. Table shows primer sequences designed for FISH probe synthesis with the help of Gene Runner software, for the selected conserved regions of centromere sequences of D. *discoideum*

Primer from 4 Centromere ClustalW						
Sl. No.	**Product Length**	**Position**	**Sequence**	**Tm**	**%GC**	**Primer Length**
1.	2292	29794-32216	FP-CCTGTACTTCGAATGTTGAGAGA	63.5	43.5	23
			RP-ATCTACAGTATCGTTTGATTTCCA	63.1	33.3	24
2.	1858	57297-59137	FP-GACAACAGCAGAGAAGCCATA	62	47.6	21
			RP-CCAAGTTACGACTATGTTCTTACA	61.6	37.5	24
3.	1555	52002-53557	FP-CAGTATTTAAGAAACCACCAGATT	62.5	33.5	24
			RP-ACCAAACAATCAGTAGAGTCGATA	62.9	37.5	24
4.	803	25202-26005	FP-TCGGTCAAATACAGATGGATCT	63.2	40.9	22
			RP-CCTAAGGAGTAACAACTGATTCAA	63	37.5	24

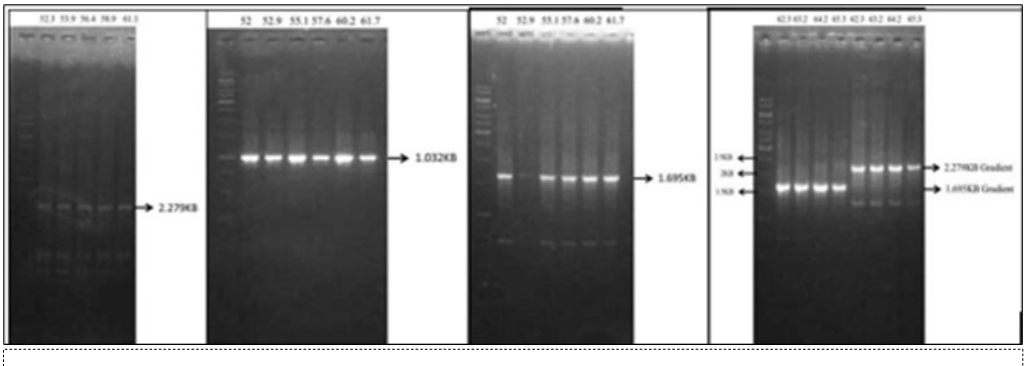

Figure 1. Agarose gel showing products of gradient PCR for the four centromere probes.

FISH probes by PCR amplification (with Dig labelled UTP) of the respective sequence according to a published standard protocol [18].

Fluorescence in situ hybridization for localization of centromeres

FISH was carried out on metaphase chromosome preparation of 46a3 strain of *Dictyostelium giganteum* bwith the help of a standard protocol used for human chromosomes, with slight modification [19].

In-house hybridization buffer (50% (v/v) formamide, 2× SSC, 10% Denhardt's solution, 0.1 M NaPO4 buffer) as described by [20] without SDS and slight variation of remaining solution components was

used for preparing probe mix. Post- hybridization washing was carried out at 45°C. Hybridization signals were amplified using Fluorescent Antibody Enhancer set for DIG Detection using the protocol described by the manufacturer (Roche Applied Science, Germany).

The FISH signals on the metaphase chromosomes, indicating the possible location of centromeres, were captured and analysed using OLYMPUS BX61 fluorescent microscope at 100x magnification and Applied Spectral Imaging software.

Results

Modal chromosome number in Dictyostelium giganteum

A minimum of 60 metaphases were analysed for each of the six strains of *Dictyostelium giganteum* (46a3, 46c6, F4, F5, F15 and F16); representative figures are shown in Fig. 2. In all six, the metaphase chromosome number ranged from 4 to 6 with a frequency that was almost the same but differed slightly

Table 3. Table shows the frequency of occurrence of cells with different number of chromosomes in six strains of D. *giganteum* In total for all six strains 1042 metaphases were analysed of which there were 452 for 46a3, 107 for 46c6, 63 for F4, 61 for F5, 222 for F15 and 137 for F16.

Number of Chromosomes	Frequency of 6 Strains of *Dictyostelium giganteum*					
	46a3	**46c6**	**F4**	**F5**	**F15**	**F16**
4	0.05	0.05	0.10	0.18	0.11	0
5	0.87	0.77	0.83	0.77	0.82	0.80
6	0.08	0.10	0.03	0.05	0.07	0.20
>6	0	0.07	0.05	0	0	0

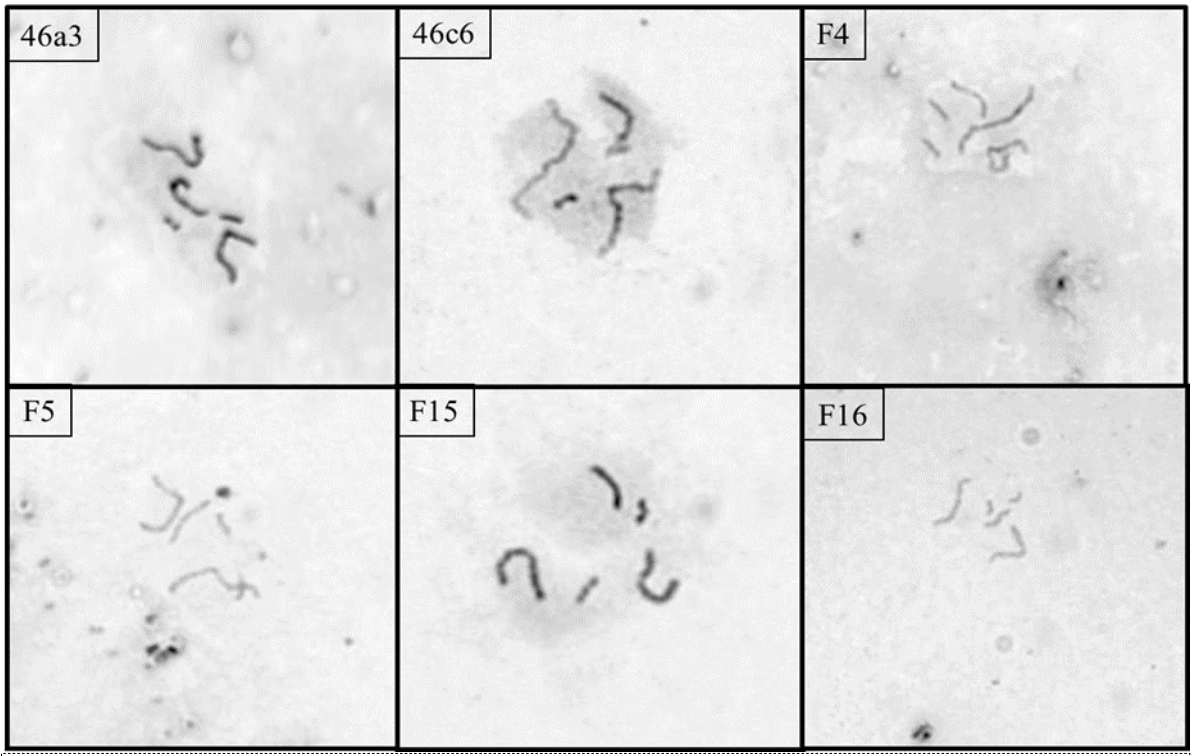

Figure 2. Representative metaphase spread of the six strains of D. *giganteum* confirming the modal number to be 5.

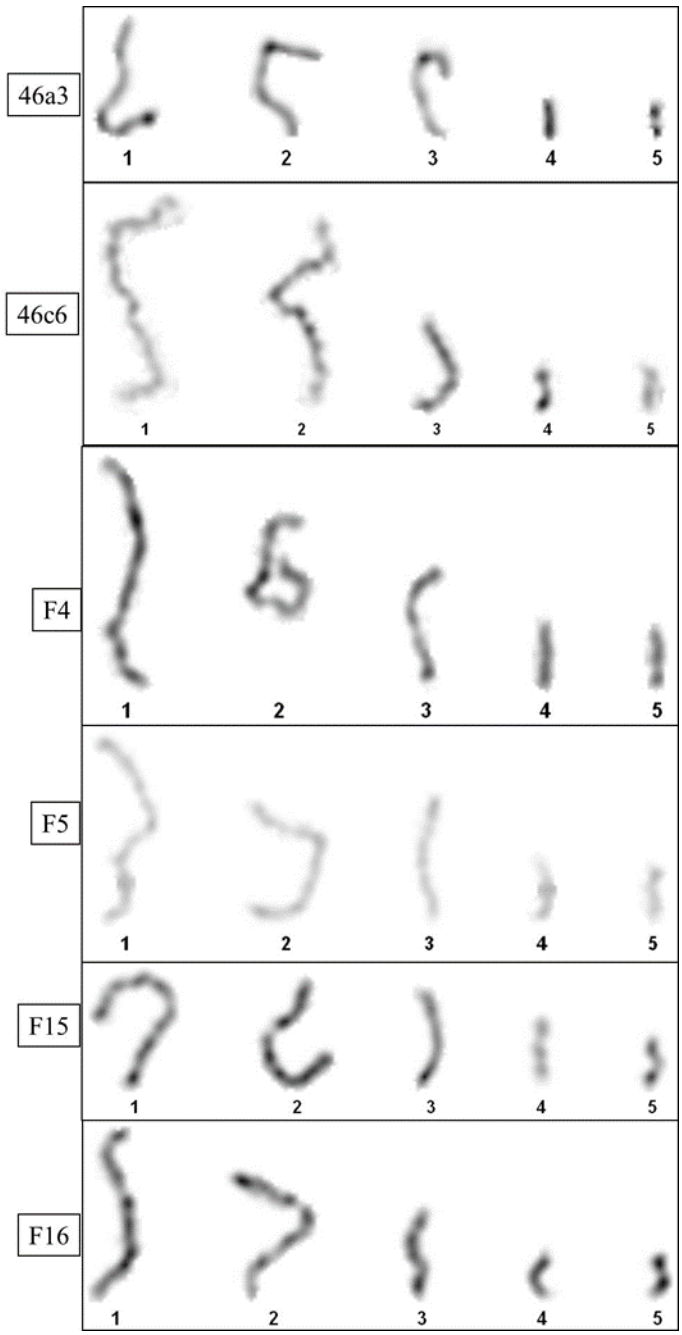

Figure 3. Classification of chromosomes in six strains of *Dictyostelium giganteum* based on size.

between them (Table 3). The modal number in each case was five. It appears reasonable to conclude that the haploid chromosome number in these strains of *Dictyostelium giganteum*, and probably in the species as a whole, is 5. No meiotic figures were seen in any preparation.

Classification of chromosomes based on size:

In all the six strains of *D. giganteum* analysed in this study, the chromosomes can be classified into 3 groups – two large chromosomes, one medium chromosome and two small chromosomes (Fig. 3).

Genome content present in each chromosome in six strains of *D. giganteum*:

Assuming the modal number to be 5 based on the data obtained, 20 well spread metaphases with modal number 5 were selected from each strain. By making use of the software Image J, the area of each

Table 4. Estimate of relative genomic content (expressed as percentages) of the five chromosomes in all six strains of D. *giganteum.*

Strains Chromosomes	**46a3**	**46c6**	**F4**	**F5**	**F15**	**F16**	**Average**
1	35.11	34.93	34.82	33.93	34.64	34.19	34.60
2	30.79	30.62	30.83	30.43	30.54	29.37	30.43
3	16.44	17.42	16.44	17.10	16.89	16.95	16.87
4	9.43	9.41	9.5	9.88	9.65	10.52	9.73
5	8.21	7.60	8.38	8.64	8.25	8.95	8.33

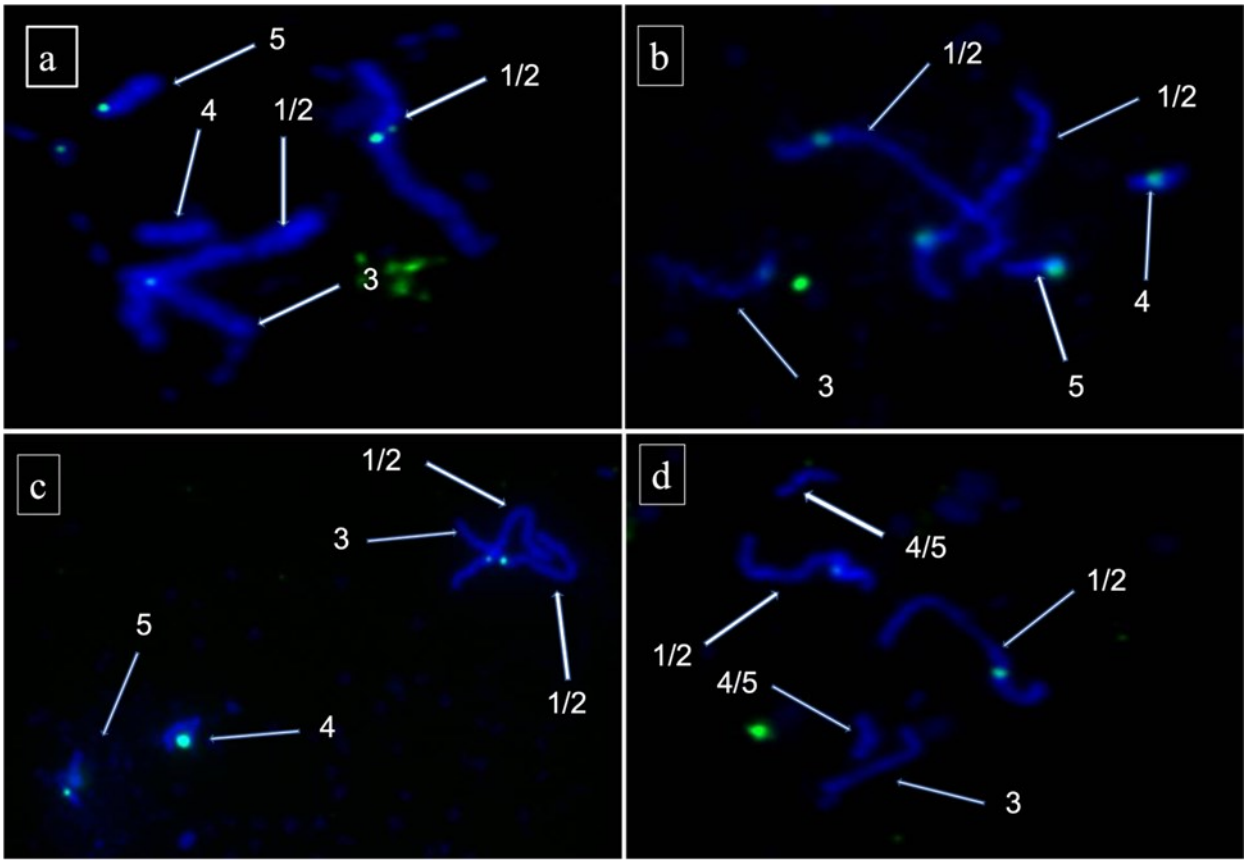

Figure 4. FISH with centromere probes (representing the 4 conserved sequences) of *Dictyostelium discoideum* on chromosomes from 46a3 strain of *Dictyostelium giganteum*, All the four panels a,b,c and d show the FISH done in 46a3 strain of *Dictyostelium giganteum* with the centromere probe (representing 4 conserved sequences). Numbers indicate the chromosome number based on size. Numbers shown as 1/2 in all the four panels and 4/5 in panel d indicate the ambiguity involved in denoting indicated chromosome exactly as 1 or 2 in all panels and 4 or 5 in panel d. 1/2 and 4/5 should be read as one or two and four or five respectively.

chromosome and the proportionate area with respect to the total area of all 5 chromosomes in a metaphase were calculated for each of the 20 metaphases for all the six strains of *D.giganteum*. This data was used to arrive at a rough estimate of proportion of genomic content of each of the 5 chromosomes in all the six strains (Table 4).

Based on the above data it can be concluded that the genomic content of chromosomes 1, 2, 3, 4 and 5 in all strains of *D. giganteum* is approximately the same, and amounts to 35%, 30%, 17%, 10% and 8% respectively. However, the small differences may be meaningful, and what they might imply will be

addressed elsewhere.

Localization of centromere by FISH and classification of chromosomes

Fluorescence in situ hybridization of probes representing conserved centromeric sequences of *D. discoideum* on the metaphase chromosomes of 46a3 strain of *D. giganteum*, indicated that two of the large chromosomes are sub metacentric, the medium chromosome is telocentric, one of the small chromosomes is metacentric and the other small chromosome is telocentric.

Discussion

To the best of our knowledge, apart from *D. discoideum*, chromosome-based studies have not been carried out on any other species of Dictyostelium except the simple karyotyping in *Dictyostelium caveatumn* [21]. Our study is also first inter-strain study. For a long time it was believed that *D. discoideum* had 7 haploid chromosomes [22]. Subsequently, pulse-field gel electrophoresis revealed 5 chromosomes [23]; the currently accepted number is 6 [24–26]. The related species *Polysphondylium violaceum* is said to have 11 or 12 chromosomes [27].

Our study involved chromosome analysis of six distinct strains of *D.giganteum* and 3 independent samples for each strain, leaving little room for any ambiguity regarding the accuracy of chromosome number arrived at. The haploid chromosome number in all six strains of *D. giganteum* analysed in our study is found to be 5. The chromosomes can be classified into 3 groups, namely group 1 with two large-sized chromosomes, group 2 with one medium-sized chromosome and group 3 with two small-sized chromosomes. The genome size of *D. discoideum* is 34.042MB and the genome of an axenic strain has been completely sequenced [25]. Preliminary results from work in progress indicate that the size of the *D. giganteum* nuclear genome is about 32 Mb (average of 6 strains; unpublished data). Based on that, and from the relative sizes of the various chromosomes in *D. giganteum*, we estimate their genomic contents as 11.07Mb, 9.73Mb, 5.40Mb, 3.11Mb and 2.66 Mb for chromosome Nos. 1,2,3,4, and 5 respectively.

FISH has been used in the past to locate the position of the centromere in human chromosomes[28,29]. Several experiments in past relating to FISH as [30,31] has shown that DIRS-1 sequences are characteristic of *D. discoideum* centromeres. But application of FISH to identify centromere has been done by[32] who used this technique to show that *Dictyostelium* centromeres contain DIRS-1, but their FISH has not been performed in proper metaphase cells. We find some studies on centromere which give the information that chromosomes appear to be acrocentric [33,34], one class of complex repeat in genome of *D. discoideum* serve as centromere [25] and sequences which compose the functional chromosomal elements like centromere are not conserved and appear to have underwent several modifications [30,35]. Our study is the first of its kind where we have localised the centromere in the chromosome of *D. giganteum* using the probe from *D. discoideum*. Data obtained from our FISH studies has indicated that two of the large chromosomes are sub metacentric, the medium chromosome is telocentric, one of the small chromosomes is metacentric and other small chromosome is telocentric.We have not carried out FISH with other *D. giganteum* chromosomes, but given the similarity in morphologies, assume that their centromeres will be similarly located.

Conclusions

The modal chromosome number in *D. giganteum* is five. On basis of size it can be classified into three groups of which first group comprises of two large chromosomes, second group contains a medium

chromosome and third group has two small chromosomes. On basis of centromere position its chromosomes of 46a3 strain of *D. giganteum* can be classified into metacentric, submetacentric and telocentric chromosomes. One of the small chromosome is metacentric, the other small chromosome along with medium sized chromosome is telocentric and two of the large chromosome is submetacentric.

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Supplementary Materials: Not applicable

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Appendix A

Not Applicable

Appendix B

Not applicable

References

1. Kaushik, S.; Katoch, B.; Nanjundiah, V. Social Behaviour in Genetically Heterogeneous Groups of Dictyostelium Giganteum. *Behav Ecol Sociobiol* **2006**, *59*, 521–530, doi:10.1007/S00265-005-0077-9/METRICS.
2. Sathe, S.; Kaushik, S.; Lalremruata, A.; Aggarwal, R.K.; Cavender, J.C.; Nanjundiah, V. Genetic Heterogeneity in Wild Isolates of Cellular Slime Mold Social Groups. *Microb Ecol* **2010**, *60*, 137–148, doi:10.1007/S00248-010-9635-4.
3. Brown, M.W.; Silberman, J.D. The Non-Dictyostelid Sorocarpic Amoebae. *Dictyostelids: Evolution, Genomics and Cell Biology* **2013**, 219–242, doi:10.1007/978-3-642-38487-5_12.
4. Raper, K.B.; Rahn A.W. The Dictyostelids. **1984**, 453.
5. Bonner, J.T. The Social Amoebae : The Biology of Cellular Slime Molds. **2009**, 144.
6. Bonner, J.T. Evolutionary Strategies and Developmental Constraints in the Cellular Slime Molds. *https://doi.org/10.1086/283930* **1982**, *119*, 530–552, doi:10.1086/283930.
7. Nanjundiah, V. The Evolution of Communication and Social Behaviour in Dictyostelium

Discoideum. *Proceedings: Animal Sciences* **1985**, *94*, 639–653, doi:10.1007/BF03191865/METRICS.

8. Strassmann, J.E.; Zhu, Y.; Queller, D.C. Altruism and Social Cheating in the Social Amoeba Dictyostelium Discoideum. *Nature* **2000**, *408*, 965–967, doi:10.1038/35050087.
9. Hayakawa, I.S.; Inouye, K. Species Recognition in Social Amoebae. *J Biosci* **2018**, *43*, 1025–1036, doi:10.1007/s12038-018-9810-1.
10. Kundert, P.; Shaulsky, G. Cellular Allorecognition and Its Roles in Dictyostelium Development and Social Evolution. *Int J Dev Biol* **2019**, *63*, 383, doi:10.1387/IJDB.190239GS.
11. Aggarwal, R.K.; Allainguillaume, J.; Bajay, M.M.; Barthwal, S.; Bertolino, P.; Chauhan, P.; Consuegra, S.; Croxford, A.; Dalton, D.L.; Den belder, E.; et al. Permanent Genetic Resources Added to Molecular Ecology Resources Database 1 August 2010-30 September 2010. *Mol Ecol Resour* **2011**, *11*, 219–222, doi:10.1111/J.1755-0998.2010.02944.X.
12. Sathe, S.; Khetan, N.; Nanjundiah, V. Interspecies and Intraspecies Interactions in Social Amoebae. *J Evol Biol* **2014**, *27*, 349–362, doi:10.1111/jeb.12298.
13. Gaudet, P.; Fey, P.; Chisholm, R. Growth and Maintenance of Dictyostelium Cells. *CSH Protoc* **2008**, *2008*, doi:10.1101/PDB.PROT5099.
14. Papadopulos, F.; Spinelli, M.; Valente, S.; Foroni, L.; Orrico, C.; Alviano, F.; Pasquinelli, G. Common Tasks in Microscopic and Ultrastructural Image Analysis Using ImageJ. *Ultrastruct Pathol* **2007**, *31*, 401–407, doi:10.1080/01913120701719189.
15. Phengchat, R.; Takata, H.; Morii, K.; Inada, N.; Murakoshi, H.; Uchiyama, S.; Fukui, K. Calcium Ions Function as a Booster of Chromosome Condensation. *Sci Rep* **2016**, *6*, doi:10.1038/SREP38281.
16. Glöckner, G.; Heidel, A.J. Centromere Sequence and Dynamics in Dictyostelium Discoideum. *Nucleic Acids Res* **2009**, *37*, 1809–1816, doi:10.1093/NAR/GKP017.
17. Djadid, N.D.; Jazayeri, H.; Raz, A.; Favia, G.; Ricci, I.; Zakeri, S. Identification of the Midgut Microbiota of An. Stephensi and An. Maculipennis for Their Application as a Paratransgenic Tool against Malaria. *PLoS One* **2011**, *6*, doi:10.1371/JOURNAL.PONE.0028484.
18. Lion, T.; Haas, O.A. Nonradioactive Labeling of Probe with Digoxigenin by Polymerase Chain Reaction. *Anal Biochem* **1990**, *188*, 335–337, doi:10.1016/0003-2697(90)90616-H.
19. Knoll, J.H.M.; Lichter, P. In Situ Hybridization to Metaphase Chromosomes and Interphase Nuclei. *Curr Protoc Hum Genet* **2005**, *Chapter 4*, doi:10.1002/0471142905.HG0403S45.
20. Wu, B.L.; Austin, M.A.; Schneider, G.H.; Boles, R.G.; Korf, B.R. Deletion of the Entire NF1 Gene Detected by the FISH: Four Deletion Patients Associated with Severe Manifestations. *Am J Med Genet* **1995**, *59*, 528–535, doi:10.1002/AJMG.1320590427.
21. Waddell, D.R.; Duffy, K.T. Breakdown of Self/Nonself Recognition in Cannibalistic Strains of the Predatory Slime Mold, Dictyostelium Caveatum. *J Cell Biol* **1986**, *102*, 298–305, doi:10.1083/JCB.102.1.298.
22. Robson, G.E.; Williams, K.L. The Mitotic Chromosomes of the Cellular Slime Mould Dictyostelium Discoideum: A Karyotype Based on Giemsa Banding. *J Gen Microbiol* **1977**, *99*, 191–200, doi:10.1099/00221287-99-1-191/CITE/REFWORKS.

23. Cox, E.C.; Vocke, C.D.; Walter, S.; Gregg, K.Y.; Bain, E.S. Electrophoretic Karyotype for Dictyostelium Discoideum. *Proc Natl Acad Sci U S A* **1990**, *87*, 8247–8251, doi:10.1073/PNAS.87.21.8247.

24. Kuspa, A.; Loomis, W.F. Ordered Yeast Artificial Chromosome Clones Representing the Dictyostelium Discoideum Genome. *Proc Natl Acad Sci U S A* **1996**, *93*, 5562–5566.

25. Eichinger, I.; Pachebat, J.A.; Glöckner, G.; Rajandream, M.A.; Sucgang, R.; Berriman, M.; Song, J.; Olsen, R.; Szafranski, K.; Xu, Q.; et al. The Genome of the Social Amoeba Dictyostelium Discoideum. *Nature* **2005**, *435*, 43–57, doi:10.1038/NATURE03481.

26. Loomis: The Genome of Dictyostelium Discoideum - Google Scholar Available online: https://scholar.google.com/scholar_lookup?title=The%20genome%20ofDictyostelium%20discoideum&pages=15-30&publication_year=1997&author=Loomis%2CW.%20F.&author=Kuspa%2CA. (accessed on 15 May 2023).

27. Williams, K.L. Examination of the Chromosomes of Polysphondylium Pallidum Following Metaphase Arrest by Benzimidazole Derivatives and Colchicine. *J Gen Microbiol* **1980**, *116*, 409–415, doi:10.1099/00221287-116-2-409/CITE/REFWORKS.

28. Marshall, R.R.; Murphy, M.; Kirkland, D.J.; Bentley, K.S. Fluorescence in Situ Hybridisation with Chromosome-Specific Centromeric Probes: A Sensitive Method to Detect Aneuploidy. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis* **1996**, *372*, 233–245, doi:10.1016/S0027-5107(96)00143-1.

29. Uroz, L.; Liehr, T.; Mrasek, K.; Templado, C. Centromere-Specific Multicolour Fluorescence in Situ Hybridization on Human Spermatocyte I and II Metaphases. *Hum Reprod* **2009**, *24*, 2029–2033, doi:10.1093/HUMREP/DEP092.

30. Wiegand, S.; Meier, D.; Seehafer, C.; Malicki, M.; Hofmann, P.; Schmith, A.; Winckler, T.; Földesi, B.; Boesler, B.; Nellen, W.; et al. The Dictyostelium Discoideum RNA-Dependent RNA Polymerase RrpC Silences the Centromeric Retrotransposon DIRS-1 Post-Transcriptionally and Is Required for the Spreading of RNA Silencing Signals. *Nucleic Acids Res* **2014**, *42*, 3330–3345, doi:10.1093/NAR/GKT1337.

31. Windhof, I.M.; Dubin, M.J.; Nellen, W. Chromatin Organisation of Transgenes in Dictyostelium. *Pharmazie* **2013**, *68*, 595–600, doi:10.1691/PH.2013.6525.

32. Dubin, M.; Fuchs, J.; Gräf, R.; Schubert, I.; Nellen, W. Dynamics of a Novel Centromeric Histone Variant CenH3 Reveals the Evolutionary Ancestral Timing of Centromere Biogenesis. *Nucleic Acids Res* **2010**, *38*, 7526–7537, doi:10.1093/NAR/GKQ664.

33. Welker, D.L.; Williams, K.L. A Genetic Map of Dictyostelium Discoideum Based on Mitotic Recombination. *Genetics* **1982**, *102*, 691–710, doi:10.1093/GENETICS/102.4.691.

34. Glöckner, G.; Eichinger, L.; Szafranski, K.; Pachebat, J.A.; Bankier, A.T.; Dear, P.H.; Lehmann, R.; Baumgart, C.; Parra, G.; Abril, J.F.; et al. Sequence and Analysis of Chromosome 2 of Dictyostelium Discoideum. *Nature* **2002**, *418*, 79–85, doi:10.1038/NATURE00847.

35. Glöckner, G. Genome Analysis of Social Amoebae. *Dictyostelids: Evolution, Genomics and Cell Biology* **2013**, 35–47, doi:10.1007/978-3-642-38487-5_2.