Are centromeric dots kinetochores?

EIBERG[1] described a Giemsa staining technique which reveals specific paired dots in the centromere region of human chromosomes in cells treated with colcemid and hypotonic KCl. He suggested that the dots may represent organelles associated with spindle fibres. Evans and Ross[2] demonstrated similar, stained or unstained dots obtained in colcemid-treated mammalian cells by other preparatory techniques. They hypothesised that these dots "may represent the kinetochores and particularly their associated proteins".

These interpretations are unlikely for several reasons. First, the location of the unstained dots shown by Evans and Ross[2] does not coincide with that of the raised dots on unstained, shadowed chromosomes. Unstained dots adjoin chromosomes in the centromere region, whereas raised dots, like Eiberg's[1] C_d dots, lie clearly within the chromosomes. Second, there is no evidence that the centromerically located spindle microtubules are always present in colcemid-treated cells. A two hour exposure of rat kangaroo cells to 0.05 μg ml^{-1} colcemid destroys all microtubules and alters the fine structure of the kinetochores[3]. Speculation that spindle proteins concentrated in the centromere regions of chromosomes could produce centromeric dots cannot, therefore, be substantiated. Third, kinetochores are rather delicate, labile organelles and they are probably affected drastically by treatments used in the preparation of chromosomes for light microscopy. For example, no kinetochores are visible in thin sections of Chinese hamster chromosomes subjected to the aceto-orcein smear technique (L. J. Journey, personal communication). Fourth, even if kinetochores were preserved by light microscopic techniques they could hardly produce prominent raised dots, for the disk-shaped kinetochores of mammalian chromosomes are only some 100 nm thick[3,4].

Optical and electron microscopic observations of rat kangaroo cells treated with hypotonic colcemid[5] suggest a different interpretation of centromeric dots. Paired dots are recognisable by phase contrast microscopy in the centromere region of favourably oriented chromosomes in embedded prometaphase cells (Fig. 1*a*). In thin sections the dots appear as patches of chromatin packed more densely than in the remainder of the chromosomes (Fig. 1*b*). The kinetochores which are less opaque to electrons lie adjacent to these patches. In metaphase cells the chromosomes are, overall, more condensed, but the chromatin is also more densely packed in the centromere region than in the arms (Fig. 1*c*). The outer layer of each kinetochore appears as a moderately electron-opaque band lying parallel to the chromosomal surface in a distinctly electron-lucent, approximately circular zone adjacent to the centromere region (Fig. 1*c* and *d*).

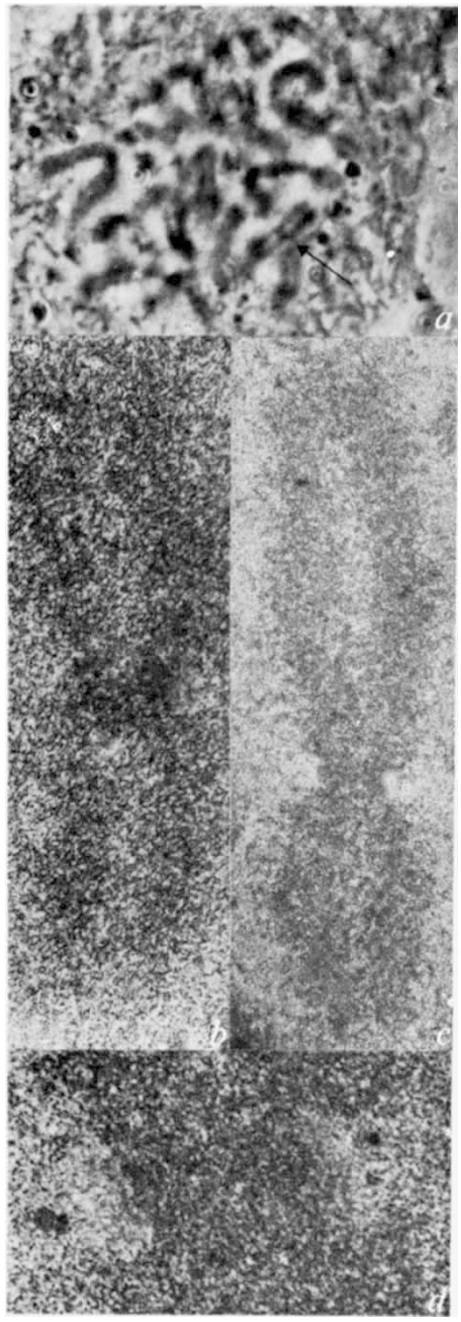

Fig. 1 Chromosomes of rat kangaroo cells (line PtK_2) treated with hypotonic colcemid (15 min at 37 °C in 0.25 μg ml^{-1} colcemid prepared by diluting an aqueous 0.5 μg ml^{-1} stock solution 1:1 with culture medium. See ref. 3 for culture conditions, fixation and embedding). *a*, Phase contrast micrograph of a prometaphase cell (×1,625). Note the paired dots in the centromere region of favourably oriented chromosomes. Single dots are visible on laterally viewed chromosomes. *b*, Thin section of the arrowed chromosome of Fig. 1*a* ×11,700. Note the two patches of densely packed chromatin in the centromere region. *c*, Metaphase chromosome ×7,475. Note the dense centromeric chromatin and the adjoining electron-lucent zones. *d*, Centromere region of another metaphase chromosome ×22,425. Note the kinetochore bands at the surface of the primary constriction and the electron-lucent zones.

These electron-lucent zones correlate with, and may be equivalent to, the unstained dots described by Evans and Ross[2]. This interpretation is supported by Journey's observations (personal communication) of "empty", circular zones adjacent to the centromere region of Chinese hamster chromosomes in thin sections of acetoorcein smears. The densely packed centromeric chromatin, on the other hand, may account for the dots revealed by Eiberg's[1] Giemsa staining technique and by the shadowing technique of Evans and Ross[2]. Dense structures reminiscent of centromeric dots are also seen in the centromere region in whole-mount preparations of Chinese hamster chromosomes treated with distilled water[6] and in whole mounts of HeLa chromosomes stained with phosphotungstic acid[7]. All these results can be explained by a greater resistance of the centromeric chromatin to the spreading forces to which chromosomes are subjected in a hypotonic medium. This resistance may reflect a specific DNA–protein composition of the centromeric chromatin, that in turn could account for the specific staining, as pointed out by Eiberg[1].

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Logic of animal conflict

MAYNARD-SMITH and Price have shown[1] that it is not necessary to invoke group selection to explain the occurrence of 'conventional' rather than 'dangerous' tactics in animal conflict. The conditions for the evolution, under individual selection, of populations in which conventional tactics predominate are, however, more stringent than they suggest. In particular, given their pay-off matrix, the population may end up consisting mainly of individuals adopting the 'dangerous' strategies, Hawk and Bully.

Consider a population consisting almost entirely of Hawk and Bully, so that nearly all conflicts are between these. Then

Hawk is the better strategy when the opponent is Bully and Bully is better when the opponent is Hawk. Thus we have a system of frequency-dependent selection, leading to a stable mixture of Hawk and Bully. At this equilibrium, Hawk and Bully will have equal fitness on average and will therefore have frequencies: Hawk 0.575652; Bully 0.424348. In nearly all conflicts, the opponent will be Hawk or Bully, with frequencies just given, so that average pay-offs will be: Mouse 19.5000; Hawk 20.4311; Bully 20.4311; Retaliator 11.3932; Prober–Retaliator 13.6357.

Therefore, types other than Hawk and Bully are at a disadvantage and will not spread. These results will not be affected by the presence of a few individuals adopting strategy Mouse for non-genetic reasons, since Hawk and Bully are the best (equally good) strategies when the opponent is Mouse.

If, for simplicity, we regard the five strategies as reproducing asexually with fitnesses given by average weighted pay-offs, we find that the Hawk–Bully equilibrium is attained from some starting points, for example, with strategies given in the order above, (0.33, 0.33, 0.33, 0.005, 0.005) or (0.9, 0.025, 0.025, 0.025, 0.025). On the other hand, starting with all strategies of equal frequency, the ultimate population consist entirely of Retaliator. These results suggest strongly that some modification of the original model is required to explain the general occurrence of conventional strategies.

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Professor Maynard-Smith replies—I am afraid that Gale and Eaves are quite right. They have found an alternative evolutionary stable strategy to the conflict which the late Dr Price and I investigated.

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Anti-Darwinism among the molecular biologists

Ohta[1] asserted that evolutionary change at the macromolecular level was caused primarily by "mutation pressure" rather than Darwinian selection. This view, upheld by an influential school of biochemists and molecular biologists (most of them named in Ohta's bibliography), strikes some of us as regressive and potentially dangerous to our science. One major danger is that it will dissuade biochemists from looking for functional significances in sequence differences (for example those of cytochrome *c* from different species). There is also a more general danger, that of encouraging ahistorical, statistical and mathematical thinking at the expense of the search for causal and historical explanations of the particularities of organisms.

It was a Scottish philosopher of science who wrote that "Only in the last resource, when the heavenly powers fail us, should resort be had to the demons of the underworld, chance and probability"[2]. In the case of macromolecular evolution, it does not seem to me that Ohta and those who think like him in any way have demonstrated the failure of the "heavenly powers" of Darwinian selection. Earlier, Kimura and Ohta[3] had coined the phrase "naive pan-selectionism" for the ideas of those who disagreed with them, and this phrase was taken up by Wills[4] in a paper, not cited in Ohta's bibliography, which criticised in some detail the arguments of Kimura and Ohta, and showed that the evidence was quite consistent with a selective origin for most, if not quite all, the protein differences met with in nature.

The major argument cited by the followers of Ohta and Kimura in favour of their attitude has been the alleged time-proportionality in the numbers of residues differing between species, for cytochrome *c* and other proteins. Reasons have been put forward, such as the "Red Queen hypothesis" of van Valen[13], why a loose proportionality of this sort might be expected on the basis of ordinary selectionist theory. Ohta, however, treats it as an established fact that there exists a proportionality far too accurate to be explained in this way. It needs to be pointed out that, in the graphs published to illustrate this proportionality, by Dickerson[5] for cytochrome c, for example, and by Wilson *et al.*[6] for haemoglobins, the apparent linearity of the relationships depends on the assignment of some very questionable ancestral ages for the taxa concerned. Thus Dickerson gives a mid-Cretaceous age (around 100 Myr) for the ancestries of the main orders of placental mammals, whereas fossil evidence[7] would place this in the very late Cretaceous or early Palaeocene, perhaps 75 Myr ago. For a common ancestry of mammals and reptiles, that is an ancestral amniote, Dickerson cites a Lower Carboniferous (around 320 Myr) age, whereas fossil evidence[7] would suggest an early Permian age of perhaps 270 Myr. For a common ancestor of Amphibia and Amniota, Dickerson's graph assigns a late Devonian age of some 350 Myr, whereas fossil evidence[7] would rather suggest the mid-Carboniferous, some 300 Myr ago. Finally, the age given by Dickerson for a common ancestor of insects and vertebrates is late Precambrian, perhaps 600 Myr old. This would be about the age of the celebrated Ediacara fauna of Australia, described by Glaessner[8] in which quite advanced annelid and possible primitive types of arthropods are represented; undoubtedly, any common ancestry of the deuterostomian line (including Vertebrata) and the molluscan–annelid one from which the insects sprang must have been very considerably older, perhaps more than 700 Myr old.

The similar graph for the haemoglobins given by Wilson *et al.*[6] assigns some even more questionable ancestral dates—for example, original separation of the cyclostome (lamprey) line from that of Gnathostomata, is placed in the Proterozoic, some 800 Myr ago, probably nearly as old as the entire metazoan line!

A further assumption of Ohta is that protein polymorphism in natural populations is non-adaptive and a result of mutation pressure. Where such polymorphism has been studied in detail for particular proteins, as pointed out by Johnson[12], the phenomena have been found to parallel closely those of polymorphism in ordinary phenotypic characters, selective control of which has been demonstrated, as pointed out by Ford[11] in another important and relevant work not mentioned in Ohta's bibliography. Selander and Kaufman[9], also not cited, draw attention to a book by Levins[10] in which the theory is developed of the long term adaptive advantages of maintaining certain critical degrees (varying with the characters and the circumstances) of heterozygosity of natural populations.

Ohta's evident pride in the mathematical sophistication of his methods of analysis prompts a scriptural gloss on his phrase "naive pan-selectionism": namely, that by adopting what he would consider as the naivety of "little children", the molecular biologists might improve their chances of entering into that "kingdom of heaven" in which the historic truths of evolution are revealed.

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