resolved — the sperm head is closer to the site of fertilisation [15,17] — this is unclear for *Onthophagus*. What are the characteristics of short sperm that contribute to fertilization success? Or approaching the problem from another angle, what drives the evolution of (large) spermatheca size? Larger spermathecae could promote increased sperm competition and relate to a greater propensity for polyandry. Genetic correlations between reproductive traits (sperm and spermatheca size) and male and female mating rates could be addressed experimentally. Artificial selection incorporating monandrous (no sexual selection) and polyandrous lines (sexual selection) could be applied to verify whether fertilisation efficiency increases with intensity of postcopulatory sexual selection. This approach could also aid in the investigation of whether inclusive fitness is higher in polyandrous than in monandrous females as predicted [8]. To specifically investigate the good sperm aspect in this system, it would be necessary to investigate offspring viability in relation to father's fertilization success. Finally, sperm number could also play a role (for example [20]), so do males with short sperm also transfer more or less sperm (depending on how costly short sperm are to produce)? Future work in this vein could help verify key predictions of sexually selected sperm processes [7,8] and further the understanding of reproductive traits central in speciation processes.

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Chromosome Bi-Orientation: Euclidian Euploidy

Establishment of proper attachments between chromosomes and microtubules is essential for the accurate division of the genome. Two recent studies indicate that these attachments are facilitated by the geometry of chromosomes and the bipolar arrangement of spindle microtubules.

Jason Stumpff and Charles L. Asbury

Cell division in eukaryotes involves interactions between microtubules of the mitotic spindle and protein complexes called kinetochores, which assemble at centromeric regions of chromosomes. Paired sister chromosomes in mitosis, or paired homologous chromosomes in meiosis I, can only be segregated properly if their kinetochores bind to microtubules that emanate from opposite spindle poles, an arrangement known as bi-orientation (Figure 1). Errors in segregation lead to aneuploidy, the cause of human trisomy disorders and a hallmark of cancer. Thus, the mechanisms promoting bi-orientation have been a subject of intense investigation for many years. Two recent studies, one of which appeared in *Current Biology*, suggest that spindle and chromosome geometry are sufficient to achieve bi-orientation [1,2].

Historically, two general mechanisms for bi-orientation have been considered (reviewed in [3]). The first relies on geometric constraints that compel paired kinetochores to face towards opposite directions. This directional bias, in conjunction with the bipolar arrangement of microtubules in the spindle, is thought to promote attachments between spindle poles and kinetochores that face each other and provide a means to avoid making attachment errors. The second mechanism relies on a widely conserved phospho-regulatory system that corrects erroneous attachments by promoting detachment when paired chromosomes are connected to the same spindle pole, an arrangement called syntelic attachment (Figure 1A). Syntelic attachments generate less tension than bi-oriented attachments, and this reduced tension is thought to activate the Aurora family of kinases, which in turn causes detachment of kinetochore–microtubules. The relative importance of these two mechanisms in ensuring bi-orientation, however, remains unclear.

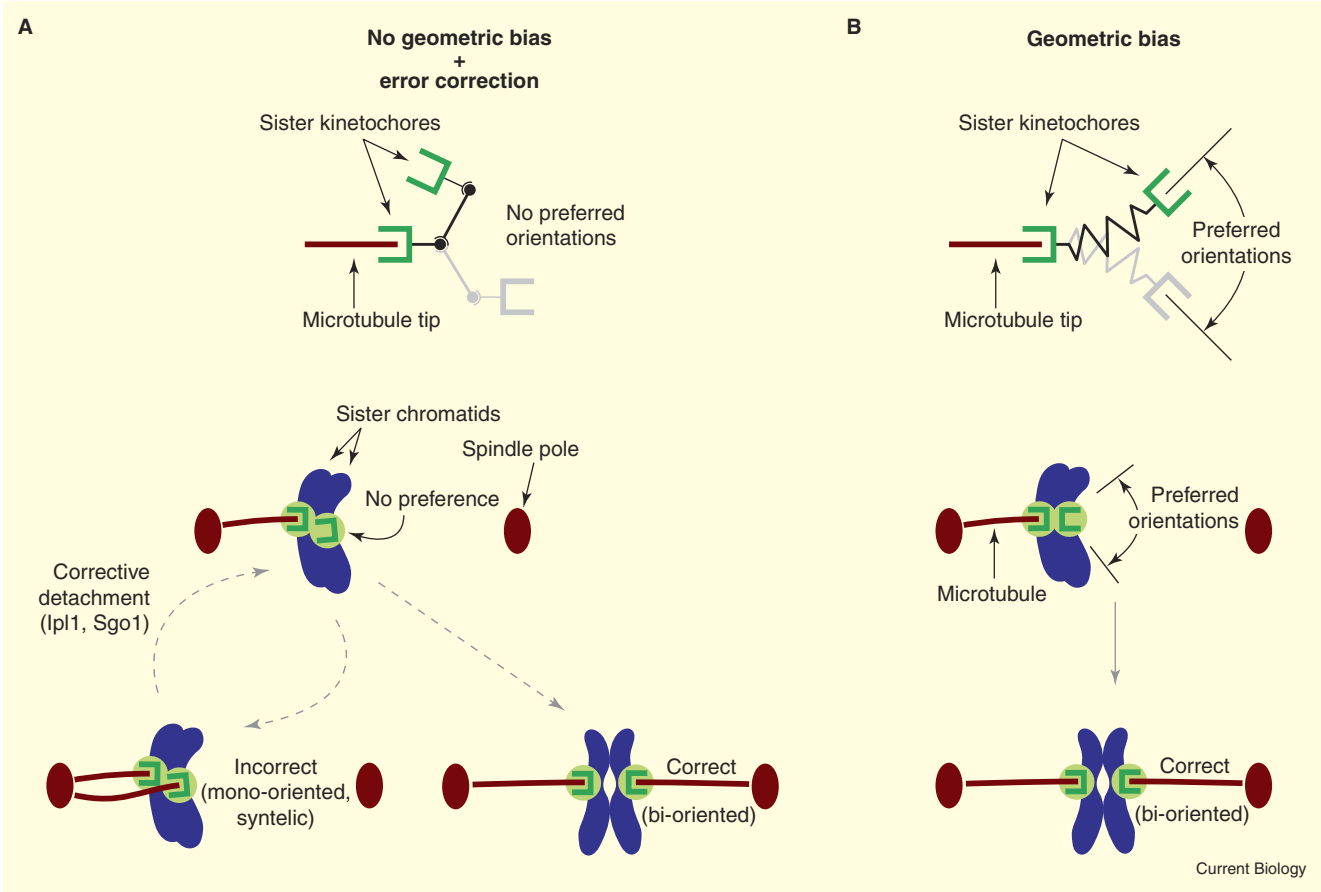

Figure 1. Two ways to promote proper attachment of sister kinetochores to spindle microtubules.

(A) If no geometric bias exists, the orientation of the sister kinetochores will be uncorrelated. After one sister has attached a microtubule tip, the other will bind microtubules emanating from either direction with equal probability. Without bias, sisters will make attachments to microtubules emanating from the same spindle pole, thus becoming mono-oriented, 50% of the time. Correction of these erroneous attachments occurs by Ipl1/Sgo1-mediated detachment of one or both kinetochores (presumably triggered by a lack of tension), allowing another attempt at bi-orientation. Given the high error rate and the fact that multiple sisters must achieve bi-orientation, numerous rounds of detachment and reattachment would have to occur for all chromosomes to achieve bi-orientation. (B) Alternatively, sister kinetochores may tend to face in opposite directions as a result of some intrinsic geometric constraint. Binding of one kinetochore to a microtubule emanating from the left would then predispose its sister to bind a filament emanating from the right. In the context of a bipolar spindle, a strong geometric bias would favor bi-orientation, without the need for corrective detachment.

Recently, powerful assays for analyzing chromosome bi-orientation and segregation have been developed in budding yeast (*Saccharomyces cerevisiae*). With advances in imaging and molecular techniques, researchers can now monitor the attachment status and subsequent segregation of individual chromosomes in live cells (reviewed in [4]). Fluorescent markers that bind near the centromeres of a specific chromosome pair appear as a single focus when the pair is attached to just one spindle pole. Once bi-orientation is achieved, the centromeres undergo transient separations and two fluorescent foci can be discerned. The marked chromosomes can then be followed through the division process for unambiguous determination of segregation errors.

A few years ago, Dewar and co-workers [5] used this bi-orientation assay to test the relative importance of chromosome geometry and error correction mechanisms during mitosis. Their study assayed bi-orientation of engineered minichromosomes containing two yeast centromeres, which presumably lack any geometric bias. When introduced into cells, these dicentric minichromosomes usually achieved bi-orientation, provided that the budding yeast aurora kinase, Ipl1, was active. In contrast, the minichromosomes rarely bi-oriented in strains with inactive Ipl1. The results of Dewar *et al.* [5] suggest that any connection that is capable of transmitting tension across sister chromosomes is sufficient for bi-orientation and that geometric constraints between sister kinetochores may be dispensable. However, a geometric contribution to bi-orientation of native chromosomes was not strictly ruled out.

Shugoshin-1 (Sgo1) is another protein implicated in the correction of improper kinetochore–microtubule attachments [6]. Similar to *ipl1* mutants, *sgo1* mutants do not respond to a lack of tension on kinetochores. However, chromosome segregation in *sgo-1* mutants is largely normal, suggesting that tension-dependent error correction is dispensable for mitotic division. Interestingly, the ability of *sgo1* mutants to segregate chromosomes can be compromised by treatment with microtubule-depolymerizing drugs [6]. Normally, cells delay mitosis when microtubules

are depolymerized but will form spindles and segregate chromosomes when the drugs are removed. Similar treatment of sgo1 mutant cells results in a normal mitotic delay, but, after drug removal, chromosome segregation is defective and the cells die.

In a recent study, Indjeian and Murray [1] examined why *sgo1* mutants exhibit this mysterious sensitivity to microtubule poisons. They assayed bi-orientation in *sgo1* mutants following incubation with microtubule-depolymerizing drugs and discovered that proper chromosome attachment and segregation were strongly correlated with the extent of spindle-pole separation at the time of drug removal. Under conditions in which spindle poles were not separated, when the drug was washed-out, bi-orientation and chromosome segregation were abnormal as previously observed, but, under experimental conditions in which spindle poles were separated at the time of drug release, proper attachments were made and cells underwent successful division. Thus, bi-orientation in *sgo1* mutants seems to depend on whether spindle poles are separated when kinetochore–microtubule attachments are established. Since spindle poles must be separated for the geometry of paired kinetochores to promote bi-orientation, this work suggests that proper spindle geometry and, by extension, chromosome geometry are sufficient for successful mitotic chromosome segregation.

Another recent study reached a similar conclusion after investigating the importance of chromosome geometry for successful meiotic division [2]. During meiosis I, paired homologs are held together by sites of recombination, which can occur far from the centromere and thus may not constrain the orientation of kinetochores. Lacefield and Murray [2] studied the correlation between successful meiotic chromosome segregation and the position of recombination events. In *ipl1* mutant cells, chromosome segregation failed more frequently when recombination sites were positioned far from the centromere. Normal segregation was restored when an artificial tether was used to hold chromosomes together near the centromere, indicating that geometric constraints imposed on meiotic centromeres are sufficient for successful division. Taken together, these two studies [1,2] suggest that tension-dependent error correction is dispensable during both mitosis and meiosis in budding yeast, provided that chromosome and spindle geometry are unperturbed.

If yeast cells with normal chromosome and spindle geometry do not rely heavily on error correction, then why do *ipl1* mutants fail to properly segregate their chromosomes the majority of the time [7]? One possible explanation is that Ipl1 function is needed both for error correction and for establishing proper spindle and chromosome geometry. Consistent with this idea, recent evidence indicates that Ipl1 has a role in bipolar spindle assembly [8]. If the relative timing of pole separation is disrupted in *ipl1* mutants, then the intrinsic geometry needed to avoid syntelic attachments may be compromised at the time when kinetochore–microtubule attachments are being made. If *Ipl1* has this additional role, but Sgo1 is involved only in error correction, this could explain why the severity of chromosome segregation defects is different in strains carrying mutations in these two genes [6,7,9,10]. Further investigation of Ipl1's role in spindle assembly may provide key information about the regulation of spindle geometry and bi-orientation. Alternatively, Ipl1 may have a direct role in creating the proper chromosome geometry. Exactly how this geometry is established remains unclear. An intriguing idea is that formation of a centromere-specific chromatin loop regulates kinetochore orientation, although it remains to be determined how this structure might be formed [11,12].

In conclusion, both intrinsic geometry and error correction appear to promote chromosome bi-orientation in budding yeast. Why would it be beneficial for cells to employ two mechanisms to ensure bi-orientation when either seems to be sufficient? Perhaps the combination is optimal for segregating chromosomes with both high fidelity and speed. Error correction ensures accuracy, but the required detachment and reattachment cycle may take a considerable amount of time. By reducing the error rate, a geometry-based mechanism that promotes bi-orientation during the initial stages of microtubule attachment could make the process far more time-efficient (Figure 1). These concepts are likely to be applicable to bi-orientation in other eukaryotes as well. In vertebrate cells, for example, paired mitotic kinetochores are presumed to be geometrically constrained, spindle poles separate before chromosomes attach to the spindle and the Aurora B kinase is required to achieve normal kinetochore–microtubule attachments [13]. Future work focused on unraveling the molecular basis of chromosome and spindle geometry and how it promotes bi-orientation should prove to be a fascinating line of investigation for years to come.

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