length is insensitive to monastrol concentration, the rate of poleward flux — the minus-end depolymerization velocity — is decreased significantly at the concentrations used [20]. These results are consistent with those of Goshima *et al*. [2], who studied both the Klp61F (Kinesin-5) depleted regime and the regime where Klp61F was overexpressed to find that spindle length remained unchanged above a threshold.

Mitchison *et al.* [19] also found evidence for an elastic spindle matrix, based on observations of kinetochore microtubule buckling during some depolymerizing conditions, which may be particular to meiotic spindles in oocytes. For a spindle matrix to work effectively as a tensile element in the mitotic spindle, it must be covalently cross-linked, or at least have a mechanical relaxation time much longer than the duration of metaphase, in order to avoid creeping and behaving effectively as a viscous element.

In summary, the new study by Goshima *et al.* [2] establishes a new approach to systematic and automated analysis of mitotic spindle length coupled to a mathematical modeling framework, and thereby identifies microtubule assembly regulation as the most sensitive means of controlling spindle length in *Drosophila* S2 cells.

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# Mate Choice: Been There, Done That

**Females may benefit from mating with several males, but how can they avoid mating with the same male repeatedly? A new study of crickets has found that females mark their mates to avoid repeat copulations.**

Tom Tregenza and
David J. Hosken

Males are born to mate; every female brings the prospect of new offspring and it is generally a case of the more the merrier. For females things are different. Females are defined by their large gametes, and generally their reproduction is limited not by mating opportunities, but by the resources they can gather to invest in their young. As a result, we expect to see males aggressively pursuing mating opportunities and females resisting their advances. This was the view promulgated by Darwin and until very recently, widely accepted. Since the advent of molecular techniques for studying paternity, however, it has become apparent that females are often much more promiscuous than had previously been imagined. The extent of multiple mating in insects has provoked a recent string of studies showing that females can benefit from mating with several males [1], but how they avoid mating with the same male over and over again has remained a mystery.

Females can benefit from extra matings for two reasons. There

Figure 1. A mating pair of crickets *Gryllodes sigillatus*.

The male (underneath) is attaching an external sperm packet to his mate. At the same time, the female is marking the male with her scent so that she can avoid mating with him in the future. (Photo courtesy of D.H. Funk.)

may be direct benefits, such as the food items that female hanging-flies are given by would-be mates, or the access to feeding areas that otherwise aggressive male birds may allow to cooperative females. Alternatively, females can benefit from matings because it gives them the opportunity to acquire better or more compatible genes for their offspring. They can do this by only re-mating when they encounter a male that they prefer over their previous mate, or by simply mating with more than one male and then either biasing their use of sperm in favour of the best male, or if better males have better sperm, by simply allowing sperm to compete.

If females mate repeatedly to allow post-copulatory differences in male fertilisation success to improve the genetic quality of their offspring, then they have a problem: they need to make sure they do not end up mating repeatedly to the same male. For an animal like a fish, with a big brain and a complex sensory system, this may not be too hard—just remember who you have already mated with and give them a wide berth next time [2]. But for an insect this may be asking too much. This has provided something of a puzzle because studies of insects such as pseudoscorpions [3], field

crickets [4], hide beetles [5] and dung flies [6] have found that females prefer novel males over their past partners. A new study by Ivy *et al*. [7] provides another example of females preferring new mates, but for the first time, reveals how they do it.

There are three ways to avoid a previous mate: you can hope you do not bump into him again; you can remember his features; or you can mark him with something you will recognise next time. It had generally been assumed that where insects are concerned, high mobility and large populations will mean that individuals do not tend to meet more than once. But this assumption has been challenged by studies showing females discriminate against previous mates [2–6], or the sperm of closely related males [8,9]. If insect populations are large and individuals move around a lot, females will not tend to encounter close relatives, so the existence of inbreeding avoidance suggests that some insect populations may have more genetic structure than we thought. This in turn means that many female insects cannot rely on never meeting their past conquests, and so to avoid previous mates they have to somehow recognise them.

To show that female *Gryllodes sigillatus* crickets (Figure 1) prefer novel males, Ivy *et al*. [7] simply

presented females with two males, one of which they had copulated with previously. Trios were observed for an hour and most females were found to mate with the new male. To examine how females achieve this feat Ivy *et al*. [7] started by creating several populations in which individuals were genetically very similar to one another. This is easy: three generations of brother–sister matings meant that individuals in a line were almost genetically identical. The authors then mated females to a male from a different inbred line and a day later gave the same females the choice of mating to a male from the same line as her first mate or to a male from a different line. Females showed no preference, suggesting that they do not remember their mates, as males that are genetically very similar and who have been reared together ought to look, feel and smell pretty similar to one another, so a female seeking to avoid a previous mate ought to also give his (almost clonal) brother a wide berth.

Next, Ivy *et al*. [7] gave females a choice of mating to a male that had previously mated to their line-sister or to a male that had previously mated to a female from a different line. Now, what they found was that over 80% of focal females avoided mating with the male that had previously mated to the female's sister. So, females avoid males that differ from one another only in the fact that one of them has previously mated to her line-sister — a behaviour that can only be explained if their sister has somehow marked the male with something that, because of the female's genetic similarity or because they were reared together, the focal female mistakes as her own. Male and female crickets are known to have substantial differences in the chemistry of their cuticle, and can use these differences to distinguish between the sexes [10]. It seems likely that the mark females place on males will turn out to be some sort of cuticular pheromone although at present exactly what it is remains unknown. Marking a male with

your own scent requires a much less sophisticated information processing system than trying to remember previous mates – a female always has her own scent available as a reference, so she can simply avoid males that smell too familiar.

The finding that females have evolved to mark their mates has two major implications. It suggests that the risk of encountering the same male twice must be large and so must the benefits of mating with more than one male. The crickets used in this study are not particularly unusual insects, they live in large populations at high densities [11] and are highly mobile. If a species like this has evolved a system for avoiding mating with the same male twice it suggests that similar abilities may be widespread. It also supports the argument that females can gain substantial benefits from mating with more than one male [12,13] and highlights the importance of what happens after a female mates. Indeed the possibility that females may often avoid mating with previous mates has such major implications that a degree of scepticism is warranted — there may be a lot of studies trying to find this phenomenon, with the danger that statistically significant results may sometimes occur by chance.

Although the sizes of the effects found in reports of female mating preference for novel males [2–7] are reasonably large, it is noteworthy that across studies the strength of the effect reported apparently decreases as sample size increases (effect sizes were calculated by converting p-values for experiments showing a mating bias to z-scores and dividing the z-score by the square root of the sample size; a linear regression of effect size against sample size gave a negative association ($r^2 = 0.727$; $F_{1,5} = 10.64$; $p = 0.031$)). This suggests that smaller studies where females fail to show discrimination against novel males may be being done but not being published, making the literature less objective than it should be. Nevertheless, Ivy *et al.* [7] provide the first study to show

that females both discriminate against males they have already mated, and that they do it by marking them as used goods. Males have been known to mark their mates for some time [14], so this sort of behaviour from females brings a little sexual equality to the mating game.

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# Cell Cycle: Bistability Is Needed for Robust Cycling

***Xenopus*** egg extracts have distinct Cdk-active and Cdk-inactive states at intermediate cyclin concentrations, a phenomenon known as bistability. A new study shows that this behavior is important for robust cell cycling.

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Cyclins and cyclin-dependent kinases (Cdks) play a central role in the cell-cycle oscillator, but they do not function in isolation. A complex network of conserved interactions generates a number of feedback mechanisms that regulate Cdk activity. For instance, the active cyclin–Cdk complex activates the anaphase-promoting complex (APC), which in turn destroys cyclin, in order to produce an alternation between interphase and mitosis (reviewed in [1]). This APC-mediated negative feedback alone could drive the cell cycle [2], but there are other conserved Cdk regulators of the cyclin–Cdk

complex. In particular, there is positive feedback on Cdk activity mediated by Cdc25 and Wee1 (reviewed in [3]). This positive feedback results in bistability in Cdk activity — two possible, stable levels of Cdk activity for a fixed cyclin concentration [4,5]. A number of investigators previously suggested that this bistability may be required for the production of distinct interphase and mitotic states in the cell cycle [6]. Recent work by Pomerening *et al*. [7] has now tested this proposal experimentally by specifically ablating the positive feedback and verifying that this indeed results in defects in the cell cycle as predicted by modelling work.