DCIS tissue has indicated that it shows the same changes in gene-expression patterns as those seen in fully invasive tumours.

Understanding DCIS transition to invasive breast cancer is of tremendous importance. In the past two decades, the reported incidence of DCIS in the Western world has increased rapidly because of regular mammography screening. But only a small fraction of patients with DCIS will develop invasive disease or die of it[10], as the vast majority have the tissue surgically removed and are then unlikely to develop subsequent tumours. This also means that additional treatment after surgery might be redundant. Nonetheless, after surgery, many women receive at least one of three regimens — radiation therapy, hormonal therapy or chemotherapy. At present, no criteria can consistently identify which women diagnosed with DCIS are most likely to benefit from these additional treatments. Identification of factors associated with subsequent invasive events could help classify women's individual risk for subsequent tumours and their response to therapies so as to avoid over- or under-treatment.

These three studies[3–5] begin to deliver on the promise of basic research in characterizing the tumour microenvironment and the application of that knowledge in the clinic. Molecular markers in epithelial cells that predict which DCIS lesions will subsequently become invasive tumours are being found[11]. By integrating such information with other data on the tissue microenvironment, researchers could identify additional molecular markers, thus improving prediction of future tumour formation and pointing the way to personalized treatment. The whisperings of molecular dialogue that go on between malignant cells and their microenvironment also hold information that can be used to categorize patients into those who need more, or less, aggressive therapy, identify patients for clinical trials, and develop new therapeutic approaches. These whisperings might even provide clinically important information before an invasive tumour can form. ■

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EVOLUTIONARY BIOLOGY

Sex ratios writ small

Jos. J. Schall

The evolutionary theory of sex ratios should apply to all creatures, both great and small. Experimental studies of the proportions of male to female sex cells of malaria parasites deliver cheering results.

Charles Darwin, the man of 'enlarged curiosity', was particularly curious about sex. He wondered, for example, why males and females are equally abundant in so many species in which males can mate with multiple females. Aren't males in surplus and a waste for such species? Darwin provided an answer, but was concerned primarily with human sex ratios. The question extends to even the single-celled protists, and on page 609 of this issue[1] Reece *et al.* revisit this venerable problem with a study of sex ratios in a protist that is both complex and lethal — the *Plasmodium* parasite that causes malaria.

Plasmodium prospers by replicating asexually within its vertebrate host, but also produces male and female gametocyte cells for transmission to a blood-feeding insect vector. Sex occurs within the vector, after each female gametocyte develops into a single female gamete, and each male yields several male gametes[2]. Intuition suggests that a *Plasmodium* infection's transmission success into the vector would be greatest when just enough male gametocytes are present to mate with all the females. Female-biased sex ratios are indeed common, but an apparent surplus of male gametocytes is routinely seen in some *Plasmodium* species, and gametocyte sex ratio varies among and even within infections over time[2–6]. Sex-ratio theory, a mainstay of modern evolutionary biology, offers explanations for these observations, but experimental verification has long been lacking.

Reece *et al.*[1] report that rodent malaria parasites follow sex-ratio theory quite well. Their elegant experiments show that each parasite clone shifts its ratio of male and female gametocytes according to the density of gametocytes in the blood, the fecundity of each male gametocyte and the likelihood of selfing (that is, union of male and female cells from the same clone). But *Plasmodium* also surprises with an additional talent — the parasite seems to detect kin and non-kin in the infection, and even the proportions of each.

Darwin provided a verbal explanation for the occurrence of equal proportions of males and females (Carl Düsing supplied the algebraic treatment a decade later)[7]. When sex ratio is biased, the less-common gender will have, on average, higher fitness, strictly because it will claim more offspring in the next generation. Mothers that produce offspring of the less-common gender would thus expect more 'grand offspring'. The equilibrium sex ratio would be 1:1. Almost a century later, W. D. Hamilton recognized that this model holds only for outbred populations[8]. In a species that reproduces in patches in which sisters mate only with brothers, a mother's fitness depends on reducing competition among her sons for mates. Thus, just enough sons should be produced to mate with all the daughters. As the degree of mating between siblings declines within patches, the sex ratio should shift towards more equal representation of males and females. Humans show a 1:1 sex ratio because we are so well outbred.

Hamilton's model fits the life history of malaria parasites[9]. All mating of *Plasmodium* gametes occurs in a single blood meal within the vector. If an infection consists of a single genotype, or clone, of parasites, the optimal sex ratio for that clone would be one male gametocyte to **f** female gametocytes, where **f** is the fecundity of the male, or the number of gametes it produces. In mixed-clone infections, the optimal sex ratio for each genotype depends on the likelihood of selfing, and will shift appropriately towards more males.

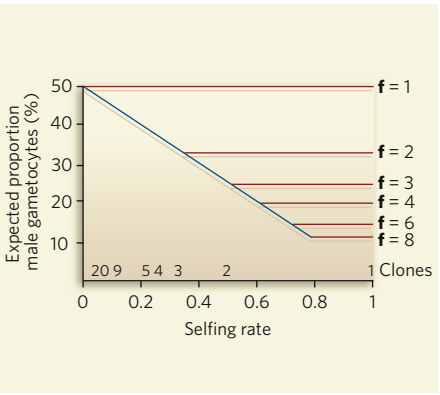

Figure 1 | A theory under test. Reece and colleagues[1] find that sex-ratio theory[2] predicts the proportion of malaria parasites' male gametocytes within a vertebrate host. The selfing rate depends on the number of genetically distinct clones and their proportions. Shown here are the expected rates of selfing with equal proportions of 1 to 20 clones. The fecundity of each male gametocyte (**f**) is the number of viable gametes produced per cell. With high fecundity (**f** = 8), and only one clone present, just enough males (11%) will be produced to mate with all the females within the insect vector. With low fecundity or many clones, the gametocyte sex ratio tilts towards higher production of males. (Figure redrawn from refs 2 and 4.)

Hamilton's simple equations allow predictions of expected sex ratios (Fig. 1).

Two additions must be mentioned to account for wrinkles in the *Plasmodium* life cycle. First, the sex ratio of several *Plasmodium* species is female-biased early in the infection, but shifts towards more males as the infection ages[5,6]. When the host mounts an immune attack against the parasite, carry-over of antibodies in the blood meal will kill many male gametes. Male fecundity will decline and more male gametocytes should be produced. Second, low-density infections may result in few gametocytes being transmitted, so male gametes cannot find a female. 'Fertility insurance' would then drive the production of more males[10].

The Reece group[1] used well-characterized clones of *Plasmodium chabaudi* originally isolated from the natural host, African thicket rats (*Thamnomys*), and then inoculated into laboratory mice to initiate experimental infections. Real-time application of the polymerase chain reaction allowed quantification of specific genetic strains and precise measurement of sex ratio.

The authors found that single-clone infections were female-biased early on, but that over time the sex ratio shifted towards males. Single-clone infections should yield 11% male gametocytes early in an infection if $\mathbf{f} = 8$ as per malariology lore. Four of the clones behaved according to theory. The two others produced more males, so we can predict the fecundity of these as 1 (the clone designated DK) and 4 (CR) (Fig. 1). Mixing all six clones should give 42% males, and this is just what was observed for the first six days of the infections. This outcome could be spurious if the DK clone dominates in infections (with its high male production), but this clone is known to be a poor competitor and to have low density in mixed infections. Mixing clones two-by-two, the expected result is 25% males, if both clones are equally abundant. But only one clone behaved as expected, with the others producing too few males.

However, Reece *et al.* determined the relative abundance of each clone, finding a negative correlation between the proportion of each parasite clone and its proportion of male gametocytes; when a clone predominated, it was more likely to self, and so produced fewer male cells. Finally, infections with a low density of gametocytes produced more males, even when only a single clone was present, which matches the expectations of fertility insurance.

These results should give cheer to fans of sex-ratio theory because the theory applies even for protist parasites dwelling within blood cells. Hamilton's equations are so simple, yet work so well. This is the real wonder of Reece and colleagues' study; it is as though this 'simple' parasite knows a little algebra.

Further questions have arisen, of course. There seems to be genetic variation for male fecundity (among isolates); why should this be? How does the parasite recognize its own density in the host, and — even more vexing — how does it monitor the presence of kin versus non-kin in other blood cells? Finally, Reece and colleagues' experiments are a study in evolutionary ecology, but in this case the parasite and host have not coevolved, and the ecology is foreign. When a parasite of thicket rats enters a lab mouse, it meets a strange environment. Yet the protist follows the rules laid down in sex-ratio theory. Getting the gametocyte sex ratio right seems to be crucial for *Plasmodium*, no matter what host it visits. Once again, when dealing with sex, it seems that getting it right is all-important. ■

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MATHEMATICAL PHYSICS

Packings close and loose

Francesco Zamponi

What determines how grains such as sand pack together to fill a space? A thoroughgoing investigation of how geometry and friction interact in such systems is a step towards a more general understanding.

How should we arrange objects to pack them as tightly as possible, making best use of all the available space? Packing problems have long fascinated both physicists and mathematicians, but have proved surprisingly tough nuts to crack. Take the 'Kepler conjecture', for instance. It was in 1611 that Johannes Kepler first suggested that the densest packing of identical spheres is achieved by cubic (face-centred cubic) and hexagonal arrangements, with a packing fraction of 74%. Carl Friedrich Gauss produced the first partial proof of this in 1831. What might be a final proof was published only in 1998. It is a 'proof by exhaustion', reached using modern computing power to crunch wearisomely through an inordinate number of possible packing configurations — and its ultimate veracity is still being checked.

Sphere packings are extremely important, not only in condensed-matter physics[1], where they describe the favoured configurations adopted by crystals, but also in computer science and mathematics[2], where they pop up in problems related to group theory, number theory and error-correcting codes. On page 629 of this issue, Song, Wang and Makse[3] take a significant stride towards a unified theory of a particular type of packing — not of the regular packings of the Kepler conjecture, but the random, amorphous packings that model the behaviour of everyday granular materials such as sand and nuts (Fig. 1).

When spherical grains are randomly thrown into a box and shaken, they form an amorphous arrangement with a packing fraction of 64%, significantly lower than the 74% of the densest possible crystalline packing. Remarkably, this final density — the signature of 'random close packing' — was found to occur however the samples were prepared: whether by throwing grains into a box, shaking them and allowing them to settle; depositing them randomly around a disordered 'seed cluster'; slowly compressing a looser arrangement; and so on.

If small regions of regular, crystalline packing are created first, a random close packing can then be continuously compacted until a denser, entirely crystalline structure is obtained[4]. When looking at individual configurations, therefore, the density value 64% does not seem to have any special importance. Its relevance must instead be related to the statistical properties of an ensemble of packings produced by a given method. Is random close packing favoured for entropic reasons, such that there are just many more ways of jumbling grains up to form a random close packing than any other configuration? Is it a well-defined 'metastable' state that can persist for a considerable time? Or is it related to a hidden critical point, such that particularly large numbers of particles must be rearranged to change the density (a quality characterized by a large 'correlation length')? Many attempts have been made to achieve the statistical description of random close packings that such questions demand[5–9]. These studies, supported by numerical simulations, revealed how important geometry, and in particular the network of particle contacts[5], was in determining the density and other structural features of the final packing.

Random loose packings are related to