Come Fly, and Leave the Baggage Behind

Richard E. Lenski

Why have some organisms evolved the capacity for sexual reproduction, whereas others make do with reproducing asexually? Since the time of Weismann (*1*), most biologists have been taught that sex produces variation and thereby promotes evolutionary adaptation. But how does sex achieve this effect, and under what circumstances is it worthwhile? On page 555 of this issue, Rice and Chippindale (*2*) provide evidence in support of one particular explanation: Sex promotes adaptation by allowing beneficial mutations to spread without being held back by the baggage of deleterious mutations in other genes.

The traditional explanation for sex is that it accelerates adaptation by allowing two or more beneficial mutations that have appeared in different individuals to recombine within the same individual (*3*). Without sexual recombination, individual clones that possess different beneficial mutations compete with one another, slowing adaptation by clonal interference (*4*). Sex allows simultaneous improvements at several genetic loci, whereas multiple adaptations must occur sequentially in clonal organisms.

But recently, this explanation has failed to satisfy for several reasons. First, sex imposes a 50% reduction in reproductive output (*5*). If a female can produce viable offspring on her own, why dilute her genetic contribution to subsequent generations by mating with a male? Second, the circumstances under which this kind of model provides sufficient advantage to offset the cost of sex are restrictive, requiring certain forms of selection and environmental fluctuations (*6*). Third, alternative models propose that the advantage of sex lies in eliminating deleterious mutations rather than in combining beneficial ones (*7*). Still another hypothesis, the one tested by Rice and Chippindale, involves an interplay between deleterious and beneficial mutations (*8*). Finally, empirical tests of these hypotheses have failed to produce a clear winner. All this leaves the area of sex ripe for good, clean experiments.

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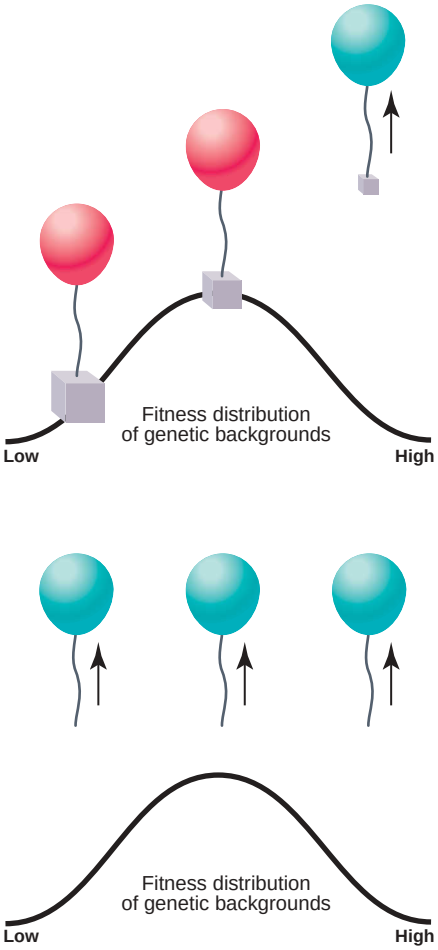
One approach has been to use microbes that can reproduce asexually and to examine the effects of manipulations that promote sexual recombination. For example, two viral genomes can recombine only when they coinfect the same cell (*9*). In their study, Rice and Chippindale take the opposite approach, converting the normally sexual fruit fly *Drosophila melanogaster* into an effectively clonal organism. They achieved this by constructing females with synthetic chromosomes containing almost the entire (haploid) genome. These females were used to propagate "asexual males" that mated each generation but could not recombine genes with their mates. As sexual controls, males mated with females whose genomes freely recombined.

To what end these tricks? Rice and Chippindale wanted to compare the fate of beneficial mutations in sexual and asexual populations under conditions that are well-defined and typical of metazoans. The model they tested predicts that fewer beneficial mutations will ultimately succeed (spread to fixation) in an asexual population compared with a sexual one, even though beneficial mutations arise at the same rate in both (see the figure). In any population, there exists genetic variation in fitness. A new beneficial mutation may thus occur on different genetic backgrounds that will influence its subsequent fate. In a sexual population, a beneficial mutation (one that avoids early extinction by random drift) moves into a new background every generation. During many generations, this mutation will experience an average of all backgrounds, regardless of whether it appeared in a particularly good or bad one. Thus, the fate of beneficial mutations in a sexual population is largely independent of background (see the figure).

In an asexual population, by contrast, each beneficial mutation remains forever associated with the background in which it appears. At first glance, this association does not matter too much because the background in which the mutation appears is determined by chance and is thus usually "about average" for the population. But being "about average" is not good enough over the long haul in an asexual population, because of the inevitable competition among all the different clones. Instead, the gain in fitness from the beneficial mutation must be large enough so that the resulting clone is at the head of its class. If the benefit is small relative to the variation in fitness among backgrounds, then this condition will be fulfilled only rarely. The other beneficial mutations, which occur in typical backgrounds, will be outcompeted by the fittest clones (even though they do not carry the beneficial mutation). In summary, this model predicts that a higher proportion of beneficial mutations will succeed in a sexual population than in one that is asexual.

To test this prediction, Rice and Chippindale imposed selection for a specific male-encoded mutation in 34 populations,



The benefits of sex. Effect of sex on the fate of beneficial mutations. Genetic variation in fitness exists in all populations. A beneficial mutation may appear in a genetic background that is more or less fit. (**Top**) Without sex, a beneficial mutation remains linked to the background in which it first appeared. If benefits are small relative to the variation in fitness among backgrounds, then only a beneficial mutation that occurs in one of the most fit backgrounds is able to rise above the other clones. (**Bottom**) With sex, a beneficial mutation moves into a new background each generation. Its fate is independent of the background in which it appeared, and it can rise on its own merit.

half of which were sexual and half effectively asexual. (In each case, the mutation started with 20 copies per population to avoid early extinctions by drift, which would be uninteresting because they should occur equally under both treatments.) They then monitored the mutation's trajectory over 10 generations. Consistent with theory, the beneficial mutation spread at similar average rates for several generations under the two treatments. In later generations, however, the beneficial mutation continued to increase in the sexual populations, but not in those that were asexual. Although the beneficial mutations gave a transient fitness boost to the backgrounds in which they landed, the resulting clones were not usually the eventual winners. (It would be nice to see most beneficial mutations go extinct in the asexual populations, as theory predicts they eventually should, but that would require the experiment to continue for many more generations.) Rice and Chippindale also showed that the initial fitness variation was sufficiently large, relative to the mutation's selective advantage, that this outcome was expected according to the theory. These experiments strongly support the hypothesis that sex is advantageous because it accelerates adaptive evolution—not by combining multiple beneficial mutations, but by freeing individual ones from the baggage of mutations at other genetic loci.

So now we know why sex evolved, right? Not so fast. As seductive as these experiments are, the big question is hardly answered, for two reasons. First, understanding the evolution of sex is really two different questions: the origin of sex, and its long-term maintenance in the face of its costs. As Rice and Chippindale conclude, their experiments are about the "persistence over geological time" of sexual recombination. Second, most competing hypotheses for the evolution of sex are not mutually exclusive, especially if one is interested in why sex is maintained (*10*). There may have been one particular advantage that got sex going, but once it evolved, several forces might make it difficult to return to an asexual state (*11*). Sex may bring beneficial mutations together, purge the genome of deleterious mutations, and—as now shown—allow beneficial mutations to fly free of the baggage of deleterious mutations. The experiments of Rice and Chippindale notwithstanding, evolutionary geneticists will continue to be very interested in sex.

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PERSPECTIVES: IMMUNOLOGY

Stress, NK Receptors, and Immune Surveillance

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Ever since the immune surveillance hypothesis proposed that the immune system could naturally recognize and eliminate tumors, investigators have been seeking cells that could serve such a purpose. Natural killer (NK) cells of the innate immune system that do not express the T cell receptor (TCR), rather than T cells of the adaptive immune system that do express the TCR, seemed to fit the bill. However, activating receptors expressed by NK cells and their associated ligands have only recently been identified, finally opening the door to the molecular dissection of NK cell involvement in immune surveillance. Now, papers by Girardi *et al.* (*1*) on page 605 of this issue and Diefenbach *et al*. (*2*) in *Nature* identify not only NK cells but also T cells expressing the γδ or αβ TCR as the prime movers in immune surveillance and natural antitumor immunity. The missing link turns out to be the NK cell receptor NKG2d, which is expressed by γδ and αβ T cells as well as by NK cells. These findings unite innate and adaptive immunity within the arena of cancer immunology.

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The first NK receptors to be clearly defined bound both classical and nonclassical MHC (major histocompatibility complex) class I molecules and blocked the killing of target cells by NK cells (*3*). This inhibition was associated with ITIM domains (immunoreceptor tyrosine-based inhibitory motifs) in the cytoplasmic tails of these NK receptors. ITIMs provide docking sites for phosphatases that oppose the activity of tyrosine kinases, enzymes that are essential for NK cell activation. However, certain members of the NK receptor family—CD16, KIR2DS, Ly49D/H, and CD94/NKG2c—do not contain ITIMs. Instead, they are associated with the adapter molecule DAP12, which has an ITAM domain (immunoreceptor tyrosine-based activating motif) capable of activating NK cells (*4*). Thus, the view is emerging that the activity of NK cells is balanced by opposing activating and inhibitory signals. But, other than the down-regulation of MHC class I molecules, it has been unclear which other molecules are likely to tip the balance in favor of NK cell activation. The discovery of ligands for the NKG2d receptor (*5*–*7*) has shed new light on NK cell activation as well as on T cell–dependent adaptive immunity.

Encoded within the NK receptor gene complex, NKG2d differs from other known NK receptors in three ways. First, it is associated with the adapter molecule DAP10, which contains neither conventional ITIMs nor ITAMs. Instead, DAP10 contains a YXXM motif in its cytoplasmic tail that binds to phosphatidylinositol (PI) 3-kinase after phosphorylation of its tyrosine residues. Second, NKG2d is expressed by all NK cells as well as by $CD8^+$ αβ and γδ T cells, suggesting that it is important in both innate and adaptive immunity. Third, at least some of the ligands for NKG2d can be induced by environmental stresses and are expressed on the surface of many tumor cells. The two best characterized NKG2d ligands are MICA and MICB in human cells, which are nonclassical MHC molecules whose expression is induced by classic stress stimuli such as heat shock (see the figure). MICA and MICB do not have known murine orthologs, but mouse NKG2d does bind to many products of the retinoic acid inducible gene family *Rae-1α–ε* as well as to the product of the *H60* gene. Although expression of *Rae-1* and *H60* genes apparently is not induced by heat shock, they are up-regulated in at least one example of genotoxic stress (application of carcinogens to mouse skin) (*1*). In common with MICA and MICB, Rae-1 and H60 appear to be up-regulated in a number of tumors, leading Girardi *et al*. and Diefenbach *et al*. to directly evaluate Rae-1 and H60 involvement in immune recognition and tumor surveillance in mice.