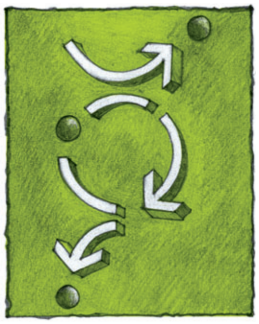
Yeast's beery smell attracts fruit flies

Fermentation



The enjoyment of a pint of your favourite beverage starts with the smell. As you inhale, the fruity odours wafting from the beer into your nose are caused by acetate esters formed while the brewer's yeast *Saccharomyces cerevisiae* ferments sugars into alcohol. Although those smells are certainly appreciated by humans, scientists have long wondered why *S. cerevisiae* produce compounds like acetate esters that don't appear to have any direct benefit to the yeast.

Noting that many insects are attracted to fermentation, Joaquin Christiaens and his colleagues at the Katholieke Universiteit Leuven in Belgium decided to investigate how acetate ester smells influence the behaviour of the laboratory fruit fly *Drosophila melanogaster*. They began by knocking out the *ATF1* gene (which is responsible for producing acetate esters during fermentation) in a strain of *S. cerevisiae*, which they called *atf1*$^{-}$. After confirming that this reduced the production of acetate esters, the authors compared how attractive smells from each of these yeasts were to *D. melanogaster*. They found that fruit flies were strongly attracted to the wild-type *S. cerevisiae* strains, but not the *atf1*$^{-}$ mutant strain.

Because it was possible that knocking out *ATF1* could have caused other changes to the yeast, the researchers then supplemented the smells from the *atf1*$^{-}$ mutant strains with the major acetate esters produced by *S. cerevisiae* – phenylethyl acetate, isoamyl acetate and ethyl acetate. They found that adding ethyl acetate to the *atf1*$^{-}$ yeast smells caused fruit flies to be equally attracted to the wild-type and *atf1*$^{-}$ mutant yeast smells. This showed that the reduced production of ethyl acetate in particular when *ATF1* was knocked out, was what made fruit flies turn up their noses at *atf1*$^{-}$ mutants.

To confirm that it was actually the fruit fly's sense of smell that caused the wild-type yeast to be so attractive, the researchers turned to *in vivo* calcium imaging of the antennal lobes of fruit flies as the insects sniffed the different smells. They found that the smell from the wild-type yeast strain caused a large brain response, whereas that from the *atf1*$^{-}$ mutant did not, and adding ethyl acetate to the *atf1*$^{-}$ mutant smell caused the fruit fly antennal lobes to respond more like they did to the wild-type yeast strain.

Finally, while the authors were convinced that the production of ethyl acetate in particular was important for the attraction of fruit flies to yeast, it remained unclear why the yeast might want to attract fruit flies. The authors hypothesized that having more fruit flies attracted to their smell might help the yeast disperse. Setting up a test arena where there were two competing yeast colonies – the nice-smelling wild-type strain, which they engineered to fluoresce green, and the less-attractive *atf1*$^{-}$ mutant, which fluoresced pink – the team then left a fruit fly in the arena overnight to see which yeasts the insect distributed around the arena. Counting the number of green and pink fluorescent colonies that had grown overnight, the authors found that the flies had dispersed the wild-type yeast much more than the *atf1*$^{-}$ mutant.

Yeast may seem like simple organisms, but by producing ethyl acetate they are able to manipulate the much more complicated fruit fly into acting like an airline – picking up yeast passengers from one area and carrying them to another. This is most advantageous for yeast wanting to swap genes, but it is especially lucky for beer-loving members of the human race.

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Paternal care is life or death to stickleback

Paternal effects

Retorts such as 'My Dad's (insert adjective here) than your Dad!' have haunted schoolyards for generations, but recent work published in the *Proceedings of the Royal Society B* suggests that there might be some biological relevance to these chants. Adequate parental care is, of course, essential to offspring well being and denying this care can permanently alter how offspring respond to stressful situations. Maternal care is well known to affect the behavioral phenotype of offspring, but we know very little about the influence of paternal care. Moreover, the adaptive significance of such non-genetic parental effects on offspring behavior is so far only speculative. Katie McGhee from the University of Illinois, USA and Alison Bell from the University of Cambridge, UK wanted to know how paternal care contributes to the behavioral phenotype of offspring, and whether or not this translates to a fitness benefit.

In an attempt to answer these questions, McGhee and Bell designed a set of experiments using three-spined sticklebacks. Stickleback dads are the sole caregivers for the clutch of eggs that they fertilize, providing essential life-supporting care to their wee ones by guarding the nest against hungry predators and fanning their eggs to help

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circulate oxygen. In the first set of experiments, the researchers removed half of a father's clutch of eggs and raised them separately as orphans, then monitored the father's behavior with the remaining eggs in order to assess the quality of care offered by a particular father. For example, while some fathers spent most of their time at the nest and frequently fanned their young (direct care), others spent more time inspecting a fake predator placed in their tank at the expense of nest guarding and fanning (defensive care). Then, when the offspring were a little older and no longer under Dad's watchful eye, McGhee and Bell tested the father-raised offspring and their orphaned siblings for anxiety-like behavior. As one might expect, orphaned fish tended to be more anxious than those that had received paternal care, but the quality of that paternal care had an even bigger effect on behavior. Orphans that missed out on the affections of a father giving direct care were considerably more anxious than their father-reared siblings, but this disparity was less pronounced when orphaned and reared siblings from defensive care fathers were compared. So, paternal care does affect offspring behavioral phenotype, but are these changes biologically relevant?

In a second experiment, McGhee and Bell used a new group of juvenile sticklebacks that were raised as orphans, without any paternal care. First, they assessed each juvenile for anxiety-like behavior as in the previous experiment, and then 2 h later they placed it in a tank with a big and scary live Northern pike fish. The fish were permitted to freely interact…for a little while at least. The researchers measured how long it took for the pike to launch its first attack on the stickleback and how long it took for the little fish to become the pike's dinner. Their results were clear: the higher an orphaned stickleback scored on its anxiety test, the sooner it became pike food, underscoring that non-genetic paternal effects on stickleback behavior are directly linked to fitness. So, in sticklebacks, there is no substitute for a father's love.

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The hormone battle behind 'eau de snake'

Pheromone signalling

There are a few steps to successful reproduction. First, you need to pick a member of the correct species. Then you have to pick the correct sex to mate with, with bonus points for picking an attractive mate. In some species, males and females are visibly different from one another, with males typically being larger or more ornamented than females. In other species, males and females are similar in appearance, and need a different way to tell each other apart. The red-sided garter snake (*Thamnophis sirtalis parietalis*) is such a species, where males and females appear almost identical. These snakes solve the problem of telling males and females apart through pheromones, secreted hormones that serve as chemical signals. These 'eau de snake' pheromones are multi-purpose, and communicate the species, the sex and even the attractiveness of an individual.

So what makes a snake smell like an attractive female? Rockwell Parker from Oregon State University, USA, and Robert Mason from Washington and Lee University, USA, decided to probe these pheromones more deeply and investigate how pheromone signals are regulated. In an initial study, the researchers learned that by injecting male snakes with estrogen, a hormone that is typically associated with females, they could induce the males to start producing female pheromones. Therefore, they knew that estrogens were important for stimulating the production of female pheromones. However, the researchers then wanted to know whether there was also a role for testosterone, a hormone that is typically associated with males. Is the absence of testosterone alone enough to activate the expression of the female pheromone?

To investigate this question, the researchers compared the pheromone production of castrated males with males that underwent a sham surgery without having their testes removed. The scientists then compared these snakes to both castrated males and intact males that received supplementary testosterone. For all of the males, they measured total pheromone production, as well as assessing the composition of the pheromones. The researchers also measured how attractive the treated male snakes were to wild male snakes, by counting how many wild males would leave a female to investigate and court the treated males.

The scientists first determined that castration reduced the total circulating testosterone compared with control males. Both castrated and intact snakes that were injected with testosterone had testosterone levels that were slightly higher than control males, but within the reasonable range of testosterone for this species. Castrated males produced more overall pheromone than intact males, and the pheromone profile was very similar to that of an attractive female. Interestingly, injecting the castrated males with testosterone cancelled this effect, and these males had a pheromone profile that was restored to that of a typical male. Finally, wild males were attracted to the castrated males, and frequently tried to court them. However, the wild males were much less interested in sham-operated males, or castrated males that were injected with supplementary testosterone.

Since castrated male snakes have no specific hormone profile, and yet produce a female pheromone, the results of this study indicate that the default signalling in red-sided garter snakes is female, and male pheromone profiles are only produced when there is active inhibition of the female signalling by testosterone. Therefore, these results suggest that pheromone signalling in snakes is regulated not by one hormone, but by an active battle of hormones.

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Different explanations for looking like the mailman

No one is overly surprised when kids look like their fathers. Children contain genes from both parents so naturally they look and act a bit like each one. But what if your offspring look less like your actual mate than your former one? Is it a scandal? Maybe, but not necessarily. In an elegant new paper in *Ecology Letters*, Angela Crean and her Australian colleagues show that this unexpected resemblance may just be due to 'telegony'.

Telegony is the long-ago discredited idea that males can influence not just their own genetic offspring, but also future offspring produced by the same female sired by different males. Most problematically, the idea lacked evidence. Equally, it lacked a mechanism and clashed fundamentally with the modern understanding of mendelian inheritance. How, after all, could a father influence offspring to which it contributed no genes?

As shown by Crean and her colleagues, the answer – at least for a group of Australian flies – turns out to be less complicated than you might think. Like many insects, young female neriid flies produce immature eggs that take several weeks to fully develop. Although males that mate with females during this interval sire no offspring, the research team hypothesized that they could still influence her future offspring. Importantly, males don't only transfer sperm during mating. They also transfer seminal fluid containing a diverse cocktail of proteins that have wide ranging effects on female physiology – and potentially on their immature eggs.

To test this idea, the team first mated immature female flies with two types of males. The first were well fed and in prime condition while the second were food deprived. In previous studies, the group found that big males tended to give rise to big offspring while food-deprived males tended to produce runts. Here, because the eggs were immature, neither treatment group was expected to sire any offspring at all. However, via their semen, these males could still modify the environment in which the eggs completed development.

Once the females had matured, they were mated for a second time with males of the two treatment groups and allowed to lay their fertilised eggs. Strikingly, the team found that the size of the offspring of these twice-mated females was better predicted by the size of their first mate than by the size of the actual genetic father. If the first mate was big, so too were the offspring, even if the second mate was a runt. And this, in a nutshell, is telegony.

Non-genetic factors, especially the maternal environment have long been recognized as key determinants of offspring phenotype. If mom drinks, smokes or has a poor diet, this can lead to syndromes in offspring that reflect the conditions they experienced *in utero*. By contrast, paternal influences have been largely ignored because dads – absent parental care – are just sperm machines, right? The results of this study add to the mounting evidence that this assumption is plainly false and the implications of this are far-reaching. Could loser males benefit from the semen of high-quality males, or high-quality males suffer from the miserable semen of losers? Can females distinguish high genetic quality males from those whose telegonic 'fathers' were high quality? More generally, what does 'quality' even mean if non-genetic factors can so easily decouple phenotype and genotype? Answering these questions, as well as clarifying the generality of telegony, remain fascinating research areas for the future.

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