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## Correspondences

### Increased outbreeding in yeast in response to dispersal by an insect vector

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Genetic diversity can be maintained in a heterogeneous environment if different alleles are favoured at different sites [1]. Under these circumstances, organisms that remain local are selected to inbreed in order to conserve locally adapted combinations of alleles [2], but those individuals that disperse should outbreed in order to increase the genetic variance among their progeny [3] and to make it more likely that some inherit combinations of alleles highly adapted to their new site. Here we report experimental results from the budding yeast *Saccharomyces cerevisiae* that fit with this theoretical prediction. We found that rates of outbreeding increased more than ten-fold when yeast spores were digested by an insect dispersal vector, the fruitfly *Drosophila melanogaster*. Outbreeding is modulated by the tetrad, a physical structure linking the four spores produced by meiosis of a diploid cell. Tetrads cause low rates of outbreeding by default, but their dissolution in the insect gut results in the liberation of spores, which is necessary for increased outbreeding rates. These findings provide new insights into the little known population biology and life history of one of the prime model organisms.

In yeasts of the genus *Saccharomyces*, sexual reproduction is triggered by adverse environmental conditions. A starved diploid cell enters meiosis and produces a tetrad of four resistant haploid

spores. When conditions improve, spores germinate and mate to restore the diploid state. Mating can occur immediately after germination, or after haploid mitotic division. This form of sexual reproduction, however, usually results in selfing between the four spores of a same mother cell, probably because the spores of a tetrad are enclosed within an envelope, the ascus, and joined by inter-spore bridges [4], maintaining them in close proximity. Outbreeding requires that the tetrad breaks up, which frees the spores and allows them to mate with spores generated from other tetrads. In the laboratory, yeast geneticists routinely use enzymes to digest the physical structures that hold spores in the tetrad in order to allow crosses between different yeast strains [5].

Yeasts have long been known to be dispersed by insects [6], in particular fruitflies, which feed on yeast [7]. This raises the intriguing possibility that the digestion of tetrads in the insect gut is the natural equivalent of the geneticist's enzyme, breaking up the tetrads and allowing increased rates of outbreeding in dispersing yeast. We have tested this idea experimentally using two well-known laboratory model species, the yeast *S. cerevisiae*, and the fruitfly *D. melanogaster*. We first performed experiments which confirmed that yeast spores, and not vegetative cells, are the primary dispersal stage for these fungal species. These experiments demonstrated that, as expected, viable spores can be recovered from fly faeces long after ingestion, while vegetative cells cannot be recovered from fly faeces (see Figure S1 in the Supplemental data available on-line with this issue). Microscopic examination also showed that spores were abundant in faeces and were separated into individual spores, in striking contrast to undigested tetrads.

We then investigated whether dissolution of the tetrad in the fly gut facilitates outbreeding in *S. cerevisiae*. To this end, we

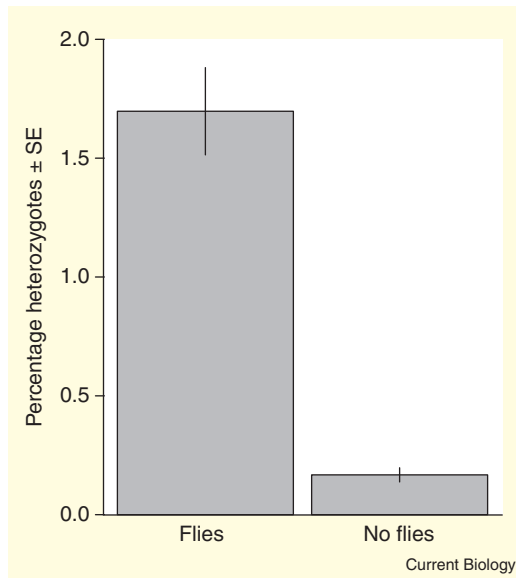


Figure 1. The effect of flies on outbreeding rates. This graph shows the mean percentage ( $\pm$ SE of mean) of heterozygotes formed from tetrads of a pair of genetically marked strains that were either fed to flies or maintained in the absence of flies. The percentage of heterozygotes differed significantly between treatments (t-test on square-root arcsine transformed proportions,  $t_{8.4} = 8.2$ ,  $P < 0.0001$ ). The outbreeding rate is twice the rate of heterozygote production, because half the between-tetrad matings will be between strains carrying the same genetic marker.

created a pair of homothallic strains carrying different genetic markers that allowed us to detect inter-strain crosses (see Supplemental data). The marked strains were mixed and sporulated, kept in the presence or absence of flies for 24 hours, and then cultured in a medium allowing germination and fertilisation. This experiment showed that outbred heterozygotes were more than ten times more frequent when flies were present than when they were absent (Figure 1).

Finally, we quantified the degree to which fly digestion results in the release of spores from tetrads (See Supplemental data, Figure S2). We fed tetrads of a heterothallic strain of yeast to flies, and spread the flies' diluted faeces onto plates, so that individual colonies grew from germinated spores. We found that a large proportion of the resulting colonies consisted of haploids which are capable of mating with tester strains. Far fewer haploids were found when colonies were grown from spores that had not been eaten by flies, implying that in this case the spores had already mated in their tetrads before forming diploid colonies. This experiment demonstrated that digestion increases the number of haploids available to mate outside the tetrad.

Our results show that the digestion of sporulated yeast by fruitflies is associated with the dissolution of tetrads and results in an increased outbreeding rate. Because yeast dispersal is known to involve insects, our findings suggest that the colonisation of novel environments is associated with a genetic diversification of dispersing cells through outbreeding, which facilitates adaptation to the conditions encountered in the new habitat. In non-dispersing tetrads, in contrast, high frequencies of inter-ascus matings can preserve local adaptation.

The association between dispersal and outbreeding fits several features of the genetic structure of natural yeast populations. A study of the population structure in the wild yeast, *S. paradoxus*, revealed a surprising absence of genetic differentiation between sampling sites which were several kilometres apart [8], suggesting that yeast undergo long-distance dispersal. Furthermore, genetic analysis of wild isolates did not show any sign of linkage disequilibrium between chromosomes, despite widespread homozygosity of strains [8]. These results indicate that wild yeast strains alternate automixis with regular events of outbreeding, thus breaking

up associations between chromosome. The outbreeding rate estimated for the wild population (1.1% per generation [8]) is in the same order of magnitude as the rate measured in our experiment (3.5%, Figure 1), although the significance of this correspondence is difficult to judge in the absence of an estimate of dispersal rate in the wild.

The results from our study also demonstrate the important role that insects play in the life history of yeast. We have shown that, in addition to serving as vectors of long-distance dispersal, insects may act as mediators of outbreeding in yeast and thus promote genetic mixing on both a geographic and a genomic scale. Besides revealing a hitherto unknown feature of yeast natural history, this finding is remarkable because it documents how a free-living organism relies on another species to perform outbreeding. Because sex and recombination have been shown to facilitate adaptation to adverse environmental conditions in yeast [9], these results show that the capacity of yeast populations to respond efficiently to selection is based on the interaction with their dispersal vectors.

The ascus is not unique to yeasts but is shared by all ascomycetes, in some of which it has given rise to elaborate devices for spore dispersal [10]. Although the association between the consumption of yeast by a dispersal vector and increased yeast outbreeding could be merely coincidental, it leaves the intriguing possibility that — despite its simple morphology — the yeast tetrad might represent a derived trait allowing yeast to adaptively modulate the outbreeding rate, thus uncoupling the decision to increase genetic variation from the decision to make spores by meiosis. Such an uncoupling of meiosis and the mating system has hitherto been thought to be reserved to higher organisms (for example, see [11]) but it might be of prime importance to sexually

reproducing microorganisms such as yeast which, because of their small size, are subject to an extremely patchy and diverse environment that favours local adaptation.

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#### Supplemental data

Supplemental data are available at <http://www.current-biology.com/cgi/content/full/17/3/R81/DC1>

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## Crayfish respond to electrical fields

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Fresh or salt water covers seventy percent of the Earth's surface. Aquatic environments are alive with electrical activity. Electrical signals carry information about the structure of the environment and the activity of other animals, and many aquatic vertebrates have evolved electroreception [1–5]. This sensitivity has been reported only in vertebrates [6], but one might predict it would be phylogenetically widespread, given its potential advantages. Here we present the first evidence that an invertebrate species, a freshwater crayfish, responds with different behaviours to a range of electrical signals.

Electrical fields from decaying organic matter and other physio-chemical sources are common in ponds and rivers [7], such as those where crayfish are found, so we designed an experiment to determine whether *Cherax destructor* could respond to such fields. First we placed two pairs of electrodes in either end of an aquarium and introduced animals singly into the arena in darkness. Following a 10 minute acclimation period we recorded the amount of time they spent in either end of the aquarium (one third) over the next 5 minutes. The animals showed no preference for either end during this control test ( $n = 10$ , paired t-test  $T = 0.357$ ,  $p = 0.729$ ).

We repeated the experiment but, after the acclimation period, a DC field ( $0.4 \mu\text{A}/\text{cm}^2$ ) was created between the electrodes in one end of the arena during the 5 minute observation period. The pair of electrodes activated was chosen randomly for each trial. If *C. destructor* cannot detect electrical fields, their behaviour should be the same as the control group. This was not the case — animals spent more time in the field end than the control end ( $n = 10$ , paired t-test  $T = 2.457$ ,  $p = 0.036$ ; see Figure S1 in the Supplemental data available on-line with this issue). Thus,

*C. destructor* can respond to a constant electric field of a type common in natural environments, and the behaviour described here suggests it may be attracted to such fields.

Because *C. destructor* responds to DC fields, it might also be able to detect dynamic signals generated by the movements of invertebrates and vertebrates [7–10]. To test this, we introduced crayfish singly into an aquarium and, following a 5 minute acclimation period, presented them with a test signal ( $0.4 \mu\text{A}/\text{cm}^2$ ) and a control signal ( $0.004 \mu\text{A}/\text{cm}^2$ ). These were presented three times each in random order and at random time intervals (30–120 seconds between stimuli). The signals were a step function generated by switching the field on for 1 s and then off again. Observations were recorded at the instant the field was turned on, which is when the greatest change in electrical current occurs.

A crayfish would not be expected to change its behaviour if it did not detect the signal. Out of the 60 stimuli presented, a behavioural change occurred 70% of the time upon receipt of the large signal (21/30), but in only 17% upon receipt of the small signal (5/30). Individual crayfish changed their behaviour significantly more in response to the large signal than to the small signal ( $n = 10$ , Wilcoxon sign rank  $Z = -2.873$ ,  $p = 0.004$ ).

To determine a threshold to the response, crayfish were exposed to multiple signals with amplitudes smaller or larger than those in the previous experiment. This time, we played the signals at random intervals after the animals were motionless. We then looked for small and immediate movements of the claws (chelipeds), antennae or legs when the signal was presented (Figure 1 inset). These were often followed by walking and occasionally by defence postures (spreading of the claws). These behavioural changes were most reliably seen in fields of  $0.4$  and  $0.8 \mu\text{A}/\text{cm}^2$ , but occurred down to  $0.2 \mu\text{A}/\text{cm}^2$  (Figure 1).

Can natural signals from moving animals also elicit a response? We presented crayfish with analogues of electrical activity from moving animals. Crayfish emit an electrical