stock for each round of mating and selection. Therefore, mate choice by the R strain is disregarded, and R preferences for mates from the experimental population cannot evolve by the flow of experimental alleles into the R population. Compared to natural populations, then, these experimental populations are to some extent set up to evolve PAM.

Nevertheless, it is interesting to see that they consistently did so, in some cases by altering the speed with which their mating reactions occurred. The genetic basis of the evolved mating preference is also interesting: mutations were selected in at least three genes, in one case, and these interacted synergistically to produce the evolved mating preference. In models of reinforcement and sympatric speciation, the fewer linkage groups involved, the easier it is for PAM and genetic isolation to evolve (Table 1). The selection of three apparently unlinked mutations in only 36 cycles is less surprising than it may seem, however, because all three alleles were selected in the same (experimental) population — not in a pair of populations with potentially two-directional gene flow as in most models.

With its reliance on inducible suicide genes and markers linked to mating type genes, the setting in which this mate choice evolved [1] is admittedly somewhat contrived. Previous studies have indicated that sequence divergence between species of *Saccharomyces* maintains genetic isolation by preventing chromosome pairing and crossing over [12], while chromosome rearrangements have been implicated in subsequent strengthening of the barriers to hybridization [13]. Leu and Murray [1] have added the observation that strong mate choice evolves rapidly when hybridization is harshly penalized. Still, in contrast to the awesome power of yeast genetics lies the awful weakness of yeast ecology. What remains now is to find an experimental system in which ecologically relevant selection against hybrids leads to mate choice and the beginning of speciation.

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DOI: 10.1016/j.cub.2006.01.029

Nematode Behavior: The Taste of Success, the Smell of Danger!

Through experience, the nematode worm *Caenorhabditis elegans* learns to distinguish high quality bacteria — food — from low quality or toxic bacteria. Increased release of the neurotransmitter serotonin onto identified interneurons determines whether *C. elegans* chooses to feed or leave.

Catharine H. Rankin

The ability to acquire food is critical for all organisms to survive. The first task is to locate food and to distinguish it from all of the other things in the environment; the second is to assess whether this is good food or whether to keep searching. If you happen to be a nematode such as *Caenorhabditis elegans* moving through the soil, your food is bacteria, and you find it by chemical cues. Several recent studies have increased our understanding of how *C. elegans* finds and makes decisions about food.

There are many different kinds of bacteria in the soil, some of them are nutritious for *C. elegans*, some of them are hard to eat and not very nutritious, while others are actually toxic. Recent work has shown that *C. elegans* learns about its food through experience. Shtonda and Avery [1] tested whether *C. elegans* is a picky eater: they found that the worm has a kind of hunting behavior that changes depending on the type of food they find. 'Hunting' is defined by two different forms of locomotion: dwelling, which is movement with frequent stops and reversals; and roaming, which is straight, rapid movement forward. When worms find themselves near what they consider 'good food' they dwell, and rarely roam; when they near what they consider bad food, roaming is very common.

How does a worm define 'good' or 'bad' food? In an earlier paper, Avery and Shtonda [2] showed that different strains of bacteria differ in their ability to support growth and reproduction in *C. elegans*. In the new paper [1], they used five strains of bacteria that differ in their ability to support *C. elegans* growth. They tested the behavioral response of wild-type worms to these five strains of bacteria, and they also looked at the responses of two feeding deficient mutant strains, *eat-2* and *eat-5*. Using measures of growth, they determined that two of the bacteria strains were 'high quality' food, two were 'mediocre quality' and one was 'poor quality'.

The difference between the types of bacteria was that some were difficult to eat, while others were not. Worms given a choice of two strains of bacteria over a 2 hour period chose the higher quality bacteria in nine out of ten tests. When bacteria tested were of the same quality, worms chose randomly. Worms used chemotaxis to find the bacteria on the plate; however, they did not appear to have innate preferences. Food preferences seem to be developed over exposure time, suggesting that worms needed to try the food to make a decision.

Interestingly, worms with mutations that made it more difficult for them to eat — the *eat-2* and *eat-5* mutants — had stronger preferences for high quality food and roamed even on mediocre quality food. From the result of tests using mutants and worms with specific laser-ablated neurons, Avery and Shtonda [2] hypothesized that control of switching between the locomotion patterns of roaming and dwelling is the key to the food-seeking strategy of *C. elegans*. Their data suggest that the interneuron AIY, the differentiation of which is promoted by the LIM domain transcription factor TTX-3, acts to extend the time a worm spends seeking food, stimulating it to leave low-quality food to seek 'greener pastures'.

Shtonda and Avery [1] showed how worms choose between high and low quality bacteria; however, some bacteria are actually toxic to worms. In their recent paper, Bargmann and colleagues [3] showed that *C. elegans* can learn to avoid odors associated with toxic bacteria. Pathogenic bacteria can proliferate in the intestine of *C. elegans* and after several days will kill the worms. Once again, worms were found to require experience to distinguish the quality of the food. Worms that had never experienced the pathogenic bacteria were as attracted to it as they were to non-pathogenic bacteria; however, worms that had had experience with the pathogenic bacteria strongly preferred non-pathogenic over the pathogenic bacteria in a choice test.

To test whether worms were learning a preference for the non-pathogenic bacteria, or an aversion to the pathogenic bacteria, Zhang *et al.* [3] developed a four choice maze which allowed them simultaneously to present odors of four different strains of bacteria and see which the worms would choose. In the maze there was a non-pathogenic bacteria and a pathogenic strain that the worm had experienced earlier, and a non-pathogenic and a pathogenic bacteria strain that the worms had never experienced. The results showed that both the proportion of worms approaching the known healthy bacteria and the proportion avoiding the known pathogenic bacteria increased compared to naïve worm choices, suggesting that olfactory learning on pathogens includes both attraction and aversion components.

Tests on adult worms showed that this learning occurs with exposures as short as 4 hours. Tests with a variety of mutant strains of worms deficient in serotonin neurotransmission led to the conclusion that serotonin is essential for pathogen-induced olfactory learning. By selectively rescuing the serotonin-deficient neurotransmission in specific identified neurons, Zhang *et al.* [3] determined that the ADF and NSM chemosensory neurons play a critical role in this olfactory learning, with AFD mediating the aversive signal and NSM mediating the attractive signal. Using immunocytochemistry, they then showed that exposure to pathogenic bacteria increases the serotonin level in the AFD neurons. The authors present data supporting the hypothesis that increased release of serotonin from the AFD chemosensory neurons activates Mod-1 serotonin receptors on the AIY and AIZ interneurons, thereby modulating the aversive learning.

It is interesting to note that both of these studies [1,3] have identified the AIY interneuron as important for the changes in food-seeking behavior after experience. It would be interesting to use the roaming and dwelling measures of Shtonda and Avery [1] on worms exposed to pathogenic bacteria to see if AIY is regulating the choice behavior in the pathogenic bacteria exposure, as it does in the food quality tests. The prediction is that the *ttx-3* mutant worms would show abnormal learning in the pathogenic bacteria assay.

In investigating how worms choose optimal food, researchers observed the behavior of worms and hypothesized about the activity of identified neurons that are implicated in the behaviors observed. Faumont and Lockery [4] have developed a new procedure that will allow for testing these hypotheses: it allows simultaneous recording of behavior and activity in an identified neuron using a genetically encoded optical probe. The technique involves using two different microscope objectives, one low enough to view the entire worm, the other high enough to image a single identified neuron. Images of activity and behavior were captured simultaneously but separately using different wavelengths of light. In this experiment Faumont and Lockery [4] recorded changes in emission from the 'cameleon' calcium sensor in ASH sensory neurons in response to changes in chemical cues, while simultaneously recording the behavior of the semi-restrained worm. They were able to show a correlation

between the probability of reverse swimming and calcium transients in ASH sensory neurons when exposed to an aversive chemical stimulus.

This technique could be used to test some of the hypotheses of Shtonda and Avery [1] and Zhang *et al.* [3], by allowing observations of activity in AIY and behavior of a semi-retrained worm exposed to different bacteria. Together these new studies show the power of combining genetic approaches with neural circuit analysis in an organism with a small tractable nervous system in which all neurons have been uniquely identified in order to determine the mechanisms of behavior.

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DOI: 10.1016/j.cub.2006.01.025

Tumor Suppressors: Control of Signaling by Endocytosis

Genetic defects of the endosomal 'ESCRT' machinery in *Drosophila* have been found to cause loss of epithelial cell polarity, accompanied by overproliferation of mutant and adjacent wild-type cells. These results can be attributed to defective endocytosis of transmembrane proteins that control cell polarity and proliferation, including Crumbs and Notch.

Bernd Giebel[1] and Andreas Wodarz[2]

Three recent and independent genetic screens [1–3] aimed at identifying genes that control epithelial organization and tissue growth in *Drosophila* have uncovered the genes *vps25* and *erupted* (*ept*). These are the fruit-fly orthologs of the yeast genes *vps25* and *vps23* (*Tsg101* in mammals), which both encode components of the 'endosomal sorting complex required for transport' (ESCRT). In clones of mutant cells in follicular and imaginal epithelia — the eye, leg and wing imaginal disc — the epithelial polarity of mutant cells is lost, leaving round shaped cells which are arranged in multilayered masses and contain expanded apical membranes. These clones of mutant cells are surrounded by wild-type cells with normal epithelial cell morphology, demonstrating that mutant cells lose their epithelial character in a strictly cell autonomous manner. Despite their normal cell morphology, the wild-type cells surrounding the mutant cell clones show massive overproliferation, resulting in outgrowths of the wing and leg and in overgrowth of the eye.

In mutant clones in the eye disc, Notch activity is dramatically increased, causing ectopic expression and secretion of the cytokine-like molecule Unpaired (Upd) [1–3]. In mutant wing disc cells, increased Notch activity leads to ectopic expression of the secreted growth factor Wingless (Wg) [2]. Both secreted Upd and Wg induce cell proliferation in surrounding wild type cells. In mutant cells of leg discs, activity of the Decapentaplegic (Dpp) receptor Thickveins (Tkv) is highly increased, resulting in the inhibition of Wg expression and, as a consequence, enhanced expression and secretion of Dpp. This ectopic secretion of Dpp induces overproliferation in ventral regions of the leg disc, causing an outgrowth of surrounding wild-type-cells [2].

What causes the cell-autonomous loss of epithelial cell polarity in the mutant cell clones? The transmembrane protein Crumbs (Crb) is essential for the establishment and maintenance of apico-basal cell polarity in ectodermal epithelia. In wild-type epithelial cells, Crb localization is restricted to the apical plasma membrane domain, whereas in clones of *ept* mutant cells, Crb is localized on the whole plasma membrane [1]. The abnormal localization of Crb in *ept* mutant cells resembles the situation where Crb is overexpressed, which also leads to loss of epithelial polarity and overproliferation [4,5].

Apart from their proliferation inducing effect, *vps25* and *ept* mutant cells normally contribute very little to the overgrown structures mentioned above. It turns out that the reason for this lies in the reduced fitness of the mutant cells when they are in competition with adjacent wild-type cells. Artificial reduction of the proliferation rate of the surrounding wild-type cells, or blocking apoptosis in the mutant cells, results in massive overproliferation of mutant cells, which go on to develop properties of metastatic cells [1–3]. In this respect, the mutant cells resemble precancerogenic cells in mammals, which also have to acquire at least one additional mutation that prevents apoptosis in order to develop a tumor.

In eukaryotic cells, a number of transmembrane proteins are endocytosed from the plasma membrane and are transported to the lysosome to be degraded. The first step on the journey to the lysosome is addition to the transmembrane protein of a single ubiquitin residue. In the early endosome, such ubiquitinated proteins are recognized by Vps27/HRS, a