A Virus in a Fungus in a Plant: Three-Way Symbiosis Required for Thermal Tolerance

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A mutualistic association between a fungal endophyte and a tropical panic grass allows both organisms to grow at high soil temperatures. We characterized a virus from this fungus that is involved in the mutualistic interaction. Fungal isolates cured of the virus are unable to confer heat tolerance, but heat tolerance is restored after the virus is reintroduced. The virus-infected fungus confers heat tolerance not only to its native monocot host but also to a eudicot host, which suggests that the underlying mechanism involves pathways conserved between these two groups of plants.

Endophytic fungi commonly grow within plant tissues and can be mutualistic in some cases, as they allow plant adaptation to extreme environments (1). A plant-fungal symbiosis between a tropical panic grass from geothermal soils, Dianthus hamum, and the fungus Curvularia protuberata allows both organisms to grow at high soil temperatures in Yellowstone National Park (YNP) (2). Field and laboratory experiments have shown that when root zones are heated up to 65°C, non-symbiotic plants either become shriveled and chlorotic or simply die, whereas symbiotic plants tolerate and survive the heat regime. When grown separately, neither the fungus nor the plant alone is able to grow at temperatures above 38°C, but symbiotically, they are able to tolerate elevated temperatures. In the absence of heat stress, symbiotic plants have enhanced growth rate compared with nonsymbiotic plants and also show significant drought tolerance (3).

Fungal viruses or mycoviruses can modulate plant-fungal symbioses. The best known example of this is the hypovirus that attenuates the plant-fungal symbioses. The best known example of this is the hypovirus that attenuates the fungus, either by the Clinical/Translational Fellowship Program of the MRCE, the W.M. Keck Foundation, and the NIH National Research Service Award (NRSA) F32 AI069698-01. P.A.P. was supported by the NIH Institutional NRSA T32 GM07067 to the Washington University School of Medicine.

Supporting Online Material
www.sciencemag.org/cgi/content/full/315/5811/509/DC1 Materials and Methods

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Fig. 1. Presence or absence of CThTV in different strains of C. protuberata, detected by ethidium bromide staining (A), Northern blot using RNA 1 (B) and RNA 2 (C) transcripts of the virus as probes, and RT-PCR using primers specific for a section of the RNA 2 (D). The isolate of the fungus obtained by sectoring was made virus-free (VF) by freezing-thawing. The virus was reintroduced into the virus-free isolate through hyphal anastomosis (An) with the wild type (Wt). The wild-type isolate of the fungus sometimes contains a subgenomic fragment of the virus that hybridizes to the RNA 1 probe (arrow).

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lyophilized, frozen at -80°C, and subcultured to cure it completely of the virus. The complete absence of CThTV in this isolate was confirmed by dsRNA extraction, Northern blotting, RT-PCR (Fig. 1), and electron microscopy (no particles were observed in four grids). We assessed experimentally the ability of the wild-type and virus-free isolates to confer heat tolerance by using thermal soil simulators (2, 11). Plants inoculated with the virus-infected wild-type isolate of the fungus tolerated intermittent soil temperatures as high as 65°C for 2 weeks (10 hours of heat per day), whereas both nonsymbiotic plants and plants inoculated with the virus-free isolate of the fungus become shriveled and chlorotic and died (Fig. 2).

To confirm that CThTV was involved in heat tolerance in the plant-fungal symbiosis, we reintroduced the virus into the virus-free fungal isolate and tested its ability to confer heat tolerance. To provide a selectable marker, the virus-free isolate was transformed with a pCT74 vector containing a hygromycin-resistance gene (13) by restriction enzyme-mediated integration (REMI) transformation (14). Virus-containing wild-type hygromycin-sensitive (Wt) and virus-free hygromycin-resistant (VF) isolates of C. protuberata were cultured on single Petri dishes and allowed to undergo hyphal fusion or anastomosis (Fig. 3A). The mycelium from the area of anastomosis was subcultured twice with single conidia grown on hygromycin-containing plates. Thirty-five hygromycin-resistant isolates obtained in this way were screened for their dsRNA profiles, but only one was found to have acquired the virus (Figs. 1 and 3B). This fungal isolate, newly infected by hyphal anastomosis with CThTV (An), was tested for its ability to confer heat tolerance by the same experimental approach indicated above. The heat-stress experiment confirmed that the isolate newly infected with CThTV confers the same level of heat tolerance as that conferred by the wild-type isolate (Fig. 2).

Previously, we found that some beneficial endophytes isolated from monocots could be transferred to eudicots and still function as mutualists (3). Thus, we tested the ability of the C. protuberata isolates to confer heat tolerance to tomato (Solanum lycopersicon). Using a slightly modified protocol for the heat-stress experiment (11), we obtained similar results to those obtained with D. lanuginosum (Fig. 4). However, it was not possible to attain 100% fungal colonization of tomato plants (11), and this may explain the higher proportion of dead plants colonized with the Wt or An fungus, compared with the experiment using D. lanuginosum. Given that C. protuberata, when infected with CThTV, provides similar mutualistic benefits to both a monocot and a eudicot, it is possible that the underlying mechanism is conserved between these two groups of plants.

Plants inoculated with C. protuberata infected with CThTV do not activate their stress-response system in the usual way. For example, the osmolyte concentration in these plants does not increase as a response to heat stress, although the levels are constitutively higher than in plants colonized with the virus-free isolate or the nonsymbiotic plants (fig. S6). It has been hypoth-
The Neural Basis of Loss Aversion in Decision-Making Under Risk

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People typically exhibit greater sensitivity to losses than to equivalent gains when making decisions. We investigated neural correlates of loss aversion while individuals decided whether to accept or reject gambles that offered a 50/50 chance of gaining or losing money. A broad set of areas (including midbrain dopaminergic regions and their targets) showed increased activity as potential gains increased. Potential losses were represented by decreased activity in several of these same gain-sensitive areas. Finally, individual differences in behavioral loss aversion were predicted by a measure of neural loss aversion in several regions, including the ventral striatum and prefrontal cortex.

Many decisions, such as whether to invest in the stock market or to accept a new job, involve the possibility of gaining or losing relative to the status quo. When faced with such decisions, most people are markedly risk averse. For instance, people typically reject gambles that offer a 50/50 chance of gaining or losing money, unless the amount that could be gained is at least twice the amount that could be lost (e.g., a 50/50 chance to either gain $100 or lose $50) (1). Prospect theory, the most successful behavioral model of decision-making under risk and uncertainty (1, 2), explains risk aversion for “mixed” (gain/loss) gambles using the concept of loss aversion: People are more sensitive to the possibility of losing objects or money than they are to the possibility of gaining the same objects or amounts of money (1, 3–5). Thus, people typically require a potential gain of at least $100 to make up for exposure to a potential loss of $50 because the subjective impact of losses is roughly twice that of gains. Similarly, people demand substantially more money to part with objects that they have been given than what they would have been willing to pay to acquire those objects in the first place (6). Loss aversion also has been used to explain a wide range of economic behaviors outside the laboratory (7, 8). Further, loss aversion is seen in trading behavior of both children as young as age five (9) and capuchin monkeys (10), which suggests that it may reflect a fundamental feature of how potential outcomes are assessed by the primate brain.

Previous neuroimaging studies of responses to monetary gains or losses have focused on activity associated with the anticipation of immediate outcomes (“anticipated” utility) (11, 12) or the actual experience of gaining or losing money (“experienced” utility) (11, 13, 14) rather than specifically investigating which brain systems represent potential losses versus gains when a decision is being made (“decision” utility). Behavioral researchers have shown that anticipated, experienced, and decision utilities often diverge in dramatic ways, which raises the possibility that the corresponding brain systems involved may also differ (15). In the current study, we aimed to isolate activity associated with the evaluation of a gamble when choosing whether or not to accept it (i.e., decision utility) without the expectation that the gamble would be immediately resolved. This allowed us to test whether neural responses during the evaluation of potential outcomes are similar to patterns previously reported in studies of anticipated and experienced outcomes.

One fundamental question for the study of decision-making is whether loss aversion reflects the engagement of distinct emotional processes when potential losses are considered. It has been suggested that enhanced sensitivity to losses is driven by negative emotions, such as fear or anxiety (16). This notion predicts that exposure to increasing potential losses should be associated with increased activity in brain structures thought to mediate negative emotions in decision-making [such as the amygdala or anterior insula; compare with (17, 18)]. Alternatively, loss aversion could reflect an asymmetric response to losses versus gains within a single system that codes for the subjective value of the potential gamble, such as ventromedial prefrontal cortex (VMPFC)/orbitofrontal cortex (OFC) and ventral striatum (11, 19, 20).

To examine the neural systems that process decision utility, we collected functional magnetic resonance imaging (fMRI) data while partici-
Reports: “A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance” by L. M. Márquez et al. (26 Jan. 2007, p. 513). On page 514, in the legend to Fig. 4, the colors of the histogram are inverted: The number of dead plants is black, and the number of alive plants is white.