## Climate change forced zombie ant fungi to adapt



"In tropical areas, zombie ants bite onto leaves, but in temperate areas, they bite twigs or bark," said David P. Hughes, associate professor of entomology and biology, Penn State.

Zombie ants are actually various species of carpenter ants that are infected with a parasitic fungus. About half the species of carpenter ants can be infected and each species has its own fungus. The zombie ant phenomena currently occurs around the globe on all continents except Europe. However, a fossil zombie ant was found in Germany, so they did once exist in Europe as well.

"They are probably not in Europe because the forests there are so managed," said Hughes. "They likely went locally extinct there."

Zombie ant spores fall on ants from above and the fungus multiplies in the ant body using it as a source of nutrition. Eventually, the fungus manipulates the ant to climb high into the branches and clamp on by biting. If an infected ant dies in the colony or on the ground it has zero chance of infecting another ant, so positioning the ant bodies where fungi can be widely distributed is essential for these fungi.

"In the late summer and early fall there are both leaves and twigs everywhere the ants reside," said Raquel G. Loreto, postdoctoral scholar in entomology, Penn State. "But in temperate areas the trees are deciduous and lose their leaves in the fall. There, the ants bite onto twigs."

Tropical forests are almost completely evergreen, remaining in leaf year round. Many temperate forest trees drop all their leaves in the fall.

The German fossil, which dates to around 47 million years ago, shows the ant biting into a leaf. During that era, wet evergreen forests ranged from the equator nearly to the North and South poles. Because all the forests were evergreen, all the zombie ants would have chosen to bite into leaves.

As the climate cooled, temperate forests grew in the northernmost and southernmost areas worldwide. Zombie ants in those areas that bit on leaves would have quickly ended up on the ground when the leaves fell. The fungal species that manipulated its host onto leaves would not successfully reproduce. This implies there was a strong force of natural selection acting on where the ant host was manipulated. Over time, the fungi evolved so that in temperate areas, zombie ants are manipulated to bite onto twigs or bark.

"Some of the ants do not simply bite the twigs, but wrap their back legs around the twig and hold on," said Loreto. "They probably do this because biting twigs in not enough to hold them on."

This manipulative behavior developed in temperate areas around the globe. The researchers report the results of their study in the current issue of *Evolution*.

They looked at three separate areas. First they determined that tropical zombie ants always bite leaves and temperate zombie ants always bite twigs and bark and that 90 percent of the dead temperate ants found have their legs wrapped around the twigs.

They then determined that leaf biting, rather than twig biting, was the ancestral trait -- the approach originally used when the world was much warmer. They also determined that the twig biting behavior evolved at different times in different places, making it a convergently evolved trait in temperate areas arising independently in different locations -- in this case North America and Japan.

Much of the work involved looking at samples of zombie ants wherever they could be found -- museums, other collections, photographs, and previously compiled datasets.

"We had a great asset here who is Kim Fleming," said Hughes. "Kim is a citizen scientist whose property in South Carolina is festooned with zombie ants hanging on trees. As both an excellent photographer and natural historian, Kim was able to collect detailed data for us on the zombie ants over 18 months by taking continual images of samples on her land. This was precious data that would have been very hard to collect.

"Kim is an author of this paper, but perhaps the greatest recognition of her importance is that the fungal species infecting carpenter ants in South Carolina is now named after her, *Ophiocordyceps kimflemingiae*."

The researchers also looked at the phylogenetic relationship of the various

fungi by examining extracted DNA from as many samples as possible. They found that genetically, twig biting and leg wrapping developed independently to adapt the fungi to temperate vegetation.

"We can estimate that these changes occurred between 40 and 20 million years ago," said Hughes. "However, because of the scarcity of zombie ant fossils, we can't be any more specific than that at the moment."

Whenever climates change -- getting either warmer or colder, wetter or dryer -- plants and animals either adapt or die out. Zombie ant fungi also adapted well to the changing environment around them and their manipulation of carpenter ants ensured their survival up to today.

"What is remarkable here is that we have shown that the complex manipulation of an animal by microbe has responded to selection pressure the climate imposes on animals and plants," said Hughes. "That was a cool finding that really excited us."

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