

### Little E/Z changes make a big difference

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This shows a caterpillar attacking corn ear. Credit: Bernd Hommel, Julius Kühn Institute

The coming of summer brings promise for humans and insects alike. The farmer planted maize for a bountiful harvest, but the European Corn Borer (ECB) is looking for a good meal right away. The caterpillars of this pest bore deep into the maize stems, where they eat the inner pith causing the weakened stalks to fall over before the ears can ripen. As if one pest were not bad enough, there are even two different races, called E and Z that have a subtle difference in the shape of their pheromones. Interfering with the pheromone communication system of pest insects is a promising means of crop protection. But for years the E/Z distinction, so obvious to the insects, has baffled researchers.

Now a team of scientists from Lund University in Sweden and the Max



Planck Institute for Chemical Ecology in Jena, Germany, have identified the gene that makes E and Z females produce their respective pheromones, causing a <u>reproductive isolation</u> that could eventually lead to formation of new species.

All over the world farmers fight the ECB with insecticides, genetically modified plants, or biological pest control. The moth with the scientific name Ostrinia nubilalis is native to Europe and was introduced to North America early in the last century, where it received its common name. The E- and Z-races were discovered in the 1970s when synthetic pheromones gave very different results in attracting males in Iowa and New York. The Z-race, now widespread in the western USA, mainly attacks maize, whereas insects of the E-race specialize on Artemisia and other plants.

# The two races don't interbreed in the field, and the chemical structure of the sex pheromone holds the key

The ECB sex pheromone, called 11-tetradecenylacetate, contains a long zig-zag tail of 14 <u>carbon atoms</u>, with one double bond. This can be in the E (trans) configuration, or alternatively the Z (cis) configuration which causes the tail to bend at that point. This seemingly small structural variation makes a big difference at mating time. When an ECB female of the Z-race is ready to mate, she releases the E and Z isomers in a ratio of 3:97, and only Z-race males are attracted. E-females emit the same isomers but in a ratio of 98:2, and this attracts only E-race males, "In evolutionary biology this is called a 'reproductive isolation barrier'; which is interesting because it can mark the beginning of the evolution of new species," Jean-Marc Lassance, first author of the study, explains.





This shows the chemical structure of sex pheromone (E- and Z-11-tetradecenylacetate). Credit: MPI Chemical Ecology

## A difference in enzymes (reductases) is responsible for the different E and Z ratios

"However, these two races are still far away from a development into new species, and this was actually a crucial advantage for our genetic analyses," says Astrid Groot from the Max Planck Institute for Chemical Ecology, who has been studying sex pheromones of moths since 2001. "Although they wouldn't find each other in the field, if we put males and females of different races together in the lab, mating occurs and fertile offspring are produced. This allowed us to map the gene controlling this difference in female pheromone production." This gene mapped to a different chromosome from the desaturase enzyme that first introduces



the E or Z double bond into the 14-carbon tail, but to the same chromosome as the reductase enzyme that later produces the final pheromone. Intriguingly, there are many differences in the amino acid sequences of the reductases isolated from the E or Z races. When Jean-Marc Lassance measured enzyme activities, he found that even though both races create the E and Z 14-carbon tail in nearly equal amounts, the reductase from E-race females mainly converted the E-form to the final pheromone, and the Z-race reductase converted mostly the Z-form. So a difference in the respective reductase enzymes is responsible for the different E and Z ratios released by the female pheromone glands.

### **Reason for the development of the two races unclear**

This new finding explains only half of the story, since the reason that E and Z males exhibit such strong preferences for E-rich and Z-rich pheromone blends produced by the females is still unknown. "We can only speculate here. We have known for a long time that the gene responsible for pheromone production in females and the gene controlling male response are different; in fact they occur on different chromosomes. And we still don't understand how selection could act on two independent genes to initiate the development of the two races," says Christer Löfstedt from Lund University, Sweden. The researchers are now turning their attention to mapping the male-response gene based on additional crosses.





This is a male and female moth of the European corn borer. Credit: Bernd Hommel, Julius Kühn Institute, Germany

#### Pheromone traps and "mating disruption"

As well as giving insight on the evolution of new species, the pheromones of insect pests can also be manipulated for crop protection. As soon as their chemical structure is elucidated, they can be chemically synthesized and applied in pheromone traps. These can be used as a sensitive method to monitor the population sizes of pest populations, or to catch and kill large numbers of males intent on mating. Another method is "mating disruption", where synthetically produced pheromones are widely dispersed in the field, swamping out the weaker signals of individual females, which the disoriented males cannot locate. These methods are especially useful when insecticides must be avoided, such as in control of codling moth in apple orchards, or trapping large numbers of bark beetles to protect forests. Because very low amounts of



species-specific sex pheromones are sufficient to lure or confuse male insects which respond even to the slightest pheromone concentrations in the air, these methods are in particular environmentally friendly.

**More information:** Lassance, J-M; Groot, AT; Lienard, MA; Antony, B; Borgwardt, C; Andersson, F; Hedenström, E; Heckel, DG; Löfstedt, C: Allelic variation in a fatty-acyl reductase gene causes divergence in moth sex pheromones. NATURE, Advance Online Publication, 30.6.2010, DOI 10.1038/nature09058

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