

Non-Mendelian inheritance in Ghent

Mi(gh)ty Tricks

Thomas Van Leeuwen and Co. wanted to reveal the mechanism of action of a certain mite pesticide. They went beyond, however, and found how the mites acquire resistance by fixing mitochondrial mutations inherited from their mothers.

Science certainly isn't short of rules. Permanent and unshakeable, every scientist dreams of giving their name to one. But rules are made to be broken as new discoveries can complicate established patterns of thought. And so it was with the discovery of non-Mendelian inheritance. Gregor Mendel's laws governing the inheritance of characteristics eventually inspired the discovery of genetic linkage and laid the foundations of modern genetics. But even before Mendel's ideas became widely understood, exceptions to the rule were already being found.

Non-Mendelian inheritance describes any pattern of inheritance which cannot be explained by these rules. Thomas Van Leeuwen and Bartel Vanholme of the Department of Crop Protection at Ghent University, Belgium, wrote a recent study into an intriguing application of non-Mendelian inheritance.

Non-Mendelian inheritance was first documented by Carl Correns, a German botanist who helped to popularise Mendel's findings, after he became interested in variegation in leaves. Correns noticed that female gametes from green-leaved plants always gave plants with green leaves, even if crossed with pollen from variegated plants. Extra-nuclear inheritance neatly explained Correns' observations as both chloroplasts (which were later found to harbour the mutation responsible) and mitochondria, which both contain their own DNA, are only present in the cytoplasm of the female gamete.

The answer lay with non-nuclear DNA

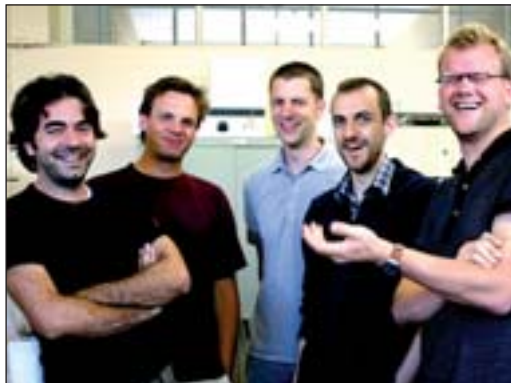
Van Leeuwen's group studies the control of crop pests, focusing on mites, or, to give them their proper name, the Acari (a family which also contains ticks). Chemicals that kill mites (acaricides) fuel a one billion dollar industry and belong to many chemically distinct groups. How these chemicals work at the molecular level is poorly understood.

The initial aim of a study recently published in *PNAS* (vol. 105 (16): 5980-5) by Van Leeuwen, in collaboration with Roth-

amsted Research (Harpenden, UK) and Bayer CropScience (Monheim, Germany), was to explain the mode of action of an acaricide called bifenazate. By examining a resistant population of the two-spotted spider mite (*Tetranychus urticae*), an economically important and promiscuous plant pest, Van Leeuwen *et al.* were able not only to reach the molecular target of bifenazate but gain a rare view of non-Mendelian inheritance in action.

Their first big clue was the observation that resistant females always produced resistant offspring and susceptible females always produced susceptible offspring.

As with Carl Correns' leaves, the answer lay with non-nuclear DNA and specifically the mitochondrial gene which encodes a



Thomas Van Leeuwen (L.) and the Ghent mite people

protein essential for ATP production, cytochrome b. Cytochrome b is the major protein of the cytochrome bc_1 complex, which transfers electrons from reduced ubiquinone to cytochrome c, and features an absolutely conserved amino acid motif across fungi, protists, animals and plants in the so-called Q_0 site. Sequencing the cytochrome b gene from the mitochondria of *T. urticae* revealed differences between susceptible and resistant mites, all of which were predicted to lead to a change in an amino acid of the Q_0 site. Remarkably, without bifenazate, no fitness cost was found in mites with these mutations. However, when bifenazate was present, mites with the modified Q_0 site were over 100,000 times more resistant. Van Leeuwen and his group had arrived at



Tetranychus urticae

Photo: Wim Grunwald

the most likely mode of action of this particular acaricide.

So what are the implications of such non-Mendelian inheritance for the selection of resistant mites in a population in the field? "Resistance management as we know it today is mostly based on Mendelian inheritance," explains Van Leeuwen, "recombination of resistance genes and the dilution of these genes through immigration of susceptible individuals: this is all in vain if [resistance is given by a] mitochondrial mutation, as resistance will always pass through the mother."

Farmers prefer to spray their crops as few times as possible in the year. It's expensive and time consuming. What's more, every time an acaricide is sprayed, the farmer is effectively selecting for mutations leading to resistant individual mites within the population, decreasing the effectiveness of future sprays. "If one of these resistance mutations occurs in the nucleus of a germ cell in which each gene has a single copy, the progeny will immediately be heterozygous," explains Van Leeuwen.

However, there can be 10,000 copies of the mitochondrial genome in every cell. "One out of 10,000 copies mutated is a completely different situation to one out of two copies mutated [as is the case for nuclear genes]." This gives a much smaller chance of the mutation ever becoming fixed, making mitochondrial genes very attractive targets not only for acaricides but insecticides too, as there can be no selection for a single mutated copy by lack of a phenotypic effect.

Theory and practice

At least, that is the theory. However as someone once said, in theory, there is no difference between theory and practice; in practice there is. And so it is with the mitochondria of two-spotted spider mites. Bifenazate resistant mites isolated from a greenhouse in which bifenazate had been sprayed regularly were found to contain only mutated mitochondrial DNA, encoding a modified Q_0 site in the cytochrome b

gene. So how did this mitochondrial allele become fixed in the mite population? As it turned out, a particular feature of the mitochondria of this *T. urticae* strain, heteroplasmy, is important for the emergence of resistance in a population. Heteroplasmy is the presence of more than one organellar genome sequence in the same individual cell, in this case the single nucleotide variation of either a cytosine or an adenine in the gene encoding cytochrome b.

The marble picture

This crucial observation prompted Van Leeuwen and his colleagues to take a closer look at the mechanism of this particular kind of non-Mendelian inheritance. Specifically, it allowed them to link mitochondrial genotype and the bifentazate resistance phenotype. The percentage of mutant haplotypes in individual mothers to a large extent determined the variation in their progeny. Once this heteroplasmy was found and characterised in different individual mites, all that remained was to estimate the average number of mitochondrial copies passed on from mother to offspring. "It's like taking ten marbles [of two different colours] from ten thousand," if the mother has a known resistance genotype, then the variation in the offspring can be measured. "With these results it is possible to calculate, as a percentage, how big the sample of marbles [of either colour] is that you have taken out of the pool."

From these models, Van Leeuwen realised that *T. urticae* mothers do not pass ten thousand DNA copies to their offspring, but only a few hundred. "The lower the

amount [of DNA] which is passed through, the higher the risk for resistance development," but also the potential for sampling error. This phenomenon, 'genetic bottlenecking', means that mitochondrial genotype frequencies can be very different from generation to generation, especially in conditions ideal for rapid selection (such as in the presence of a chemical like bifentazate). So Van Leeuwen can explain not only how *T. urticae* is targeted by bifentazate, but also why resistant mites quickly gain a foothold in field populations.

So what's next for Van Leeuwen and his group? Understanding why bifentazate specifically targets spider mites will be an important milestone. "If we can understand why it is so specific, we can use this technology to make other molecules which are similarly specific." Another project for the future is a larger study to find out how widespread resistance to bifentazate is in the field. For farmers and growers, who need to know how best to use the battery of chemicals at their disposal to make the most money, this kind of information is like gold dust.

Genome sequence in progress

T. urticae has been chosen as a model organism for Chelicerates and its genome is now being sequenced, to be unveiled in early 2009. Its release will fuel interest in research on spider mites and their chemical control, particularly on the nuclear-encoded target-sites and enzymes mites use to degrade acaricides. Exciting times ahead, then, for agricultural sciences in Ghent.

WILLIAM TEALE

ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER

