...and discovered on Mount Kilimanjaro

The endocyttoplasmic bacterium Wolbachia causes the death of arthropod embryos, when present in reproductive organs, by cytoplasmic incompatibility. Wolbachia is harboured in both sexes but transmission is maternal only. Cytoplasmic incompatibility occurs when infected males inseminate uninfected females or females bearing a different variant of Wolbachia. Two kinds of Wolbachia have been described: (mod−resc−) which induces cytoplasmic incompatibility by modifying sperm but can rescue this phenotype when in the egg, and (mod−resc+) which neither induces cytoplasmic incompatibility nor rescues from it. Theory predicts a third kind (mod−resc+) that would not induce cytoplasmic incompatibility but would rescue from it. We have found such a Wolbachia variant in a Drosophila simulans population on Mt Kilimanjaro, Tanzania. The existence of a mod−resc+ Wolbachia shows that the modification and rescue functions can be dissociated with regard to the cytoplasmic incompatibility process.

We detected this Wolbachia variant (wKi) in isofemale lines from flies captured in March 1996. Of 49 lines, 9 showed a positive PCR signal with primers for the Wolbachia 16S RNA gene. Infected (Kili+) and uninfected (Kili−) Kilimanjaro males were crossed with Kili+ females. No cytoplasmic incompatibility was detected. The percentage of unhatched eggs did not differ significantly whether the males were infected or uninfected (12.3 ± 2.6% and 10.7 ± 3.3%, respectively).

We tested the compatibility of Kili+ flies with individuals infected by each of the three natural mod−resc+ Wolbachia strains (wKi, wHa, or wNo). Kili+ males were compatible with females infected by wKi (12.0 ± 2.3% unhatched eggs), wHa (14.0 ± 4.1%) or wNo (8.5 ± 1.3%). Kili+ females were incompatible with males infected by wKi (100.0% unhatched eggs) or wHa (84.7 ± 3.3%). Surprisingly, the Kili− females were completely compatible with wNo-infected males (8.5 ± 2.1% unhatched eggs), whereas Kili− females were incompatible (70.2 ± 3.5%). Wki thus behaves as a mod−resc+ variant with regard to the wNo type.

Cytoplasmic incompatibility helps Wolbachia in invading previously uninfected populations, because it eliminates uninfected eggs, giving a higher fitness to infected females. However, during the invasion process, any mod− variant arising by mutation will also increase in frequency as long as it is still resc−. Under certain conditions, these mod−resc− variants would completely replace the mod−resc+ wild type. If this happened, the population would again become vulnerable to invasion by uninfected cytoplasm, because mod− variants do not exert any selective pressure against uninfected eggs. The infection might then be lost with time. Meanwhile, because mod+ variants have been eliminated from the population, the rescue capability becomes useless and resc− mutants can be expected to arise.

The Wolbachia variants discovered in wild D. simulans populations have been of two types: mod−resc− (wKr, wHa, wNo) and mod−resc+ (wMa, wJa). wHa has been found both on the American and Australian continents, whereas wMa is known only in Madagascar. We established that wKr and wKi are different because wKr cannot rescue from the wNo cytoplasmic incompatibility phenotype. The status of wMa is unknown in this respect, so wMa and wKi might be the same variant. Moreover, the 16S rDNA amplification product of wKi has one VspI restriction site, as do wMa and wNo sequences, but such a site is absent from wKr, wHa and wJa sequences.

Eastern Africa is thought to be the centre of origin of D. simulans, so the Wolbachia infection of the Kilimanjaro population could be one of the oldest in this species, implying that mod−resc+ variants derive from a mod−resc− ancestor with time. In contrast, wNo is known only in time in the Seychelles and in New Caledonia, so perhaps wNo-infected populations originate from migrants that left East Africa bearing the mod−resc− ancestor. After geographic isolation, the Indo-Pacific variant could have remained mod−resc− while the ancestral variant has evolved into mod−resc+ in Africa. The maintenance of the rescue function implies that it might have a further role.

The proportion of insect species potentially infected by Wolbachia is estimated at 16% (ref. 1), so further research might well yield other mod−resc+ variants. Mt Kilimanjaro is probably just the tip of an iceberg.