modification and rescue might involve multiple *Wolbachia* genes.

The existence of *Wolbachia* strains that do not modify sperm but can rescue the cytoplasmic incompatibility phenotype has been predicted by theory^{13,14}. Our work suggests that previously classified *mod⁻resc⁻* strains of *Wolbachia* can in fact rescue cytoplasmic incompatibility and can therefore be considered *mod⁻resc⁺*. This finding helps explain how *Wolbachia* *mod⁻* strains could have spread into insect populations without the action of cytoplasmic incompatibility. *Wolbachia* strains that can rescue but not cause cytoplasmic incompatibility can be expected to increase in frequency in populations by essentially parasitizing the cytoplasmic incompatibility sperm modification effect of a related *Wolbachia* strain.

The potentially lower fitness costs and perfect maternal transmission of these strains^{7,9} might explain how they could displace the initial strain that they parasitized in order to invade the host population. This is in accordance with models predicting that *Wolbachia* will evolve towards neutrality with respect to cytoplasmic incompatibility as long as maternal transmission frequency can be increased¹³.

Kostas Bourtzis*, Stephen L. Dobson, Henk R. Braig, Scott L. O'Neill

Section of Vector Biology,
Department of Epidemiology and Public Health,
Yale University School of Medicine,
60 College Street, New Haven, CT 06510, USA
e-mail: scott.oneill@yale.edu

*Alternative address: Institute of Molecular Biology
& Biotechnology, Foundation for Research and
Technology — Hellas, Heraklion, Crete, Greece

...and discovered on Mount Kilimanjaro

The endocytosymbiotic bacterium *Wolbachia* causes the death of arthropod embryos, when present in reproductive organs, by cytoplasmic incompatibility¹. *Wolbachia* is harboured in both sexes but transmission is maternal only. Cytoplasmic incompatibility occurs when infected males inseminate uninfected females² or females bearing a different variant of *Wolbachia*^{3,4}. Two kinds of *Wolbachia* have been described¹: (*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 it⁵⁻⁷. 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 rRNA gene⁸. 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 ($12.3 \pm 2.6\%$ and $10.7 \pm 3.3\%$, respectively).

We tested the compatibility of *Kili⁺* flies with individuals infected by each of the three natural *mod⁺resc⁺* *Wolbachia* strains (*wRi*, *wHa* or *wNo*)⁹. *Kili⁺* males were compatible with females infected by *wRi* ($12.0 \pm 5.1\%$ unhatched eggs), *wHa* ($14.0 \pm 4.1\%$) or *wNo* ($8.5 \pm 1.3\%$). *Kili⁺* females were incompatible with males infected by *wRi* (100.0% unhatched eggs) or *wHa* ($84.7 \pm 3.3\%$). Surprisingly, the *Kili⁺* females were completely compatible with *wNo*-infected males ($8.5 \pm 2.1\%$ unhatched eggs), whereas *Kili⁻* females were incompatible ($70.2 \pm 3.5\%$). *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⁻* mutants 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^{9,10} have been of two types: *mod⁺resc⁺* (*wRi*, *wHa*, *wNo*) and *mod⁻resc⁻* (*wMa*, *wA*). *wA* has been found both on the American and Australian continents, whereas *wMa* is known only in Madagascar. We established that *wA* and *wKi* are different because *wA* cannot rescue from the *wNo* cytoplasmic incompatibility phenotype¹⁰. 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* sequences⁸, but such a site is absent from *wRi*, *wHa* and *wA* sequences.

Eastern Africa is thought to be the centre of origin of *D. simulans*¹¹, 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 the Seychelles and in New Caledonia¹⁰, 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.

Hervé Mercot, Denis Poinot

Laboratoire Dynamique du Génome et Évolution,
Institut Jacques Monod, CNRS-Université Paris 7,
75251 Paris, France
e-mail: mercot@ccr.jussieu.fr

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