25 Years of the *Onchocerca ochengi* Model

Trends box.

- The *Onchocerca ochengi* system has consistently shown that strong, albeit incomplete, natural immunity exists in cattle and that partial protection can be induced by both irradiated and recombinant vaccines.
- In accordance with human data, the *O. ochengi* system has demonstrated that *Wolbachia* induce a profound local neutrophilia within nodules. Recent proteomic data obtained from nodule fluid show that adult worms are 'bathed' in liberated neutrophil granule contents, especially antimicrobial proteins.
- The *Wolbachia* endosymbiont of *O. ochengi* has a highly reduced genome that can produce very few vitamins and cofactors, although it may generate energy in the form of ATP for its host. The most abundant *Wolbachia* proteins induce neutrophil activation and chemokinesis.
- Small RNAs with diagnostic potential are released by *Onchocerca* spp. into nodule fluid and human (and bovine) blood.

25 Years of the *Onchocerca ochengi* Model

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Abstract:

Although of limited veterinary significance, *Onchocerca ochengi* has become famous as a natural model or 'analogue' of human onchocerciasis (river blindness), which is caused by *Onchocerca volvulus*. On the basis of both morphological and molecular criteria, *O. ochengi* is the closest extant relative of *O. volvulus* and shares a number of key natural history traits with the human pathogen. These include exploitation of the same group of insect vectors (blackflies of the *Simulium damnosum* complex) and formation of collagenous nodules with a very similar histological structure to human nodules. Here, we review the contribution of this natural system to drug and vaccine discovery efforts, as well as to our basic biological understanding of *Onchocerca* spp., over the past quarter-century.

Keywords: Filariasis, *Wolbachia*, macrofilaricide, biomarkers, vaccines.

History and basic biology

In 1992, *Parasitology Today* published an article by Alexander (Sandy) Trees entitled "*Onchocerca ochengi*: Mimic, Model or Modulator of *O. volvulus*?" that introduced a

relatively obscure filarial species to a wider audience [1]. In our view, the intervening two-and-a-half decades have clearly demonstrated that *O. ochengi* has lived up to its initial promise and fulfilled its potential as a superlative natural system for the study of onchocerciasis.

Onchocerca ochengi was first described by Bwangamoi [2] from bovine skins collected from an abattoir in Uganda. The nodules were reported to damage hides at several locations across East Africa and reduce their market value (the sole veterinary impact of this disease) [3]. Interestingly, this effect on the leather reflected one of the few significant biological differences between *O. ochengi* and *Onchocerca volvulus:* adults of the former are located in intradermal nodules, whereas human *O. volvulus* nodules are usually subcutaneous. Several years later, Bussieras *et al*. [4] described a similar *Onchocerca* spp. from cattle in Togo that had shorter **microfilariae** (see Glossary), which was named *Onchocerca dermata.* Subsequent investigations by Bain *et al*. [5] determined that Bwangamoi had published incorrect measurements for *O. ochengi* microfilariae, and thus *O. dermata* was abandoned as a junior synonym of *O. ochengi*.

For over a decade, *O. ochengi* was neglected for onchocerciasis research in favour of other bovine *Onchocerca* species from temperate regions, such as *Onchocerca gibsoni* and *Onchocerca lienalis*. However, as entomological monitoring to assess the efficacy of onchocerciasis control came to the fore in the late 1980s, the potential for larvae of bovine *Onchocerca* spp. in blackflies to complicate the calculation of transmission potentials was recognised. This drew increasing attention to *O. ochengi*, as it is challenging to discriminate

between the **L3** of this species and that of *O. volvulus* without recourse to molecular assays [6-8].

In the early 1990s, the remarkable parallels between the natural history of *O. ochengi* and *O. volvulus* began to attract more international interest, and through collaborations between the Liverpool School of Tropical Medicine, Eberhard Karls University (Tübingen) and veterinary researchers from Cameroon, *O. ochengi* eventually supplanted the other bovine *Onchocerca* spp. as the model of choice for chemotherapeutic and immunological investigations. Almost everything we know about *O. ochengi* in cattle has been derived from data collected at a single field site adjacent to the River Vina du Sud, a blackfly breeding site located near the town of Ngaoundéré in the Adamawa Region of Cameroon (Figure 1). The first experiment in naturally-infected cattle examined the relationship between nodule load and microfilarial density in animals of different ages, exploiting the accessibility of the nodules, which are easily numerated by palpation and can be removed surgically under local anaesthesia. This study suggested that immunity to microfilariae, but not adult worms, may develop in older animals [9]. Other aspects of the natural history of *O. ochengi* in its natural host were rapidly established, such as the pre-patent period for microfilarial appearance in the skin (a minimum of 10 months) and their predilection site (the posterior ventral region) in comparison with other bovine *Onchocerca* spp. found in Cameroon [10,11]. Subsequently, longer-term experiments were conducted that revealed fascinating patterns of differential susceptibility to infection among cattle, with bulls exhibiting significantly higher nodule and microfilarial loads than did cows, and some animals maintaining very low levels of parasitosis despite intensive exposure [12]. Importantly, these disparities in susceptibility could not be accounted for by differences in attractiveness to the blackfly vector, indicating

that natural immunity to *O. ochengi* can develop under conditions of hyperendemicity [12,13]. This has important implications for vaccine development for human onchocerciasis (see below).

In parallel with its exploitation for the screening of drugs and vaccine candidates, the natural history of *O. ochengi* continues to be explored in depth. Insights into the reproductive biology of the parasite have been provided by genotyping of individual adult worms and intrauterine microfilariae collected from different nodules. This demonstrated that >50% of gravid worms contained progeny which were sired by more than one male, and some nodules contained females that had mated with males that were no longer present in the nodule [14]. Intriguingly, the genotyping of individual developing larvae from *Simulium damnosum sensu lato* and additional adult worms from nodules revealed that 16 – 17% of the "*O. ochengi*" specimens actually belonged to the taxon *Onchocerca* sp. "Siisa" [15]. This enigmatic member of the genus was previously only known from two developing larvae obtained from *S. damnosum s. l.* in Uganda [16]. Subsequent genotyping studies of adult worms, dermal microfilariae and embryonic stages obtained from a single infected cow from Ngaoundéré showed that *O. ochengi sensu stricto* and *Onchocerca* sp. "Siisa" freely interbreed, producing progeny that are viable (although whether hybrids are fertile is yet to be confirmed) [17]. Thus, these taxa can be considered strains of the same species that may have recently reencountered each other after a period of geographic separation. Surprisingly, this same study also reported preliminary evidence for hybridisation between *O. ochengi* and *Onchocerca dukei*, a species that normally forms nodules on the deep fasciae [11,17].

Here, we review the unique contribution of the *O. ochengi* system to onchocerciasis research over the past quarter-century, highlighting important insights it has provided in the realms of drug and vaccine evaluation, in addition to the role it has played in deepening our understanding of the basic biology of *Onchocerca* spp.

Contribution to vaccine development

In one of the first field studies of *O. ochengi* in Cameroon, comparisons of the prevalence of human onchocerciasis around a settlement with a high density of cattle, versus a similar area with few cattle, strongly suggested that *O. ochengi* L3 can induce **zooprophylaxis** against *O. volvulus* infection by naturally immunising the human host [18]. Subsequently, cross-protection was evaluated in the opposite direction by immunising cattle with *O. volvulus* L3, followed by a challenge with *O. ochengi* L3, which demonstrated almost 100% protection [19]. In a series of logistically challenging experiments, cryopreserved *O. ochengi* microfilariae were transported to the UK and injected into a surrogate blackfly vector (*Simulium ornatum*) in order to generate L3 for experimental infections of British cattle under controlled conditions. Antibodies from these animals were shown to recognise recombinant *O. volvulus* vaccine candidates, supporting the use of the *O. ochengi* system for onchocerciasis vaccine research [20]. In parallel, studies in Cameroon involving long-term follow-up of putatively immune animals under intense field exposure revealed that they remained relatively refractory to infection compared with naïve or drug-cured cattle [13]. Furthermore, under both experimental and natural conditions, irradiated L3 were demonstrated to confer significant reductions in nodule (experimental challenge) or microfilarial (natural challenge) loads [13].

Once these foundational experiments were completed, a bovine field trial of recombinant antigens that had induced significant levels of protection against filarial nematodes in rodent models was undertaken [21]. Eight prioritised vaccine candidates were selected and each immunised calf received all eight *O. ochengi* antigens, which were administered at different anatomical sites in the optimal adjuvant for each individual antigen (Freund's or alum). The calves were protected from exposure to blackflies until the immunisation schedule was completed, then they were maintained next to the River Vina du Sud for 22 months. Although 92% of the vaccinated animals developed nodules and had similar nodule loads to the controls (which received adjuvant only), 42% of the vaccinated animals did not develop **microfilaridermia**, whereas all control animals were microfilaria-positive. A limitation of the study design was that for logistical reasons, the antigens could not be evaluated separately in cattle. However, there was a strong trend for the microfilaria-negative animals to have higher IgG2 antibody levels to a particular antigen representing a moiety from tropomyosin [21].

Role as a macrofilaricidal drug screen

In 1991, the extremely close relationship between *O. ochengi* and *O. volvulus* and the accessibility of bovine nodules for assays on adult worms led the World Health Organisation to select *O. ochengi* as a tertiary drug screen (*i.e.*, a system to evaluate compounds following earlier rounds of prioritisation using *in vitro* assays and small animal models). This project, named MACROFIL, was supported by the Special Programme for Research and Training in Tropical Diseases (TDR) throughout the 1990s and was focused on the search for a nontoxic **macrofilaricidal** drug, which has been the "Holy Grail" of filariasis research for decades (and continues to this day). Early experiments demonstrated that the susceptibility

of *O. ochengi* to anthelminthics is similar to that of *O. volvulus*. Indeed, ivermectin at 200 μg/kg exhibited excellent activity against microfilariae but no significant macrofilaricidal effects [22]; whereas a flubendazole derivative (UMF-078) was highly macrofilaricidal [23] (although it induced neurological toxicity in cattle if repeated doses were administered orally [24]). One of the key strengths of the *O. ochengi* system is the ability to perform a triple assay on excised nodules, comprising measurement of adult worm motility, cellular metabolism (assessed by reduction of a tetrazolium salt to a formazan dye), and quantification of embryonic stages in female worms [22]. In contrast, for logistical and ethical reasons regarding the number of nodules that can be surgically removed from a human patient, the triple assay is rarely performed on *O. volvulus* today. Pharmacokinetic analyses of drug concentrations within nodules may also be performed [25]. This combined readout is easier to interpret than assessment of worm damage in fixed histological sections; albeit the latter method has performed well for human trials of macrofilaricidal drugs following expert training in the pathology of *O. volvulus* nodules [26,27]. Through the application of tattoo ink and/or subcutaneous implantation of microchip transponders in experimental animals, it is also straightforward to ensure that only nodules that were present from the onset of treatment are evaluated in the triple assay [24].

An ongoing controversy in the onchocerciasis field has been the question of whether macrocyclic lactones have any significant macrofilaricidal activity. In the *O. ochengi* system, even monthly doses of ivermectin or doramectin at 500 μg/kg (>3 times higher than the annual dose used for preventive chemotherapy of human onchocerciasis) were not found to have any overt macrofilaricidal effects, although they induced prolonged sterilisation of female worms [28]. This result was reminiscent of a clinical trial in humans where a single

dose of ivermectin of up to 1.6 mg/kg failed to kill adult *O. volvulus* [29]. Moreover, moxidectin at 200 μg/kg (a single dose, or seven consecutive monthly doses) was similarly inefficacious against adult *O. ochengi* [30]. Accordingly, although assessment of moxidectin in human clinical trials is ongoing, initial data suggest that it does not kill adult *O. volvulus*, at least after a single dose [31].

More recently, natural products used in traditional African medicine and veterinary medicine have been evaluated against adult *O. ochengi*, using worms obtained from abattoir material maintained in short-term *in vitro* culture systems [32,33]. This approach has also been applied for the screening of existing FDA-approved drugs to identify macrofilaricidal activity. Consequently, auranofin (a gold-containing compound used in the treatment of rheumatoid arthritis) has been prioritised as a drug that has the potential to be repurposed for onchocerciasis control [34].

Contribution to the discovery of antibiotic efficacy against adult filariae

During the MACROFIL programme, an animal heavily infected with *O. ochengi* developed a co-infection with the dermal bacterium *Dermatophilus congolensis* and was treated intermittently over several months with a long-acting formulation of oxytetracycline. It was noted that following antibiotic chemotherapy, all *O. ochengi* nodules resolved. Since *O. ochengi* has an estimated lifespan of 5 – 10 years, this outcome was entirely unexpected, although reports of intracellular bacteria in *O. volvulus* published 20 years prior suggested a potential mechanism of action [35]. A controlled trial of oxytetracycline against *O. ochengi* was initiated using an intermittent regimen administered over a six-month period. Remarkably, this regimen led to killing of all adult worms, resolution of nodules, and

clearance of microfilariae from the skin nine months after the treatment commenced [30]. Electron microscopic observations revealed that intracellular bacteria within adult worm tissues (Figure 2), identified by DNA sequencing as *Wolbachia* endosymbionts, became degenerated in treated worms several months before the parasites were killed. In parallel, other researchers working with other filarial nematodes in rodent models demonstrated that shorter tetracycline regimens led to prophylactic effects, growth retardation and blockage of embryogenesis [36-38]. Clinical trials of doxycycline against *O. volvulus* in human clinical trials rapidly followed and reported that daily therapy for six weeks was profoundly **embryostatic** [39].

Since these seminal discoveries, a major objective of macrofilaricidal drug research has been to shorten the antibiotic regimen required for permanent sterilisation or killing of adult *O. volvulus*, as treatments requiring compliance over periods of several weeks have been considered too protracted for mass drug administration programmes. In the *O. ochengi* system, intermittent regimens have been consistently shown to be more efficacious than continuous treatment for potent macrofilaricidal effects. Thus, a total dose of 140 mg/kg administered daily for 14 days is completely ineffective, whereas the same dose delivered monthly for six months kills the majority of adult worms within one year [40-42]. Importantly, although the 14-day regimen induced a transient depletion of *Wolbachia* from adult worm tissues, endosymbiont populations recrudesced to pre-treatment levels within six months [40]. However, considering the logistics of drug distribution and ensuring compliance, protracted intermittent regimens are not ideal for human use. In cattle, long-acting oxytetracycline administered by intramuscular injection provides therapeutic coverage for several days, such that a twice-weekly regimen is effectively a continuous

treatment. Condensing the total dose of 140 mg/kg into three weeks rather than six months revealed an efficacy against adult female worms of approximately 50% (as measured at one year post-treatment) [41]. The shortest regimen of doxycycline used in a human trial that showed significant macrofilaricidal effects was four weeks (at 200 mg/day) [43], and recent modelling data indicate that the briefest theoretically effective regimen is between two and four weeks [44]. These observations highlight the strong similarities in the response of *O. ochengi* and *O. volvulus* to antibiotics *in vivo.*

A possible alternative antibiotic for the chemotherapy of filarial infections is rifampicin, which has some advantages over doxycycline, including the potential for use in young children (doxycycline is contraindicated in children <8 years) and a bactericidal mode of action. Rifampicin did not exhibit clear superiority to doxycycline in human trials [45,46], although there has been intense interest in combining doxycycline with rifampicin on the basis of significantly enhanced efficacy compared with doxycycline alone in a small animal model [47]. However, in the *O. ochengi* system, rifampicin alone or rifampicin plus oxytetracycline administered for three weeks showed no benefit over oxytetracycline monotherapy [41]. Indeed, there was a trend for rifampicin to reduce both the macrofilaricidal and embryostatic effects of oxytetracycline when administered in combination, perhaps because of pharmacokinetic interference between these drugs, which has been reported for doxycycline and rifampicin combinations in humans [48]. Disappointingly, more than 16 years after the first clinical trials of an antibiotic for treatment of onchocerciasis, this approach is yet to be implemented on a wide scale; although through the efforts of the Anti-*Wolbachia* Consortium (A·WOL), several other repurposed antibiotic combinations are currently in clinical trials [49]. Future studies in *O.*

ochengi-infected cattle could further our understanding of the mechanisms of antibiotic tolerance in *Wolbachia,* which to date have only been investigated *in vitro* [50].

What is the role of *Wolbachia* in *O. ochengi*?

In the early years after the discovery of *Wolbachia* in filarial nematodes, our understanding of the symbiosis was shaped largely by the sequencing of the genome of *Brugia malayi* (a cause of lymphatic filariasis in humans) and that of its *Wolbachia* symbiont, *w*Bm [51,52]. In particular, the absence of the haem and riboflavin metabolic pathways in the nematode genome and their completeness in the symbiont genome suggested that the basis of the obligate dependency was nutrient provisioning, which remains the leading hypothesis today. However, several lines of data challenge this interpretation, such as the universal absence of the haem pathway in the Nematoda [53] (most described members of which do not harbour obligate symbionts) and the widespread retention of haem autotrophy in bacteria. Moreover, the genome of a naturally **aposymbiotic** filarial parasite of humans, *Loa loa*, did not reveal any obvious differences in metabolic capacity compared with *B. malayi* [54]. While there are experimental data indicating that exogenous riboflavin can partially rescue the deleterious effects of antibiotic treatment of *B. malayi in vitro* [55], it is likely that *Wolbachia* has different roles in filarial nematodes instead of, or in addition to, nutrient provisioning.

Several years before the *Wolbachia* and filarial nematode genomes were available, it was already known that antibiotic treatment led to a profound reduction in neutrophil infiltration in *O. volvulus* nodules, and that only *Onchocerca* extracts containing *Wolbachia* exhibited chemotactic activity for neutrophils *in vitro* [56]. Subsequent studies in *O. ochengi*-infected cattle involving serial nodulectomies after short-term (ineffective) or prolonged (macrofilaricidal) oxytetracycline treatments revealed that intranodular neutrophil dynamics mirrored *Wolbachia* density in adult worms [57]. Thus, short-term treatment caused a transient reduction in neutrophil numbers in the nodule, but recrudescence of *Wolbachia* led to restoration of the neutrophil population and worm survival. Strikingly, decreases in intranodular neutrophils were accompanied by a corresponding increase in local eosinophilia, involving degranulation on the adult worm cuticle (Figure 3). This was ephemeral after the short, continuous regimen but continued for several months during and after prolonged chemotherapy, such that degranulating eosinophils substantially outnumbered neutrophils in regions adjacent to the adult worms [57].

While these data were suggestive that degranulating eosinophils contributed to killing of adult worms and that the attraction of neutrophils to *Wolbachia* may be protecting the worms, it was also possible that eosinophils only degranulate on worms after they are already dead (or terminally moribund). To test this scenario, *O. ochengi*-infected cattle were treated with an organoarsenical macrofilaricide, melarsomine, or the prolonged oxytetracycline regimen [58]. Importantly, initial *in vitro* studies demonstrated that melarsomine had no effect on the viability of *Wolbachia*. Statistically significant increases in intranodular eosinophil populations were only observed in antibiotic-treated animals, and a

significant elevation in degranulating eosinophils was also restricted to this group. Further histological analyses revealed that eosinophil degranulation was initiated on worms that were morphologically normal, with unchanged gene expression for a marker of viability. Finally, ultrastructural studies showed that in antibiotic-treated but not melarsomine–treated cattle, eosinophil degranulation caused frank damage to the worm cuticle and these cells were even observed free in the parasite pseudocoelom. Taken together, these data strongly supported the hypothesis that *Wolbachia* has a defensive mutualist role in *O. ochengi*, protecting adult worms from eosinophil degranulation by stimulating an ineffective neutrophil response [58].

While the precise mechanisms by which the attraction of neutrophils by *Wolbachia* prevent eosinophil degranulation on adult worms are unclear, it is known that *Wolbachia* products can be exposed to the immune system by several routes (in addition to worm death), such as release of bacteria from the reproductive tract [59,60] and secretory pore [61], and even localisation of *Wolbachia* surface protein (WSP) in the nematode cuticle [62,63] (Figure 4, Key Figure). Plausible molecular interactions with eosinophils in the periphery of the nodule that could prevent degranulation on worms under normal circumstances include lactoferrin from neutrophils (present in high concentrations in *O. ochengi* nodule fluid [64]) and/or release of lipopeptides (from *Wolbachia*), which have been shown to inhibit eosinophil migration in humans and mice, respectively [65,66].

Almost certainly, “one size does not fit all” when it comes to the symbiosis between *Wolbachia* and filarial nematodes. Highly motile or migratory filarial species, including the lymphatic filariae and non-nodule-forming *Onchocerca* spp. (such as *Onchocerca gutturosa*)

are very unlikely to depend on this particular immune evasion mechanism as they are not a 'sitting target' for eosinophils. Intriguingly, *Onchocerca flexuosa*, which is the only known aposymbiotic member of the genus, fails to attract neutrophils and resides in nodules with a high density of eosinophils [56,67]. Unlike *O. ochengi*, *O. flexuosa* has an estimated lifespan of only one year [68], which is approximately the same length of time it takes for *O. ochengi* to be killed by eosinophils following antibiotic chemotherapy.

O. ochengi in the genomic era

Although some high-throughput studies of *O. ochengi* were attempted using mass spectrometric methods prior to release of an *O. ochengi* genome (for instance, to identify diagnostic biomarkers [69]), these analyses were constrained by the phylogenetic distance between this species and its nearest sequenced relatives. However, in the past five years, there has been an explosion of *Onchocerca* genome data made freely available (see WormBase ParaSite http://parasite.wormbase.org), including a very high-quality reference genome for *O. volvulus* [from the Wellcome Trust Sanger Institute (WTSI) using material from Cameroon], a draft genome for *O. flexuosa* (WTSI sequencing of material from Spain), and two draft genomes for *O. ochengi* (sequencing by University of Edinburgh and WTSI using material from Cameroon).

The advent of next-generation sequencing also enabled further insights into *Wolbachia* genomics, with the complete symbiont genome from *O. ochengi* representing the first from its phylogenetic clade (strain *w*Oo from supergroup C). The *w*Oo genome is the smallest *Wolbachia* genome sequenced to date (11% smaller than the supergroup D genome, strain *w*Bm, from *B. malayi*) and lacks the riboflavin metabolic pathway, although the capacity for

haem biosynthesis is retained [70]. Recent comparative genomics of supergroup C, including a new *Wolbachia* genome from the dog heartworm, *Dirofilaria immitis*, have revealed that clade C exhibits unique features, such as pronounced GC skew, a lack of transposable elements, and significant long-range **synteny** [71]. Expression studies (transcriptomics and proteomics) of strain *w*Oo showed that it does not resemble what would be expected for a classical nutritional symbiont, in that genes for vitamin and cofactor synthesis were poorly expressed [70]. The only highly expressed proteins with a potential role in metabolic provisioning were enzymes involved in the synthesis of purine nucleoside triphosphates, suggesting that *Wolbachia* may act as a 'supplementary mitochondrion' and provide ATP for the energy requirements of its host. Subsequent studies on *B. malayi* also supported such a role for *Wolbachia* [72,73]. Perhaps more importantly, several of the most highly expressed proteins in *w*Oo [64,70] are known or suspected to activate neutrophils, especially WSP [74] and peptidoglycan-associated lipoprotein [75,76].

The availability of the *O. ochengi* genome (and the *O. volvulus* genome as a comparator) has recently enabled the first high-throughput analysis of an *Onchocerca* spp. across the major stages of the lifecycle [64] using a proteomics approach. In addition, it has also facilitated unprecedented insights into the composition of the tiny volume of interstitial fluid that surrounds adult worms in the nodule. As might be expected for a nematode with such a long lifespan, the major parasite-derived products in nodule fluid are antioxidant enzymes (such as peroxiredoxins) and immunomodulatory proteins (including transforming growth factor-β homologues and a member of a novel family of ShK-domain proteins). In accordance with the classical histological studies of *Onchocerca* nodules described above,

the most abundant host-derived proteins in nodule fluid are antimicrobial effectors released by neutrophils [64].

One of the major priorities for human onchocerciasis research is to greatly improve diagnostics by becoming less dependent on insensitive skin snipping methods to detect microfilariae. Indeed, the ability to detect secreted or excreted products from a single viable adult female worm by a non-invasive method could revolutionise the surveillance of onchocerciasis and post-control evaluation. The cattle system has made an important contribution to this goal by the identification of *Onchocerca*-derived small RNAs released into nodule fluid and bovine serum *in vivo* [77,78], and the stage-specific proteomic analyses also have a key role to play in this regard. Thus, in *O. ochengi*, quantitative analysis of protein expression (Figure 5) has demonstrated that very few proteins are uniquely expressed by adult female worms (at least in robustly detectable quantities). However, one such female-specific protein (containing a bactericidal permeability-increasing superfamily domain) had not been reported previously from filarial worms [64] and could represent a target for new diagnostics.

Concluding remarks

In 1981, Odile Bain [79] hypothesised that *O. volvulus* speciated from *O. ochengi* following a host-switching event during the domestication of cattle. If this hypothesis is correct (see Outstanding Questions), the evolutionary distance between these species is miniscule in the wider context of nematode evolution, since domesticated cattle only entered sub-Saharan Africa 5 000 years ago [80]. The availability of the *O. volvulus* and *O. ochengi* genomes now allow this hypothesis to be formally tested, but whatever the outcome of this analysis, the

past 25 years have consistently shown that these sister species are extremely close in almost every major aspect of their biology. Therefore, as the drive to evaluate macrofilaricidal drugs, vaccines and novel diagnostics increases in urgency, *O. ochengi* is set to continue to provide a unique preclinical system for onchocerciasis research.

Glossary

Aposymbiotic: Lacking symbiotic relationships in the natural state.

Embryostatic: An outcome of drug or vaccine action defined by the cessation of embryonic development in the female reproductive tract.

L3: The third-stage infective larva.

Macrofilaricidal: Capable of killing adult filarial worms.

Microfilariae: The highly motile, transmissible first-stage larvae of filarial nematodes, which are born live and accumulate in either the skin or the blood depending on the species.

Microfilaridermia: The presence of microfilariae in the skin.

Synteny: Conservation of blocks of gene order between different chromosomes.

Zooprophylaxis: Reduced disease transmission to humans due to the diluting effect of animal populations.

Acknowledgments

We acknowledge the pioneering work of Sandy Trees and Alfons Renz that established the *O. ochengi* model in the early 1990s; the vision of Ted Bianco and David Taylor who brought it to the fore for vaccine research; and long-term funding support for this system from the European Commission.

Figure legends

Figure 1. Key Field Site for *Onchocerca ochengi* Research under Natural Conditions. A. Location of the field site (purple star) on the Adamawa Plateau of northern Cameroon. B. The River Vina du Sud near Ngaoundéré where the main vector for *Onchocerca ochengi* in the region, *Simulium squamosum* [13,81], breeds in high densities.

Figure 2. Localisation of *Wolbachia* Endosymbionts in *Onchocerca ochengi*. A. A cross-section of an adult female *O. ochengi* surrounded by neutrophils (n; Giemsa stain) inside a bovine intradermal nodule. *Wolbachia* endosymbionts (stained with an anti-*Wolbachia* surface protein antibody, brown deposits) are located in the somatic hypodermal cords (yellow arrow) and within developing microfilariae (yellow arrowhead) inside the uteri(u). The location of the worm intestine is also indicated (i). Scale bar, 50 μm. Reproduced from [58] under a CC-BY license. B. Numerous *Wolbachia* cells (arrows) are shown at higher magnification within host-derived vacuoles (v) in a somatic hypodermal cord beneath the cuticle (c). The locations of glycogen deposits (g) and mitochondria (m) are also shown. Scale bar, 1 μm.

Figure 3. Breakdown of Mutualism after Antibiotic Chemotherapy Triggers Eosinophil Degranulation. Numerous bovine eosinophils release toxic granules (Giemsa stain; black arrows) onto the surface of an adult female *Onchocerca ochengi* (asterisk) eight weeks after commencement of oxytetracycline treatment. Note the absence of neutrophils (cf. Figure 2A). The locations of the worm epicuticle (ec) and a vacuolated hypodermal cord cell (vhc) are indicated. Scale bar, 10 μm.

Figure 4 (Key Figure). Routes by which *Wolbachia* Products Interact with the Host Immune System. *Wolbachia* can be liberated from viable adult female filariae by a number of routes, including expulsion of free bacteria from the vulva along with microfilariae during parturition (1) [60], escape via the secretory pore (2) [61], and secretion of *Wolbachia* surface proteins into the cuticle (3) [62,63]. In addition, natural or drug-induced death of all parasite stages within the definitive host will result in substantial releases of endosymbionts [82-84] (not shown). Intact bacteria and secreted or excreted *Wolbachia* products attract neutrophils (5), forming a barrier around adult *Onchocerca ochengi* in the nodule [56,57]. Following prolonged antibiotic chemotherapy, *Wolbachia* is depleted, leading to a sharp decline in local neutrophilia and a corresponding influx of eosinophils (6), which degranulate on the worm cuticle [57,58]. It is not known how *Wolbachia* might prevent intranodular eosinophilia, but high concentrations of neutrophil-derived lactoferrin (7), or lipoproteins released by *Wolbachia* (8), are plausible mechanisms [65,66].

Figure 5. Heat Map of Protein Abundance across the *Onchocerca ochengi* Lifecycle. Proteins were quantified by label-free mass spectrometry and dendrograms were generated by hierarchical clustering based on pair-wise distance. Stage-specific clusters of expression and selected proteins of interest are highlighted for intrauterine microfilariae (iuMf, purple), vector-derived infective larvae (vL3, red), nodule fluid (NF, black), adult males (AM, blue) and adult females (AF, pink). UNC, uncoordinated; G protein, guanine nucleotide-binding protein; ASP, activation-associated secreted proteins; ACAD, acyl-CoA dehydrogenase; TGF, transforming growth factor; MSP, major sperm protein; PSP, protein serine-threonine phosphatase; CBS, cystathionine beta-synthase; BPI, bactericidal permeability-increasing.

Numbers in parentheses represent the number of proteins quantified in each family. Figure reproduced from [64] under a CC-BY licence.

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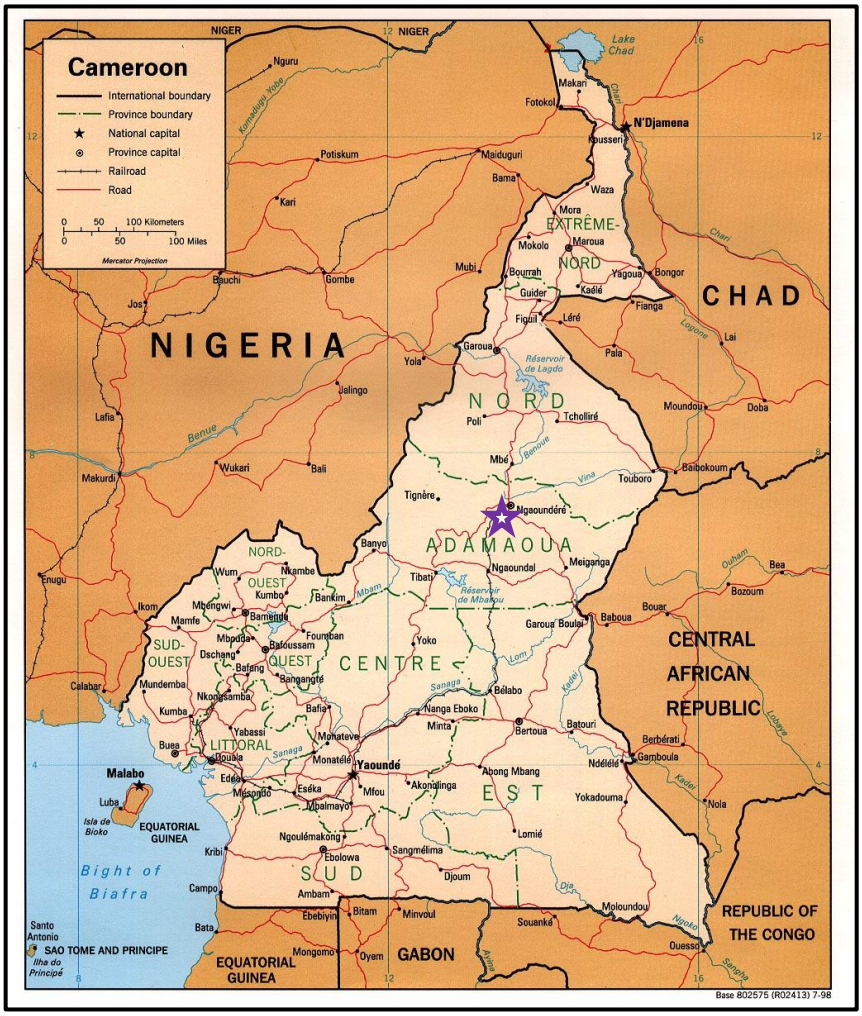
25 Years of the *Onchocerca ochengi* model.

Outstanding Questions box

- What is the precise status of *Onchocerca* sp. “Siisa” in terms of its taxonomic relationship with *O. ochengi* and *O. volvulus*? Does it represent an East African strain of *O. ochengi* that has only recently arrived in West Africa? Are hybrids of “Siisa” and *O. ochengi sensu stricto* equally fertile as the offspring arising from within-strain matings? Similar questions pertain to the putative hybridization reported between *O. ochengi* and *O. dukei* in Cameroon.
- Why do all antibiotics evaluated to date require several weeks to irreversibly deplete *Wolbachia* from *O. ochengi* and other filarial worms? What are the molecular mechanisms underlying antibiotic tolerance and the ability of *Wolbachia* to recrudesce after relatively short drug regimens?
- How does the neutrophil recruitment stimulated by *Wolbachia* prevent eosinophil influx and degranulation in the nodule? What are the relative roles of physical exclusion, immunomodulation of granulocyte function and cell death? Could an immunotherapeutic approach boost the efficacy of short-term antibiotic treatments?
- What is the evolutionary history of *O. ochengi* and *O. volvulus* and did they really diverge as little as 5 000 years ago? What is the molecular basis of host specificity? On a continent-wide scale, how does the diversity of the two species compare and can the origin of the putative host switch be pinpointed?
- Will it be possible to accelerate vaccine development for human onchocerciasis by finding a practicable combination of antigens and adjuvants that induces substantive and long-lasting protection in the *O. ochengi* system? What are the mechanisms underlying prevention of microfilaridermia in the original recombinant vaccine trial in cattle?

Figure 1

A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National capital
Province capital
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REPUBLIC OF THE CONGO
EQUATORIAL GUINEA
GABON
SAO TOME AND PRINCIPE
Bight of Biafra
Lake Chad
N'Djamena
Maroua
Garoua
Ngaoundéré
Yaoundé
Malabo
Bata
Kousseri
Makari
Fotokol
Maiduguri
Potiskum
Kano
Jos
Bauchi
Gombe
Yola
Mubi
Bongor
Pala
Lai
Moundou
Doba
Baibokoum
Touboro
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Banyo
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Kadéi
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Chari
Ouham
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Bertoua
Batouri
Abong Mbang
Yokadouma
Nola
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Djoum
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Souanké
Ouesso
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Ambam
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B

Figure 2

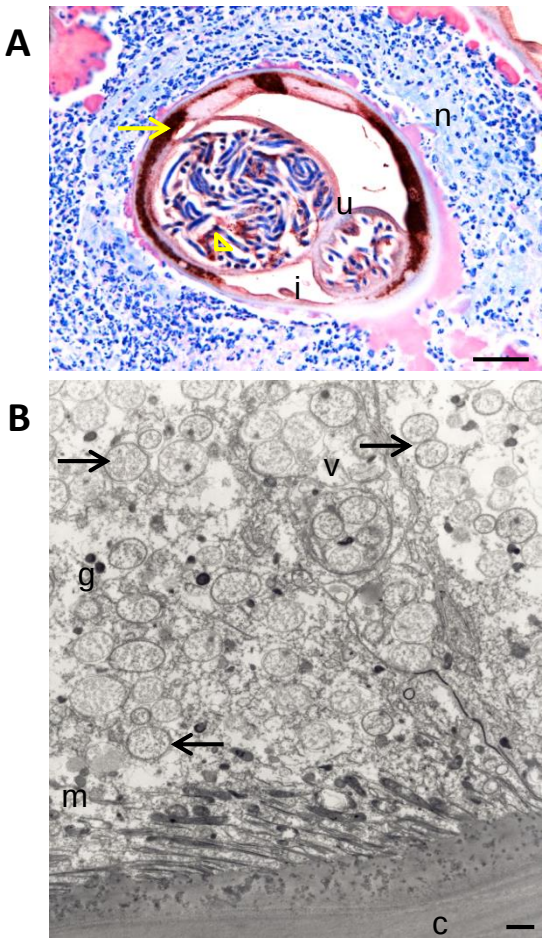
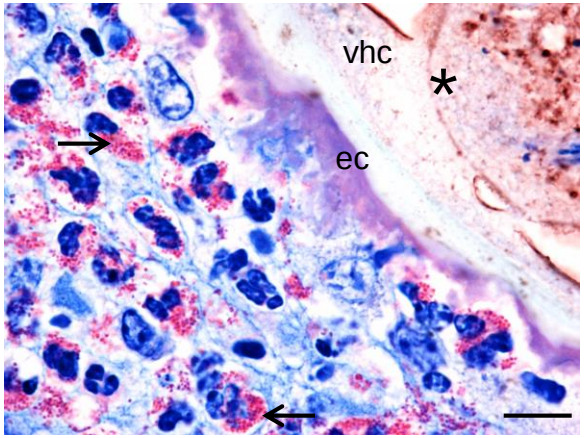


Figure 3



Key Figure - Figure 4

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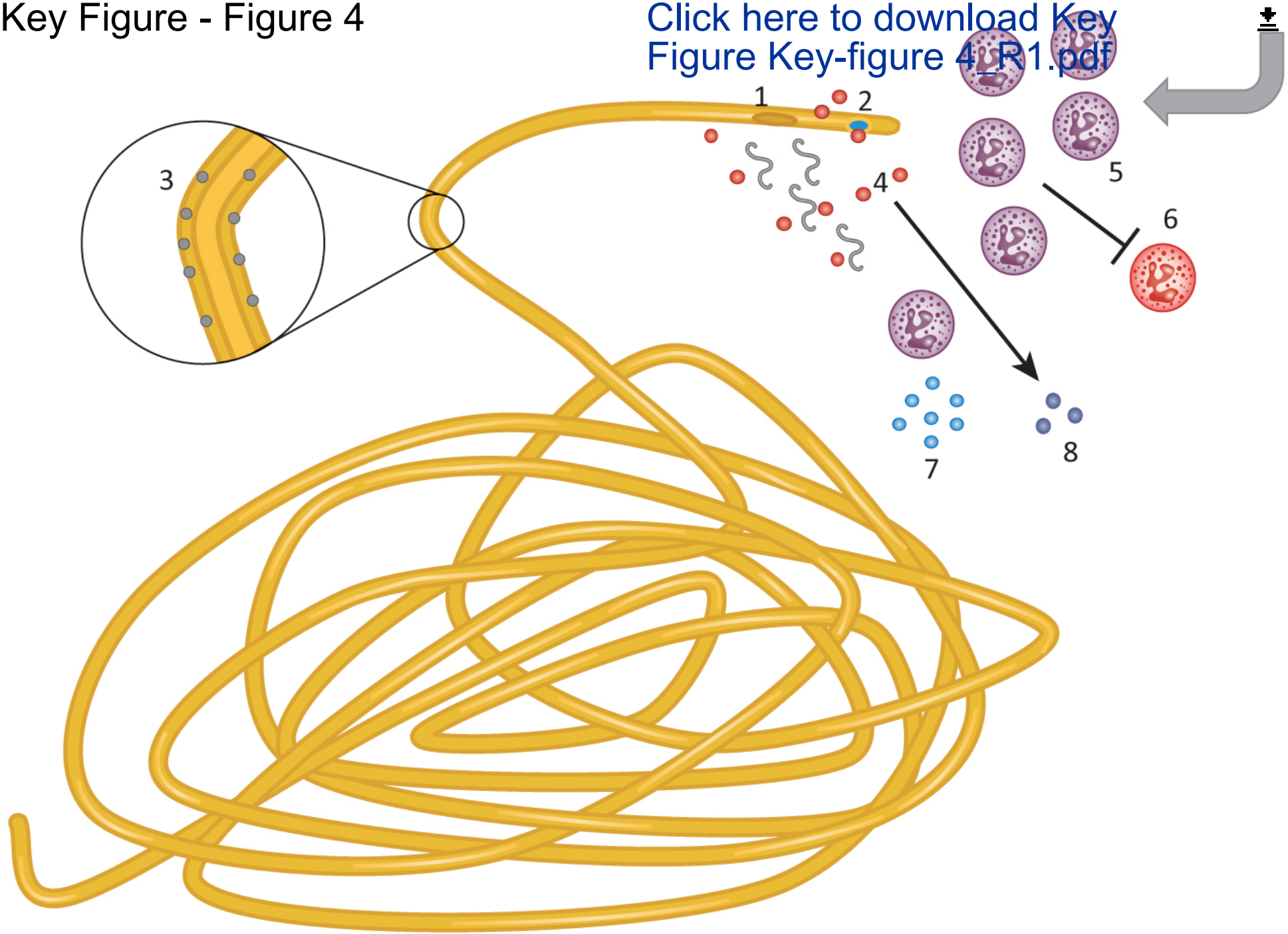
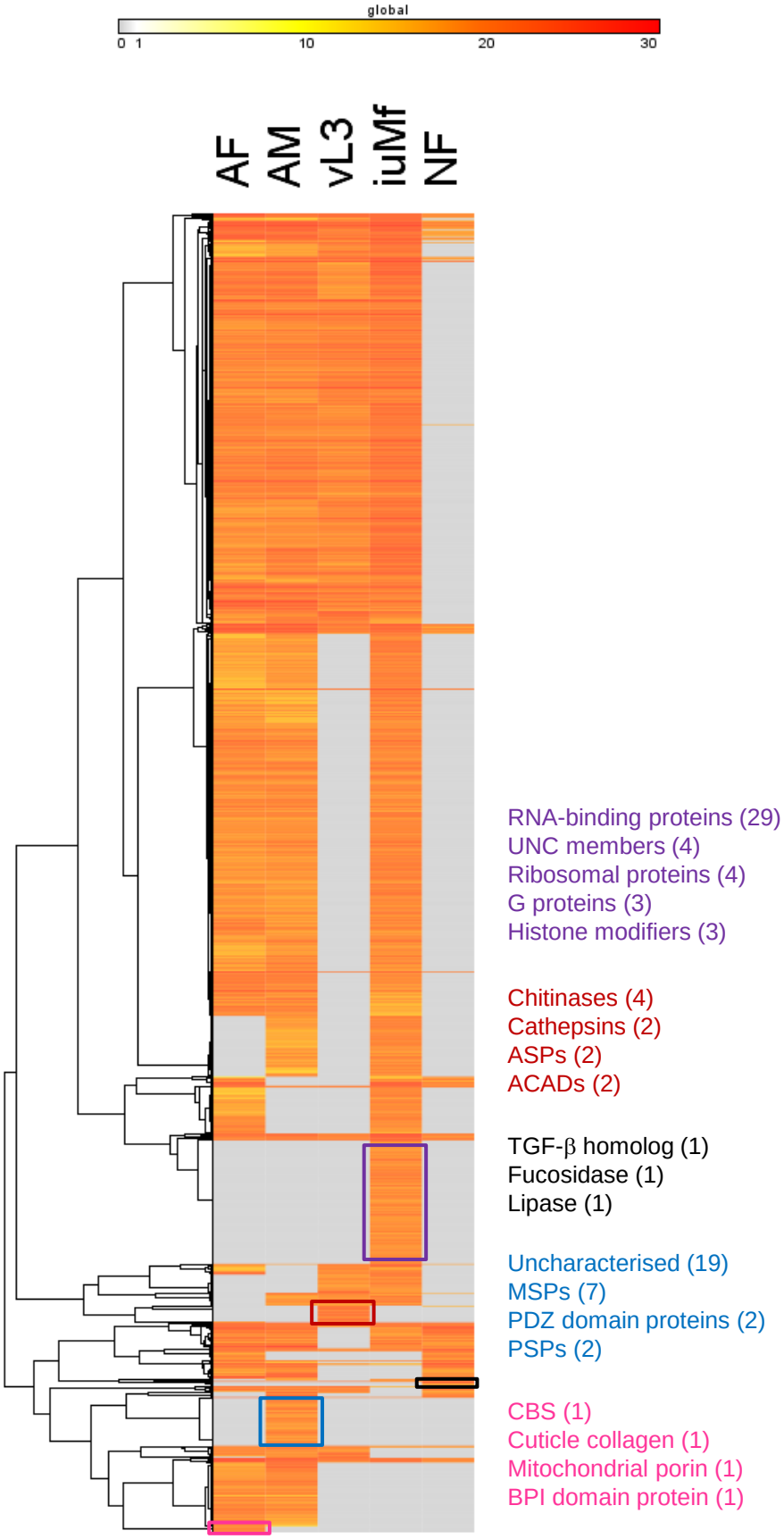


Figure 5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G proteins (3)
Histone modifiers (3)
Chitinases (4)
Cathepsins (2)
ASPs (2)
ACADs (2)
TGF-β homolog (1)
Fucosidase (1)
Lipase (1)
Uncharacterised (19)
MSPs (7)
PDZ domain proteins (2)
PSPs (2)
CBS (1)
Cuticle collagen (1)
Mitochondrial porin (1)
BPI domain protein (1)