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Investigations into the isolation of the Tukuyu focus of onchocerciasis (Tanzania) from *S. damnosum* s.l. vector re-invasion

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ABSTRACT

As part of the feasibility study for an onchocerciasis vector elimination project we investigated the isolation of the Tukuyu focus in Tanzania from possible vector re-invasion. This was achieved by examining the distribution of the *Simulium damnosum* complex vector cytospecies outside the focus to look for potential sources of re-invasion. Besides cytotaxonomic identifications of the aquatic stages, we applied morphotaxonomic and molecular techniques to identify *S. thyolense* and confirm it as the anthropophilic species in both the Tukuyu and the neighbouring Ruvuma foci. We detected significant differences in chromosome inversion frequencies between the Tukuyu populations and those breeding to the south-west in the adjacent Songwe river basin and in northern Malawi (where there is no man-biting and no onchocerciasis), suggesting that there is not normally a great deal of migration in either direction. By contrast, populations of *S. thyolense* from the Tukuyu and Ruvuma foci (150 km southeast of Tukuyu) were much more similar in terms of their chromosomal polymorphisms, indicating a higher possibility of re-invasion, although migration is still restricted to some extent, as indicated by some differences in chromosome polymorphisms between the two foci. Future migratory events which might be associated with vector control operations can be monitored by vector cytospecies identification, the frequency of polymorphic inversions which characterise the different vector populations, and the identification of accompanying non-vector cytospecies (e.g. *S. plumbeum* and cytotype Kasyabone occur exclusively in the two foci, and hence their re-appearance in Tukuyu could have only one outside source). The morphology of the scutal pattern of neonate males may act as a quick test for vector species identification where chromosome squashes are unavailable.

1. Introduction

Human onchocerciasis (river blindness) is a widespread disease in sub-Saharan Africa caused by infection with *Onchocerca volvulus* (Leuckart) (Spirudida: Onchocercidae) which is transmitted by *Simulium* blackflies (Diptera: Simuliidae), and causes blindness and skin disease. A global total of some 37 million people are currently thought to be infected (Basáñez et al., 2006). In eastern Africa, two different groups of vectors are known, the *Simulium damnosum* Theobald complex and the *Simulium neavei* Roubaud group (Raybould and White, 1979). However, the flies have a much wider geographical distribution than the disease, which is more focal, because there are many non-anthropophilic (and hence non-vector) populations of *S. damnosum* s.l. This observation led to the discovery of many chromosomally distinct entities (cytospecies and cytotypes) within the *S. damnosum* complex (Post et al., 2007), which can differ in their host preferences and vectorial importance.

Modern onchocerciasis control is usually based upon the distribution of the anthelminthic drug ivermectin to affected communities, and in Africa this is being achieved with the assistance of the World Health Organisation African Programme for Onchocerciasis Control (APOC). APOC was established in 1995 with the aim of eliminating onchocerciasis as a public health problem throughout the entire continent. However, the conditions under which ivermectin might interrupt transmission were not clear, and so APOC also included a number of vector elimination (local eradication) projects in foci which were thought to be isolated from vector immigration and were sufficiently accessible to be

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cost-effective (Remme, 1995). This included one of Africa's southernmost onchocerciasis foci, found in the Tukuyu valley of southern Tanzania at the northern end of Lake Nyasa (=Lake Malawi).

Onchocerciasis was first recorded in the area by Fischer (1932), but the disease seems to have gone largely unnoticed until the epidemiological and transmission studies which began in the late 1970s (Pedersen and Maegga, 1985; Pedersen and Kolstrup, 1986), and early evidence suggested that "the focus was small and well isolated" (Raybould and White, 1979). Le Berre (1979) more or less agreed with this assessment, but Guillet (1981) considered it unlikely, and both authors considered that a cytotaxonomic survey would be an important part of any assessment of isolation. *Simulium nyasalandicum* De Meillon (a member of the *Simulium neavei* group) had been recorded biting people at high altitudes (above 1,300 m) but none of the flies were infected and only sporadic cases of onchocerciasis occurred in the local communities (Pedersen and Maegga, 1985). *Simulium damnosum* s.l. was found to be transmitting onchocerciasis throughout the focus, but very little was known about the cytospecies involved. Dunbar had identified a single larva from the Mbaka river as *Simulium kilibanum* Gouteux (Raybould and White, 1979; Maegga and Cupp, 1993), but he identified subsequent collections from the rivers Mbaka, Lufilyo and Kiwira as Sanje form (Pedersen and Maegga, 1985). This was surprising, because *S. kilibanum* is a known vector, but Sanje form is not. Subsequent cytotaxonomic surveys (Maegga, 1990; Maegga and Cupp, 1993, 1994) also identified *Simulium kilibanum*, from low altitude sites in the Tukuyu area, but throughout the main body of the focus a new cytotype (the Kiwira form) was identified. This new form showed some cytotaxonomic similarities with Sanje form, and there is no doubt that previous identifications of Sanje form from Tukuyu focus were misidentifications (Maegga and Cupp, 1993). A second new cytotype (the Kasyabone form) was also described from the focus (Maegga and Cupp, 1994), but it was found in only a single river, where Kiwira form was still the most abundant cytotype. There were also nine other specimens which could not be assigned to any known cytotype. There was no doubt that the Kiwira form was the major vector (or possibly the only vector) in the Tukuyu focus, because it was the only cytotype found throughout the focus and cytotaxonomic identification of laboratory-reared larval progeny of 70 wild adult female flies allowed to feed to repletion on human bait were all Kiwira form, including progeny from two infected females (Maegga and Cupp, 1994). A vector elimination feasibility study (Walsh and Maegga, 1996) concluded "that there is a very high degree of isolation otherwise a unique form of *S. damnosum* could not have evolved and become so dominant in the area".

Nevertheless, insufficient was known about the populations of cytospecies and cytotypes in south-central Tanzania, around the Tukuyu focus, which might act as sources of reinvasion, such as the Ruvuma onchocerciasis focus some 150 km southeast along Lake Nyasa. We report an entomological survey carried out from 2002 to 2005 to investigate the isolation of the Tukuyu onchocerciasis focus and to infer whether vector elimination might be feasible, by determining geographical variation in the cytotaxonomic, morphotaxonomic and molecular characteristics differentiating the Tukuyu vector population from the *S. damnosum* complex populations in the surrounding areas (Ruvuma focus, south-central Tanzania, northern Malawi). Some related and supplementary data have already been published (Krüger et al., 2004; Krueger, 2006; Krueger et al., 2006a, 2006b; Post et al., 2007).

2. Materials and methods

2.1. Study area

Mbeya Region in south-west Tanzania harbours the small Tukuyu (=Itete) focus (3000 km^2) near the northern tip of Lake Nyasa covering Rungwe and Kyela districts, and some communities in Ludewa district. As part of the Rift Valley, the focus is fairly well defined by physical relief, having mountain barriers to the north (Mporoto Mountains and Mt. Rungwe volcano), west (Undali Mountains) and east (Livingstone Mountains). To the south it is bordered by the Songwe Valley, which forms the border with Malawi, and by Lake Nyasa. The three main rivers, Kiwira, Mbaka and Lufilyo, drain the slopes of Mt. Rungwe towards Lake Nyasa. The area records the highest rainfall in Tanzania (annual mean above 2000 mm), with a dry season from June to October (with prevailing winds blowing along the lake from the SSE) and a rainy season from November to May (with prevailing winds blowing over the Mporoto Mountains and the Mbeya Range from the NNW). Most of the area's vegetation is either miombo woodland or diverse cultivation including tea plantations in the elevated parts. The nucleus of the onchocerciasis focus appears to be in the mid-altitudes of the three major rivers (Lufilyo, Mbaka and Kiwira) and some of their tributaries (Pedersen and Maegga, 1985; Pedersen and Kolstrup, 1986). About 30 km SE of the main focus there is a subfocus where *S. damnosum* s.l. breeds in the Lumbira river near where it flows into Lake Nyasa and transmits onchocerciasis (Pedersen and Kolstrup, 1986; Maegga, 1991).

150–200 km SE of the main Tukuyu focus lies the Ruvuma (=Songea) onchocerciasis focus. This is the largest focus of onchocerciasis in Tanzania (endemic area roughly estimated 15,000 km^2), bordering Mozambique in the south and Lake Nyasa in the west (Maegga, 1991). It is mostly contained within Ruvuma Region, but to the northwest, Ludewa district of Iringa Region is also involved. To the north of the Ruvuma focus, there was a small focus historically documented at Wino (Maegga, 1991). However, biting or breeding *S. damnosum* s.l. have never been found there (Walsh and Maegga, 1996; Maegga, unpublished observations) and it may no longer be active. There are no other onchocerciasis foci around Lake Nyasa (Maegga, 1991; Krüger et al., 2004), although there are man-biting *S. damnosum* s.l. in the Livingstone Mountains at Njombe town near the River Ruhudji (Iringa Region), and apparently non-anthropophilic populations of *S. damnosum* s.l. to the West of Lake Nyasa (in northern Malawi) around the Misuku Mountains and the Nyika Plateau (Krüger et al., 2004).

2.2. Field sampling

More than 100 sampling sites were surveyed, mostly during the dry and wet seasons 2002–2005, to cover the Tukuyu and Ruvuma foci of onchocerciasis and the surrounding areas (including northern Malawi). Approximately 50% of these sample sites were found positive for immature stages of *S. damnosum* s.l. The sampling locations and cytotaxonomic identifications are listed in Table 1, and summarised in Figs. 1 and 2. For the majority of collections it was possible to obtain sufficient material so that some could be preserved in both Carnoy's (3:1 absolute ethanol: glacial acetic acid) and 100% ethanol. In addition, from a number of sites living pupae were collected for subsequent emergence of adult flies (in the Tukuyu laboratory) for morphological screening.

The failure to find *S. damnosum* s.l. (or any other blackflies) at some 44 riverine sites was due to a variety of environmental factors. In Karonga district of northern Malawi some riverbeds were found to be completely dry and hence not suitable for breeding, whereas recent flooding prevented successful sampling at other sites, particularly along the Tanzania–Malawi border. At other localities, streams proved to have little and rather warm water (up to 31 °C), or were slow-moving and swampy. Occasionally, where sampling failed during the dry season trip it was subsequently possible to obtain specimens in the wet season.