The Potential for Dispersal of Onchocerciasis in Ecuador in Relation to the Distribution of the Vector *Simulium exiguum* (Diptera:Simuliidae)

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The future dispersal of onchocerciasis in Ecuador is dependent on the distribution of cytotypes of the vector species complex Simulium exiguum. *Over the last 14 years, collections of larvae have been made from over 25 rivers, between 80-1600 m altitude, from various sites on both sides of the Andes. Analysis of larval polytene chromosomes was used to determine the distributions of each cytotype. On the western side of the Andes, the Cayapa cytotype (the only cytotype directly incriminated as a vector) has a distribution from Santo Domingo de los Colorados northwards. The Quevedo and Bucay cytotypes occur from Santo Domingo de los Colorados southwards. On the eastern side of the Andes, the Aguarico cytotype occurs in the Rio Aguarico and a new cytotype is present in the tributaries of the Rio Napo. Whether the disease will spread south of Santo Domingo and on the eastern side of the Andes depends on vector capacity of the cytotypes and the dispersal patterns of individuals infected with onchocerciasis. At present the Aguarico, Bucay and Quevedo cytotypes are known to be efficient hosts, but their biting preferences and biting densities have not yet been evaluated.*

Key words: *Simulium exiguum* - vector cytotaxonomy - onchocerciasis - oncocercosis - dispersal - Ecuador

The distribution of a vector species is paramount in determining the natural dispersal of a particular vector-borne disease. The widespread distribution of *Simulium exiguum* in Ecuador has often been seen as being predictive of the spread of onchocerciasis from the Esmeraldas Province (Shelley 1988, Guderian & Shelley 1992; Fig. 1). However, this might not be the case where a vector is actually a complex of sibling species. In this instance the sibling species might possess different vector capacities and the disease might not establish in areas where only a poor vector occurs, so that the disease might not spread to cover the range of the complex.

S. exiguum has been described as being a species complex of three cytotypes: Aguarico, Cayapa and Bucay (Procunier et al. 1985). A fourth cytotype, Quevedo, has also been discovered (Procunier 1989) which is closely related to the Bucay cytotype. However, it is not clear whether the X-linked chromosome difference represents an interspecific polymorphism (Charalambous et al. 1993a, b), and so it is probably best to consider the Bucay and Quevedo cytotypes to be conspecific.

At present it is thought that only the Cayapa and Aguarico cytotypes are vectors as they alone occur in the Esmeraldas Province. The occurrence of *S. exiguum* sensu lato between altitudes of 50-2000 m on both sides of the Andes (Fig. 1) has been documented (Shelley et al. 1989). However, the distribution of cytotypes has not been clear, because cytotyping has not been performed in detail until recently, and because sampling has been performed from a limited area, it is possible that other cytotypes occur in Ecuador.

In this paper we present an up-to-date distribution map of all known cytotypes of *S. exiguum* in Ecuador, review the distribution of cytotypes in relation to the possible spread of onchocerciasis, and indicate future areas of research.

MATERIALS AND METHODS

Collections of larvae were made between 1981 and 1993 in rivers on both sides of the Andes but concentrated along the Pacific side, as most

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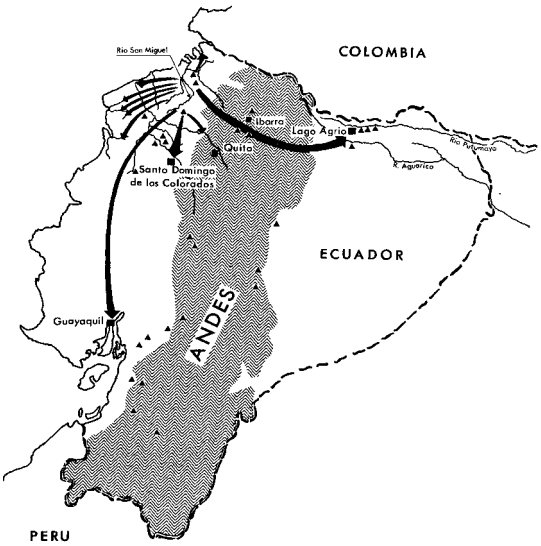

Fig. 1: distribution of *Simulium exiguum* s.l. (triangles) in Ecuador in relation to human migration patterns from the Esmeraldas Province (indicated by arrows) (After Guderian & Shelley 1992).

of the migration of infected people from Esmeraldas is towards Santo Domingo de los Colorados and Guayaquil.

Routine methods of sampling larvae and analyzing the polytene chromosomes of their silk glands were employed. Larvae of *S. exiguum* s.l. were collected from submergent and trailing vegetation in rivers over 5 m wide. The larvae were placed immediately into Carnoy's fixative (3:1 glacial acetic acid : 100% ethanol). The fixative was changed at least twice within 3 hr of collecting before the larvae were stored at 4°C.

Polytene chromosome preparations were made using the Feulgen method as described in Charalambous et al. (1993a) which also allows the sex of the larva to be determined. Identification of each larva to cytotype was made using the chromosome maps in Procunier et al. (1985) and Charalambous et al. (1993a, b). In addition, data on the distribution of *S. exiguum* s.l. were taken from The Natural History Museum [formerly the British Museum (Natural History)] collection, which includes permanent polytene chromosome slides (determined by Dr WS Procunier) and adults, pupal skins and larvae mounted on slides and in spirit.

RESULTS AND DISCUSSION

Cytology - Approximately 850 larvae of *S. exiguum* s.l. were identified to the cytotype level. The distributions of four cytotypes discovered are shown in Fig. 2. There is the possibility of a fifth cytotype occurring in the tributaries of the Rio Napo.

As the majority of sampling has been performed in the altitude range where *S. exiguum* s.l. appears to be most abundant (80-1600 m), new cytotypes could potentially be found either in areas or at altitudes not sampled before, eg further south towards Peru or at altitudes between 50-80 m or 1600-2500 m.

The distributions of the known cytotypes on the western side of the Andes are quite marked. The Cayapa cytotype (in which chromosome IIL is standard; Procunier et al. 1985) occurs primarily in the north of the country and diminishes in density towards central Ecuador where it occurs sympatrically with the Quevedo cytotype (in which inversion IIS-A is X-linked and inversions IIL-5+6 are fixed; Charalambous et al. 1993a). The distributions of these two cytotypes are essentially parapatric with one another. The Quevedo cytotype mainly occurs in central Ecuador, where it is possibly sympatric with the Bucay cytotype (in which inversions IIL-5+6 are fixed; Procunier et al. 1985). Three collections in central Ecuador (marked B/Q in Fig. 2) were found to contain single females which were heterozygous for IIS-A, the remaining males and females were of the

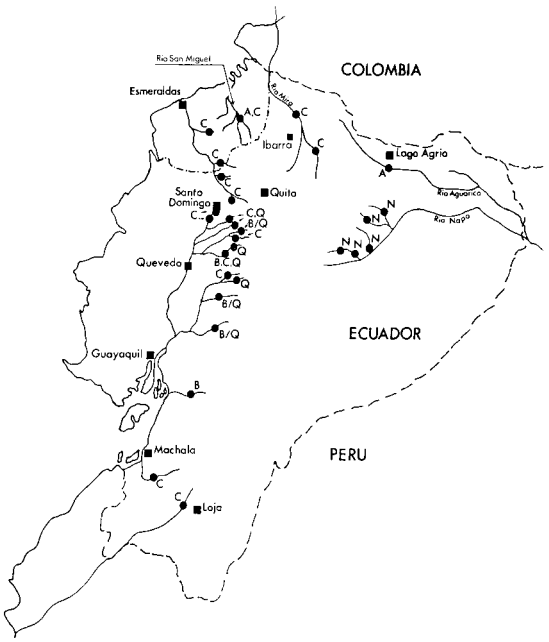

Fig. 2: distribution of the known cytotypes of *Simulium exiguum* species complex in Ecuador. Sample sites (filled circles) together with the cytotypes occurring at each site are shown. Cytotypes are A = Aguarico, B = Bucay, C = Cayapa, Q = Quevedo, N= possible new cytotype and B/Q = Quevedo cytotype definitely and possibly hybrids through interbreeding with the Bucay cytotype (see text for further details). Santo Domingo de los Colorados is abbreviated to Santo Domingo.

Quevedo cytotype. These heterozygous females could be the product of hybridization between the Bucay and Quevedo cytotypes or be the result of a balanced polymorphism of IIS-A occurring on the X chromosome in the Quevedo cytotype (Charalambous et al. 1993b). The Bucay cytotype replaces the Quevedo cytotype further south. The two most southern samples collected in the area near Machala and Loja have been identified as the Cayapa cytotype by Dr WS Procunier [one and three permanent slides, respectively, in The Natural History Museum (formerly the British Museum (Natural History) collection]. It has been noted above that the Cayapa cytotype has, essentially, a northern distribution. As such there is the possibility that the southern identifications of the Cayapa cytotype are mistaken and, as the chromosome slides are now unreadable, more sampling will be needed to confirm or refute its occurrence there.

On the eastern side of the Andes, only collections from the Rio Aguarico had been cytotyped when the species complex was first uncovered and in this river only the Aguarico cytotype (in which inversions IIL-3.4 are fixed and inversions IIL-1 and IIS-1 are Y-linked; Procunier et al. 1985) was found. Collections were made in the tributaries of the Rio Napo in June and September 1993. It was expected that these collections would contain the Aguarico cytospecies as the Rio Aguarico itself flows into the Rio Napo in Brazil. However, an extremely polymorphic population was present instead. In these collections inversions IIL-3.4 are polymorphic, inversions IIL-1 and IIS-2 do not appear to be Y-linked and a new inversion, IIIL-L, appears to be fixed. It is possible therefore (subject to further analysis to establish the stability of IIIL-L) that this population represents a new cytotype (indicated as N in Fig. 2). Unfortunately, collections were not made from the Rio Aguarico in 1993 (as the river levels were much too high for sampling in June), and so it is not clear whether the present populations there are Aguarico cytotype or whether they belong to the putative new cytotype. Furthermore, it remains a possibility that the fixed inversion of the Aguarico cytotype might be IIIL-L and not IIL-3.4. If this is the case then the Rio Napo populations and the Rio Aguarico population belong to the same cytospecies with inversions IIL-3.4 occurring in all larvae only in the Rio Aguarico. To address these questions, more collections need to be made from the Rio Aguarico and the tributaries of the Rio Napo at the same time of year. The finding that inversions IIL-3.4 may be polymorphic in the Aguarico cytotype raises the possibility that the Aguarico individuals found in the main focus of onchocerciasis in the Rio San Miguel in Esmeraldas (marked A,C in Fig. 2; Procunier et al. 1985) have been misidentified. This is because sibling species often share the same inversions, so that these individuals might actually have belonged to the Cayapa cytotype and possessed IIL-3.4 as polymorphic inversions. However, eventhough no populations so far analyzed from the western side of the Andes have possessed IIL-3.4 as polymorphic inversions, this does not exclude the possibility that these inversions may occur in the Rio San Miguel population of the Cayapa cytotype. Again, we envisage that further collections will resolve this point.

Possible dispersal of onchocerciasis - Guderian and Shelley (1992) have predicted that, because of logistical problems in distributing ivermectin, reducing onchocerciasis to low public health importance will only be successful if some degree of vector control is performed at some localities. Consequently, knowledge of the distribution and breeding grounds of *S. exiguum* s.l. in Ecuador will yield information vital for disease control.

Of the four known members of the *S. exiguum* species complex, only the Cayapa cytotype has been directly incriminated as a vector of onchocerciasis (Shelley et al. 1986). Although all four cytotypes have been shown experimentally to be capable of hosting the parasite to the L3 infective stage, it is not clear whether the vector capacities of the other three cytotypes are equal to that of the Cayapa cytotype (Shelley et al. 1990). Until such studies are conducted, the Cayapa cytotype must remain the main target for possible vector control.

The confirmed distribution of Cayapa is northerly, ranging from north of Ibarra to Quevedo (Fig. 2). The collections made along the Rio Mira were near the altitudinal limit of *S. exiguum* s.l. (at 900 m and 1600 m), which is usually to be found in greatest abundance between 80-500 m. The Rio Mira drains into the Pacific ocean in Colombia and, therefore, as migration along rivers occurs, it is likely that the Cayapa cytotype may occur in the lower altitudes of the Rio Mira in Colombia and be responsible for onchocerciasis transmission amongst Awa Indians. Whether the Cayapa cytotype is present in the Rio Micay focus of onchocerciasis in Colombia (Tidwell et al. 1980) is not known and should be investigated, although a less efficient vector cytospecies seems to be implicated (Shelley 1988).

Apart from the reported occurrence of the Aguarico cytotype in the Rio San Miguel, all collections made in the Esmeraldas Province have contained only the Cayapa cytotype. Furthermore,

collections further south reveal that the Cayapa cytotype remains the only member of the *S. exiguum* complex north of Santo Domingo de los Colorados. As people are migrating to Santo Domingo de los Colorados from Esmeraldas Province, it is very likely that transmission could occur in this vicinity if the Cayapa cytotype occurs at a high enough biting density.

The Quevedo cytotype occurs in central Ecuador and is most abundant in the region south of Santo Domingo de los Colorados. The Bucay cytotype may be sympatric with the Quevedo cytotype in some central areas (B/Q in Fig. 2), but replaces it further south (Fig. 2). Whether or not the disease could spread south of Quevedo depends on the vectorial capacity of the Bucay and Quevedo cytotypes and similarly whether the disease will spread in the region around Lago Agrio and Rio Napo depends on the vector capacity of the Aguarico and the putative new cytotypes. The only information known in this respect is that the three confirmed cytotypes can act as efficient hosts to the parasite and will bite humans during collections made in the river for larvae and pupae (personal observation). However, information is needed on the biting preferences of the cytotypes and their biting densities. Until such baseline entomological studies are performed it will be difficult to assess and inaccurately predict whether the disease could spread further south than its present limit.

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