Molecular population studies of *Simulium damnosum* s.l. (Diptera: Simuliidae) using a novel interspersed repetitive DNA marker

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Simulium damnosum s.l., the vector of West African onchocerciasis, has been the target of a major insect control initiative for the past 20 years. However, attempts to study the migration patterns of reinvading infective flies into controlled areas have been restricted by the lack of suitable genetic markers. Here, the results of the first population-level study of *S. damnosum* s.l. using a repetitive DNA marker, pSO11, are presented. Sequence analysis of pSO11 revealed a complex internal pattern of repetition and an open reading frame with 56.8 per cent similarity to a mouse retrotransposon protein. Combined with an interspersed genomic distribution, the internal structure of pSO11 suggested that it represents the 5' half of a transposable element. The genomic diversity of the sequence was analysed using Southern blot analysis of genomic DNA. Data were collected from 475 individuals from the island of Bioko (in Equatorial Guinea), Cameroon, Côte d'Ivoire and Sierra Leone. The results indicate that pSO11 is useful as an indicator of genomic diversity and that the experimental design used here permits comparison of an approximately random sample of chromosomal loci. Making this assumption, estimates of homozygosity based on pSO11 diversity were used to study inter- and intraspecific variability. The results indicate that pSO11 is potentially useful for studying population-level processes in *S. damnosum* s.l.

Keywords: DNA markers, migration, natural populations, onchocerciasis, repetitive DNA, *Simulium damnosum*

Introduction

In West Africa human onchocerciasis is a highly debilitating blinding disease caused by infection with the filarial nematode *Onchocerca volvulus*. The parasite is transmitted between people by the blood-sucking fly *Simulium damnosum sensu lato*. However, *S. damnosum* s.l. is a complex of sibling species which differ in their epidemiological significance (Wilson & Post, 1994). The riverine, immature stages of the vectors make them particularly good targets for population control (Philippon *et al.*, 1990), and coordinated studies of the biology and systematics of *S. damnosum* s.l. with the epidemiology of the disease have culminated in the World Health Organisation Onchocerciasis Control Pro-

gramme (OCP). The OCP has attempted to interrupt disease transmission by managing vector populations through treatment of breeding sites with insecticides (Le Berre *et al.*, 1990) and distribution of the drug ivermectin to the human communities.

The OCP has been successful in controlling the disease in many areas, but operations have often been hampered by regular but unexpected natural movements of vectors over hundreds of kilometres to reinvade controlled areas (Baker *et al.*, 1990). The response to these reinvasions has usually been to extend the controlled area, but this is costly in terms of time and resources. Attempts to investigate these problems have been hindered by the practical difficulties of conducting population surveys over large areas of continental West Africa and by a lack of suitable markers to study population genetics in *S. damnosum* s.l. Allozymes have been used for the study of migration rate and gene flow in other groups (Raab & Kennedy, 1980; Slatkin, 1985), but

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previous studies have failed to uncover sufficiently useful levels of polymorphism in *S. damnosum s.l.* (Townson *et al.*, 1987).

The paucity of conventional genetic markers in *S. damnosum s.l.* makes the group an ideal target for molecular techniques. Molecular methods have already been applied to the development of identification of *S. damnosum s.l.* species with some success (Post & Flook, 1992). Some molecular systematics has also been carried out at the inter-specific level in North American blackfly species (Jacobs-Lorena *et al.*, 1988), including comparative sequence analysis of mitochondrial DNA (Xiong & Kocher, 1993). However, no studies have been undertaken within species and this paper describes the results of the first population-level study of *S. damnosum s.l.* to use DNA markers.

The specific aims of this work were: (i) to characterize a candidate repetitive DNA probe, pSO11; (ii) to assess its suitability as a genetic marker by generating information on its genomic distribution; and (iii) to apply the probe in an uncomplicated population scenario (compared with most *S. damnosum s.l.* populations) consisting of an allopatric island population in Bioko, Equatorial Guinea. The vectors of onchocerciasis on the island of Bioko consist of a single chromosome form called the Bioko form of the *S. squamosum* subcomplex, which is itself part of the *S. damnosum* complex. The Bioko form is endemic, undoubtedly shows some isolation from mainland populations, but the precise degree of isolation is not known and so its specific status remains uncertain. The vagility of the *S. squamosum* subcomplex is not considered as high as those species which normally inhabit the savannahs (Garms & Walsh, 1987).

Materials and methods

Collection and preservation of material

Larvae were collected in Carnoy's solution for cytotoxic identification. Pupae available from 10 collections at nine sites were kept in a dark humid cage at room temperature until adults emerged. The neonate adults were preserved in 100 per cent ethanol and stored at 4°C (Post *et al.*, 1992). A single sample (number 3) of biting adult female flies was collected at human bait and also stored in ethanol. Sites are listed in Table 1 and locations indicated on Fig. 1. For comparisons additional collections were used.

1 Three samples of *S. squamosum s.str.* neonates from Cameroon (sample numbers 10, 11 and 12,

respectively). These specimens were identified by correlated larval cytotoxicity. The taxonomic status of the Bioko form is not yet clear (authors' unpubl. data), but even if the Bioko form is not the same species as *S. squamosum* they are geographically and taxonomically close within the *S. squamosum* subcomplex.

2 Two samples of neonates from the *S. sanctipauli* subcomplex identified by correlated cytotoxicity: a mixed sample of *S. soubrense/S. leonense* from River Rokel at Makpankaw, Sierra Leone (8°44'N, 11°56'W) (sample number 13), and a sample of *S. sanctipauli* from River Sassandra at Koperagui, Côte d'Ivoire (5°38'N, 6°38'W) collected by M. D. Wilson.

Sequencing of pSO11

The repetitive DNA probe pSO11 was originally isolated from an *S. leonense* genomic DNA library for its potential use in sibling species identification (Post & Flook, 1992). The 3.27 kb *EcoRI* insert of pSO11 was digested with restriction enzymes, transferred to a nitrocellulose membrane by Southern blotting and hybridized with radiolabelled genomic DNA. Analysis of the autoradiographs indicated that the repetitive region of the clone was confined to a 1.37 kb *EcoRI/HindIII* fragment. This fragment was isolated, digested with *AluI* and *HaeIII* restriction endonucleases and subcloned into M13. The subfragments were sequenced on both strands using the Sanger dideoxy method (Sanger *et al.*, 1977). The pSO11 sequence was assembled from the partial sequences and analysed using the GCG package (Devereux *et al.*, 1984). The final sequence has been deposited in the EMBL data library with the accession number X70645.

Genomic DNA extraction and restriction digestion

DNA was extracted from individual flies using a previously described protocol (Flook *et al.*, 1992). The DNA was ethanol precipitated three times, resuspended in a final volume of 15 µL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) + RNase (50 mg mL⁻¹) and stored at -20°C. Genomic DNA was digested with *EcoRI* at a low concentration (0.5 U µL⁻¹) and, to avoid partial digestion of DNA, reactions were incubated overnight at 37°C. In a random sample of membranes, probe DNA was stripped and new hybridizations performed with a mosquito ribosomal RNA probe, AaR1 (Gale & Crampton, 1989). This probe hybridizes to a single *EcoRI* fragment in *S. damnosum s.l.* and conse-

Table 1 Results of pSO11 Southern hybridization analysis of individual flies from different species and localities. Figures are calculated from pSO11 hybridizing fragments in the size range 3.27–0 kb. For collection details see text. Distance values were calculated only between samples on the same autoradiograph, and so for some populations more than one average pairwise similarity (APS) value was calculated. Results are not shown for the *S. sanctipauli* population from Côte d'Ivoire because the samples were run on a number of different gels and no direct comparisons comprising sufficient individuals were available from which to calculate APS values. Coll. No. refers to the collection number for a given site. Season refers to whether the sample was collected in the first trip at the end of the dry season (in May) or in the second, at the beginning of the rainy season (in November). ΣH = number of different haplotypes observed; ΣB = number of different band sizes observed; AvH = Average number of haplotypes ($= \Sigma H/n$); APS = average pairwise similarity; MAPS = mean average pairwise similarity ($= \Sigma APS/NS$, where NS = number of samples)

Coll. No.	Season	Co-ordinates	<i>n</i>	ΣH	ΣB	AvH	APS	MAPS
Bioko								
1	Rainy	3°23'N,8°46'E	10	8	11	0.8	35.3	35.3
2	Rainy	3°28'N,8°35'E	21	15	11	0.7	13.7	
			12	10	9	0.8	15.3	
			12	9	7	0.8	17.1	15.4 ± 1.7
3	Rainy	3°34.5'N,8°37'E	21	17	15	0.8	10.7	
			22	15	13	0.7	8.6	
			25	19	14	0.8	19.8	13.0 ± 4.2
			26	21	16	0.8	13.7	
			12	11	8	0.9	12.3	
4	Dry	3°30.5'N,8°36.5'E	6	6	27	1.0	23.8	19.1 ± 6.6
			9	8	9	0.9	14.4	
5	Rainy	3°37.5'N,8°37.5'E	11	11	8	1.0	22.0	22.0
6	Dry	3°41'N,8°39'E	14	7	12	0.5	9.2	
			11	11	9	1.0	17.1	13.2 ± 5.6
7	Rainy	3°41'N,8°39'E	24	15	13	0.6	19.3	
			25	14	11	0.6	17.3	
			12	7	11	0.6	5.6	14.1 ± 7.4
8	Rainy	3°41'N,8°39.5'E	6	6	7	1.0	25.0	
			10	10	12	1.0	19.7	22.4 ± 3.7
9	Rainy	3°43'N,8°43.5'E	11	11	13	1.0	11.9	
			8	5	7	0.6	26.0	
			6	4	3	0.7	22.2	20.0 ± 7.3
			Σ = 314				17.3	
Cameroon								
10	Dry	4°37'N,9°21'E	16	15	20	0.9	17.8	17.8
11	Dry	4°43'N,9°29'E	12	11	16	0.9	9.0	
			12	10	12	0.8	8.3	
								8.7 ± 0.5
12	Dry	4°43'N,9°29'E	13	13	28	1.0	15.6	
			22	21	25	1.0	11.4	13.5 ± 3.0
			Σ = 75				12.4	
Sierra Leone								
13	Rainy	8°44'N,11°56'W	11	10	24	0.9	53.1	51.6 ± 5.8
			12	12	21	1.0	56.6	
			25	25	38	1.0	45.2	
			Σ = 48				51.6	

quently complete digestion of genomic DNA could be confirmed.

Agarose electrophoresis and Southern blotting

Agarose gels (0.9 per cent, FMC SeaKem) were prepared using $1 \times$ TAE buffer (40 mM Tris-acetate, 1 mM Na_2EDTA) and run overnight at $<2 \text{ V cm}^{-1}$. Postelectrophoresis, gels were prepared for Southern blotting by denaturation and neutralization (Southern, 1975; Sambrook *et al.*, 1989). Limited exposure to ultraviolet light on a transilluminator was used to depurinate the DNA prior to denaturation (FMC-Bioproducts, 1990). DNA was transferred to nitrocellulose filters (Biotrace NT) by capillary transfer.

Hybridization

Insert DNA from *Eco*RI-digested pSO11 was recovered from agarose gels and radiolabelled with [$\alpha\text{-P}^{32}$]-dCTP using random-primed labelling. Filters

were prehybridized in ($5 \times$ SSC, 50 per cent deionized formamide, $5 \times$ Denhardt's, 0.05 M phosphate buffer, 50 mg mL^{-1} denatured herring sperm DNA) at 42°C in prehybridization solution ($5 \text{ mL } 100 \text{ cm}^{-2}$ filter) and the buffer was exchanged for fresh buffer ($5 \times$ SSC, 50 per cent deionized formamide, $5 \times$ Denhardt's, 0.02 M phosphate buffer, 100 mg mL^{-1} denatured herring sperm DNA) immediately before hybridization. Labelled probe was denatured by heating to 100°C and added at a concentration of $1 \times 10^7 - 5 \times 10^8 \text{ c.p.m. } 100 \text{ cm}^{-2}$. All hybridization reactions were continued overnight at 42°C in a hybridization oven. Filters were washed in various concentrations of SSC, 0.1 per cent SDS at different temperatures and exposed to Fuji RX film at -45°C or -70°C .

Data analysis

The observed banding profiles for pSO11 were so variable that an appropriate statistic for the comparison of different specimens was a similarity

Fig. 1 Map of Bioko indicating mean average pairwise distance values (MAPDs) and standard errors (where more than one APD was available) for the different collecting sites. MAPDs were only calculated for sites where sufficient numbers of pupae were collected. Asterisks indicate samples collected in the dry season.

coefficient and in this study the DNA-fingerprint similarity index, S (Lynch & Crease, 1990; Yuhki & O'Brien, 1990), was used. For two individuals i and j the index is calculated as

$$S_{ij} = \frac{2F_{ij}}{F_i + F_j},$$

where F_i and F_j are the numbers of bands in each individual, and F_{ij} is the number of shared bands. To summarize the similarity between large numbers of individuals, this statistic is extended to cover all pairwise comparisons and is referred to here as the average pairwise similarity (APS), where for $i < j$ and N is the population size

$$APS = \frac{1}{\binom{N}{2}} \sum_i \sum_j S_{ij} \times 100.$$

This statistic essentially treats the banding patterns as phenotypic characters and does not directly estimate important genetic parameters. It ranges from 100 (complete identity) to 0 (complete dissimilarity).

Although both statistics could be calculated unambiguously for individual specimens run on the same gel, it was more difficult to estimate them among individuals on different gels because of the problem of identifying homologous bands. This precluded the direct comparison of discrete bands from individual DNA samples size-fractionated on different gels and consequently the comparison of different populations by standard parameters such as G_{ST} was not possible in this study. Instead, values of APS were calculated for individuals from the same population run on the same gel, and the indices compared between populations. For populations where more than one filter was available, the mean APS (MAPS) was also calculated.

Results

Characterization of pSO11

The sequence of the pSO11 *HindIII/EcoRI* fragment was 67.7 per cent A+T and dot matrix analysis revealed a complex pattern of repetition, consisting of two adjacent sets of repeats (Fig. 2). First, three tandem repeats with a consensus length of 276 bp (LR1, LR2 and LR3) were detected at the 5' end of the sequence. No internal pattern of repetition could be detected within these repeats. Differences were detected between the repeats at 64 positions (23.2 per cent), although a significant amount of this heterogeneity was accounted for by a 25 bp deletion

Fig. 2 Gene map of pSO11. The dashed line corresponds to the beginning of the unsequenced 3' region of the original pSO11 *EcoRI* insert. LR1, LR2 and LR3 correspond to the positions of three 276 bp repeats. SR1, SR2 and SR3 correspond to the positions of three 63 bp repeats. ORF indicates the position of the open reading frame discussed in the text and the arrow indicates the sense of the ORF.

in LR2. Secondly, three tandem repeats of length 63 bp (SR1, SR2 and SR3) were identified 8 bp downstream of the 3' end of LR3. These repeats were 12 per cent heterogeneous and showed some similarity to the 5' region of the 276 bp repeats. The unsequenced and nonrepetitive region of pSO11 was downstream of SR3.

A search was made for open reading frames (ORFs) located in the nonrepetitive regions of the sequence and a 152 bp candidate was found upstream of LR1 (see Fig. 1). The 3' end of the ORF coincided with the 5' end of the complete pSO11 fragment and no further sequence could be obtained. This sequence was translated and used to search the PROSITE sequence database with the PROSRCH program (Collins & Coulson, 1987). Among several similarities found was a match to the C terminal end of an L1 retrotransposon protein sequence from *Mus domestica* (Loeb *et al.*, 1986). The L1 and pSO11 ORF sequence amino acid sequences were aligned and found to share 17.6 per cent identity (56.8 per cent similarity).

As a second stage of its characterization, the genomic distribution of pSO11 was analysed by Southern blot analysis of a limited number of individual flies. Using restriction enzymes that did not cut the repetitive region of the pSO11 clone (*EcoRI*, *HindIII*, *HaeIII*), genomic DNA preparations were digested, size-fractionated on agarose gels and transferred to nitrocellulose membranes. Filters were hybridized with radiolabelled pSO11 as described under Materials and methods. Similar banding profiles were generated for the different restriction enzymes, consisting of multiple (30–50), discrete hybridizing fragments of uniform intensity. These patterns also varied between individuals and are consistent with an interspersed repetitive distribution of pSO11. If copies of the repeat were localized

in the genome, for example if pSO11 belonged to a family of clustered tandem repetitive DNA, or was a rare mobile element inserted within a region of clustered repetitive DNA, distributions of band sizes would be expected to be relatively uniform. Taken together, the information concerning the sequence and genomic distribution of the pSO11 *HindIII/EcoRI* fragment strongly suggests that it is the 5' half of a transposable element.

Size distribution of pSO11 hybridizing EcoRI fragments

To examine the genomic distribution of pSO11 in more detail, 475 *EcoRI*-digested nuclear DNA samples from individual flies were examined in Southern blot hybridizations. In the resulting autoradiographs, the lengths of different hybridizing fragments were interpreted as corresponding to the distance between the internal *EcoRI* site of a pSO11 hybridizing fragment and the nearest adjacent *EcoRI* site in the flanking genomic DNA at the insertion site. If pSO11 is a transposable element and is randomly inserted into different regions of the genome, the distribution of pSO11 hybridizing *EcoRI* fragment sizes should therefore approach the distribution for all *EcoRI* genomic DNA fragments. To examine this prediction, the distribution of the sizes of pSO11 hybridizing fragments was compared with the distribution of *EcoRI* fragment sizes estimated from densitometer scans of ethidium bromide stained genomic smears. Only fragments smaller than 3.27 kb were included because a hybridizing band of this size occurred at high frequency in the Bioko population and served as a convenient marker on the gels. Also, because the number of bands greater than 3.27 kb was very high (see Fig. 3), the hybridization was usually too intense to score bands accurately. A graph summarizing the results is presented in Fig. 4 and the plots show that the cumulative distribution of pSO11 fragment sizes is very similar to that obtained for the *EcoRI*-digested genomic DNA fragments.

A problem arises in the comparison of these cumulative distributions of fragment size because the pSO11 distribution is biased owing to the presence of the 1.37 kb pSO11 copy in all fragments. This decreases the probability that an *EcoRI* site will occur within the first 1.37 kb of an *EcoRI* fragment containing a copy of pSO11. However, because this probability will be the same in all pSO11 hybridizing fragments the observed similarity in the shape of the distributions indicates that the distance between the end of pSO11 copies and adjacent *EcoRI* repeats is

Fig. 3 Autoradiograph showing the hybridization pattern of ^{32}P -labelled pSO11 probe to individual *EcoRI*-digested, individual fly DNA preparations. Restriction fragments were size-fractionated on a 0.9 per cent 1 $\times$ TAE agarose gel. Figures refer to the size of molecular weight marker bands in kb.

distributed in a similar way to *EcoRI* sites in the entire genome. It follows from this observation that the chromosomal distribution of the pSO11 sequences is random with respect to the distribution of *EcoRI* recognition sites.

Estimation of copy numbers

Taking the distribution of pSO11 to be approximately random, the samples of hybridizing *EcoRI* fragments measured in the different individuals constitute random samples from which it should be possible to make an estimate of relative copy number between species. Thus, the distribution of band numbers in individuals of different species was examined in our sample and the number of bands in different size categories (within the range 0–3.7 kb) averaged across all samples within species. The results are summarized in Table 2 and the estimated copy numbers relative to *S. squamosum* Bioko were 1.5 in the Cameroon *S. squamosum*, 3.1 in *S. sanctipauli* s.str and 4.4 in *S. soubrense/S. leonense*. Although these estimates can only be very approximate, the apparent equality of band numbers between the two *S. squamosum* s.l. taxa, and the increased numbers in *S. sanctipauli* and *S. soubrense*,

both concur with the results of various hybridization analyses which suggest that the copy number of pSO11 is species-specific (authors' unpubl. data). This observation is important because it is possible that changes in the structure of pSO11 in different host genomes might bias the distribution of observed fragment sizes between species. Because there is a degree of correspondence between melting curve estimates of copy number and estimates derived here, this could be taken as a further indication of the randomness of the 0–3.27 kb sample of pSO11 hybridizing fragments.

Fig. 4 Comparison of observed cumulative frequencies of pSO11 hybridizing fragment sizes and *Eco*RI-digested *S. squamosum* Bioko genomic DNA smear. Only bands in the range 0–3.2 kb were scored. ▲ = *Eco*RI-digested genomic DNA fragments; ■ = pSO11 hybridizing *Eco*RI fragments.

Interspecific variation in banding and heterozygosities

The APS estimates for the Bioko, Cameroon and Sierra Leone populations are presented in Table 1. As a preliminary step, the relationship between sample size and APS was examined by calculating the product-moment correlation coefficient for these two variables. A negative correlation of 0.33 was obtained but this correlation was not statistically significant ($P = 0.13$) indicating that the APS values were independent of sample size.

The highest intraspecific APS values were observed in the Sierra Leone population (sample 13) and the difference between these APS values and the Cameroon and Bioko *S. squamosum* s.l. was statistically significant (unpaired *t*-test, $P < 0.01$). Differences were also apparent between the *S. squamosum* subcomplex samples with the Cameroon samples demonstrating considerably lower APS values (MAPS = 12.42) than the Bioko populations (MAPS = 17.27). However, this difference was not statistically significant at the 5 per cent level. Hence, if the assumption that pSO11 is an unbiased indicator of genomic diversity holds, and its mobility is similar in these taxa, the results imply that the Cameroon sample is no more heterozygous than the Bioko sample.

Bioko form intrapopulation variation

The results of the intrapopulation surveys are presented in Table 1 and differences in APS values calculated for the different populations can be detected. Figure 1 shows the distribution of the MAPS values of different Bioko populations. The MAPS for different populations collected in the rainy season ranged from 13.02 ± 4.7 (sample 3) to 35.3 (sample 1). Comparing the MAPS values using *t*-tests, some of the estimates of variability were statistically significant ($P < 0.05$) between samples collected in the rainy season; for example, using this approach, MAPS values between samples 2 and 8 and samples 3 and 8 were found to be significantly different. However, in two of the samples (7 and 9) the standard errors of the MAPSs were particularly high and so differentiation was not shown to be statistically significant in most comparisons.

Discussion

Interspecific variation

Two kinds of interspecific variation were identified in the present study. First, interspecific variation in

Table 2 Average numbers of pSO11 bands. For collection and experimental details see main text. Figures refer to average number of pSO11 hybridizing fragments in the size range 3.27–0.0 kb. The copy numbers are calculated relative to *S. squamosum* Bioko. In *S. squamosum* Bioko flies, the 3.27 kb high frequency band was not included in calculations. In *S. sanctipauli* subcomplex flies two high frequency hybridizing fragments, 2.3 kb and 0.8 kb bands, are included in the calculation

Species	<i>n</i>	Average band number	SD	Relative copy number
<i>S. squamosum</i> Bioko	334	1.37	1.12	1.0
<i>S. squamosum</i> Cameroon	75	2.10	1.42	1.5
<i>S. sanctipauli</i> s.l.	18	4.22	1.48	3.1
<i>S. soubrense</i> / <i>S. leonense</i>	48	6.00	1.22	4.4

copy number was observed. Such variation had been previously observed in quantitative hybridization analyses (Post & Flook, 1992) and the estimates obtained here coincide well with those results. Secondly, large differences were noted in the APS values recorded for the different taxa examined in this study. Notably, the *S. sanctipauli* subcomplex sample from Sierra Leone showed far less diversity than the two *S. squamosum* s.l. samples. The significance of this observation is difficult to interpret because the *S. sanctipauli* s.l. sample is unrelated to the other two. However, it is notable that the Makpankaw population was very large and so the low level of population diversity recorded was unexpected. It is possible that species of the *S. sanctipauli* subcomplex simply showed less genomic diversity than the other two taxa. They are not thought to be strongly migratory and breed in large rivers, which might be considered ecologically more uniform. It is also possible that this particular population may show reduced diversity as a result of some unknown founder effect such as recolonization after the dry season or insecticide treatment. A third explanation for the low variability might be connected to the genomic distribution of pSO11. As the pSO11 copy number is much higher for the two species sampled here, the pSO11 sequence might be approaching saturation level in the genome. Observations on transposable elements in other species indicate that deterministic forces counter the spread of elements (Charlesworth & Langley, 1989) and that the mobility of sequences is often decreased as copy numbers approach a limiting value.

In contrast to the above, the lack of difference in the level of diversity between the two *S. squamosum* subcomplex populations is interesting. Cytogenetic analyses of the *S. squamosum* Bioko population have

clearly identified some degree of genetic isolation from the mainland populations in Cameroon (Post *et al.*, 1997), and field observations during the collecting trips indicate large seasonal fluctuations in population size. Under these circumstances, population bottlenecks and decreased genetic variability might be predicted in the *S. squamosum* Bioko populations, resulting in decreased genetic variability compared to the Cameroon populations. However, the similarity of the APS between Bioko and Cameroon populations may indicate that the underlying processes of population colonization and extinction are actually very similar.

Structure of the Bioko S. damnosum s.l. population

One of the aims of this study was to make inferences about the population structure of the Bioko *S. squamosum* and in this respect the results were able to provide information about specific features of the Bioko population; for example, the high APS value for sample 1 from River Ruma is notable because it was from the eastern side of the island where very few potential breeding sites were observed. *Simulium damnosum* s.l. is not recorded either biting or breeding from the middle of the island and it is likely that the two sides show some degree of isolation. The relative homogeneity of this population suggests that it is smaller and more inbred, a situation consistent with these observations. In contrast, sample 3 showed the highest level of variability. This sample consisted of adult females caught at human bait, and is probably a mixture of flies emerging from a number of sites, including different rivers. Appetitive dispersal of flies (coming to humans for a blood-meal) was frequently observed between rivers, and thus a sample of wild-caught, adult females

might be expected to be more heterogeneous. Therefore, both these observations are significant because they represent situations where there exists a strong expectation concerning the population variability, and where levels of pSO11 diversity concur with that expectation.

Also of interest were the comparative MAPS values estimated for the populations sampled in the rainy season (1–3, 5, 7–9) where there is weak evidence for a decrease in variability in the more northerly populations compared to those further south on the west side of the island. This was initially examined by ranking populations according to their latitude and MAPSs, and computing the Spearman rank correlation, but no significant correlation was detected ($P > 0.1$). However, using *t*-tests to compare MAPS estimates, significant differences were detected between the most southern population (sample 2) and two northern populations (samples 8 and 9). Any trend linking variability with latitudinal position is potentially important if real because it would be consistent with a northward recolonization of the island from perennial rivers in the south as the seasonal rivers begin to flow after the dry season. Such behaviour would have important consequences for population control, because sites in the south of the island would become obvious targets for larvicidal treatment. However, it is evident from inspecting the geographical distribution of collection data (Fig. 1) that more extensive population sampling is needed to explore this possibility in detail.

Suitability of pSO11 as a population genetic marker

The results of this study showing pSO11 to exhibit high levels of intraspecific variability make it a potentially useful marker for future studies of *S. damnosum* population biology. The apparently random chromosomal distribution of pSO11 is of particular importance, because it allows homogeneity in observed pSO11 haplotypes to be equated with population homogeneity. If pSO11 is a transposable element, as the sequence analysis suggests, the above assumption has an experimental precedent in work conducted on *Drosophila*, where differences between founder populations have been detected using *in situ* hybridization studies of the *mdg1* TE polymorphism (Terzian & Biémont, 1988).

A remaining difficulty with the experimental design used here is the statistical treatment of the Southern blot banding profiles. This is a technical problem associated with the identification of homologous bands on different gels. In the field of DNA

fingerprinting various solutions to this problem have been developed including analytical approaches ('binning' strategies: Budowle *et al.*, 1991) and novel experimental procedures specifically designed to prevent ambiguous interpretations (Jeffreys *et al.*, 1991). These specific approaches are inappropriate for the pSO11 Southern blot data, but some similar improvement in procedure is necessary to generate reliable estimates of population genetic parameters from pSO11. A potential strategy might be to use PCR to detect inserted copies of pSO11 at specific sites using primers homologous to different chromosomal flanking regions.

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