Comparison of chromosome and isoenzyme polymorphism in geographical populations of *Leishmania* (*Viannia*) *peruviana*

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SUMMARY

Five chromosomes and 17 isoenzyme loci were analysed in 4 allopatric populations of *Leishmania* (*Viannia*) *peruviana*, and molecular distances calculated with 2 estimators, Chromosomal Size Difference Index and Jaccard Distance. Chromosome and isoenzyme data were in overall concordance: 13/30 isolates clustered similarly on the dendrograms constructed from the different estimators, and a significant correlation ($P < 0{\cdot}001$) was observed between the molecular distances calculated from the two sets of characters. This indicates an evolutionary association between chromosomal size polymorphism and isoenzymes. Chromosomes have a faster molecular clock than isoenzymes; twice as many genotypes were identified by chromosome analysis and significant size differences (for a total of up to 500 kb for 5 chromosomes together) were observed within a given zymodeme. Chromosomes most likely represent better indicators of genetic drift than isoenzymes, as suggested by the higher correlation between both estimators of chromosomal size-polymorphism and eco-geography. Some chromosomes might present an adaptive response to environmental variation.

Key words: *Leishmania* (*Viannia*) *peruviana*, karyotype, isoenzymes, drift, selection.

INTRODUCTION

Isoenzymes constitute the gold standard for genetic characterization of many parasites (WHO, 1990) but other genetic characters have also been analysed by restriction analysis of kDNA (or schizodeme analysis; Pacheco *et al*. 1986), RAPD (Tibayrenc *et al*. 1993), or RFLP (Victoir *et al*. 1995). These characters corroborate one another and the resulting classifications (Tibayrenc & Ayala, 1987; Bañuls, 1993; Tibayrenc *et al*. 1993).

Development of pulsed field electrophoresis (Schwartz *et al*. 1983) gave access to another type of intrinsic characters, the (re-)arrangement of DNA sequences (further called genomic characters). The high resolving power of molecular karyotyping demonstrated an extensive polymorphism among trypanosomatid populations, and useful epidemiological applications among others (Dujardin *et al*. 1987, 1993*a*, *b*, *c*; Saravia *et al*. 1990; Blaineau, Bastien & Pagès, 1992; Lighthall & Giannini, 1992).

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A preliminary comparison of genomic and genetic polymorphism in *Trypanosoma cruzi* found agreement between karyotype and isoenzyme classification (Henriksson, Petterson & Solari, 1993). However, quantitative comparison of both polymorphisms in trypanosomatids has, to our knowledge, not been reported. Difficulties have arisen from the lack of a method for numerical analysis of chromosomal size-polymorphism. Therefore, we recently introduced the calculation of the Chromosomal Size Difference Index (CSDI), a method based on measuring the absolute size differences among homologous chromosomes (Dujardin *et al*. 1995*a*). Application of CSDI to *Leishmania* (*Viannia*) *peruviana* isolates originating from different Peruvian biogeographical units (Lamas, 1982) revealed a strong eco-geographical structuring of populations along a north-south cline (Dujardin *et al*. 1993*a*, 1995*a*).

In the present paper, we extended the karyotype data obtained from the previous analysis of 30 isolates of *L*. (*V*.) *peruviana* and compare them with results of a parallel study on isoenzyme polymorphism (Bañuls, 1993) both quantitatively and in relation to the eco-geographical context.

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MATERIALS AND METHODS

Parasites

Thirty parasites previously characterized isoenzymatically (Bañuls, 1993; Guerrini, 1993) and karyotypically (Dujardin *et al.* 1993*b*, 1995*a*) as *L.* (*V.*) *peruviana* were considered in this study (see Table 1 in Dujardin *et al.* 1993*b*). These isolates originated from 4 bio-geographical units (BGUs, according to the classification of Lamas, 1982) situated along a North–South transect through the Peruvian Andes: Huancabamba, Surco-North, Surco-Centre and Surco-South (Dujardin *et al.* 1995*a*). After measuring the geographical distances separating the different isolation sites, 7 distance classes (0, 150, 482, 621, 632, 771 and 1253 km) were defined. Promastigotes of each isolate were subcultivated up to 20 times (following isolation) in blood agar medium (Tobie, Von Brand & Mehlman, 1950) and were harvested during log phase to prepare samples for the 2 methods of characterization (Ben Abderrazak *et al.* 1993; Dujardin *et al.* 1993*a*, *b*).

Molecular karyotyping

All procedures are described elsewhere (Dujardin *et al.* 1987, 1993*a*, *b*). Karyotype variability was explored at the level of 5 chromosomes recognized by random genomic probes isolated from *L.* (*V.*) *braziliensis* M2904 (Dujardin *et al.* 1993*a*): pLb-22, pLb-134Sg, pLb-134Sp (a portion of the gp63 gene Dujardin *et al.* 1994), pLb-168 and pLb-149, the latter probe was not considered in the previous report (Dujardin *et al.* 1995*a*). Chromosomes were further named after the number of the probe used to recognize them. Sizing of chromosomal bands was performed as described elsewhere (Dujardin *et al.* 1995*a*).

Multilocus enzyme electrophoresis

Fifteen enzyme systems (17 genetic loci) were run in cellulose acetate electrophoresis (Bañuls, 1993), namely: aconitase (ACON: EC 4.2.1.3), glucose-6-phosphate dehydrogenase (G6PD: EC 1.1.1.49), glucose phosphate isomerase (GPI: EC 5.3.1.9), glutamate oxaloacetate transaminase (GOT: EC 2.6.1.1), glutamate pyruvate transaminase (ALAT: EC 2.6.1.2), isocitrate dehydrogenase (IDH: EC 1.1.1.42), malate dehydrogenase NAD+ (MDH: EC 1.1.1.37), malate dehydrogenase NADP+ or malic enzyme (ME: EC 1.1.1.40), mannose phosphate isomerase (MPI: EC 5.3.1.8), nucleoside hydrolases 1 and 2 (NH1: EC 2.4.2.1 and NH2: EC 2.4.2.*), peptidases 1 and 2 (PEP1 and PEP2: EC 3.4.4.11 or 13), 6 phosphogluconate dehydrogenase (6PGD: EC 1.1.1.44), and phosphoglucomutase (PGM: EC 2.7.5.1). Electrophoresis and staining procedures were carried out according to Ben Abderrazak *et al.* (1993).

Estimators of chromosome and isoenzyme polymorphism

Two methods were used for quantifying chromosomal size-polymorphism. First, the absolute Chromosomal Size Difference Index (aCSDI) which considers chromosomes as a continuous variable and introduces a weighing of size-variation: 2 chromosomes showing a 25 kb size-difference are considered to be genomically more similar than 2 chromosomes presenting a 200 kb size difference. This index was calculated with a programme developed previously (Dujardin *et al.* 1995*a*). Secondly, in order to compare chromosome and isoenzyme polymorphism with a same method, the Jaccard Distance (Jaccard, 1908) was used: $D_{ij} = 1 - a/(a+b+c)$ where $a =$ number of bands that are common to the stocks i and j; $b =$ number of bands present in the first genotype and absent in the second; $c =$ number of bands absent in the first genotype and present in the second. Jaccard Distance was calculated on disjunctively encoded chromosomal data (JDc): therefore, size-classes of 25 kb (for chromosomes sizing from 400 to 800 kb, i.e. those recognized by probes pLb-134Sp, -134Sg, -149 and -168) or 50 kb (for chromosomes sizing from 1100 to 1500 kb, those recognized by probes pLb-22) were defined (Giannini *et al.* 1990) and all chromosomes falling in the same size-class were considered as being similar sized. In this procedure, chromosomes are considered as a discrete variable and there is no weighing of size variation: 2 chromosomes differing by 25 kb are considered genomically as different as 2 chromosomes differing by 200 kb.

For isoenzyme data, Jaccard distance was calculated from all the 17 loci (Jdi_{17}) or from the 7 polymorphic ones (Jdi_7, excluding (i) monomorphic loci and (ii) enzymatic systems which were impossible to reveal for 3 stocks but otherwise monomorphic). The latter procedure also allowed us to avoid possible biases in the comparison between isoenzymes, loci taken at random and monomorphic in their majority, and chromosomes, selected for their polymorphism. Parasites were considered as belonging to the same zymodeme if they were identical for the 17 loci considered (Godfrey, 1979). Jaccard distances and all dendrograms were computed with the program TAXO (E. Serres, Laboratoire d'Ecologie médicale et de Pathologie parasitaire, Faculté de Médecine, Montpellier, France).

Correlations

Analysis of the correlation between the molecular distances described here above and with the ge-

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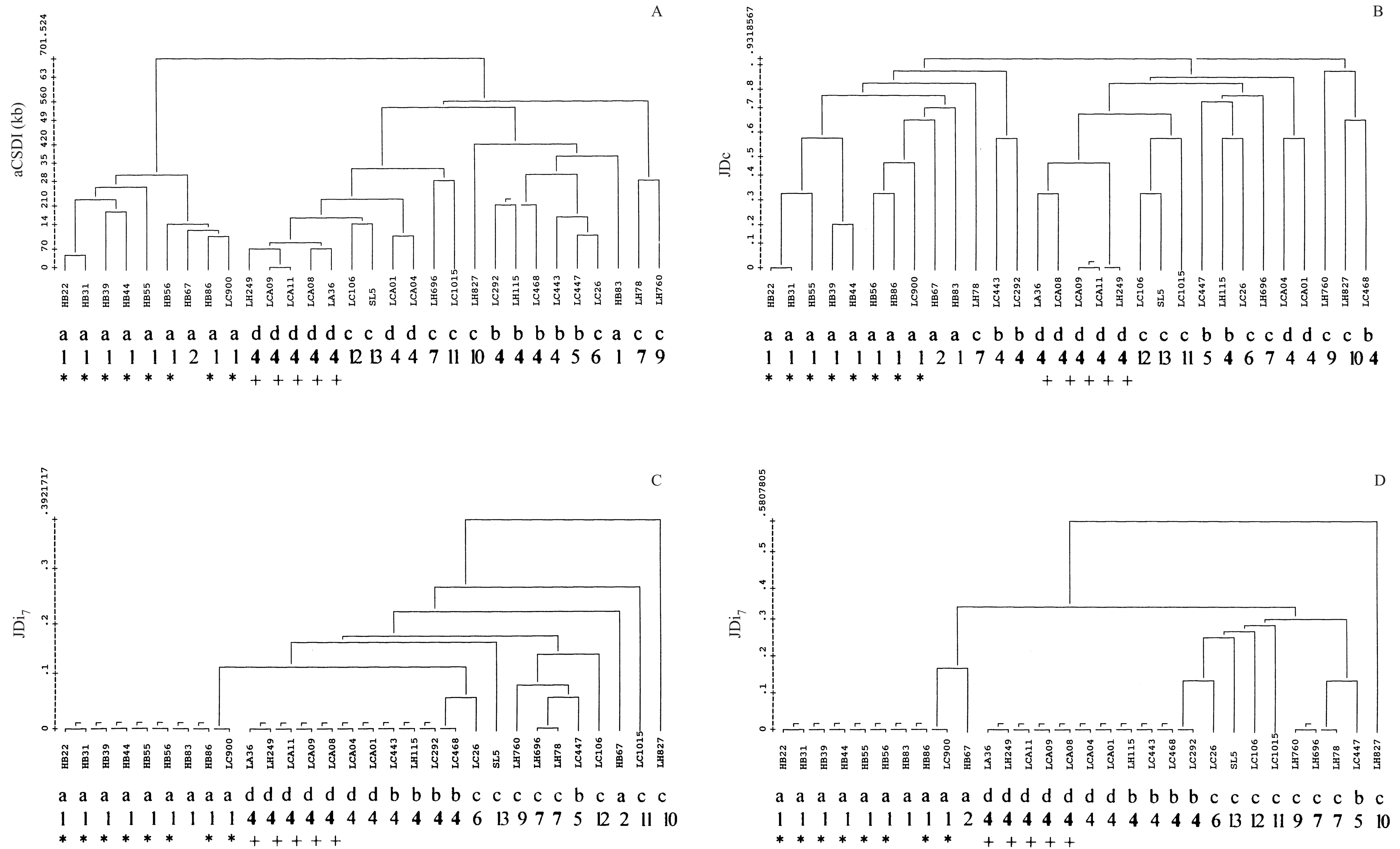

Fig. 1. Molecular polymorphism in *Leishmania* (*Viannia*) *peruviana*: dendrograms constructed from chromosomal data (A: aCSDI, and B: Jaccard distance, JDc) and isoenzyme data (C: Jaccard distance on 17 loci, JDi_{17} and D: 7 polymorphic loci, JDi_7). Under the dendrograms are given: the codes of the isolates, their BGU of origin (a, Huancabamba, b, Surco-North, c, Surco-Centre and d, Surco-South), the zymodeme numbers (after Bañuls, 1993); * and +, 2 sets of isolates clustered together in the 4 dendrograms. Isolates belonging to a same zymodeme, but chromosomally very different are indicated in bold.

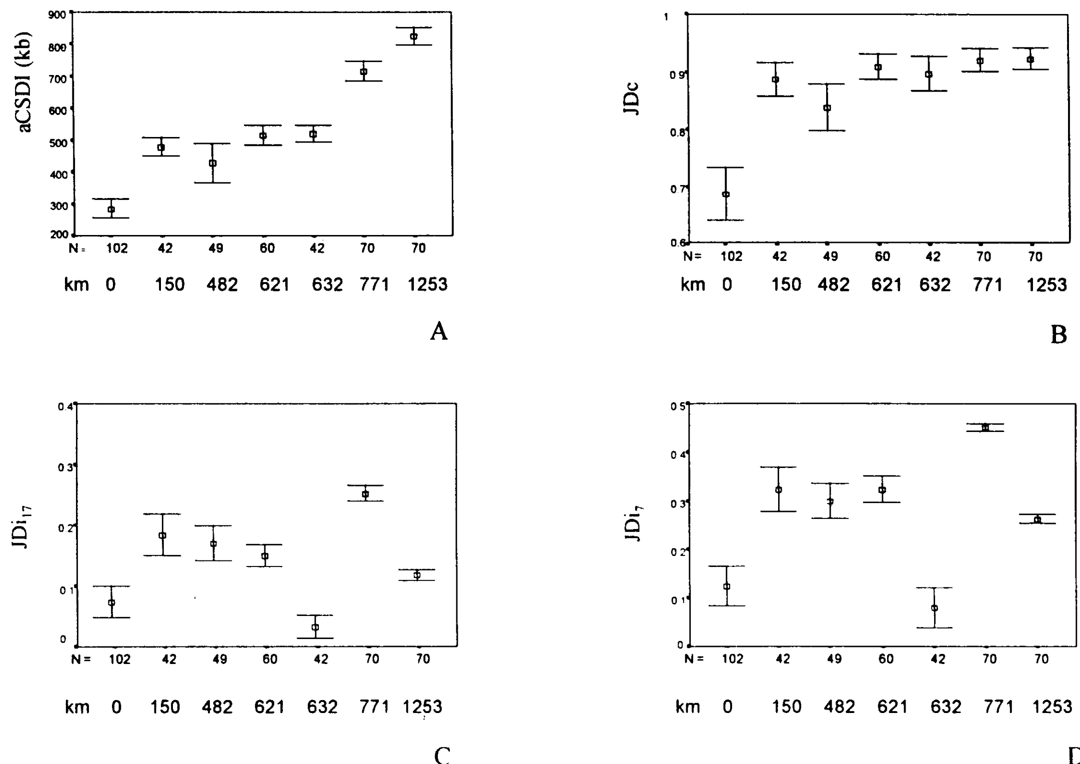

Fig. 2. Relationship between molecular and geographical distances. Average molecular distance and 95% confidence interval, calculated for N pairs of isolates separated by the respective geographical distance (expressed in km): A, aCSDI; B, JDc; C, JDi_{17}; D, JDi_7.

ography was performed by a non-parametric Mantel test (Mantel, 1967). Briefly, this test relies on a Monte Carlo simulation with 10^4 iterations which randomly permutes the different cells of one of the matrices. Contrary to the classical correlation test, this randomization procedure does not need any assumption about the number of degrees of freedom.

RESULTS

Dendrograms from chromosome and isoenzyme data

Four estimators of molecular polymorphism were calculated between all pairs of isolates and dendrograms constructed: a CSDI (Fig. 1A) and Jaccard distance (JDc, Fig. 1B) were considered for chromosomes and, Jaccard distance for 17 (JDi_{17}, Fig. 1C) and 7 polymorphic (NH2, G6PD, GOT, IDH, ME1, PGM and PEP2; JDi_7, Fig. 1D) isoenzyme loci. Analysis of the dendrograms revealed similarities as well as differences.

A relative convergence between chromosome and isoenzyme data was illustrated by the fact that 13/30 isolates clustered the same way on dendrograms constructed from the respective estimators. For instance, most isolates from the Biogeographical Unit (BGU) of Huancabamba (labelled * on Fig. 1) were gathered together. Another example was a group of 5 isolates coming from the BGU of Surco-South (labelled + on Fig. 1).

Differences concerned the degree of genetic diversity revealed by the 2 sets of markers. On dendrograms built up from chromosome data processed by aCSDI (Fig. 1A) and Jaccard distance (Fig. 1B), 29 and 27 different genotypes respectively were individualized. This value was much lower, when using isoenzyme data, as 11 different genotypes only were evidenced by the respective dendrograms (Fig. 1C and D). In addition, some members of the same zymodeme (indicated in bold, in Fig. 1) presented important chromosomal size-differences: for instance an aCSDI value of ± 500 kb (for 5 chromosomes together) was observed between LH696 and LH78 (both zymodeme 7) or between 2 groups of zymodeme 4 (La36, LH249, LCA11, LCA09 and LCA08 on one hand and LH115, LC292 and LC468 on the other hand, Fig. 1A).

Comparison of distances calculated on chromosome and isoenzyme data

In order to analyse chromosome and isoenzyme polymorphism more finely, correlations were calculated by a Mantel test (i) between the 4 previous molecular distances and, (ii) between the latter and the geographical distance separating the foci of origin of the isolates. All correlations were found to be significant ($P < 0.001$). However, the degree of relationship with geography varied strongly from one distance to the other, being the more regular for aCSDI (Fig. 2). This was further illustrated by the

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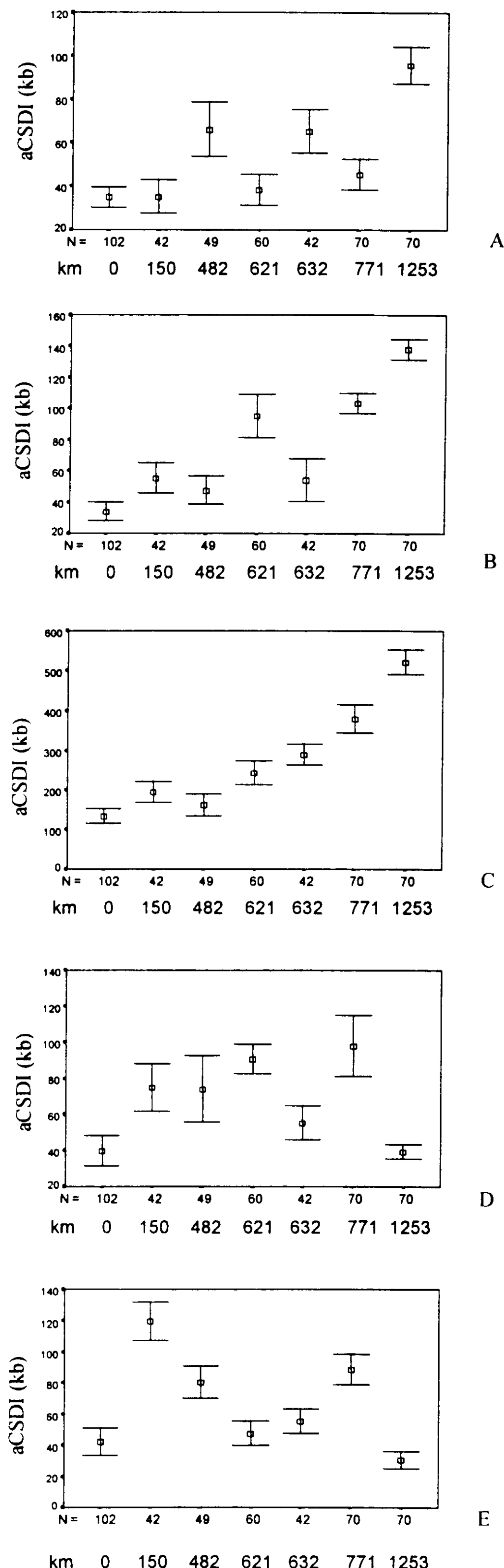

Fig. 3. Size variation of individual chromosomes according to geography. Average genomic distance (aCSDI) and 95% confidence interval, calculated for N pairs of isolates separated by the respective geographical distance (expressed in km): A, 134Sg; B, 168; C, 22; D, 149; E, 134Sp.

Table 1. Size variation of individual chromosomes (named according to the probe used to recognize them) and geography: coefficient of linear correlation (r_{lin}); R-squared value for linear (r^2_{lin}) and second order polynomial (r^2_{pol}) trend

Chromosome	r_{lin}	r^2_{lin}	r^2_{pol}
134Sg	0·473	0·22	0·25
168	0·673	0·45	0·46
22	0·732	0·54	0·56
149	0·060	0·0036	0·1479
134Sp	−0·139	0·02	0·15

r values on the respective linear regressions, that ranked as following: aCSDI (0·76) > Jdc (0·44) > JDi_7 (0·28) > JDi_{17} (0·16).

Finally, in order to determine whether size variation of each chromosome was equally correlated with geography, a Mantel test was performed between aCSDI of each of them and geographical distance. Results were highly significant ($P < 0{\cdot}001$) for each of the 5 chromosomes. However, the type of relationship differed. Three chromosomes showed a progressive size increase with geographical distance (variation in cline: 134Sg, 168 and 22) as shown on Fig. 3A–C and illustrated by the positive and high coefficient of linear correlation (Table 1). In contrast, such a cline was not observed for chromosomes 134Sp and 149. Indeed, their size first increased together with geographical distance and then decreased (Fig. 3D, E) and followed more closely a second-order polynomial trend as indicated by a higher r^2 for this function than for a linear one (Table 1).

DISCUSSION

Two types of molecular events are mostly responsible for DNA polymorphism in eukaryotes, in the absence of genetic exchange: point mutations within DNA sequences and rearrangement of DNA sequences, respectively referred to in present paper as genetic and genomic variation. Our aim was to compare quantitatively polymorphism due to these 2 types of events, in 4 allopatric populations of *L.* (*V.*) *peruviana*, by comparing through different estimators, the polymorphism in size of 5 chromosomes with the isoenzyme polymorphism at 17 loci.

Similarity was illustrated by 2 observations. First, 13/30 isolates clustered similarly on dendrograms constructed from the respective estimators. Secondly, a significant correlation ($P < 0{\cdot}001$) was revealed by a Mantel test (i) between chromosome and isoenzyme distances and (ii) between chromosome and isoenzyme distance on one hand and geographical distance on the other hand. This implies that rearrangements responsible for the chromosome size-polymorphism observed here and

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point mutations associated with isoenzyme polymorphism are evolutionarily correlated. Such a congruence between genomic and genetic characters could be explained by the strong eco-geographical structuring of the 4 populations under study (Dujardin *et al*. 1993*b*).

However, further analysis highlighted interesting differences between chromosome and isoenzyme polymorphisms. Indeed, there were twice as many genotypes defined by chromosome analysis than by isoenzyme analysis, and these chromosomal genotypes could be very different (up to 500 kb size difference for 5 chromosomes together, within a single zymodeme), Such a difference in genetic diversity was also observed in *L*. (*L*.) *infantum* (Blaineau *et al*. 1992). It could be due to difference in the resolving powers of the 2 characterization methods, but it likely indicates a faster molecular clock for the chromosomes under study than for the isoenzymes. This is supported by previous observations. Indeed, in unicellular organisms, recorded point mutation rates/gene/generation range between 10^{-9} and 10^{-6} (Dobzhansky *et al*. 1977), while in *Sacharomyces cerevisiae*, the frequency of amplification of ribosomal DNA (leading to chromosome size polymorphism) was evaluated at about 5×10^{-3}/generation (Szostak & Wu, 1980). Furthermore, size polymorphism of some chromosomes can already be observed in *Leishmania* strains cultivated over a period of 4 years (Dujardin, 1995*b*).

An additional difference between chromosome and isoenzyme data concerned the relationship between molecular polymorphism and geographical distance. Indeed, even if genomic and genetic distances were significantly correlated with geography, this relationship was more pronounced for both estimators of chromosome polymorphism. This observation is not unique to *Leishmania*: contrasts between geographical patterns of allelic variation at loci coding for proteins and patterns of chromosomal variation have been reported in other organisms (Ayala, Powell & Tracey, 1972; Nevo *et al*. 1974, 1994). In the leishmanial populations under study, several factors should be taken into consideration for interpretation of this phenomenon. First, the type of molecular distance might play a role. Among estimators of chromosome dissimilarity, aCSDI indicated a better relationship to geography than Jaccard distance did. This confirms previous reports on the more informative power of estimators that consider chromosomes as a continuous (like aCSDI, Dujardin *et al*. 1995*a*) rather than a discrete (like Jaccard's distance) variable. Secondly, differences between chromosomes and isoenzymes versus geography are certainly influenced by the different molecular clocks mentioned above. Thirdly, differences might also come from factors sorting out molecular polymorphism. A close relationship between molecular and geographical distances (as here observed for chromosomes) may be due to genetic drift (Tabachnick & Black, 1995). Optimal conditions for genetic drift certainly occur in the present case: the small size of parasite populations (Nei, 1987) and the insulated nature of the Andean valleys where *L*. (*V*.) *peruviana* is endemic. Accordingly, our results suggest that chromosomes, thanks to their faster molecular clock, show the effect of genetic drift on *L*. (*V*.) *peruviana* populations better than isoenzymes.

Genetic drift, however, is apparently not the only factor responsible for the chromosomal cline. Indeed, the type of relationship with geography varied from one chromosome to the other. A similar observation in natural populations of *Drosophila melanogaster* was explained by the action of selection, as genetic drift alone would affect all loci similarly (Singh & Rhomberg, 1987). In the case of *L*. (*V*.) *peruviana*, different selective factors might be identified: the different eco-geographical origin of the populations studied (Lamas, 1982), and their transmission by different sandfly vectors all along the cline (A. Caceres & J. C. Dujardin, unpublished observations; Perez *et al*. 1991; Davies *et al*. 1993; Villaseca *et al*. 1993). Intrinsic chromosomal characteristics also support the hypothesis of an adaptative variation: indeed, the variation of 3 of the chromosomes considered here (134Sp, 22 and 149) was associated with amplification/deletion of essential repeated genes (respectively coding for (i) gp63 (Dujardin *et al*. 1994; Victoir *et al*. 1995) the major surface glycoprotein of *Leishmania* involved in virulence, (ii) rRNA, Inga *et al*. 1998, and (iii) mini-exon, unpublished data) with potential phenotypic and adaptative consequences due to positional effects or gene dosage. In contrast, isoenzyme variation is classically considered to reflect neutral mutations (Nei, 1987) even if contradictory results have been obtained (Karl & Avise, 1992). In this context, the difference observed between variation of chromosomes and of isoenzymes might also reflect differences in selective pressures on the respective characters.

The hypothesis of adaptative significance of genomic polymorphism is not new nor specific to *Leishmania*. Studies on rates of chromosomal, protein and anatomical evolution in mammals has already led to the hypothesis that gene rearrangements might be more important than point mutations as sources for evolutionary changes (Wilson, Sarich & Maxson, 1974). This hypothesis is certainly worthwhile exploring further in *Leishmania* and other parasitic protozoa as it might generate novel answers to the numerous questions raised by the phenotypic diversity of these organisms.

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REFERENCES

AYALA, F. J., POWELL, J. R. & TRACEY, M. L. (1972). Enzyme variability in the *Drosophila willistoni* group. V. Genic variation in natural populations of *Drosophila equinoxialis*. *Genetics Research* **20**, 19–42.

BAÑULS, A. L. (1993). Analyse génétique d'isolats naturels sud-américains de *Leishmania* montrant de possibles phénomènes de recombinaison. DEA thesis, Université de Montpellier II.

BEN ABDERRAZAK, S., GUERRINI, F., MATHIEU-DAUDE, F., TRUC, P., NEUBAUER, K., LEWICKA, K., BARNABE, C. & TIBAYRENC, M. (1993). Isozyme electrophoresis for parasite characterization. In *Protocols in Molecular Parasitology* (ed. Walker, J. M.). vol. 21, pp. 361–382. Humana Press, Totowa, New Jersey, USA.

BLAINEAU, C., BASTIEN, P. & PAGES, M. (1992). Multiple forms of chromosome I, II and V in a restricted population of *Leishmania infantum* contrasting with monomorphism in individual strains suggest haploidy or automixy. *Molecular and Biochemical Parasitology* **50**, 197–204.

DAVIES, C. R., FERNANDEZ, M., PAZ, L., RONCAL, N. & LLANOS-CUENTAS, A. (1993). *Lutzomyia verrucarum* can transmit *Leishmania peruviana*, the aetiological agent of Andean cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 603–606.

DOBZHANSKY, T., AYALA, F., STEBBINS, G. L. & VALENTINE, J. W. (1977). *Evolution*. W. H. Freeman and Company, San Francisco.

DUJARDIN, J. C. (1995*b*). Significations de la variabilité caryotypique chez les leishmanies néotropicales. Ph.D. thesis, VUB, Brussels.

DUJARDIN, J. C., AREVALO, J., LLANOS-CUENTAS, A., CACERES, A. & LE RAY, D. (1993*c*). From Pulsed Field to Field: contribution of molecular karyotyping to epidemiological studies on New World Leishmaniasis. *Archives de l'Institut Pasteur de Tunis* **70**, 505–515.

DUJARDIN, J. C., DE DONCKER, S., VICTOIR, K., LE RAY, D., HAMERS, R. & AREVALO, J. (1994). Size polymorphism of chromosomes bearing gp63 genes in *Leishmania* of the *braziliensis* complex: indication of a rearrangement of the gp63 genes in *L. braziliensis* and *L. peruviana*. *Annals of Tropical Medicine and Parasitology* **88**, 445–448.

DUJARDIN, J. C., DUJARDIN, J. P., TIBAYRENC, M., TIMPERMAN, G., DE DONCKER, S., JACQUET, D., AREVALO, J., LLANOS-CUENTAS, A., GUERRA, H., BERMUDEZ, H., HAMERS, R. & LE RAY, D. (1995*a*). Karyotype plasticity in Neotropical *Leishmania*: an index for measuring genomic distance among *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* populations. *Parasitology* **110**, 21–30.

DUJARDIN, J. C., GAJENDRAN, N., AREVALO, J., LLANOS-CUENTAS, A., GUERRA, H., GOMEZ, J., ARROYO, J., DE DONCKER, S., JACQUET, D., HAMERS, R. & LE RAY, D. (1993*a*). Karyotype polymorphism and conserved characters in the *Leishmania* (*Viannia*) *braziliensis* complex explored with chromosome-derived probes. *Annales de la Société belge de Médecine tropicale* **73**, 101–118.

DUJARDIN, J. C., GAJENDRAN, N., HAMERS, R., MATHIJSEN, G., URJEL, R., RECACOECHEA, M., VILLAROEL, G., BERMUDEZ, H., DESJEUX, P., DE DONCKER, S. & LE RAY, D. (1987). Leishmaniasis in the Lowlands of Bolivia. VII. Characterization and identification of Bolivian isolates by PFG karyotyping. In *Leishmaniasis: the First Centenary* (*1885–1985*). *New Strategies for Control* (ed. Hart, D.), NATO ASI series A, vol. 163, pp. 137–148. Plenum Press, New York.

DUJARDIN, J. C., LLANOS-CUENTAS, A., CACERES, A., ARANA, M., DUJARDIN, J. P., GUERRINI, F., GOMEZ, J., ARROYO, J., DE DONCKER, S., JACQUET, D., HAMERS, R., GUERRA, H., LE RAY, D. & AREVALO, J. (1993*b*). Molecular karyotype variation of *Leishmania* (*Viannia*) *peruviana* evidences geographical populations in Peru along a North–South cline. *Annals of Tropical Medicine and Parasitology* **87**, 335–347.

GIANNINI, S. H., CURRY, S. S., TESH, R. & VAN DER PLOEG, L. H. T. (1990). Size-conserved chromosomes and stability of molecular karyotype in cloned stocks of *Leishmania major*. *Molecular and Biochemical Parasitology* **39**, 9–22.

GODFREY, D. G. (1979). The zymodemes of trypanosomes. In *Problems in the Identification of Parasites and their Vectors* (ed. Taylor, A. E. R. & Muller, R.), **17**, pp. 31–54. Academic Press, London.

GUERRINI, F. (1993). Génétique des populations et phylogénie des *Leishmania* du Nouveau Monde. Thèse de doctorat, Université de Montpellier II.

HENRIKSSON, J., PETTERSSON, U. & SOLARI, A. (1993). *Trypanosoma cruzi*: correlation between karyotype variability and isoenzyme classification. *Experimental Parasitology* **77**, 334–348.

INGA, R., DE DONCKER, S., GOMEZ, J., LOPEZ, M., GARCIA, R., LE RAY, D., AREVALO, J. & DUJARDIN, J. C. (1998). Relation between variation in copy number of ribosomal RNA encoding genes and size of harbouring chromosomes in *Leishmania* of subgenus *Viannia*. *Molecular and Biochemical Parasitology* **92**, 219–228.

JACCARD, P. (1908). Nouvelles recherches sur la distribution florale. *Bulletin de la Société vaudoise de Sciences naturelles* **44**, 223–270.

KARL, S. A. & AVISE, J. C. (1992). Balancing selection at allozyme loci in oysters: implications from nuclear RFLP's. *Science* **256**, 100–102.

LAMAS, G. (1982). A preliminary zoogeographical division of Peru, based on butterfly distributions (Lepidoptera, Papilionoidea). In *Biological Diversification in the Tropics* (ed. Prance, T. P.), pp. 336–357. Columbia University Press, New York.

LIGHTHALL, G. K. & GIANNINI, S. H. (1992). The chromosomes of *Leishmania*. *Parasitology Today* **8**, 192–199.

MANTEL, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**, 209–220.

NEI, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.

NEVO, E., FILIPUCCI, M. G., REDI, C., KOROL, A. & BEILES, A. (1994). Chromosomal speciation and adaptative radiation of mole rats in Asia Minor correlated with increased ecological stress. *Proceedings of the National Academy of Sciences, USA* **91**, 8160–8164.

NEVO, E., KIM, Y. J., SHAW, C. R. & THAELER, C. S. JR. (1974). Genetic variation, selection and speciation in

https://doi.org/10.1017/S0031182098003357 Published online by Cambridge University Press

Thomomys talpoides pocker gophers. *Evolution* **28**, 1–23.

PACHECO, R. S., LOPES, U. G., MOREL, C. M., GRIMALDI, G. & MOMEN, H. (1986). Schizodeme analysis of *Leishmania* isolates and comparison with some phenotypic techniques. In *International Symposium on Taxonomy and Phylogeny of Leishmania* (ed. Rioux, J. A.), pp. 57–65. IMEEE, Montpellier.

PEREZ, E., VILLASECA, P., CACERES, A., LOPEZ, M., ZOLESSI, A., CAMPOS, M., GUERRA, H. & LLANOS-CUENTAS, A. (1991). *Leishmania* (*Viannia*) *peruviana* isolated from the sandfly *Lutzomyia peruensis* (Diptera: Psychodidae) and a sentinel hamster in the Huayllacallan valley, Ancash, Peru. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 60.

SARAVIA, N. G., WEIGLE, K., GIANNINI, S. H., PACHECO, R., LABRADA, L. A. & GONCALVES, A. (1990). Recurrent lesions in human *Leishmania braziliensis* infection-reactivation or reinfection. *Lancet* **336**, 398–402.

SCHWARTZ, D. C., SAFRAN, N., WELSH, J., HAAS, R., GOLDENBERG, M. & CANTOR, C. R. (1983). New techniques for purifying large DNAs and studying their properties and packaging. *Cold Spring Harbour Symposium in Quantitative Biology* **47**, 189–195.

SINGH, R. S. & RHOMBERG, L. R. (1987). A comprehensive study of genic variation in natural populations of *Drosophila melanogaster*. 2. Estimates of heterozygosity and patterns of geographic differentiation. *Genetics* **117**, 255–271.

SZOSTAK, J. W. & WU, R. (1980). Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*. *Nature*, *London* **284**, 426–430.

TABACHNICK, W. J. & BLACK, W. C. (1995). Making a case for molecular population genetics studies of arthropod vectors. *Parasitology Today* **11**, 27–30.

TIBAYRENC, M. & AYALA, F. J. (1987). Forte corrélation entre classification isoenzymatique et variabilité de l'ADN kinétoplastique chez *Trypanosoma cruzi*. *Comptes Rendus de l'Académie des Sciences de Paris* **304**, Série III, 89–92.

TIBAYRENC, M., NEUBAUER, K., BARNABE, C., GUERRINI, F., SKARECKY, D. & AYALA, F. J. (1993). Genetic characterization of six parasitic protozoa: parity between random-primer DNA typing and multilocus enzyme electrophoresis. *Proceedings of the National Academy of Sciences*, *USA* **90**, 1335–1339.

TOBIE, E. J., VON BRAND, T. & MEHLMAN, B. (1950). Cultural and physiological observations on *Trypanosoma rhodesiense* and *Trypanosoma gambiense*. *Journal of Parasitology* **36**, 48–54.

VICTOIR, K., DUJARDIN, J. C., DE DONCKER, S., BARKER, D. C., AREVALO, J., HAMERS, R. & LE RAY, D. (1995). Plasticity of gp63 gene organization in *Leishmania* (*Viannia*) *braziliensis* and *Leishmania* (*Viannia*) *peruviana*. *Parasitology* **111**, 265–273.

VILLASECA, P., LLANOS-CUENTAS, A., PEREZ, E. & DAVIES, C. (1993). A comparative field study of the relative importance of *Lutzomyia peruensis* and *Lutzomyia verrucarum* as vectors of cutaneous leishmaniasis in the Peruvian Andes. *American Journal of Tropical Medicine and Hygiene* **49**, 260–269.

WILSON, A. C., SARICH, V. M. & MAXSON, L. R. (1974). The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein and anatomical evolution. *Proceedings of the National Academy of Sciences*, *USA* **71**, 3028–3030.

WORLD HEALTH ORGANIZATION (1990). *Lutte contre les leishmanioses: rapport d'un Comité OMS d'experts*. Série des rapports techniques OMS. No. 793. WHO, Geneva.

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