water which was renewed every 24 h and aerated continuously by bubbling air (10–20 ml/min). The roots, still attached to the bulbs, were immersed in the different treatment solutions. 1 h treatment with 0.1% colchicine was used to produce tetraploid and aneuploid nuclei. 0.1% caffeine treatment for 2 h produced a binucleate population which entered interphase synchronously[10]. 10^{-4} M 3'-deoxyadenosine (3'AdR) or 100 µg/ml ethidium bromide (EB) were used to inhibit RNA synthesis. The roots were fixed in 10% formol:1% hydroquinone (1:1) for 1–2 h at room temperature, and the silver impregnation technique[11] was used for the staining of nucleoli and prenucleolar bodies.

Results and discussion. *Allium cepa* L. has only 1 pair of NOR which form 2 nucleoli. Nucleologenesis kinetics in control binucleate cells (figure 1) is obtained by scoring the frequency of binucleate cells with fully organized nucleoli, and then without any prenucleolar bodies, at different times after the end of caffeine labelling. Figure 3,a, shows a control cell in the process of reorganization, with the 2 incipient nucleoli and a large number of small prenucleolar bodies which appear to cover the chromosomes. When postmitotic RNA synthesis is inhibited by continuous treatment with 3'AdR or EB (as shown in figure 1) the nucleolus fails to form. The posttelophase nuclei can be seen to be full of prenucleolar bodies (figure 3,b) which are fewer but larger than prenucleolar bodies in control cells. Segregation of the prenucleolar bodies as well as the appearance of internal vacuoles are seen in some cells. Colchicine treatment induces tetraploid and multinucleated meristematic cells (figure 2). Cells with only 1 single tetraploid nucleus have extremely delayed nucleologenesis kinetics, since only 50% of the tetraploid mononucleate cells have fully organized nucleoli 12 h after the end of the colchicine treatment (see figure 2). Nevertheless, as seen in figure 3,c, these cells have small prenucleolar bodies that are non-fused even after 8 h of being in the nuclei. Moreover, in multinucleated tetraploid cells in which one of the nuclei does not contain any nucleolar organizer, chromosomes still have small non-fused prenucleolar bodies 24 h after colchicine as seen in figure 3,e. Hence the time prenucleolar bodies remain scattered in a cell does not modify their fusion properties. Tetraploid cells also treated with colchicine but submerged in a 3'AdR solution from the 6th h of the recovery (figure 2), had enlarged prenucleolar bodies (figure 3,d) when compared with cells not treated with the inhibitor after the same number of h. Figure 3,f, shows a multinucleate tetraploid cell 24 h after the end of colchicine treatment, where prenucleolar fusion is also evident when compared with control conditions (figure 3,e).

These data suggest that treatment with RNA synthesis inhibitors produces adhesiveness in prenucleolar bodies between themselves. This functional characteristic may be related to the finding that these prenucleolar bodies show ultrastructural features which make them different from those found in untreated meristems. Hence, the prenucleolar bodies of 3'AdR-treated cells show a cap formed by granules, resembling a sort of segregation where the granular component is scarce[12]. These changes in fusion properties of the prenucleolar bodies pose an intriguing question about the role of newly synthesized RNA in their assembly properties.

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2 A. Lepoint and G. Goessens, Exp. Cell Res. *117*, 89 (1978).
3 G. Morcillo, C. de la Torre and G. Giménez-Martín, Exp. Cell Res. *102*, 311 (1976).
4 G. Morcillo and C. de la Torre, Biol. cell. *36*, 1 (1979).
5 D.D. Brown and J.B. Gurdon, Proc. natl Acad. Sci. USA *70*, 1955 (1973).
6 S.G. Philips and D.M. Philips, Exp. Cell Res. *120*, 295 (1979).
7 D. Hernández-Verdún and M. Bouteille, VI Eur. Nucleolar Workshop Weimar, 1979, p.28.
8 G. Giménez-Martín, C. de la Torre, M.E. Fernández-Gómez and A. González-Fernández, J. Cell Biol. *60*, 502 (1974).
9 J.C. Stockert, M.C. Fernández-Gómez, G. Giménez-Martín and J.L. López-Sáez, Protoplasma *60*, 265 (1970).
10 G. Giménez-Martín, A. González-Fernández and J.F. López-Sáez, J. Cell Biol. *26*, 305 (1965).
11 M.E. Fernández-Gómez, J.C. Stockert, J.F. López-Sáez and G. Giménez-Martín, Stain Technol. *44*, 48 (1969).
12 A.M. Sacristán-Gárate and J.C. Stockert, Experientia *30*, 294 (1974).

Satellite DNA sequences and reproductive isolation in the *Drosophila willistoni* group[1]

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Summary. Satellite DNAs of the *Drosophila willistoni* group have been analyzed by Hae III endonuclease digestion. Differences were observed between the 2 species *D. willistoni* and *D. paulistorum* and also between semispecies of *D. paulistorum* and, to a lesser extent, between subspecies of *D. willistoni*. Differences did not appear to be consistently greater between strains producing sterile hybrid males than between those producing fertile hybrids.

Satellite DNAs are highly repeated sequences mainly located in heterochromatin but also in telomeric sites of eukaryotic chromosomes. The different hypotheses proposed about their function include 1. that they hinder recombination in the chromosome sections where they are located[2] and 2. that they play a role in speciation by hindering the pairing of homologous chromosomes during meiosis in interspecific hybrids[3–5]. The present work tests this 2nd hypothesis by studying highly-repeated satellite sequences in populations at different stages of evolutionary divergence.

The *Drosophila willistoni* group of species provides ideal material for the test, because the main stages of the speciation process can be identified within the group[6]. We have examined the satellite DNA sequences in 8 different strains: 1. *D. willistoni willistoni* from Santa Marta (Colombia); 2. *D. w. willistoni* from Tame (Colombia); 3. *D. willistoni quechua* from Lima (Peru); 4. *D. w. 'quechua'* from

Belize; 5. *D. paulistorum* semispecies Amazonian from Caicara (Venezuela); 6. *D. paulistorum* Amazonian from Tame; 7. *D. paulistorum* semispecies Andean-Brazilian from Caicara; and 8. *D. paulistorum* Andean-Brazilian from Mirassol (Brazil). 4 levels of evolutionary divergence are represented in these strains: a) local populations; b) subspecies - *D. w. willistoni* and *D. w. quechua* - which produce sterile male hybrids but exhibit no sexual isolation and thus represent the first stage of speciation[6]; c) semispecies or incipient species - Amazonian and Andean-Brazilian of *D. paulistorum* - which produce sterile male hybrids and exhibit some sexual isolation and thus represent the second stage of speciation; d) fully reproductively isolated but closely related species - *D. willistoni* and *D. paulistorum*.

DNA was extracted from purified nuclei of 7 to 8 g of each sex of each strain using whole bodies of adult individuals, essentially according to Cordeiro-Stone and Lee[7]. The DNA was finally purified by preparative CsCl gradient centrifugation. DNA molecular weight was determined by band sedimentation in a Spinco model E ultracentrifuge according to the method of Studier[8].

Satellite DNA bands could not be isolated from the main band DNA of the different *Drosophila* species by the conventional methods of fractionation of total native DNA in CsCl, Hg^{++}-Cs_2SO_4 and Ag^{+}-Cs_2SO_4 preparative gradients. Therefore highly repeated satellite DNAs were isolated by DNA denaturation and reannealing to a Cot of 2 or 10 and subsequent fractionation on a preparative Ag^{+}-Cs_2SO_4 gradient. With this technique and using a Cot of 10, as previously shown for human and mouse DNA[9,10], unique, middle repeated intermediate DNA, and satellite highly repeated DNAs can be separated as distinct peaks because they bind different amounts of Ag^{+} according to the extent of their double-strandedness. At a Cot of 2, satellite DNA can be separated from unique and intermediate DNA. The satellite DNA appears to be lighter in Ag^{+}-Cs_2SO_4 because, having completely reannealed, it binds less Ag^{+} than the unique and intermediate DNA (figure 1).

This technique offers the advantage of separating all the satellite sequences, since no satellite DNA remains in the middle repeated and unique DNA peaks as long as the DNA size is sufficiently smaller than the presumed length of satellite DNA blocks in the genome. For this reason, *Drosophila* DNA of a size of 6000-7000 nucleotide pairs was used. That the satellite DNA obtained by this technique is in the reannealed, rather than native, state is not a disadvantage with respect to restriction enzyme digestion, since satellite DNA reacquires its full double-strandedness after reannealing to a Cot of 2 or higher, as judged from melting curves of reannealed DNA and increase in CsCl density on denaturation.

Hae III restriction endonuclease digestion was carried out on unique, middle repeated, and satellite DNAs of male and female individuals of each of the 8 *Drosophila* strains. Only a smear with no discrete bands was observed in the agarose gel electrophoresis pattern of DNA fragments after Hae III digestion with unique and middle repeated DNA. This confirms that no satellite sequence had remained bound to unique and middle repeated DNA. Agarose gel electrophoresis patterns of satellite DNA fragments of 4 *Drosophila* strains after Hae III endonuclease digestion are shown in figure 2. The relative amount in the fragments was estimated from the area under the peak corresponding to each fragment on microdensitometer tracings.

In *D. willistoni* the pattern of digestion manifests 11 fragments. The 2 major peaks have a size of 600,000 daltons and 410,000 daltons. There are no consistent differences between the strains or subspecies of *D. willistoni* in the amount of these 2 fragments.

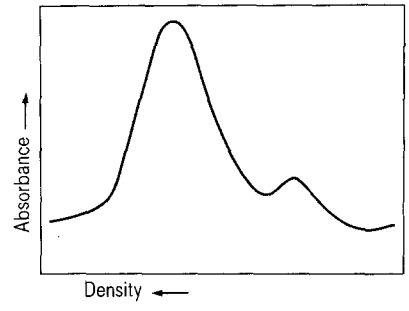

Fig. 1. Microdensitometer tracing of total nuclear DNA from female *Drosophila paulistorum* Andean-Brazilian (Caicara). The DNA was denatured and renatured to Cot 2, then centrifuged in an analytical Ag^{+}-Cs_2SO_4 equilibrium density gradient at a DNA concentration of 35 µg/ml and an Ag^{+}/DNA-P molar ratio of 0.2.

Summary of the differences between *Drosophila* strains in the relative amounts of Hae III endonuclease-digested satellite DNA fragments

Strain	Fragment size in thousands of daltons					
	1200	900	760	550	510	450
D. w. willistoni Santa Marta	–	–	+	4%	–	7–8%
D. w. willistoni Tame	–	–	+	< 1%	–	1–2%
D. w. quechua Lima	–	–	+	4%	–	7–8%
D. w. quechua Belize	–	–	+	< 1%	–	7–8%
D. paulistorum Amazonian Caicara	+	–	–	10%	5%	1%
D. paulistorum Amazonian Tame	+	+	–	15%	1%	1%
D. paulistorum Andean-Brazilian Caicara	+	–	–	15%	1%	13%
D. paulistorum Andean-Brazilian Mirassol	+	–	–	10%	5%	1%

+, present; –, absent.

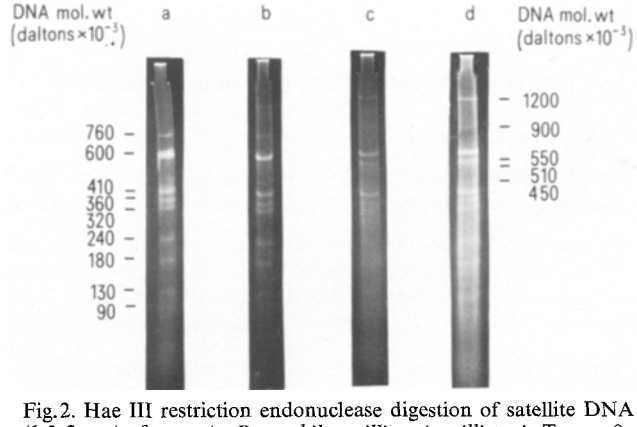

Fig. 2. Hae III restriction endonuclease digestion of satellite DNA (1.5-2 µg) from a) *Drosophila willistoni willistoni* Tame ♀; b) *D. w. quechua* Belize ♂; c) *D. paulistorum* Amazonian Caicara ♀; and d) *D. paulistorum* Amazonian Tame ♂. DNA was digested by Hae III endonuclease and run on 2.5% agarose gel electrophoresis, as reported by Southern[11]. Fragments were sized by comparison with the mobilities of the Hae III restriction digests of lambda phage and SV40 DNA[12].

However, 2 other fragments with 550,000 and 450,000 daltons mol.wt vary considerably in relative amount in strains and subspecies. The 550,000-daltons fragment accounts for 1% or less of the total satellite DNA in *D. w. willistoni* Tame and in *D. w. quechua* Belize, whereas it accounts for about 4% of the total satellite in *D. w. willistoni* Santa Marta and in *D. w. quechua* Lima. The 450,000-daltons fragment accounts for a relative amount of 7–8% in *D. w. willistoni* Santa Marta and in the 2 strains of *D. w. quechua*, while it accounts for only 1–2% in *D. w. willistoni* Tame.

The 11 fragments observed in *D. willistoni* are present with the same mol.wts in the *D. paulistorum* strains, with the exception of the largest fragment which has a mol.wt of 1,200,000 daltons in *D. paulistorum*, instead of 760,000 daltons in *D. willistoni*. Besides this difference, the *D. paulistorum* Amazonian from Tame has a fragment with a mol.wt of 900,000 daltons that is absent in the other *D. paulistorum* strains.

The 550,000 daltons fragment is present in *D. paulistorum* satellite DNA in a relative amount of about 10% in the Amazonian semispecies from Caicara and Andean-Brazilian semispecies from Mirassol, but of 15% in the other 2 strains; these amounts are all consistently higher than those observed in *D. willistoni*. Furthermore an additional fragment of 510,000 daltons is present in *D. paulistorum* Amazonian Caicara and Andean-Brazilian Mirassol in a relative amount of 5%, and in a relative amount of 1% in the other 2 strains of *D. paulistorum*. The relative amount of the 450,000-daltons fragment, which is variable in *D. willistoni*, varies in *D. paulistorum* as well; it accounts in Andean-Brazilian Caicara for 13%, and for about 1% of the total satellite in the other 3 strains. Moreover, the 410,000-daltons fragment is present in slightly different amounts in the *D. paulistorum* strains (see table for a summary).

In conclusion, even though the similarity in satellite-DNA digestion patterns in the strains studied reflects their close phylogenetic relationship, substantial differences exist between the 2 species, *D. willistoni* and *D. paulistorum*. Moreover, clear differences also exist between the strains of *D. paulistorum* and lesser differences between the strains of *D. willistoni*. However, strains of different subspecies or semispecies, which produce sterile hybrid males, are not consistently more different than strains of the same subspecies or semispecies, which produce fertile males and females. Hence, the sterility of the hybrid males cannot simply be explained in terms of the observed satellite DNA differences.

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2 M. Yamamoto and G.L.G. Miklos, Chromosoma *66*, 71 (1978).
3 P.M.B. Walker, Nature *229*, 306 (1971).
4 G. Corneo, Experientia *34*, 1141 (1978).
5 G. Corneo, Evol. Theory *1*, 261 (1976).
6 F.J. Ayala, M.L. Tracey, D. Hedgecock and R.C. Richmond, Evolution *28*, 576 (1975).
7 M. Cordeiro-Stone and S.C. Lee, J. mol. Biol. *104*, 1 (1976).
8 F.W. Studier, J. mol. Biol. *11*, 373 (1965).
9 E. Ginelli and G. Corneo, Chromosoma *56*, 55 (1976).
10 E. Ginelli, R. Di Lernia and G. Corneo, Chromosoma *61*, 215 (1977).
11 E.M. Southern, J. mol. Biol. *94*, 51 (1975).
12 P. Lebowitz, W. Siegel and J. Sklar, J. molec. Biol. *88*, 105 (1974).

Absorption of glycine and proline from the small intestine of rats infected with *Eimeria nieschulzi*

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Summary. The absorption of glycine and proline through the jejunum and ileum of rats with an *Eimeria nieschulzi* infection was impaired when the amino acids were presented to the mucosal surface as either a mixture or the dipeptide, glycyl-proline.

Coccidial infections in chickens affect the absorption of proteins, amino acids, lipids, carbohydrates and certain minerals (see reviews by Turk[1] and Ruff[2]). Amino acid studies in infected chickens have been restricted to presenting the compounds in a free form. However, it is now generally accepted that a substantial proportion of dietary amino acid is absorbed from the gut in dipeptide form and hydrolysed to amino acid within the mucosal cells of the small intestines[3]. Therefore, we have studied the absorption from both free amino acid and dipeptide through the small intestines of rats infected with *Eimeria nieschulzi*, hitherto not previously examined.

Materials and methods. The amino acids used were glycine and proline, and the peptide was glycyl-proline. These substances were chosen because both glycine and proline are well absorbed when presented to the rat small intestine either as free amino acid[4] or as glycyl-proline[5,6]. In addition, proline and glycine can be quantitatively distinguished in experimental samples due to their different spectra after reaction with ninhydrin reagent, and although some glycyl-proline can escape hydrolysis in the gut wall, the proportion in rats is small[6].

Male Wistar rats (200–250 g) were each orally inoculated with approximately 200,000 sporulated oocysts of a strain of *E. nieschulzi* supplied by Dr Dawn Owen, and 5 days later absorption from the small intestine was assessed in both infected and an equal number of uninfected controls, using the everted sac method[7]. Samples were analyzed by a ninhydrin-based method. Infection was confirmed by microscopical examination and pieces of intestine were taken from some rats for histological examination by light- and stereoscan electron microscopy. There observations showed that by the 5th day of infection many epithelial cells of the villi contained schizonts (figure 1, e) and that a change had occurred in villous architecture, from a spatulate shape (figure 1, a and b) to a more shortened and thickened appearance (figure 1, c and d) but with little or no cellular breakdown.

The effects of infection upon absorption can be seen in figure 2. In 6 of the 8 comparisons made, poorer absorption