Insect Molecular Biology (2004) **13**(6), 581–585

Tiny genomes and endoreduplication in Strepsiptera

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Abstract

Using flow cytometry, the genome sizes of two species of Strepsiptera were studied: that of male *Caenocholax fenyesi texensis* Kathirithamby & Johnston (Myrmecolacidae) at 108 Mb, which is the smallest insect genome documented to date; and those of male and female *Xenos vesparum* Rossi (Stylopidae), which are 1C = 130 and 133 Mb, respectively. The genome sizes of the following were analysed for comparative purposes: (a) the Hessian fly, *Mayetiola destructor* (Say), which was previously reported to be the smallest among insects: the male measured at 1C = 121 Mb and the female at 1C = 158 Mb; and (b) the female parasitic, haplodiploid, microhymenopteran wasp, *Trichogramma brassicae* Bezdenko, which measured at 1C = 246 Mb. The hosts of the strepsipterans were also measured: male *Solenopsis invicta* Buren, the red imported fire ant (host of male *C. f. texensis*), which is 1C = 753.3 Mb, and female *Polistes dominulus* Christ, the paper wasp (host of *X. vesparum*), is 1C = 301.4 Mb. Endoreduplication (4C) of the genome of the thorax of the male strepsipteran, and higher levels of endoduplication (4, 8, 16C) in the body of the larger female was observed. In contrast, little or no endoreduplication was observed, either in the Hessian fly, or in the parasitic wasp.

Keywords: Strepsiptera, genome size, endoreduplication, flow cytometry.

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Introduction

What is known of the cytogenetics of Strepsiptera (Insecta) is limited to a few chromosome counts (Hughes-Schrader, 1924; Noskiewicz & Poluszynski, 1928; Silvestri, 1941, 1942; Lauterbach, 1954; Baumert, 1959). Most Strepsiptera are found as free-living males that are attracted to traps, and the short-lived adult male has little or no material suitable for chromosome preparations. Accordingly, we decided to examine the cytogenetics of Strepsiptera quantitatively, using flow cytometry.

Strepsiptera are koinobionts (parasitoids that allow the host to continue to develop after parasitization), and present an interesting mix of forces that could potentially impact upon genome size evolution: including (1) small (1.5–6 mm), free-living, short-lived adult males (*c.* 5–6 h) and females which are relatively large (1.5–3.9 mm), neotenic, totally endoparasitic, cylindrical and devoid of all adult insect characters (such as eyes, antennae, mouthparts, legs, wings and external genitalia) (Kathirithamby, 1989); (2) very high reproductive rates (the ovoviviparous, neotenic females are among the most fecund insects known, producing over a million 1st instar larvae in some species); and (3) long life span of host (although the host loses its reproductive potential, it remains alive until all the free-living 1st instar larvae have emerged from the endoparasitic mother).

We measured the genome sizes of two strepsipteran species because they are unknown in this group, and we found that like every other aspect of strepsipteran development, this has unusual features (Kathirithamby, 1989, 2000). The two species measured were: male *Caenocholax fenyesi texensis* Kathirithamby & Johnston (Myrmecolacidae), and *Xenos vesparum* Rossi (Stylopidae). For comparative purposes we re-examined the genome size of the Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyidae), whose male genome was thought to be the smallest among insects. We also examined the genome of the parasitic wasp, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), to determine if its small size and parasitic lifestyle was associated with a small genome. Finally, we measured the genome sizes of the hosts of the strepsipterans to check for contamination and these are given for the first time:

Solenopsis invicta Buren (Hymenoptera: Formicidae), the red imported fire ant (host of male *C. f. texensis*), and *Polistes dominulus* Christ (Hymenoptera: Vespidae), the paper wasp (host of *X. vesparum*).

Results

Mixtures of nuclei from *C. elegans* and from the head of a single male *C. f. texensis* produced a single broadened 2C peak and a second 4C peak above the 4C of *C. elegans.* The respective 2C peaks of *C. elegans* and *C. f. texensis* were not resolved, in part because they were near one another, and in part because the *C. f. texensis* 2C nuclei were in considerable excess. This was not a problem with the 4C cells, where the peak representing 4C of *C. f. texensis* appeared immediately to the right of the only slightly smaller peak provided by the 4C nuclei from *C. elegans*. This difference provided the first evidence that the strepsipteran genome is close to that of the 100.4 Mb genome of *C. elegans* and, as such, should appear well to the left of the 2C peak of *D. melanogaster* Iso-1 (1C = 175 Mb) (Bennett *et al.*, 2003). Flow cytometric runs of PI stained nuclei from Strepsiptera using a *Drosophila* Iso-1 strain female standard confirmed this observation. Compared with *D. melanogaster*, the genome size of male *C. f. texensis* from College Station, Texas was 1C = 107.6 Mb. Nearly as small were the male and female genomes of *X. vesparum*: 1C = 129.9 and 133.0 Mb, respectively (Table 1).

The genome size of *S. invicta* (the host of the male *C. f. texensis*) is 1C = 753.3 Mb, and *P. dominulus* (the host of male and female stylopid *X. vesparum*) is 1C = 301.4 Mb (Table 1). No evidence of contamination of Strepsiptera by the hosts was observed.

Table 1. Genome size (1C) of Strepsiptera, Hessian fly, parasitic wasp and their hosts

Species	Sex	*N*	Average	SE
Caenocholax fenyesi texensis	M	2	107.6 Mb	0.7
Xenos vesparum	M	4	129.9 Mb	4.1
	F	4	133.0 Mb	5.2
Solenopsis invicta	M	4	753.3 Mb	1.8
Polistes dominulus	F	4	301.4 Mb	2.1
Trichogramma brassicae	F	4	245.6 Mb	0.9
Mayetiola destructor	M	3	120.9 Mb	1.2
	F	3	157.9 Mb	1.2

Endoreduplication varies between different insects and in parts of an insect. As is typical for most insects, nuclei scored from the head of the *D. melanogaster* Iso-1 female gave a major 2C (G_1) genome size peak and a greatly reduced 4C (G_2) peak (Fig. 1A,C). In contrast, nuclei scored from the head of the male strepsipteran produced G_1 and G_2 peaks of nearly equal height, with the number of nuclei in G_1 and G_2 nearly equal (Fig. 1B,C). The thorax of male *X. vesparum* gave only the 4C nuclei, indicative of either G_2 or endoreduplicated DNA (Fig. 1D). No 2C nuclei could be detected in the thorax, nor was there evidence of 8C, or higher ploidy nuclei. Nuclei scored from the *X. vesparum* female (shown on a log scale in Fig. 2) were 2C, 4C, 8C, 16C, often with nearly equal numbers of each. Immature females (not shown) produced 1C nuclei from

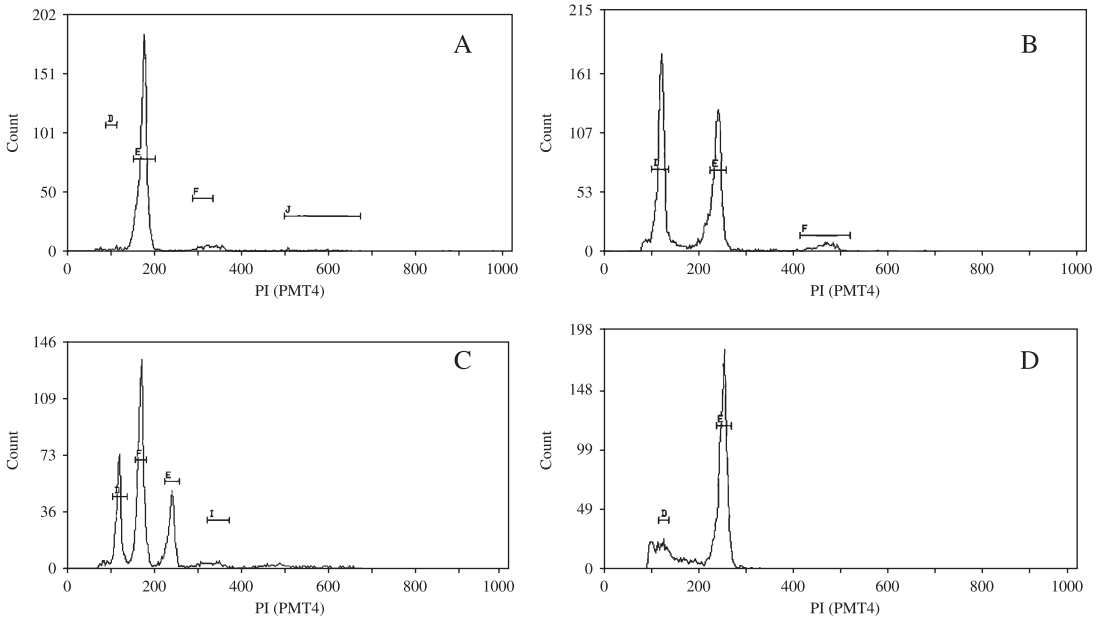

Figure 1. Diagrams showing the number of nuclei with differing levels of red fluorescence corresponding to binding of propidium iodide to the DNA of 2C and 4C nuclei in: (A) head of female *D. melanogaster* Iso-1 (1C = 175 Mb); (B) head of male *X. vesparum*; (C) co-prepared heads of male *X. vesparum* (1C = 129 Mb) and *D. melanogaster*; and (D) thorax of male *X. vesparum*.

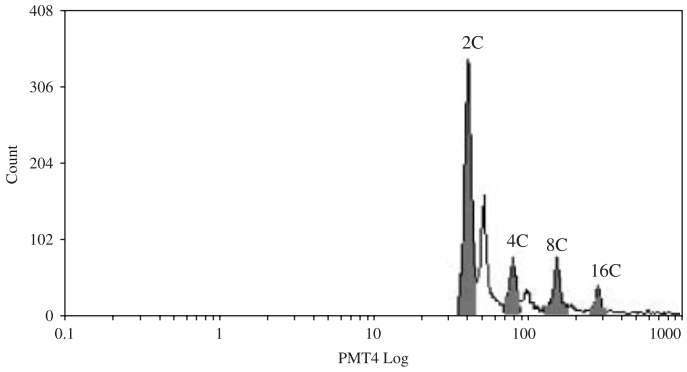

Figure 2. Propidium iodide fluorescence (PMT4) of DNA in nuclei from female *X. vesparum* (shaded) and *D. melanogaster* nuclei (unshaded) showing large numbers (8000 in total) of 2C, 4C, 8C and 16C nuclei in female *X. vesparum*.

meiocytes, as expected. The many nuclei that were present, 1C (if present), 2C and 4C, included those from large numbers of meiocytes and developing embryos within the *X. vesparum* female. Unlike the 8C and 16C nuclei, which were unique to the female, 32C and higher ploidy nuclei were scored in both the male and female.

Two other insects, which had been reported or were suspected to have small genomes, provided comparisons with the above. The genome size of male Hessian fly was 1C = 120.9 Mb, while the female was 157.9 Mb. The Hessian fly has a haploid set of four chromosomes and X1X2/Y sex determination (Stuart & Hatchett, 1990), and the genomes differ by almost 30%. The genome of the tiny parasitic wasp, *T. brassicae*, is 1C = 245.6 ± 0.9 Mb (Table 1). One *T. brassicae* replicate included nuclei from the head of a single male (1C), yielding nuclei whose 1C DNA value was exactly half that of 2C in the females, as expected of this haplodiploid insect. Unlike Strepsiptera, the nuclei isolated from the Hessian fly and *T. brassicae* heads and the Hessian fly thorax were primarily 2C (95% or more were 2C as in *D. melanogaster*), with few 4C (< 5%), and almost no 8C or 16C nuclei (< 1%). The thorax of haploid male *T. brassicae* was not scored, so the extent of endoreduplication in this region remains to be determined.

Discussion

The strepsipteran genomes are the smallest insect genomes reported to date, and are associated with the unique morphology and life history of these insects. Strepsiptera are extreme koinobionts: a strepsipteran completes its entire life cycle in a living host, and the lifecycle of a host parasitized by a viviparous female strepsipteran is extended. All other endoparasitic koinobionts undergo only their larval life history in a live host, and pupation is carried either in or outside the host. Whether these unique traits constrain the genome size of Strepsiptera remains to be determined.

It is unlikely that the small genome scored for Strepsiptera is an artefact of DNA degradation, or chromosome condensation. The 2C peaks from male neutral tissue were stable over 24 h stain time and showed no evidence of degradation. Similarly, the 1C and 2C peaks in the female, while producing greater coefficients of variation (CV) than the neural tissue in the male, were stable over 24 h and showed no degradation. If compaction of chromatin were a problem, it might be expected that PI staining would accumulate slowly over time. However, staining increased rapidly for the first 20 min, and remained constant between 1 and 24 h staining time. It is also unlikely that the abundance of 4C and 8C and the higher peaks observed in the female strepsipterans are artefacts. Comparable amounts of ploidy were not observed in all the tissues, nor was ploidy produced as an artefact in the *Drosophila* nuclei prepared along with strepsipteran tissue. The observed difference in genome size between the host and the parasitic strepsipteran means that contamination from the host would have been easily recognized.

While genome sizes have been reported for relatively few insects to date, the great majority of the smallest insect genomes (below 200 Mb) in the insect genome database (Gregory, 2001) are within 10% of the genome of *D. melanogaster*, at 1C = 175 Mb. An exception is the midge, *Prodiamesa olivacea* (Diptera: Chironomidae) at 1C = 130 Mb (Petitpierre, 1996). Another exception, the Hessian fly genome, was reported to be 1C = 100 Mb (W. C. Black, unpublished observation, given in Ma *et al.*, 1992), and would be even smaller than Strepsiptera. In this study we reanalysed the genomes of the male and female Hessian fly, and the male was found to be 120.9 Mb, while the female was 157.9 Mb. The Hessian fly genome is therefore no longer the smallest reported insect genome, when compared with *C. f. texensis* at 107.6 Mb. The parasitic lifestyle and the extremely small size of microhymenoptera means that they might have been expected to have small genomes, like Strepsiptera. However, the genome of *T. brassicae* is more than twice that of *C. f. texensis* at 1C = 245.6 ± 0.9 Mb. The genomes of two other *Trichogramma* were measured independently, and found to be the same as, or larger than, reported here for *T. brassicae* (J. van Vugt, pers. comm.).

In Strepsiptera, there is a relationship between body size and endoreduplication. That endoreduplication is associated with small genome size was anticipated by Nagl (1978), who suggested that endoreduplication is a mechanism to increase cell size and mRNA production in specialized cells. The strepsipteran male thorax is almost entirely 4C, which is consistent with the unusually large metathorax, as it accommodates the flight muscles that power the hind wings (the mesothoracic wings are reduced) (Smith & Kathirithamby, 1984) – a unique morphological feature that separates strepsipteran males from other insects. In the female, a large number of cells are 4C or greater in the cephalothorax, and to an even greater extent in the body. This reflects not only the larger size of the female, but also the large number of cells at G_2 that constitute the developing embryos. Whether females utilize endoreduplication as a means to produce large quantities of mRNAs, or genome amplification is a means to increase cell volume and thus total size, or both, awaits investigation with other strepsipteran species. Equally interesting, and remaining to be examined, is the progression of endopolyploidy during embryonic development, which occurs in the abdomen of the viviparous female.

It is of interest that the Hessian fly, which has a small genome (particularly in the male), does not show any of the endoreduplication observed in the thorax of the strepsipteran male and/or the abdomen of the female. The simplest explanation for the difference is that male and female Hessian flies are small, and neither has the unusually large thorax of the male strepsipteran nor the larger size of the strepsipteran female. In contrast, the haplodiploid system of *T. brassicae* means that the male and female genomes differ by the equivalent of one round of endoreduplication. One of the *T. brassicae* replicates produced a peak associated with haploid nuclei on the head of a single *T. brassicae* male, and the same samples showed no 4C or higher levels of endoreduplication. The haploid male and the male and female thoraces were not independently scored, so the extent of endoreduplication from 1C to 2C in the males, and from 2C to 4C in the females, remains to be determined.

Discussing insect genome size, and developmental rate and complexity, Gregory (2002) notes that the endopterygote (insects with complete metamorphosis) orders examined have genome sizes less than 2 pg, while the exopterygote (insects with incomplete metamorphosis) orders examined are not so constrained, with the exception of aphids which frequently have genome sizes under 2 pg. In the endopterygote Strepsiptera examined here, the male undergoes complete metamorphosis, has a shorter life cycle, and is extremely short-lived (~5–6 h) as a free-living adult; in contrast, the female is neotenic, has no pupal instar (Kathirithamby, 2000), and is viviparous with a long life cycle (also extending the life cycle of the host). Therefore, developmental complexity between the sexes is very different. The genome sizes of male and female *X. vesparum*, however, are similar (129 Mb and 133 Mb, respectively), presumably because (unlike the Hessian fly), there are no sex chromosomal differences, and the chromosomes pair and assort in both sexes. Thus the tiny genome size in Strepsiptera, where one sex (the male) has rapid metamorphosis, supports the developmental complexity hypothesis. The small body size (excepting the 4C thorax) and short life cycle of the male is also consistent with the proposed constraints on genome size. Interestingly, the larger and more slowly developing female has what is, in effect, a larger genome because much of her tissue is 4C, 8C 16C, and higher ploidy.

As Gregory (2002) points out, the C-value enigma is a 'complex and multifaceted puzzle, immune to one-dimensional explanations'. The Strepsiptera studied here have tiny genomes, but whether the small genome of Strepsiptera represents a reduction or constraint in genome size remains to be determined. The myrmecolacid *C. f. texensis* genome is reduced relative to the stylopid *X. vesparum*, and the life cycle of the former is more complex, the sexual dimorphism more extreme, and the female more productive. Yet reduction is not the only possible reason why the genome may be small. Another possibility is that they started out small. Petrov (2002) says that the small genome sizes reflect high rates of DNA deletions; and that these high deletion rates have, over millions of years of evolution, produced small genomes quite independently of adaptation. The tiny strepsipteran and the relatively tiny Hessian fly genomes provide an opportunity to test this possibility in the future.

Experimental procedures

Male *C. f. texensis* were reared from male *S. invicta* collected from Bee Creek Park, College Station, TX, USA. Males and females of *X. vesparum* were reared from female *P. dominulus* collected near Florence, Italy. *Caenorhabditis elegans* variety Bristol strain N2 was provided by the Caenorhabditis Genetics Center. *Drosophila melanogaster* strain Iso-1 was obtained from Gerald Rubin, University of California at Berkeley, CA, USA. Male and female Hessian flies, *M. destructor*, were reared from culture at the University of Notre Dame, South Bend, Indiana, USA. The parasitic wasp *T. brassicae* was reared from culture at Beneficial Insectary, Redding, CA, USA.

Samples were prepared for flow cytometry as described in Bennett *et al.* (2003). A single head of male *C. f. texensis* or *X. vesparum* plus a single head of female *D. melanogaster* (the size standard) were placed in 1 ml Galbraith buffer, stroked 15 times with a pestle in a Kontes Dounce tissue grinder, and filtered through 20-μm nylon mesh. Similar preparations, both with and without a single *D. melanogaster* head (standard) using: (a) combined heads of 4–5 *T. brassicae,* (b) a single head and/or thorax of *M. destructor*, (c) a single head of worker *S. invicta*, (d) brain pulled from single heads of *P. dominulus*, and (e) the posterior 1/4, the two middle portions, and the anterior 1/4, including the brain, of female *X. vesparum*, with the five parts of the female, were

prepared separately. *C. elegans* was chopped with a razor blade, ground, filtered, and then combined with the head and thorax of *C. f. texensis*, following Bennett *et al*. (2003).

Propidium iodide was added to each sample to a final concentration of 5 μg/ml for a known duration (usually 1–9 h) of up to 24 h. The mean fluorescence of stained nuclei in replicate samples of each comparison was quantified, using a Coulter Epics Elite (Coulter Electronic, Hialeah Fl) flow cytometer with a laser tuned at 514 nm and 300mW. Fluorescence at > 615 nm was detected by a photomultiplier screened by a long pass filter. To ensure that scoring included only intact nuclei (which are free from cytoplasmic tags) counting was activated by red fluorescence (discrimination), and only (gated) nuclei with the low forward and low side scatter were included in the analysis. The positions of the 2C sample peak relative to the 2C *Drosophila* peak were verified by running at least one insect of each species with no standard. This was particularly important for Strepsiptera, where the very small genome was well below (to the left of) the expected peak for any insect measured to date, and where the 2C peak fell on top of that of *C. elegans*.

Acknowledgements

We thank P. W. H. Holland for helpful suggestions, and T. R. Gregory and one other anonymous reviewer who made useful suggestions to improve the manuscript. The project was funded by the Royal Society Joint Project Grant (22335).

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