NOTE

Genome size of the northern walkingstick, *Diapheromera femorata* (Phasmida: Heteronemiidae)

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Abstract: The haploid genome size (C value) of the northern walkingstick, *Diapheromera femorata* (Say), was estimated to be 1C = 2.55 pg using Feulgen image-analysis densitometry of haemocyte and sperm nuclei. This relatively large genome is similar in size to the genomes of the few other phasmids studied so far, and is consistent with hypotheses regarding an upper limit to the size of many insect genomes imposed by the process of metamorphosis, which is relaxed among hemimetabolous orders. Comments on sperm morphology in *D. femorata* are also provided, and another possible relationship between genome size and the organismal phenotype in insects is suggested.

Résumé : La taille du génome haploïde (valeur de C) du phasme *Diapheromera femorata* (Say) a été estimée à 1C = 2,55 pg par analyse densitométrique d'images Feulgen des noyaux des hémocytes et des spermatozoïdes. Cette taille relativement élevée du génome est semblable à celles des génomes de quelques autres phasmides déjà étudiés et s'accorde avec les hypothèses sur la limite supérieure de la taille du génome de plusieurs insectes, limite imposée par le processus de métamorphose qui est moins contraignant chez les ordres d'hémimétaboles. On trouvera ici quelques commentaires sur la morphologie des spermatozoïdes de *D. femorata* qui soulignent la possibilité d'une relation entre la taille du génome et le phénotype organismique chez les insectes.

[Traduit par la Rédaction]

Introduction

Stick insects of the order Phasmida (= Phasmatodea) are intriguing for a variety of reasons. Most obviously, their uncanny resemblance to the vegetation upon which they live as adults is among the most impressive examples of mimicry in the animal kingdom. In some species (e.g., *Extatosoma tiaratum*) the eggs resemble plant seeds that ants carry into their nests (where they are afforded protection from predators), and which then hatch into nymphs that are mimics of their hymenopteran hosts. The Phasmida also contains the largest (or at least the longest, at over 36 cm) insect in the world, *Pharnacia kirbyi*. Many species undergo parthenogenetic reproduction, which makes them interesting from the standpoint of reproductive and developmental biology.

Phasmids are also of interest from a cytogenetic point of view. For example, some species of stick insects exhibit interspecific hybridization and polyploidy (e.g., Marescalchi et al. 1990; Marescalchi and Scali 2001). In addition, the aforementioned *E. tiaratum* (family Phasmatidae) may have one of the largest genomes (i.e., haploid nuclear DNA content, or "C value") yet reported for an insect (1C = 8.0 pg; Nagl and Schäffner 1981). Note, however, that this value was originally reported to be 1C = 24.0 pg but had to be corrected because of an inaccurate C value for the standard, *Acheta domestica* (and based on the cell type measured, there may be reason to believe that 8.0 pg actually represents the 2C value). Nevertheless, few insects have a genome larger than this (notable exceptions include various orthopterans; Gregory 2001, 2002). Whether large genomes such as this are typical of phasmids remains to be seen. Thus far, the genomes of representatives from only one other genus of stick insects (*Bacillus*, family Phasmatidae) have been measured, and range in size from about 1C = 2 pg to 1C = 3 pg (four species and seven subspecies in total; Marescalchi et al. 1990, 1998). Unfortunately, this lack of data is symptomatic of insects at large, of which a mere 300 species have been analyzed in terms of genome size, the majority of them flies, beetles, grasshoppers, and aphids (Gregory 2001).

The northern walkingstick (*Diapheromera femorata* Say, family Heteronemiidae) is the only species of stick insect found in Canada, where it is restricted primarily to southern regions of Ontario and Quebec (Vickery and Kevan 1985). Adult *D. femorata* are defoliators of oak trees, and are therefore considered potential, and occasionally actual, ecological pests (Wilson 1971; Giese and Knauer 1977; Vickery and Kevan 1985). The present study provides an estimate of the

Received 14 January 2002. Accepted 20 June 2002. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 23 August 2002.

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 DOI: 10.1139/Z02-106

genome size of *D. femorata*, thereby marking the first such examination of a North American stick insect, and only the third genus (and second family) from the order Phasmida so far studied.

Materials and methods

Twelve specimens of *D. femorata* were collected in early August 2001 from oak trees in Turkey Point, Ontario, near the shore of Lake Erie. The sample included six adult males, two adult females, and two juveniles of each sex. Following a lethal exposure to chloroform vapour, haemolymph samples were taken from each specimen by severing the legs and antennae with scissors and allowing the fluid to exude and air-dry onto standard microscope slides. The testes of five of the adult males were also dissected under insect Ringer saline and their sperm dispersed onto microscope slides with a pipette and then air-dried.

The slides with haemolymph and sperm, along with several internal reference standards, were stained by the Feulgen reaction (Hardie et al. 2002). Briefly, slides were postfixed for 24 h in MFA solution (85 parts methanol : 10 parts formalin : 5 parts glacial acetic acid), rinsed for 10 min under running tap water, and then hydrolyzed for 2 h in 5 M HCl at room temperature. A brief dip in 0.1 M HCl prevented the carryover of strong acid prior to immersion for 2 h in freshly prepared Schiff's reagent. The slides were then rinsed in three changes of fresh bisulfite solution, for 10 min in running tap water, and in three changes of distilled water.

At the time of measurement, slides were mounted in refractive-index liquid (n_D = 0.154) to eliminate glare caused by cell membranes. Integrated optical densities (IODs, green channel) of the Feulgen-stained nuclei were analyzed using the Bioquant True Color Windows 98 image-analysis software package (R&M Biometrics Inc., Nashville, Tenn.). Image acquisition was accomplished with the use of an Optronics DEI-750 CE three-chip CCD camera along with a Leica DM/LS compound microscope (100× oil-immersion lens) connected via a BQ6000 frame-grabber board to a Pentium II® 350 MHz PC. A neutral-density filter was used to homogenize the incident light, but no other wavelength filtration was necessary with this system (Hardie et al. 2002). IODs in arbitrary units were converted to absolute DNA content by comparison with the mean IOD of chicken erythrocyte nuclei (*Gallus domesticus*, 1C = 1.25 pg). Blood cells of rainbow trout (*Oncorhynchus mykiss*) and Siamese fighting fish (*Betta splendens*) as well as sperm and (or) haemocytes from fruit fly (*Drosophila melanogaster*), yellow mealworm beetle (*Tenebrio molitor*), and house cricket (*Acheta domestica*) were also included in the staining run as additional internal "checks" (Hardie et al. 2002).

Results and discussion

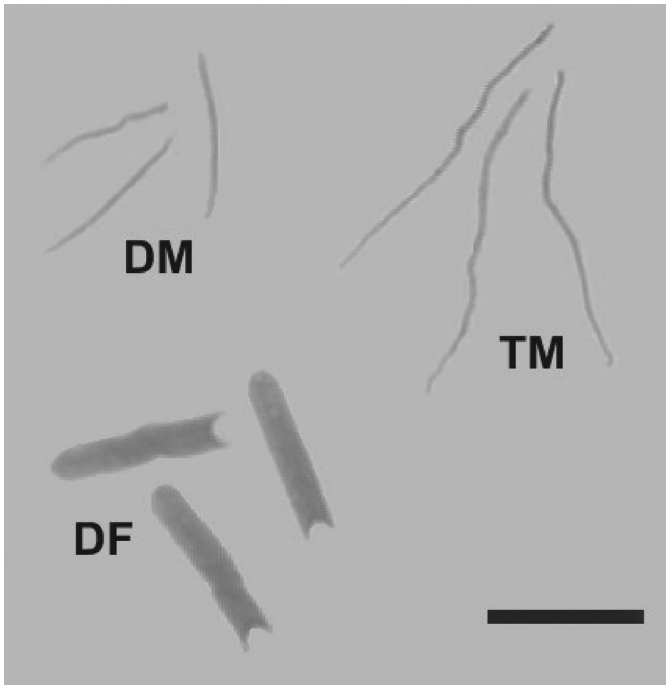
The sperm nuclei measured in the present analysis gave IODs slightly less than half those of the haemocytes, which is to be expected, owing to differences in DNA compaction levels between the two cell types (Hardie et al. 2002). Based on an average of all values calculated from both the haemocyte (n = 12) and sperm (n = 5) samples, the genome size of *D. femorata* is estimated at 1C = 2.55 ± 0.06 pg (mean ± standard error). This is within the range of 1C values, 1.95–2.94 pg, for representatives of the genus *Bacillus* obtained by means of flow-cytometric measurement of midgut cells versus a mouse thymocyte standard (Marescalchi et al. 1990, 1998). The possibility that phasmid genomes are consistently large across different families is supported by this observation, though much work remains to be done before any generalizations can be made regarding a "typical" genome size for the order as a whole.

European populations of *Bacillus atticus* consist of both diploid and triploid parthenogenetic strains, and among the former there is significant latitudinal variation (~20%) in nuclear DNA content, which decreases gradually from east to west (Marescalchi et al. 1998). By contrast, *D. femorata* is bisexual, and the consistent DNA contents of male and female somatic cells and sperm indicate a likely diploid state in this species. Geographical variation within *D. femorata* was not evaluated in the present study, but at least within the single population sampled here there was no detectable intraspecific variation in genome size. In both *Bacillus grandii* and *Bacillus rossius*, the only two bisexual species of this genus studied, the sex-determination system results in females (XX) with one more chromosome and about 10–20% more DNA than males (XO) (Marescalchi et al. 1990). Again in contrast to these European species, *D. femorata* showed no differences in haemocyte DNA content between males and females, nor between juveniles and adults (t tests, all p > 0.30).

It was recently suggested that because more DNA means slower cell division, the intensive tissue differentiation which occurs during metamorphosis may place a ceiling of about 1C = 2 pg on the genome sizes of holometabolous insects (Gregory 2002). As members of a hemimetabolous order, phasmids develop by a series of nymphal moults (usually five in *D. femorata*; Giese and Knauer 1977) and undergo no complete metamorphosis, thereby freeing them of this constraint. The relatively large genome of 2.55 pg reported here for *D. femorata* is consistent with this hypothesis.

It may also be that while insect genome size is constrained by developmental lifestyle (and (or) vice versa), certain phenotypic traits may be affected by variation in DNA content. In insects from orders as diverse as the Coleoptera, Dermaptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Plecoptera, Siphonaptera, and Trichoptera, sperm are highly elongated and contain similar hairlike nuclei (T.R. Gregory, unpublished data). Figure 1 shows Feulgen-stained sperm nuclei from two such species of insects with the commonly elongated nuclear form (*D. melanogaster* and *T. molitor*) along with those of *D. femorata*, which displays a very different type of compact sperm nucleus. Intriguingly, nearly identical compact nuclei have also been found in the Embiidina (T.R. Gregory, unpublished data). Figure 2 shows the shape of the entire sperm cells of *D. femorata*, which appear superficially more similar in shape to those of vertebrates than to those of most other insects. It could be that sperm morphology in groups such as walkingsticks and webspinners is dictated in part by their relatively large genomes. If this is so, it would clearly have implications also for the morphology of the reproductive organs in these species, and perhaps by extension for their reproductive strategy. More study will be

Fig. 1. Feulgen-stained sperm nuclei from the northern walkingstick, *Diapheromera femorata* (DF), alongside those of the fruit fly, *Drosophila melanogaster* (DM), and the yellow mealworm beetle, *Tenebrio molitor* (TM). Scale bar = 10 μm; 100× oil-immersion objective.

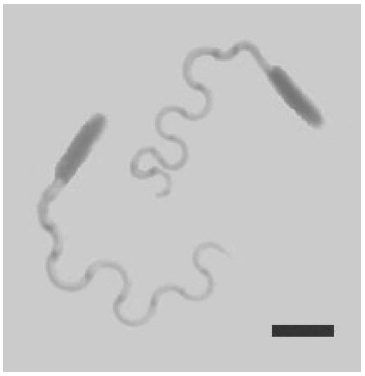


required to determine the relationship (if any) between genome size and sperm morphology in insects, but if such a pattern holds, then the case of *D. femorata* can be taken as yet another example of the complex evolutionary feedback loop that exists between the genome, the cell, and the organism.

Acknowledgments

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Post-Graduate and University of Guelph Alumni Doctoral scholarships to T.R.G. and a NSERC research grant to Paul Hebert. Sincere thanks are extended to Sarah Adamowicz for assistance with specimen collection and to Dave Hardie for help with the staining and image-analysis protocols. Thanks are also extended to the two anonymous reviewers whose comments helped to improve the manuscript.

Fig. 2. Wright-stained cells of *D. femorata*, showing that sperm morphology in this species is very different from the highly elongate sperm with no prominent head which is characteristic of most insect orders. Scale bar = 10 μm; 40× objective.



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