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European Journal of Histochemistry 2022; volume 66:3400

Genome size evaluations in cockroaches: New entries

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ABSTRACT

In this paper, we report genome size (GS) values for nine cockroaches (order Blattodea, families Blattidae, Blaberidae and Ectobiidae, ex Blattelidae), three of which are original additions to the ten already present in the GS database: the death's head roach (*Blaberus craniifer*), the Surinam cockroach (*Pycnoscelus surinamensis*) and the Madeira cockroach (*Leucophaea maderae*). Regarding the American cockroach (*Periplaneta americana*), the GS database contains two contrasting values (2.72 *vs* 3.41 pg); likely, the 2.72 pg value is the correct one as it is strikingly similar to our sperm DNA content evaluation (2.80 ± 0.11 pg). Also, we suggest halving the published GS of the Argentine cockroach *Blaptica dubi*a and the spotted cockroach (the gray cockroach) *Nauphoeta cinerea* discussing i) the occurrence of a correlation between increasing 2n chromosome number and GS within the order Blattodea; and ii) the possible occurrence of a polyploidization phenomenon doubling a basic GS of 0.58 pg of some termite families (superfamily Blattoidea, epifamily Termitoidae).

Key words: genome size, C-DNA content, cockroaches, Blattodea.

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Contributions: MM, CAR, conceived the study and performed the experiments; MM, CAR, EC, wrote the manuscript. All the authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Funding: This work was supported with a grant of Fondazione Veronesi for scientific research to MM.

Ethics statement: All the animals used were reared in the animal house of the former Department of Animal Biology of the University of Pavia.

Introduction

Cockroaches (Blattodea) constitute one of the major and most representative groups of the Invertebrata and are characterised by some specific biological features that render their study particularly appealing: a wide geographical distribution, the commensalism, and group behaviour of many species. Furthermore, they make incomparable contributions as animal models to aid the understanding of invertebrate physiology, as well as fundamental additions (together with crustaceans) to comparative endocrinology.[1] Moreover, cockroaches play a major role in human health as vectors of pathogens (viruses, bacteria, nematodes, cestodes) thus favouring the transmission of human diseases[2] and provide opportunities for food production as a cheap source of animal protein (*B. dubia, P. surinamensis* and others species, are contributing to meet food demands, not only in Eastern countries) proposed as an alternative to meat industry: they can be raised for human consumption or as an ingredient as feed for non-human animals.[3] Consultation of several genome size (GS) databases shows that, despite these features, cockroaches constitute one of the least represented groups: there are only 12 records listed in the main GS database[4] even though it is estimated that there are more than 4,000 existing species.[5,6] New GS entries can benefit several research fields such as phylogenomics and sequencing projects.[7] New GS data can contribute, as a useful preliminary step, both to achieving greater knowledge of the general biological features of these animals and, in a broader view, to a better understanding of the evolutionary role played by GS.

Another potential benefit from enlarging the cockroach GS database is its possible contribution as a cyto-taxonomical tool to favour one of the current systematic groupings achieved after decades of discussion. Until recently, some authors favoured a direct link between Blattoidea and Mantoidea, whereas others considered Blattoidea much more closely related to termites, order Isoptera,[8-11] thus creating a specific order (Blattaria) to include the three, namely Blattoidea, Mantoidea, and Isoptera, as a sub-order. Thanks to mitochondrial genome sequencing, the debate was largely resolved. The vast majority of authors now accept that termites are roaches,[12-14] so that Beccaloni (accessed January 12, 2022)[6] suggested the following grouping and systematic ranking: super-order Dictyoptera; order Blattodea which includes three superfamilies: Corydioidea, Blaberoidea, and Blattoidea. The Blaberoidea superfamily includes two families: Blaberidae and Ectobiidae (ex Blattellidae) while the Blattoidea superfamily includes the Blattidae family and the epifamily Termitoidea (which includes all of the termites). The Dictyoptera includes another order grouping all the mantis species, Mantoidea. A detailed description is provided in: http://cockroach. speciesfile.org/HomePage/Cockroach/HomePage.aspx.[6]

Despite this, two names are still preferentially in use to refer to the cockroach group at the systematic level of orders, Blattaria and Blattodea, as reported even in the main GS database[4] (accessed January 12, 2022). This is because the International Commission of Zoological Nomenclature has no rules for the construction of the names of orders. We decided to use the name Blattodea (as explained) even though only three GS entries (of the 12 recorded in the database) are referred to as pertaining to the Blattodea order (while the remaining nine are referred to Blattaria due to the contributors' original systematic attribution) namely those of *Pachlora nivea* (Blaberidae), *Parcoblatta pensylvanica* (Blattidae) and one of the two reported values for *Periplaneta americana* (Blattidae). To avoid any confusion, in the present paper, we will refer to the family systematic level of the animals we studied; at this level, there are no ambiguities.

Making use of sperm DNA-Feulgen scanning microdensitometry we present here the GS of nine cockroach species from the three families of the Blattodea order: Ectobiidae (ex Blattelidae: *Blattella germanica*), Blattidae (*Blatta orientalis*, *Periplaneta americana*), and Blaberidae (*Blabera fusca, Blaberus cranifer, Blaptica dubia, Pycnoscelus surinamensis, Nauphoeta cinerea*, *Leucophaea maderae*). Comparing our values with those present in the main GS database revealed that three of them are new entries (*B. cranifer, P. surinamensis* and *L. maderae*), which can now be added to the GS database thus increasing the number of GS records to 15. Three other values support those already listed (*B. germanica*, *B. orientalis* and *B. fusca*) while for *P. americana* we suggest resolving the two conflicting values for GS (2.72 pg *vs* 3.41 pg) by favouring the 2.72 pg value. In addition, we propose halving the existing GS values for the Argentinian wood cockroach *B. dubia* and that of the wood speckled roach *N. cinerea* (as explained in the Results).

Finally, we briefly suggest two speculative hypotheses that need to be validated by increasing the available GS records: i) the occurrence of a positive correlation between 2n chromosome number and GS within the order Blattodea; and ii) the possible occurrence of a polyploidization phenomenon, doubling a basic GS of roughly 0.5 pg (in termites, epifamily Termitoidae) up to a maximum GS value of 3.24 for the Blaberidae family. Mining the GS database for mantis GS records revealed five GS listed, with values spread around 3 to 4.5 pg.

Materials and Methods

Cell preparation

Three air-dried sperm slides were prepared for each of the two males of the following species: *Blabera fusca, Blaberus cranifer, Blaptica bubia, Blatta orientalis, Blattella germanica, Leucophaea maderae, Nauphoeta cinerea, Periplaneta americana,* and *Pycnoscelus surinamensis.* All the animals came from the animal house of the Department of Animal Biology, University of Pavia (Italy), and were reared under standard conditions as regard temperature, humidity, and food access. Animals were anaesthetised by insufflating carbon dioxide for 30 s into the bottles in which they were housed. Once anaesthetised, they were decapitated and dissected. Sperm were collected into Ringer's solution for cockroaches (NaCl: 12.2 g/1000 mL; KCl: 0.21 g/1000 mL; $CaCl_2$: 0.20 g/1000 mL) as described in 1963[15] and immediately smeared allowing them to air-dry.

Feulgen procedure

Air-dried specimens were fixed in 10% formaldehyde aqueous solution for 20 min. The Feulgen reaction included hydrolysis in 5 N HCl at room temperature for 60 min and staining with Schiff's reagent (basic fuchsin; BDH) for 45 min. Given that several batches had to be processed, it was important that each batch comprised slides bearing DNA standards. The standards were erythrocytes of the chicken (*Gallus gallus*) and sperm and lymphocytes of *Mus musculus domesticus* with 2.54, 3.4, and 6.8 pg nuclear DNA, respectively. Advantages of Feulgen staining include limited fading and minor sensitivity to DNA base composition.

Microphotometry and statistical analysis

Fifty sperm nuclei were evaluated from each of two Feulgen-treated slides (randomly selected from three prepared) for each of the two animals examined. Thus, for each species, we measured 200 sperm nuclei so that both technical (inter-slide) and biological (inter-individual) variability were taken into account.

Nuclear DNA contents were recorded with a scanning micro-

scope photometer 03 and the APAMOS program (Zeiss). The wavelength for maximum absorbance was determined at 550±5 nm instead of the expected 560 nm. A planapochromat 100x objective (n.a. 1.3) opened the measuring diameter to 0.5 µm and the illuminated field to 10 µm, both in the plane of the specimen. Therefore, scanning steps were set to 0.5 µm in both dimensions and for all measurements. Photometric errors due to glare and non-specific light loss were evaluated; since they proved to be constant and negligible (<3%), no instrument adjustment was made.

Statistical analyses were carried out using Microsoft Excel and SigmaStat software. The significance of differences among mean DNA contents was evaluated by one-way analysis of variance (ANOVA). Multiple *a posteriori* comparisons among means were performed using Tukey's test.

Results

Table 1 reports the sperm DNA contents measured by scanning microphotometric absorption of Feulgen-stained sperm nuclei pertaining to nine cockroach species. As shown, these GS values (namely the haploid C-DNA content) are scattered from a minimum of 2.09 ± 0.24 pg for *B. germanica* (Ectobiidae) to a maximum of 3.24 ± 0.21 and 3.23 ± 0.19 for *B. cranifer* and *B. fusca* (Blaberidae), respectively. Each of the possible mean DNA content comparisons was statistically significant with the exception of the two Blattidae GS (*B. orientalis* 2.95 ± 0.32 *vs P. americana* 2.80 ± 0.11) and the three possible comparisons among *B. dubia* (2.53 ± 0.34 pg), *P. surinamensis* (2.65 ± 0.28 pg) and *N. cinerea* (2.65 ± 0.26 pg).

We then mined the GS database[4] (accessed January 12, 2022), which provides 1345 GS values for insects, ten of which pertain to cockroach families: four Blaberidae, three Ectobiidae, and three Blattidae. Considering the GS we measured, the database can be improved with the three new Blaberidae entries for *B. cranifer, P. surinamensis* and *L. maderae*. Thus, at present we know the GS for 13 of the 4,622 currently named cockroach species.[6,16,17] Comparing the GS values already known with those we measured led to further interesting considerations. To facilitate these comparisons Table 1 reports the GS we found and, in parentheses, those already provided by other authors (see the reference section of the GS database). There is clearly a very good concordance between our values and the data already reported for *B. germanica*, *B. orientalis* and *B. fusca* cockroaches. Interestingly, the GS database presents two contrasting GS values for *P. americana*: 2.72 and 3.41 pg. We suggest favouring the 2.72 pg value (originally presented, but not published, in 1953 by Elen Rash) since it is strikingly similar to the sperm DNA content we measured (2.80 ± 0.11 pg). In addition, it must be noted that the 3.41 pg value was obtained from Feulgen image analysis of hemocytes, cells for which ploidy is not firmly established (they were probably cells in the S phase of the cell cycle). Finally, we suggest halving two of the existing GS values: those for the Argentinian wood cockroach *B. dubia* and the wood speckled roach *N. cinerea*.

At present, it is not possible to infer any correlation between maximum and minimum GS values and the systematics allocation of the species considered given both the paucity of the current data and the fact that there are statistically significant mean GS differences both within and intra-systematic groups. However, Table 1 reports the 2n chromosome number[18] for the species we considered. It does seem that there is a moderate correlation between increasing 2n chromosome number and increasing GS values within the cockroaches species we analyzed: 2n 23 – 24 (2.09, 2.20); 2n 33-34 and 37 (2.80, 2.65, 2.65); 2n 47 – 48 (2.95); 2n 73 – 74 (3.24, 3.23). However, many more GS data entries are required before this idea can be supported or evaluated by robust statistical analysis.

Discussion

At present, the GS database[4] (accessed January 12, 2022) contains a total of 1345 GS values for insects. Very few of them, just 33, are related to the super-order Dictyoptera: 28 to the order Blattodea (16 termites; 12 cockroaches' *sensu strictu;* 2 wood roaches of the family Cryptocercidae) and five to the order

Table 1. Mean sperm DNA content ($\bar{x}$ + SD) expressed as pg. The DNA pg were calibrated versus chicken erythrocytes and murine sperm and lymphocytes (see *Materials and Methods*). The systematic allocation of the cockroach species is according to Beccaloni[6] while the 2n chromosome numbers are those reported in White.[29] We were unable to find any literature reference for the *B. dubia* 2n number.

Species	Family	Common name	GS C-DNA content $\bar{x}$ ± SD (pg)	2n
Blatta orientalis, Linnaeus 1758	Blattidae	Oriental cockroach	2.95 ± 0.32 (3.03)	47 – 48[29]
Periplaneta americana, Linnaeus 1758	Blattidae	American cockroach	2.80 ± 0.11 (2.72 – 3.41)	33 – 34[29]
Blaberus cranifer, Burmeister 1838	Blaberidae	Death's head roach	3.24 ± 0.21	73 – 74[29]
Blabera fusca, Brunner von Wattenwyl, 1865	Blaberidae	giant Mexican cockroach	3.23 ± 0.19 (3.36)	73[29]
Blaptica dubia, Serville 1839	Blaberidae	Argentinian wood cockroach	2.53 ± 0.34 (4.54)	Not found
Pycnoscelus surinamensis, Linnaeus, 1758	Blaberidae	Surinam cockroach	2.65 ± 0.28	37[29]
Nauphoeta cinerea, Olivier 1789	Blaberidae	Wood speckled roach gray cockroach	2.65 ± 0.26 (5.15)	37[29]
Leucophaea maderae, Fabricius, 1781	Blaberidae	Madeira cockroach	2.20 ± 0.11	23[29]
Blattella germanica, Linnaeus 1767	Ectobiidae (ex Blattellidae)	German cockroach	2.09 ± 0.24 (2.00)	23 – 24[29]

Mantoidea. This is very strange considering that nearly 8,000 of a total of nearly 12,000 insect species already described, pertain to the super-order Dictyoptera. Even stranger is the fact that there are just ten values for the Ectobiidae, Blaberidae and Blattidae families all together, which comprise 4,622 of the cockroach species described.[6] As described in the Results, we add three new GS records pertaining to the order Blattodea, family Blaberidae (i.e., *B. cranifer, P. surinamensis, L. maderae*). Furthermore, we had the opportunity to measure the GS of six other species, finding an excellent concordance between our measurements and those already present in the database for *B. germanica* (Ectobiidae), *B. orientalis* (Blattidae), and *B. fusca* (Blaberidae). Our values for *B. dubia* and *N. cinerea* (both belonging to the Blaberidae family) are nearly half of those already present in the database.

We suggest that these conflicting data (5.15 *vs* 2.65 for *N. cinerea* and 4.54 *vs* 2.53 for *B. dubia*, respectively) are not the result of a technical bias (due to the different techniques employed, *i.e.*, scanning Feulgen microdensitometry *vs* Feulgen image analysis) but rather to the "choice" of the cells used for the measurement of DNA content: sperm (in the present paper) *vs* haemocele circulating cells.[19] Since we measured the sperm DNA content, we suggest considering our values as the actual haploid GS for these two species. The two GS previously reported by Koshikawa and coworkers[17] probably reflect the diploidy of somatic haemocele circulating cells. With regard to the GS of *P. americana* (Blattidae), we suggest that this should be taken as 2.80 pg (the value we recorded for sperm DNA) and not the 3.41 pg value reported by both He *et al*.[19] and Hanrahan *et al*.[20] The discrepancy between the *P. americana* GS values calculated by these authors and the value that we measured is probably due to the different cell types employed (as for *N. cinerea* and *B. dubia*). While He and co-workers, as well as Hanrahan and co-workers,[19,20] measured the DNA content of nuclei obtained from grinding the heads of the animals (and then filtering the cell suspension through 20 µm and 38 µm meshes, respectively, to obtain nuclei for the flow cytometric measurements), we directly evaluated the GS as sperm C-DNA content. In support of the idea that the actual GS of *P. americana* is 2.80 pg, there is an additional early GS evaluation provided by the "mother" of GS research, Elen Rash: in 1953 she measured the GS of *P. americana*, finding a DNA content of 2.72 pg (a value strikingly similar to the one we measured). Since we evaluated the sperm DNA content, we suggest that our results represent the correct *B. dubia*, *N. cinerea,* and *P. americana* GS values. Likely, the animals used in older studies were polyploid: i.e., animals from parthenogenetically derived polyploid clones (parthenogenesis is very frequent in cockroaches.[17]

In summary, we consider that the actual GS data for the three families we studied comprise the following 13 records (mean values):

Blaberidae: 1.52 - 2.20 – 2.53 – 2.65 – 2.65 – 3.23 –3.24

Ectobiidae: 1.05 - 2.00 – 2.09

Blattidae: 2.80 – 2.95 – 3.41

The scattering of the GS values within a systematic group is not unexpected since the GS of several taxa varies over broad ranges. What is a paradox is that this does not correlate with the organism's complexity (the C-value paradox). Decades of attempts to solve this intriguing aspect of living organisms (linking the molecular level of genome expression with its phenotypic traits then exposed to environmental selection) have generated several hypotheses.[21,22] However, the study of GS (and genome composition/organization) and its phenotypic correlates (regardless of whether nucleotypic, nucleoskeletal or whatever other causative or co-evolutionary relationship) has not yet reached a satisfactory conceptual conclusion. Even today, it constitutes an integrated sphere of analysis bringing together cytology, cytogenetics, physiology, and ecology, in an interesting research field named "eco-physiological cytogenetics",[23] in which the major achievements have been at the level of relating GS with nuclear and cellular volumes, metabolic rates, developmental time and population size.[22-28] As regards any possible role played by GS in diversifying the Blattodea species, the picture we now have for cockroach GS values is a scattered distribution throughout the families, with some very large GS values present together with smaller values within each family. In the light of the new GS data, we speculate that GS exhibits a moderate (positive) correlation with the 2n chromosome number:[18] from minimum GS values at around 2 pg with 2n 23-24 up to 3.2 pg associated with 2n 73-74 (as detailed in the *Results* section). To support or refute this hypothesised correlation, many more GS data and 2n numbers are needed in order to be able to carry out a rigorous statistical analysis.

In search of a more general overview of a possible role played by GS, we looked at the GS records for termites and the related order of Mantoidea. Before discussing any possible inferences, it should be mentioned that cockroach systematics has been, and still is, a field of intense debate (see *Introduction*), so that cockroach phylogenetic relationships remain a topic of active discussion. In recent years, thanks to the molecular analysis of the mitochondrial genome, some taxonomic molecular studies were performed showing that termites are cockroaches that developed eusociality and split from the main cockroach group no later than 200 million of years ago (end of the Triassic) while the Mantodea and Blattodea (*e.g.*, super-order Dictyoptera) are estimated to have diverged in the mid-Permian (roughly 270 million years ago). In our search of a possible role of GS in determining phylogenomic relationships within the super-order Dictyoptera we surveyed the GS data present in the GS database[4] (accessed January 12, 2022). There are only a few records (28 Blattodea and 5 Mantoidea; plus the 3 new Blattodea that we are adding): despite such limited numbers, the GS values are scattered throughout possible successive duplications of a basic Termitoidea GS value of 0.58. In fact in each of the groups it seems that a duplication phenomenon gives rise to the present values: Termitoidea: min 0.58 pg – max 1.90 pg (two possible duplications), Blattodea: min 1.05 pg – max 3.36 pg (three possible duplications) and Mantoidea: min 2.92 pg – max 4.53 pg (four possible duplications). It is clear that we need many more molecular studies dissecting the genome constitution of cockroaches, termites and mantis and many more GS data in order to corroborate the occurrence of a polyploidization phenomenon multiplying the basic termite GS value (Figure 1).

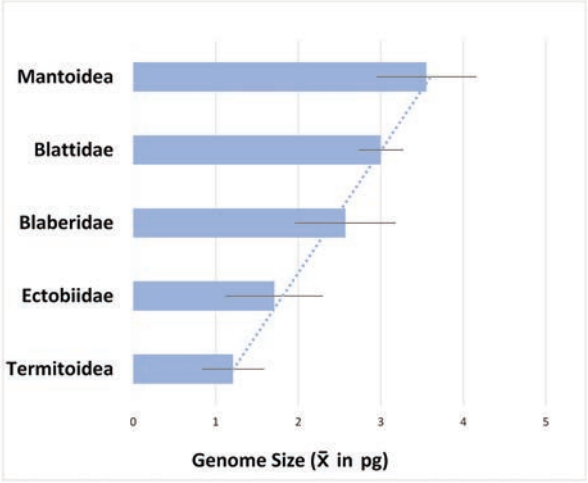

Figure 1. Cockroaches (all systematic groups) mean GS values. The trend line shows linear increasing values from Termitoidea to Mantoidea.

Acknowledgements

We thank Prof. Piergiorgio Rubini for sample preparation.

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Received for publication: 24 February 2022. Accepted for publication: 21 March 2022.

European Journal of Histochemistry 2022; 66:3400
doi:10.4081/ejh.2022.3400