Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: M. Husemann, D. Sadílek, L.-S. Dey, O. Hawlitschek, M. Seidel (2020) New genome size estimates for band-winged and slant-faced grasshoppers (Orthoptera: Acrididae: Oedipodinae, Gomphocerinae) reveal the so far largest measured insect genome. *Caryologia* 73(4): 111-120. doi: 10.13128/caryologia-966

Received: June 10, 2020

Accepted: September 24, 2020

Published: May 19, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

New genome size estimates for band-winged and slant-faced grasshoppers (Orthoptera: Acrididae: Oedipodinae, Gomphocerinae) reveal the so far largest measured insect genome

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Abstract. Grasshoppers, specifically those of the family Acrididae are known to have the largest genomes of all insects. However, less than 100 species of Orthoptera have their genome size estimated so far. In the present study, we measured the genome size of five acridid species belonging to the two subfamilies Oedipodinae and Gomphocerinae. All of the genomes measured are large and range between 1C = 11.31 pg in the female of *Chorthippus dorsatus* and 1C = 18.48 pg in the female of *Stethophyma grossum*. The latter represents the so far largest measured insect genome. We further provide a summary of genome size estimates available for Orthoptera.

Keywords: C-value, flow cytometry, *Stethopyhma, Oedipoda, Sphingonotus, Chorthippus.*

INTRODUCTION

The genome has become one of the most important targets of interest for biologists. In times of high throughput sequencing, projects like i5k generate data of entire genomes are at a daily base (Robinson et al. 2011; Li et al. 2019). However, we still have little data and a limited understanding of the variance in genome size across organisms. Especially for insects, the most diverse group of organisms on earth, data of only about 1,300 of the expected diversity of several million species are available (Sadílek et al. 2019a; Gregory 2020). Generating new data on genome sizes is important, e.g., for choosing the adequate NGS applications for genomic sequencing (Rodríguez et al. 2017). Yet, genome size can also be a taxonomic feature

and can be used for species determination (Sadílek et al. 2019b). For many applications taxa with specifically large genomes still remain a difficult target, especially if no complete genome sequence is available. Further, in order to understand why some species or species groups have specifically large genomes, whereas others are rather small requires comprehensive data across a large range of taxa.

While the so far largest genome of any organism was estimated in a plant, the monocot *Paris japonica* Franchet with 1C = 152.23 pg (Pellicer et al. 2010), the largest genome sizes in insects have been measured in Orthoptera, specifically Caelifera, with 1C values of 16.93 pg in *Podisma pedestris* (Linnaeus, 1758) (Podismi-nae) and 16.34 pg in *Stauroderus scalaris* (Fischer von Waldheim, 1846) (Gomphocerinae) (Gregory 2020 for a list). However, there is also a lot of variation within Orthoptera with genome sizes as small as 1C = 1.55 pg found in the cricket *Hadenoecus subterraneus* (Scudder, 1861) (Rasch and Rasch 1981). Nevertheless, a clear trend for larger genomes in the short-horned grasshoppers is observed, and specifically in the family Acrididae. In the present study, we were able to locate only 85 published genome size estimates from all Orthoptera (e.g. Gregory 2020).

To better understand the evolution of genome size in Orthoptera, especially the huge genomes of grasshoppers of the Acrididae family, it is obligatory to generate additional information. Hence, we provide new genome size information for members of the Acrididae family, i.e. three species of the subfamily Oedipodinae and two species of the Gomphocerinae. We present, to our knowledge, the so far largest genome size of any insect and summarize the knowledge on genome sizes in Orthoptera.

MATERIAL AND METHODS

Sampling

Eight specimens from five species (Table 1), all of the family Acrididae, were collected for our analyses in September 2019 in Hamburg, Georgswerder (Germany, 53.5097°N 10.0301°E). Specimens were collected by hand and kept alive until further processing. We included two species of the subfamily Gomphocerinae: *Chorthippus dorsatus* (Zetterstedt, 1821) and a species of the *Chorthippus biguttulus* (Linnaeus, 1758) group (a group of three species *C. biguttulus*, *C. brunneus* (Thunberg, 1815), *C. mollis* (Charpentier, 1825), which can only be identified with certainty by male song patterns; our specimen is a female, but according to morphological traits most likely represents *C. biguttulus*), as well as three species of the subfamily Oedipodinae: *Oedipoda caerulescens* (Linnaeus, 1758), *Sphingonotus caerulans* (Linnaeus, 1767), and *Stethophyma grossum* (Linnaeus, 1758) (Table 1, 2).

Reference specimens are deposited in the Zoological Museum Hamburg (ZMH), part of the Center of Natural History (CeNak) under the accession ZMH 2019/21.

Genome size analysis

Nuclear DNA content (2C) was measured by the flow cytometry method (FCM) as in Sadílek et al. (2019a, b) at the Department of botany of Charles University, Prague. The muscle tissue of one hind femur was used for FCM analysis against the plant-internal standard *Pisum sativum* L. "Ctirad" (Fabaceae) with 2C = 9.09 pg (Doležel et al. 1998; Doležel and Greilhuber 2010). Fresh tissue was homogenized and mixed with a leaf of

Table 1. Diploid chromosome number, 2C genome size, sample/standard ratio of both DAPI- and PI-stained samples and GC content of grasshopper species studied. Samples were measured against *P. sativum* standard with 2C = 9.09 pg. F = female, M = male, 2n = male diploid chromosome number, 2C = nuclear DNA content for nuclei with diploid chromosome number, CV = average coefficient of variation for each stain used.

Species	2n	Sex	2C (pg)	Sample/ standard DAPI ratio	Sample/ standard PI ratio	GC content (%)	Sample CV DAPI - PI
Sphingonotus caerulans	22+XX	F	26.63	2.424	2.930	42.14	2.70 - 2.95
Sphingonotus caerulans	22+X0	M	25.12	2.321	2.764	41.87	2.71 - 2.81
Oedipoda caerulescens	22+XX	F	28.39	2.621	3.123	41.88	3.71 - 5.62
Chorthippus dorsatus	16+XX	F	24.14	2.359	2.656	40.82	2.58 - 2.64
Chorthippus biguttulus	16+XX	F	22.62	2.149	2.488	41.35	2.50 - 4.07
Stethophyma grossum	22+XX	F	36.95	3.326	4.065	42.35	3.41 - 4.23
Stethophyma grossum	22+X0	M	34.72	3.172	3.820	42.08	2.19 - 2.84

Table 2. Genome sizes of Orthoptera so far measured. The template of the table was extracted from Gregory (2020); it was complemented with original references and additional studies. References with an * indicate that the original reference could not be accessed and data are extracted only from Gregory (2020). [1]relative genome size - measured with the DAPI - from Morgan-Richards (2005). M= male, F = female, 2C = genome size of the diploid cell, 2n = diploid chromosome number (if sex is not determined, karyotype of the male is presented; in all species the sex determining system is XX/X0, only males of *Podisma pedestris* can be variable with XY/X0), n.a. = not available; FD = Feulgen densitometry, FCM = flow cytometry method; AN = antenna, BR = brain, HE = haemocytes, MS = muscle, OV = ovaries, S = sperm, TS = testes; AC = *Allium cepa* (1C = 16.50 pg), BO = *Bos taurus* (1C = 3.70 pg), BP = *Bellis perennis* (1C = 1.76 pg), DM = *Drosophila melanogaster* (1C = 0.18 pg), DV = *Drosophila virilis* (1C = 0.34 pg), GD = *Gallus domesticus* (1C = 1.25 pg), HS = *Homo sapiens* (1C = 3.50 pg), LM = *Locusta migratoria* (1C = 5.50 pg), MD = *Musca domestica* (1C = 0.90 pg), MM = *Mus musculus* (1C = 3.30 pg), OM = *Oncorhynchus mykiss* (1C = 2.60 pg), PA = *Periplaneta americana* (1C = 3.41 pg), PS = *Pisum sativum* (1C = 4.55 pg), SG = *Schistocerca gregaria* (1C = 8.70 pg).

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Suborder: Caelifera									
Acrididae	Acridinae	*Acrida conica*	n.a.	12.55	23	FD	HE	GD, OM	Rasch 1985*
Acrididae	Acridinae	*Acrida conica*	M	10.82	23	FD	TS	GD	Rees et al. 1978
Acrididae	Acridinae	*Caledia captiva*	M	10.9	23	FD	TS	GD	Rees et al. 1978
Acrididae	Acridinae	*Cryptobothrus chrysophorus*	M	9.37	23	FD	TS	GD	Rees et al. 1978
Acrididae	Acridinae	*Schizobothrus flavovittatus*	M	7.5	n.a.	FD	TS	GD	Rees et al. 1978
Acrididae	Catantopinae	*Macrotona australis*	M	8.49	23	FD	TS	GD	Rees et al. 1978
Acrididae	Catantopinae	*Peakesia hospita*	M	10.47	23	FD	TS	GD	Rees et al. 1978
Acrididae	Catantopinae	*Phaulacridium vittatum*	M	10.73	23	FD	TS	GD	Rees et al. 1978
Acrididae	Cyrtacanthacridinae	*Schistocerca cancellata*	M	9.49	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	n.a.	8.96	23	FD	V	MM	Fox 1970*
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	M	8.71	23	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	M	8.55	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Cyrtacanthacridinae	*Schistocerca gregaria*	M	8.74	23	FD	S	n.a.	Camacho et al. 2015
Acrididae	Cyrtacanthacridinae	*Schistocerca paranensis*	M	8.63	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Cyrtacanthacridinae	*Valanga irregularis*	M	9.44	23	FD	TS	GD	Rees et al. 1978
Acrididae	Eyprepocnemidinae	*Eyprepocnemis plorans*	M	9.7	23	FD	S	LM	Ruiz-Ruano et al. 2011
Acrididae	Eyprepocnemidinae	*Heteracris adspersus*	M	6.34	23	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Gomphocerus sibiricus*	M	8.95	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus apicalis*	n.a.	12.61	17	FD	TS	GD	Belda et al. 1991*
Acrididae	Gomphocerinae	*Chorthippus biguttulus*	F	11.31	18	FCM	MS	PS	**this study**
Acrididae	Gomphocerinae	*Chorthippus binotatus*	n.a.	10.91	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus cf. binotatus*	n.a.	10.35	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus brunneus*	M	10.15	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus brunneus*	M	9.46	17	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Gomphocerinae	*Chorthippus brunneus*	M	8.55	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Chorthippus dorsatus*	n.a.	8.34	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus dorsatus*	F	12.07	18	FCM	MS	PS	**this study**
Acrididae	Gomphocerinae	*Chorthippus jacobsi*	n.a.	10.84	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus jucundus*	n.a.	11.88	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus longicornis*	M	8.58	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus nevadensis*	n.a.	11.53	17	FD	TS	GD	Belda et al. 1991

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	n.a.	14.72	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	n.a.	13.83	17	n.a.	n.a.	n.a.	Petitpierre 1996
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	M	13.36	17	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Gomphocerinae	*Pseudochorthippus parallelus*	M	12.31	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Chorthippus scalaris*	n.a.	14.72	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Chorthippus vagans*	M	8.68	17	FD	TS	AC	Gosalvez et al. 1980
Acrididae	Gomphocerinae	*Chorthippus vagans*	n.a.	8.64	17	FD	TS	GD	Belda et al. 1991
Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	n.a.	13.38	17	n.a.	n.a.	n.a.	Petitpierre 1996
Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	M	12.66	17	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Gomphocerinae	*Myrmeleotettix maculatus*	M	12.14	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Omocestus viridulus*	M	13.16	17	FD	TS	LM	John and Hewitt 1966
Acrididae	Gomphocerinae	*Stauroderus scalaris*	n.a.	16.34	17	n.a.	n.a.	n.a.	Petitpierre 1996
Acrididae	Melanoplinae	*Campylacantha olivacea*	F	6.98	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Campylacantha olivacea*	M	6.15	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Melanoplus differentialis*	M	6.79	23	FCM	BR	PA	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Melanoplus differentialis*	n.a.	6.23	23	FD	HE	GD, OM	Rasch unpubl. *
Acrididae	Melanoplinae	*Melanoplus differentialis*	n.a.	3.84	23	FD	OV, TS	BO	Swift and Kleinfeld 1953*
Acrididae	Melanoplinae	*Melanoplus differentialis*	F	7.26	24	FCM	BR	PA	Hanrahan and Johnston 2011
Acrididae	Melanoplinae	*Melanoplus sanguinipes*	n.a.	5.83	23	FD	HE	GD, OM	Rasch unpubl. *
Acrididae	Melanoplinae	*Podisma pedestris*	M	16.93	23/24	FD	S	SG	Westermann et al. 1987
Acrididae	Oedipodinae	*Ailopus thalassinus*	M	6.68	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Austroicetes pusilla*	M	6.29	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Gastrimargus musicus*	M	9.01	n.a.	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Humbe tenuicornis*	M	8.21	23	FD	TS	LM	John and Hewitt 1966
Acrididae	Oedipodinae	*Chortoicetes terminifera*	M	7.22	23	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Oedipodinae	*Chortoicetes terminifera*	M	5.99	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Locusta migratoria*	F	6.44	24	FCM	n.a.	MM	Wang et al. 2014
Acrididae	Oedipodinae	*Locusta migratoria*	n.a.	6.35	23	FD	HE	GD, OM	Rasch 1985
Acrididae	Oedipodinae	*Locusta migratoria*	n.a.	6.27	23	FD	V	MM	Fox 1970
Acrididae	Oedipodinae	*Locusta migratoria*	M	6.09	23	FD	TS	MM	Wilmore and Brown 1975
Acrididae	Oedipodinae	*Locusta migratoria*	M	5.47	23	FD	TS	GD	Rees et al. 1978
Acrididae	Oedipodinae	*Locusta migratoria*	n.a.	5.28	23	FD	S	MD	Bier and Müller 1969*
Acrididae	Oedipodinae	*Oedipoda caerulescens*	F	14.2	24	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Sphingonotus caerulans*	M	12.56	23	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Sphingonotus caerulans*	F	13.32	24	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Stethophyma grossum*	M	17.36	23	FCM	MS	PS	**this study**
Acrididae	Oedipodinae	*Stethophyma grossum*	F	18.48	24	FCM	MS	PS	**this study**
Morabidae	Morabinae	*Warramaba virgo*	n.a.	4	15	FD	BR	GD	White and Webb 1968

Family	Subfamily	Species	Sex	1C [pg]	2n	Method	Cell Type	Standard Sp.	References
Morabidae	Morabinae	*Warramaba virgo*	n.a.	3.75	15	n.a.	n.a.	n.a.	Petitpierre 1996
Suborder: Ensifera									
Anostostomatidae	Deinacridinae	*Hemideina crassidens* [1]	M	5.4	15	FCM	AN	BP	Morgan-Richards 2005
Anostostomatidae	Deinacridinae	*Hemideina crassidens* [1]	F	6.01	16	FCM	AN	BP	Morgan-Richards 2005
Anostostomatidae	Deinacridinae	*Hemideina thoracica* [1]	M	5.95	15	FCM	AN	BP	Morgan-Richards 2005
Anostostomatidae	Deinacridinae	*Hemideina thoracica* [1]	F	6.53	16	FCM	AN	BP	Morgan-Richards 2005
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2.38	11	FIA	HE	DM	Koshikawa et al. 2008
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FD	HE	GD, OM	Rasch 1985
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FD	OV, TS	MM, HS	Lima-de-Faria et al. 1973
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FCM	BR	DM	Gregory unpubl.
Gryllidae	Gryllinae	*Acheta domesticus*	n.a.	2	11	FIA	HE	GD	Gregory unpubl.
Gryllidae	Gryllinae	*Gryllus pennsylvanicus*	n.a.	2.68	11	n.a.	n.a.	n.a.	Petitpierre 1996
Gryllidae	Gryllinae	*Gryllus pennsylvanicus*	n.a.	2.06	21	FD	S	MD	Bier and Müller 1969
Gryllidae	Gryllinae	*Gryllus pennsylvanicus*	n.a.	2	21	FD	HE	GD, OM	Rasch 1985
Gryllidae	Oecanthinae	*Oecanthus niveus*	n.a.	1.71	n.a.	FCM	BR	DV	Hanrahan and Johnston 2011
Gryllotalpidae	Gryllotalpinae	*Neoscapteriscus borellii*	n.a.	3.41	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Rhaphidophoridae	Ceuthophilinae	*Ceuthophilus stygius*	n.a.	9.55	n.a.	FD	HE	GD, OM	Rasch and Rasch 1981
Rhaphidophoridae	Ceuthophilinae	*Hadenoecus subterraneus*	n.a.	1.55	n.a.	FD	HE	GD, OM	Rasch and Rasch 1981
Tettigoniidae	Conocephalinae	*Conocephalus sp.*	M	2.65	33	FCM	BR	GD	Hanrahan and Johnston 2011
Tettigoniidae	Conocephalinae	*Conocephalus sp.*	F	3.03	34	FCM	BR	GD	Hanrahan and Johnston 2011
Tettigoniidae	Conocephalinae	*Neoconocephalus triops*	M	7.29	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Tettigoniidae	Conocephalinae	*Neoconocephalus triops*	F	7.93	n.a.	FCM	BR	GD	Hanrahan and Johnston 2011
Tridactylidae	n.a.	unknown sp.	n.a.	2.63	n.a.	FCM	BR	DV	Hanrahan and Johnston 2011
Trigoniidae	Trigonidiinae	*Laupala cerasina*	n.a.	1.93	n.a.	FCM	BR	GD	Petrov et al. 2000

the standard in 500 µl of 4°C cold Otto buffer I. The suspension of released cells was then filtered through a 42 µm nylon mesh and divided in two parts. One part was stained with 1,000 µl DAPI solution (stock: 25 ml Otto buffer II, 1 ml DAPI (0.1 mg/ml), 25 µl 2-mercaptoethanol (2 µl/ml)); the second part was stained with 1,000 µl propidium iodide (PI) solution (stock: 25 ml Otto buffer II, 1 ml RNase (1 mg/ml), 1 ml PI (1 mg/ml), 25 µl 2-mercaptoethanol) (Doležel et al. 2007).

For DAPI analysis, the Partec CyFlow instrument (Partec GmbH, Münster, Germany) with UV LED chip and for PI analysis the Partec SL instrument with a green solid-state laser (Cobolt Samba, 532 nm, 100 mW) were used. Each sample was stained for several minutes before measurement, and 3,500 to 5,000 particles were recorded in each FCM analysis. FCM data were analysed with the Partec FloMax v. 2.52 software (Partec GmbH, Münster, Germany).

Combined DAPI and PI measurement results of the same sample express the AT/GC ratio of the genome of the species, the GC content (e.g. Šmarda et al. 2008; Sadílek et al. 2019a, b). The GC content of *P. sativum* is 38.50% (e.g. Barrow and Meister 2002; Šmarda et al. 2008) and the GC content of the analysed samples was calculated with the Microsoft Excel macro from Šmarda et al. (2008).

RESULTS

DAPI-stained samples yielded a lower coefficient of variation (CV) than PI-stained samples, on average CV = 2.83% and 3.59% respectively. All the analysed species of Oedipodinae reached higher genome size values than the analysed species of Gomphocerinae. We were able to measure the genome size of both sexes only in two species (*S. caerulans* and *S. grossum*). There, the female/male genome size values clearly reflected the XX/X0 sex determination system differences. Due to this sex determination system it is generally preferred to report genome size in 2C values rather than the commonly used 1C value. However, to allow for better comparability, we here report both values.

All analysed species of Oedipodinae had distinct genome size (Table 1). The male of *S. caerulans* had 2C = 25.12 pg (1C = 12.56 pg); the female had 2C = 26.63 pg (1C = 13.32 pg). The female specimen of *O. caerulescens* exhibited a 2C value of 28.39 pg (1C = 14.20 pg). The largest genome size was recorded in *S. grossum*, where the male reached 2C = 34.72 pg (1C = 17.36 pg) and the female 2C = 36.95 pg (18.48 pg). Both closely related Gomphocerinae species showed very similar genome sizes (Table 1): 2C = 22.62 pg (1C = 11.31 pg) in the *C.* cf. *biguttulus* female and 2C = 24.14 pg (1C = 12.07) in the female of *C. dorsatus*.

The sample/standard ratio of samples stained with PI was always higher than in DAPI-stained samples of the same specimen, ranging from 11% difference in the female of *C. dorsatus* to 18% difference in the female of *S. grossum*. This trend is observable also in the GC content, where *C. dorsatus* had only 40.82% and the female of *S. grossum* had 42.35% (Table 1). However, the GC content differences among all species analysed were minimal.

DISCUSSION

We present new genome size estimates for five species of Acrididae, one of which represents the largest genome of all insects measured so far, the genome of the female of *Stethophyma grossum* with 2C = 36.95 pg (1C = 18.48 pg). We also measured a female of *C. dorsatus* with 2C = 24.14 pg (1C = 12.07 pg). This species was measured before using the Feulgen densitometry method with 1C = 8.34 pg (Belda et al. 1991). However, the more recent method of flow cytometry we used is considered more accurate for genome size estimations (e.g. Doležel and Greilhuber 2010). Furthermore, we collected all previous estimates from Gregory (2020) and added few additional resources to provide some basic visualization of the genome size variation in the different subfamilies of Orthoptera (Fig. 1).

In total, we gathered 92 (our new data included) estimates of genome sizes belonging to 54 species (Table 2, Fig. 1). These data included 68 estimates for Caelifera (43 species) and 17 for Ensifera (11 species). They ranged from 1C = 3.75 pg for *Warramaba virgo* (Key, 1963) (Morabidae) (Petitpierre 1996) to 1C = 18.48 pg for *Stethophyma grossum* (Oedipodinae, present study) in Caelifera and from 1C = 1.55 pg for *Hadenoecus subterraneus* to 1C = 9.55 pg for *Ceuthophilus stygius* (Scudder, 1861) (both cave Rhaphidophoridae) in Ensifera (Rasch and Rasch 1981). Average 1C values in Ensifera and Caelifera are 3.16 pg (± 2.18 pg) and 9.83 pg (± 3.32 pg) respectively. Further analyses at the family and subfamily level are difficult, as most data comes from Acrididae with 66 measurements (78%). The average genome size in Acrididae is 10.01 pg (± 3.19 pg). Within Acrididae, most estimates came from 26 measurements of Gomphocerinae and 17 of Oedipodinae with average genome sizes of 1C = 11.52 pg (± 2.17 pg) and 9.13 pg (± 4.20 pg) respectively (Table 2, Fig. 1).

Generally, the short-horned grasshoppers (Caelifera) appear to have larger genomes compared to the long-

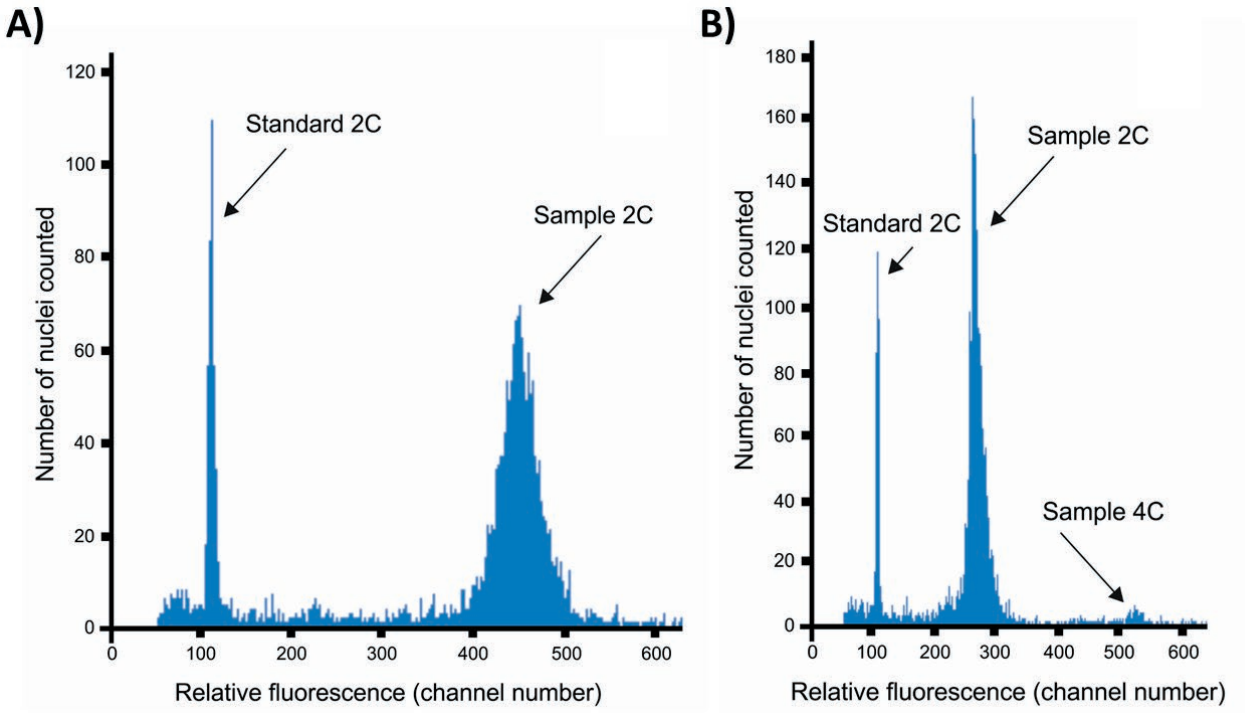

Figure 1. Relative fluorescence histograms for samples stained with PI. 2C peaks represent diploid cells and 4C peaks represent cells in the G2 phase of the cell cycle. with replicated DNA. Standard used: *P. sativum* 2C = 9.09 pg. (**A**) *S. grossum* female with 2C = 36.95 pg. (**B**) *C. biguttulus* female with 2C = 22.62 pg.

horned grasshoppers (Ensifera). However, this is not correlated with the number of chromosomes. Despite their relatively low male number of chromosomes of 2n = 17 (most of other Acrididae have 2n = 23; e.g. Sylvester et al. 2019), Gomphocerinae have some of the largest genome sizes. Their average genome size is 1C = 11.52 pg ranging from 1C = 8.34 pg in *C. dorsatus* (Belda et al. 1991) to 16.34 pg in *Stauroderus scalaris* (Petitpierre 1996; Gregory 2020). Moreover, they show large intraspecific variation in genome size evident from different studies (Table 2), for example: 1C = 12.31 pg to 14.72 pg for *Pseudochorthippus parallelus* (Zetterstedt, 1821) (John and Hewitt 1966; Wilmore and Brown 1975; Belda et al. 1991; Petitpierre 1996) or 1C = 8.55 to 10.15 pg for *C. brunneus* (John and Hewitt 1966; Wilmore and Brown 1975; Gosalvez et al. 1980). All studies of the two species mentioned above share the method of Feulgen densitometry and used testes to measure genome size. Hence it remains unclear whether this variation is natural or the result of methodological differences. However, it is more likely that the large intraspecific differences are a result of a combination of multiple factors: different populations analysed, lack of chromosome observations, various standards used and also different instrumentation could play some role.

The variation in genome size is even higher in Oedipodinae with a minimum of 1C = 5.28 pg for *Locusta migratoria* (Linnaeus, 1758) (Bier and Müller 1969) and a maximum of 1C = 18.48 pg in *Stethophyma grossum*. Hence, *S. grossum* represents the so far largest measured confirmed insect genome. A study by Schielzeth et al. (2014) measured much larger genome sizes for the Gomphocerinae species *C. biguttulus* with 1C up to 236.05 pg. Due to the enormous variation of the estimates in the study and critical methodological issues, Camacho (2016) suggested that these estimates cannot be considered reliable. Hence, we consider our estimate of the *S. grossum* genome size as the current upper size of insect genomes. Since only very few species have been measured so far, it is expected that this is not the upper bound for genome sizes in grasshoppers or for insects in general.

The reasons for the large size of Caelifera genomes remain largely unknown. However, a recent paper by Shah et al. (2020) suggests that repetitive DNA and especially the expansion of satellite DNA may be a main reason for the large genomes in Orthoptera. The most likely causes are genome duplications at the basis of the Acrididae, which would also explain their specifically high rates in nuclear mitochondrial pseudogenes (numts,

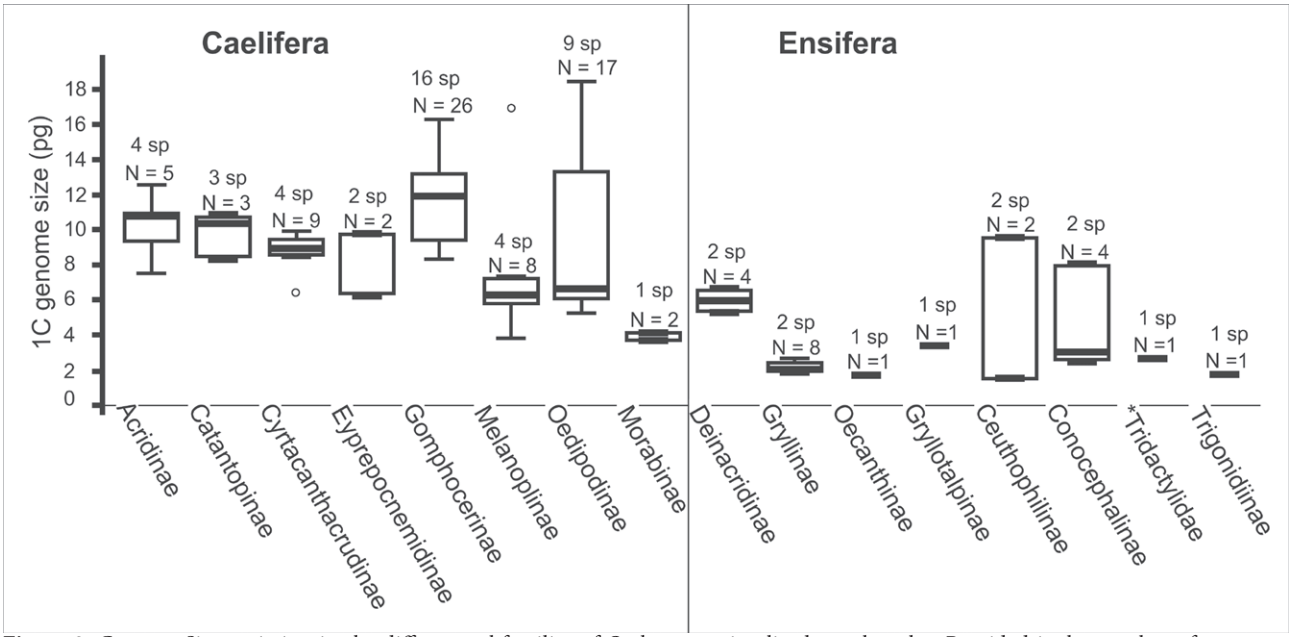

Figure 2. Genome Size variation in the different subfamilies of Orthoptera visualized as a boxplot. Provided is the number of measurements (N) and the number of species (sp) these measurements were derived of (some of the species were measured repeatedly by different authors). Most of the data excerpted from database Gregory (2020) completed with another original data comprehended in Table 2. *unknown species genome size was analysed, determined only on family level.

Bensasson et al. 2000; Song et al. 2008) posing difficulties to species identification using DNA barcoding and to phylogenetic reconstruction (Hawlitschek et al. 2017, Song et al. 2018). It may also explain why only a single incomplete genome is available to date (Wang et al. 2014). Grasshopper genome sizes remain a major obstacle to genomic research, and many further studies will be required to understand genome size variation and evolution in Orthoptera.

ACKNOWLEDGEMENT

We thank Torsten Demuth for providing locality access and help with sampling. We also thank Martin Fikáček (Charles University, Prague, Czech Republic) for financial support for processing the samples in FCM laboratory of Tomáš Urfus (Charles University, Prague, Czech Republic) from the botany department.

DATA AVAILABILITY STATEMENT

All data generated and used in this article is included as tables and figures.

GEOLOCATION INFORMATION

All sampling for this study was performed 2019 in Hamburg, Georgswerder (Germany, 53.5097°N 10.0301°E).

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