C-value estimates for 31 species of ladybird beetles (Coleoptera: Coccinellidae)

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This study provides C-value (haploid nuclear DNA content) estimates for 31 species of ladybird beetles (representing 6 subfamilies and 8 tribes), the first such data for the family Coccinellidae. Despite their unparalleled diversity, the Coleoptera have been very poorly studied in terms of genome size variation, such that even this relatively modest sample of species makes the Coccinellidae the third best studied family of beetles, behind the Tenebrionidae and Chrysomelidae. The present study provides a comparison of patterns of genome size variation with these two relatively well-studied families. No correlation was found between genome size and body size in the ladybirds, in contrast to some other invertebrate groups but in keeping with findings for other beetle families. However, there is some indication that developmental time and/or feeding ecology is related to genome size in this group. Some phylogenetic patterns and possible associations with subgenomic features are also discussed.

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The extensive variation in genome sizes (haploid nuclear DNA contents, or "C-values") observed among taxa (> 3,300-fold in animals alone) has been a puzzle in molecular biology for more than half a century. While the issue is gaining increasing attention because of the theoretical and practical importance of genome size information (HARDIE et al. 2002), many important groups remain overlooked. In particular, insects – the most diverse types of animals on the planet – have been badly neglected in past work on genome size.

Ladybirds (family Coccinellidae) are some of the most familiar of all beetles, and are represented by some 5,000 species worldwide (i.e. more than mammals). Yet, to date none has been studied from the perspective of nuclear DNA contents. In fact, most of the current beetle genome size dataset derives from only a half-dozen families (GREGORY 2001). The present study provides C-value measurements for 31 species of coccinellids collected in central Europe and southern Ontario, Canada, or sampled from laboratory or commercial colonies. While relatively small, this survey nevertheless makes the Coccinellidae the third best studied family of beetles, behind the Tenebrionidae (64 species) and Chrysomelidae (56 species). Amazingly, no other coleopteran families have received anywhere near even this rudimentary level of coverage (all others ≤ 6 species). Clearly, a great deal of work remains to be done in the genomic study of this most speciose of all animal orders, of which the present report is only a beginning.

MATERIAL AND METHODS

Most of the specimens included in the present study were collected from wild populations in southern Ontario, Canada (by TRG and SJA) and the Czech Republic (by ON), with some additional specimens from laboratory colonies kept in the Czech Republic or commercial colonies maintained by Natural Insect Control of Niagara Falls, Ontario (Table 1).

C-values were assessed using the Feulgen image analysis densitometry equipment and protocols described in detail by HARDIE et al. (2002). While most insects, including most beetles, possess elongated spermatozoa (GREGORY 2002a), those of many coccinellids are particularly long and lightly staining, which greatly complicates their measurement. When possible, sperm were used in comparison to a *Drosophila melanogaster* (1C = 0.18 pg) sperm standard, but in cases where these cells could not be used, haemocytes were analyzed relative to a *Tenebrio molitor* (1C = 0.52 pg) haemocyte standard (Table 1). Chicken and rainbow trout erythrocytes were also included in each staining run for use as internal controls (HARDIE et al. 2002), but were not used to calculate genome size for the beetles.

RESULTS AND DISCUSSION

Summary of the results

Table 1 provides a list of the genome size estimates for the various species of ladybirds studied here. Overall, these representatives of the Coccinellidae range 9-fold in C-value, from 0.19 pg in *Coleomegilla maculata* to 1.71 pg in *Exochomus quadripustulatus*, with a mean of 0.53 pg $\pm$ 0.05 SE and a median of 0.43 pg. Apart from a highly anomalous value of 3.69 pg in the leaf beetle *Chrysolina carnifex* (PETITPIERRE et al. 1993) and a value 2.71 pg in the chafer *Rhizotrogus lepidus* (BOSCH et al. 1989), all beetle C-values reported so far fall below 2 pg (GREGORY 2001). In the Tenebrionidae, genome sizes range about 6-fold (0.16–0.87 pg) with an average of 0.37 pg $\pm$ 0.02. The Chrysomelidae are more variable, ranging about 21-fold if *C. carnifex* is included and 11-fold if this species is omitted (0.17–1.98 pg) and displaying an average of 0.75 pg $\pm$ 0.07 SE (0.81 pg with *C. carnifex*). The Dermestidae, which have not been nearly as well studied (n = 6), range about 2-fold (0.90–1.98 pg) with an average of 1.30 pg $\pm$ 0.17 SE. In this regard, the Coccinellidae are among the more variable in terms of overall range and have a mean C-value intermediate between the comparatively well studied Tenebrionidae and Chrysomelidae.

Intraspecific variation in genome size has been reported for some tenebrionid beetles, most notably in members of the genus *Tribolium*, but this was relatively minor and contributed far less to the total variance in the measurements than error within individual samples (ALVAREZ-FUSTER et al. 1991). Within one species, *T. anaphe*, the variation reached 25 % (ALVAREZ-FUSTER et al. 1991), but this seems likely to be due to experimental error since these were all animals from the same laboratory strain and not from geographically variable populations. Moreover, the absolute variation amounted to a mere 0.07 pg. Other examples in which statistically significant intraspecific variation has been reported, such as *Phylan semicostatus* from different Mediterranean islands, actually involve even less absolute variability (0.04 pg in total). Given the sources of error inherent in genome size estimation (RASCH 1985; HARDIE et al. 2002), it would be prudent to expect a wider margin of deviation before ascribing this to real biological variation. Taking this more realistic approach, variation among conspecifics was negligible in the present study, even when specimens were collected from multiple localities (Table 1). As a prime example, individuals of *Propylea quatuorodecimpunctata* collected from wild populations in North America (where it is an introduced species) and Europe gave nearly identical C-value estimates. On the other hand, at least one coccinellid, *Chilocorus stigma*, has been reported to exhibit pronounced intraspecific karyotypic variation (SMITH 1956), and would be interesting to study in more detail from a genome size perspective in the future.

Taxonomy and phylogeny

Based on a variance component analysis (nested ANOVA), it is apparent that most ($\sim$ 75 %) of the total variance in the dataset is derived from differences among subfamilies within the family Coccinellidae (Table 2). However, it must be borne in mind that the subfamily Coccinellinae makes up the majority of the species included here and that many of the tribes are represented by only one or a few species, such that more detailed sampling may change this distribution. A further 19 % of the variance in the present dataset was contributed by differences among congeneric species. Note, however, that in reality there were only a few examples of species from the same genera available here, and that the bulk of this variation was added by two genera with members differing by about two-fold (*Psyllobora* and *Hippodamia*, but not *Coccinella*, *Harmonia*, or *Anatis*; Table 1).

Figure 1 shows the phylogenetic relationships among the coccinellid tribes included in the present study, based on the reconstruction by KOVÁR (1996). While many more data will be required before any strong conclusions can be made, some patterns are apparent. For example, without resorting to the questionable practice of assigning actual numerical values to ancestral genome size states, it appears that relatively small genome sizes have probably been typical throughout much of coccinellid evolution, with only a few groups experiencing what are probably secondary increases. Most notably, the single representative of the tribe Chilochorini (*Exochomus quadripustulatus*), which has by far the largest genome size, is clearly part of a derived group (Fig. 1).

Body size

In addition to genome size data, Table 1 provides information on body sizes in the various species of ladybirds (expressed as mid-range values according to BIELAWSKI 1959, IABLOKOFF-KHNZORIAN 1982 and GORDON 1985). Although genome size and body size are positively correlated in various invertebrates (GREGORY et al. 2000), there is no association between these parameters in ladybirds taken together ($r^2 = 0.003$, $p > 0.77$, $n = 31$), nor within the best represented tribe Coccinellini ($r^2 = 0.06$, $p > 0.33$, $n = 18$; data log-transformed prior to analysis). Similarly, no correlation with body size has been found in

Table 1. *Summary of the ladybird data, including taxonomic information, haploid genome size (GS, in pg), standard error (SE) of genome size measurements, body size (BS, mid-range in mm), the cell type (CT) used in the measurement, the number of individuals (n) studied, the standard (ST) used to calculate genome size, and the source location (LOC) of the specimens. Abbreviations used for cell types and standards, and a numbered list of collection localities, are given at the end of the table. Taxonomy follows* BIELAWSKI *(1959),* IABLOKOFF-KHNZORIAN *(1982),* GORDON *(1985), and* KOVÁR *(1996), with updates according to* KOVÁR *(pers. comm., 2003).*

Species and taxonomic information	GS	SE	BS	CT	n	ST	LOC
Family Coccinellidae							
Subfamily Chilocorinae							
Tribe Chilocorini							
Exochomus quadripustulatus (*Brumus quadripustulatus*, auct.)	1.71	0.03	4.0	HC	2	TM	1
Subfamily Coccidulinae							
Tribe Coccidulini							
Coccidula rufa	0.74	0.003	3.0	HC	2	TM	1
Lindorus lophanthae	0.57	0.02	2.5	S	5	DM	2
Rhyzobius litura	0.88	–	3.0	HC	1	TM	1
(Tribe uncertain)							
Cryptolaemus montrouzieri	0.80	0.01	4.0	S	4	TM	2
Subfamily Coccinellinae							
Tribe Coccinellini							
Adalia bipunctata	0.36	0.04	4.5	HC	3	TM	1
Anatis labiculata	0.67	–	8.5	S	1	TM	3
Anatis ocellata	0.74	–	9.0	HC	1	TM	1
Ceratomegilla undecimnotata (*Semiadalia undecimnotata*, auct.)	0.36	0.001	6.0	HC	2	TM	1
Coccinella quinquepunctata	0.49	0.02	4.0	HC, S	2	DM, TM	4
Coccinella septempunctata	0.33	0.01	7.0	HC, S	7	DM, TM	1,3
Coccinella trifasciata	0.43	0.02	5.0	S	4	DM	4
Coleomegilla maculata lengi	0.19	0.02	5.5	S	2	DM	3
Cycloneda sanguinea limbifer	0.30	–	5.0	HC	1	TM	1*
Cycloneda munda	0.51	0.005	4.5	S	3	DM	4
Harmonia axyridis	0.34	0.008	8.0	HC	2	TM	1**
Harmonia dimidiata (*Leis dimidiata*, auct.)	0.32	0.004	9.0	HC	2	TM	1***
Hippodamia convergens	0.43	0.01	6.0	HC	4	TM	7
Hippodamia variagata (*Adonia variegata*, auct.)	0.29	–	4.5	HC	1	TM	3
Myzia pullata	0.87	–	7.5	S	1	DM	5
Neoharmonia venusta	0.39	0.007	6.0	S	2	DM	3
Oenopia conglobata (*Synharmonia conglobata*, auct.)	0.43	–	4.5	S	1	DM	1
Propylea quatuorodecimpunctata	0.50	0.03	4.0	HC, S	5	DM, TM	13
Tribe Psylloborini							
Psyllobora (*Thea*) *vigintiduopunctata*	0.99	–	3.5	HC	1	TM	1
Psyllobora vigintimaculata	0.42	0.006	2.5	S	3	DM	3
Tribe Tytthaspidini							
Anisosticta bitriangularis	0.34	0.01	3.5	S	4	DM	6
Coccinula quatordecimpustulata	0.46	–	3.5	HC	1	TM	1
Tytthaspis sedecimpunctata	0.24	0.05	3.0	HC, S	2	DM, TM	1
Subfamily Epilachinae							
Tribe Cynegetinini							
Cynegetis impunctata	0.51	–	4.0	S	1	DM	1
Subfamily Scymninae							
Tribe Hyperaspidini							
Brachiacantha ursina	0.64	–	3.5	S	1	DM	4
Subfamily Sticholotidinae							
Tribe Serangiini							
Delphastus catalinae	0.31	0.01	1.5	S	2	DM	2

Abbreviations: cell types (CT): HC = haemocytes, S = spermatozoa.
Standards (ST): DM = *Drosophilia melanogaster* spermatozoa (1C = 0.18 pg), TM = *Tenebrio molitor* haemocytes (1C = 0.52 pg).
Locations (LOC): 1) Czech Republic; 2) commercial colony, Natural Insect Control, Niagara Falls, Ontario; 3) Hamilton, Ontario; 4) Elgin, Ontario; 5) Beausoleil Island, Ontario; 6) Guelph, Ontario.
Location notes: * lab colony, originally from Cuba; ** lab colony, originally from Vancouver Island, British Columbia; *** lab colony, originally from South Asia.

Table 2. *Variance component analysis showing the contribution of different taxonomic levels to the total variance in the coccinellid genome size dataset. Most of the variation follows a higher taxonomic pattern, being based on differences among subfamilies within the family Coccinellidae. However, note that the taxonomic sampling in this study was limited, with few congeneric examples available (i.e. 31 species from 24 genera) and most of the species being members of the subfamily Coccinellinae (Table 1).*

Taxonomic level	n	% variance
Subfamilies within the family	6	74.5
Tribes within subfamilies	8	4.1
Genera within tribes	24	2.2
Species within genera	31	19.2

either the Tenebrionidae or Chrysomelidae taken as a whole (JUAN and PETITPIERRE 1991; PETITPIERRE and JUAN 1994). At lower taxonomic scales, no correlation was found among a small number of species in the genus *Tribolium* (ALVAREZ-FUSTER et al. 1991), but negative correlations with body size have been reported in other tenebrionids, including within the genus *Pimelia* (PALMER et al. 2003) and intraspecifically within *Phylan semicostatus* (PALMER and PETITPIERRE 1996). However, in the former case the relationship is only significant following complex phylogenetic correction, and again in the latter, the absolute difference in genome size among populations was a mere 0.04 pg, thereby raising considerable doubts about the real biological (vs statistical) significance of such relationships.

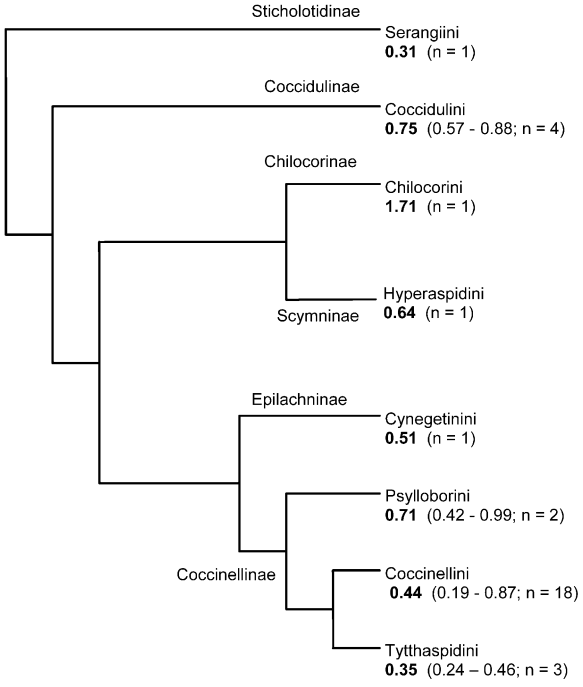

Fig. 1. Phylogeny of coccinellid tribes included in the present study, as presented by KOVÁR (1996). Mean genome sizes (in pg) are given for each tribe, with the range (where applicable) and number of species included in the dataset provided in brackets. Subfamilial divisions are also indicated. Based on the available data, it would appear that small genome size has been typical of coccinellid evolution, with only one case of significant secondary increase.

Development

In their discussion of genome size variation among a few members of the Chrysomelidae, PETITPIERRE and JUAN (1994) noted that species with one generation per year possessed C-values greater than 0.6 pg, while those with multiple generations had genome sizes less than 0.5 pg. In this sense, it is interesting to consider a possible relationship between genome size and developmental rate in particular groups of beetles, as has been reported in many other taxa (GREGORY 2002b). To this end, coccinellid genome sizes were compared with available published data on two developmental parameters in the present study, lower developmental threshold (LDT, in °C) and sum of effective temperatures (SET, in degree-days) for both eggs ($n = 10$) and pupae ($n = 9$) (HONEK 1996). While there was no apparent correlation with LDT for either eggs or pupae ($p = 0.61$, and 0.86, respectively), SET was positively correlated with genome size in pupae ($p = 0.016$) and may also be significant in eggs if more taxa are added (currently, $p = 0.14$). Figure 2 shows the strong positive relationship between pupal development time and genome size when the develop-

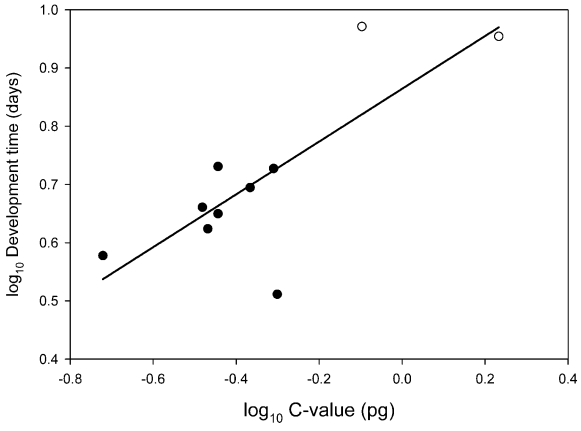

Fig. 2. The correlation between temperature-corrected pupal developmental time (in days at 24°C) and haploid nuclear DNA content (C-value, in pg) for 10 species of coccinellids. The relationship is highly significant ($r^2 = 0.61$, $p < 0.008$). The two species indicated by open circles are coccidophagous and are not subject to the same developmental time constraints as the aphidophagous species indicated by filled circles.

mental data were standardized to 24°C. Unfortunately, data were insufficient for a comparison with larval or total development time, and of course such comparisons are complicated by differences in food sources and other features among species, but it is clear that further analysis along these lines is warranted.

Based on these preliminary results, it seems that either longer development time permits an increase in genome size, or that larger genome sizes slow development. Part of the constraint on genome size that is evident in most ladybirds may relate to rapid development. A rapid developmental rate is certainly selected for by aphidophagy and cannibalistic behaviour. Species that prey on aphids, in particular, must develop rapidly because of the short lifespan of the typical aphid colony (KINDLMANN and DIXON 1999a). Moreover, most aphidophagous species also prey upon their conspecifics (eggs, larvae or pupae) and may also feed on other ladybird species (MAJERUS and HURST 1997). In some cases, the first instar larvae may even prey on their own siblings (OSAWA 2003), which forces the eggs and pupae to develop extremely rapidly.

On the other hand, ladybirds preying upon scale insects (coccids) do not face these two evolutionary pressures and develop much more slowly (KINDLMANN and DIXON 1999b). Notably, the eight species indicated by filled circles in Fig. 2 are aphidophagous, and the standardized development time of their pupae at 24°C ranges from 3.5 to 5.5 days. The two species indicated by open circles at the right end of the distribution (i.e. with the largest genomes), *Cryptolaemus montrouzieri* and *Exochomus quadripustulatus*, are both coccidophagous, and the calculated pupal developmental time of both species at 24°C is 9 days or more (HONEK 1996; O. Nedvěd, unpubl. data).

These observations aside, it is apparent that a very small genome size is not needed in order to successfully invade new environments. Thus, invasive species like *Coccinella septempunctata*, *Harmonia axyridis*, and *Propylea quatuorodecimpunctata* do not have substantially smaller C-values than the endemic North American species that they have largely replaced in a very short period of time. However, it seems that other properties besides rapid development may contribute more to invasive success, especially fecundity, voracity (LUCAS et al. 2002), and intraguild predation (HEMPTINNE et al. 2000).

In a more general sense, the Coccinellidae studied here lend support not only to the developmental hypotheses based on developmental rate per se, but also on the complexity of development. Specifically, it has been suggested that insects with complete metamorphosis, including beetles, will possess genome sizes below a threshold of roughly 2 pg (GREGORY 2002b). All of the ladybirds included in this survey live up to this expectation, and indeed of the more than 160 coleopteran genome size estimates that have now been reported, only two appear to exceed this hypothetical threshold (the afore-mentioned leaf beetle *Chrysolina carnifex* at 3.69 pg and the chafer *Rhizotrogus lepidus* at 2.71 pg; BOSCH et al. 1989; PETITPIERRE et al. 1993). Much broader taxonomic sampling will be required for a more convincing test of this hypothesis in beetles, but for the most part the existing data seem to support it well.

Karyotypic features

Within the tenebrionids and chrysomelids there appears to be a positive relationship between C-value and chromosome number, but there are also notable (and common) cases of substantial variation in DNA content despite karyotypic constancy (JUAN and PETITPIERRE 1991; PETITPIERRE et al. 1993).

The diploid chromosome numbers of the Coccinellidae range from $2n = 10$ (two species in the tribe Coccinellini; LYAPUNOVA et al. 1984) to $2n = 28$ (*Ortalia pectoralis* in the subfamily Ortaliinae, not represented in this study; YADAV and GAHLAVAT 1994). However, most of the species display the typical karyotype of $2n = 20$ found in beetles of the suborder Polyphaga, with others possessing $2n = 18$ chromosomes. Only the tribe Hyperaspidini consistently exhibits fewer chromosomes than this ($2n = 14$ or 16; LYAPUNOVA et al. 1984). While there is a 9-fold difference in nuclear DNA contents in the present dataset, there is no evidence for polyploidy in any ladybird species studied karyologically. Similarly, there is no apparent correlation between the number of chromosomes and genome size. Thus, *Psyllobora vigintiduopunctata*, with a genome size of 0.99 pg, has the same $2n = 20$ chromosome compliment as *Hippodamia variegata* at only 0.29 pg. Likewise, *Tytthaspis sedecimpunctata*, which has an increased number of chromosomes ($2n = 24$; ROZEK and HOLECOVA 2002) has an even smaller genome than these two species (0.24 pg).

One karyotypic feature that seems to relate to genome size in at least some ladybirds is the fusion of chromosomes. For example, centric fusion among non-homologous (sex and autosomal) chromosomes is common among North American species of the genus *Chilocorus* (SMITH 1959; SMITH and VIRKKI 1978), and is often accompanied by the loss of heterochromatic arms and hence leads to a decrease in genome size. However, this mechanism cannot provide a general explanation for the variation in genome size within the entire family Coccinellidae.

Other genomic characteristics

As with eukaryotes in general, most of the variation in genome size among beetles must be explained by variability in the percentage of non-coding DNA contained within the chromosomes. Amounts of C-band heterochromatin were not found to correlate with genome size in tenebrionids (JUAN and PETITPIERRE 1989), but the present study has revealed an intriguing potential link with a specific non-coding portion of the coccinellid genome. In a recent study, von der SCHULENBURG et al. (2001) reported substantial variation in the sizes of the first ribosomal internal transcribed spacers (ITS1) of different species of coccinellids. A comparison of the 7 tribes of coccinellids for which both ITS1 lengths (von der SCHULENBURG et al. 2001) and genome sizes (Table 1) are available, revealed a suggestive but non-significant trend for the two to be positively associated ($r^2 = 0.49$, $p < 0.08$). Obviously, more data will be required in order to determine whether a relationship exists between genome size and this particular type of non-coding DNA, but it does bear noting that by far the largest ITS1 spacer was found in *Exochomus quadripustulatus* (von der SCHULENBURG et al. 2001), and that this species also has the largest C-value so far reported in the family by a wide margin (Table 1). Again, the large genome size of *E. quadripustulatus* is almost certainly a derived feature relative to other coccinellids (Fig. 1). It would be of substantial interest to examine other non-coding segments, especially transposable elements (which are probably the largest contributor to genome size), in this and other coccinellids.

Concluding remarks

Although the study of animal genome size variation has been ongoing for more than 50 years, little progress has been made in the study of the most diverse of all animal groups. With the addition of the present study, only three families of beetles have now been examined to the tune of 30 species or more. Nevertheless, some interesting patterns are beginning to emerge in the beetle genome size dataset, a fact that should prompt a much more intensive effort to study these important animals in the future.

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