Genome size variation in lepidopteran insects

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Abstract: Little information is available on genome size diversity among insects, even in otherwise well-studied groups such as the Lepidoptera. In fact, only six lepidopteran species have been studied to date. The present study therefore represents the first attempt to survey genome size variation in this group, giving estimates for more than 50 species and increasing the coverage of the order to 15 families. Based on this expanded data set, some interesting patterns of variation can be observed, albeit only in a preliminary way. By providing the first large survey of lepidopteran genome sizes, as well as some methodological guidelines and highlights of interesting future work, it is hoped that this study will stimulate further analysis of this diverse group of insects.

Résumé : On connaît mal la diversité de la taille du génome chez les insectes, même chez des groupes, comme les lépidoptères, qui sont, par ailleurs, bien étudiés. En réalité, seules six espèces de lépidoptères ont été étudiés sous cet angle. Notre étude est donc la première tentative pour caractériser la variation de la taille du génome chez ces insectes; elle procure des estimations pour plus de 50 espèces et augmente les connaissances de manière à couvrir 15 familles de l'ordre. D'après ces données élargies, on peut déceler quelques patterns intéressants de variation, bien que de façon bien préliminaire. Notre étude suscitera, nous l'espérons, une analyse plus poussée de ce groupe diversifié d'insectes en fournissant le premier inventaire d'importance des tailles des génomes chez les lépidoptères, ainsi que des conseils méthodologiques et des pistes intéressantes de recherche future.

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Introduction

Moths and butterflies have been collected and studied with great fervour for centuries, but many aspects of their biology remain almost entirely unknown. As a prime example, the genome sizes (haploid nuclear DNA contents or "C-values") of only six lepidopterans have so far been determined. These values include two economically important species (the silkworm moth, *Bombyx mori*, at 0.5 pg and Chinese oak silkmoth, *Antheraea pernyi*, at 1 pg), three pest species (the gypsy moth, *Lymantria dispar*, at 1 pg; wax moth, *Galleria melonella*, at 0.5 pg; and tobacco budworm moth, *Heliothis virescens*, at 0.4 pg), and the geometrid *Lycia pomonaria* at about 1.5 pg (Table 1).

Although insect genome size data are quite limited in general (Gregory 2001), it is surprising that lepidopterans have been so poorly studied. To remedy this situation and to provide preliminary insights concerning the patterns of genome size variation in lepidopterans, this study provides C-value estimates for more than 50 species from this order. Patterns of variation and some potential correlates of genome size are discussed. As well, some methodological insights are provided to aid future genome size studies on this group. Some suggestions for future research are also given in the hopes of stimulating further study of this large and familiar group of insects.

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Materials and methods

Collection and identification

Most of the species included in this survey were collected at ultraviolet lights near Guelph, Ontario, with a few additional specimens collected elsewhere (Table 1). Species identifications and taxonomy followed Handfield (1999).

Sample preparation

Genome sizes were estimated by Feulgen image analysis densitometry of spermatozoa (Hardie et al. 2002). In most insects, mature sperm are thin and highly elongated and lack a distinct head (Gregory 2002*a*). Sperm of this type ordinarily form in bundles, which must be dispersed so that individual nuclei can be measured. In some groups, this can be accomplished easily by gentle mixing with dissecting pins or a pipette, but the tight bundles in moths require more substantial efforts to separate individual sperm. Lepidopterans also possess two types of sperm, eupyrene and apyrene, but only the former of these is nucleated (Friedländer 1997). Under natural conditions, lepidopteran sperm bundles are activated by a secretion of the male reproductive tract and separate following insemination of the female (Shepherd 1974*a*, 1974*b*, 1975). Thus, the spermathecae of mated females can be dissected to provide dispersed sperm (Morrow and Gage 2000), although this can be somewhat difficult. Moreover, most of the individuals collected by the simplest methods (e.g., at ultraviolet light traps) are male.

Two methods have been developed for the separation of moth sperm bundles, both of which begin with the dissection of the testes and their associated tubules under insect

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Table 1. Genome sizes of roughly 60 species of lepidopterans (phylum Athropoda, subphylum Hexapoda, class Insecta, subclass Pterygota, order Lepidoptera), most of them from the present study and based on Feulgen image analysis of sperm versus a sperm standard from *Drosophila melanogaster* (1 C = 0.18 pg).

Species	Common name	GS	SE	*N*	Source
Family Arctiidae					
Ctenucha virginica	Virginia ctenucha	0.50	0.007	7	Guelph, Ont.
Euchaetes egle	Milkweed tiger moth	0.79	—	1	Guelph, Ont.
Grammia (*Apantesis*) *virgo*	Virgin tiger moth	0.98	0.03	2	Guelph, Ont.
Halysidota tesselaris	Tiger moth	0.61	0.02	2	Guelph, Ont.
Hyphantria cunea	Fall webworm moth	0.66	0.02	2	Guelph, Ont.
Hypoprepia fucosa	Painted lichen moth	1.13	0.04	8	Guelph, Ont.
Spilosoma virginica	Virginian tiger moth	0.64	—	1	Guelph, Ont.
Family Bombycidae					
Bombyx mori	Silkworm moth	0.52			Rasch 1974
" "		0.53			Gage 1974
Family Danaidae					
Danaus plexippus	Monarch butterfly	0.29	0.01	2	Guelph, Ont.
Family Drepanidae					
Drepana arcuata	Arched hooktip moth	0.31	—	1	Guelph, Ont.
Drepana bilineata	Two-lined hooktip moth	0.34	—	1	Guelph, Ont.
Family Geometridae					
Ennomine sp.	Geometer moth	0.45	—	1	Guelph, Ont.
Ennomos subsignaria	Elm spanworm moth	0.40	—	1	Guelph, Ont.
Euchlaena irraria	Least-marked Euchlaena	1.94	—	1	Guelph, Ont.
Gueneria similaria	Geometer moth	0.40	—	1	Guelph, Ont.
Lycia (*Poecilopsis*) *pomonaria*	Geometer moth	1.46			Petitpierre 1996
Metanema determinata	Dark Metanema	1.01	—	1	Guelph, Ont.
Nepytia canosaria	False hemlock looper	0.77	0.02	7	Turkey Point, Ont.
Prochoerodes transversata	Maple spanworm moth	0.85	0.05	2	Guelph, Ont.
Scopula limbodata	Larger lace border moth	0.46	0.009	2	Guelph, Ont.
Semiothisa bisignata	Red-headed inchworm moth	0.34	0.02	2	Guelph, Ont.
Xanthotype sospeta	Geometer moth	0.32	0.01	2	Guelph, Ont.
Unidentified sp. 1	Geometer moth	0.48	—		Guelph, Ont.
Unidentified sp. 2	Geometer moth	0.49	—		Guelph, Ont.
Unidentified sp. 3	Geometer moth	0.51	—		Guelph, Ont.
Unidentified sp. 4	Geometer moth	0.84	—		Guelph, Ont.
Family Lasiocampidae					
Malacosoma americana	Eastern tent caterpillar moth	0.51	0.005	2	Guelph, Ont.
Malacosoma disstria	Forest tent caterpillar moth	0.57	0.01	4	Guelph, Ont.
Family Lymantriidae					
Lymantria dispar	Gypsy moth	1.03			Petitpierre 1996
Family Noctuidae					
Caenurgina crassiuscula	Owlet moth	1.50	0.03	2	Guelph, Ont.
Calophasia lunula	Owlet moth	0.69	0.03	2	Guelph, Ont.
Cerma cerintha	Tufted bird dropping moth	1.05	—	1	Guelph, Ont.
Condica videns	White-dotted groundling moth	0.54	—	1	Guelph, Ont.
Cucullia intermedia	Owlet moth	0.46	—	1	Guelph, Ont.
Eudryas grata	Beautiful wood nymph moth	0.38	0.02	2	Guelph, Ont.
Heliothis virescens	Tobacco budworm moth	0.41			Taylor et al. 1993
Lacinipolia renigera	Owlet moth	0.60	—	1	Guelph, Ont.
Macronoctua onusta	Iris borer moth	0.67	—	1	Guelph, Ont.
Noctua pronuba	Large yellow underwing moth	0.57	—	1	Guelph, Ont.
Panthea pallescens	Tufted white pine caterpillar moth	0.86	0.02	3	Guelph, Ont.
Papaipema nepheleptena	Owlet moth	0.66	—	1	Guelph, Ont.
Raphia frater	Brother moth	0.74	0.007	2	Guelph, Ont.
Xestia (*Amathes*) *c-nigrum*	Spotted cutworm moth	0.78	0.05	2	Guelph, Ont.
Unidentified sp. 1	Owlet moth	0.64	—	1	Guelph, Ont.
Unidentified sp. 2	Owlet moth	0.90	—	1	Guelph, Ont.
Unidentified sp. 3	Owlet moth	0.99	—	1	Guelph, Ont.

Table 1 (*concluded*).

Species	Common name	GS	SE	*N*	Source
Family Notodontidae					
Gluphisia septentrioniz	Common Gluphisia	0.36	—	1	Guelph, Ont.
Heterocampa bilineata	Prominent moth	0.60	0.009	5	Turkey Point, Ont.
Nadata gibbosa	White-dotted prominent moth	0.33	—	1	Guelph, Ont.
Peridea basitriens	Oval-based prominent moth	0.36	0.01	5	Turkey Point, Ont.
Unidentified sp.	Prominent moth	0.46	0.02	2	Guelph, Ont.
Family Papilionidae					
Papilio canadensis	Canadian tiger swillowtail butterfly	0.44	<0.001	2	Courtesy of Mark Scriber, Michigan State University (haemocytes versus a standard from *Tenebrio molitor* at 0.52 pg)
Papilio glaucus	Eastern tiger swillowtail butterfly	0.44	0.02	5	Courtesy of Mark Scriber, Michigan State University (haemocytes versus a standard from *Tenebrio molitor* at 0.52 pg)
Family Pterophoridae					
Unidentified sp. 1	Plume moth	0.88	0.02	2	Guelph, Ont.
Unidentified sp. 2	Plume moth	0.59	—	1	Guelph, Ont.
Family Pyralidae					
Galleria melonella	Wax moth	0.50			Rasch 1985
Family Saturniidae					
Antheraea pernyi	Chinese oak silkmoth	1.00			Efstratiadis et al. 1976
Family Satyridae					
Bicyclus anynana	African butterfly	0.49	0.01	4	Courtesy of Antonia Monteiro, State University of New York, Buffalo
Family Sphingidae					
Pachysphinx modesta	Modest sphinx	0.46	0.02	2	Guelph, Ont.
Paonias excaecatus	Blinded sphinx	0.58	—	1	Guelph, Ont.
Paonias myops	Small-eyed sphinx	0.63	—	1	Guelph, Ont.
Smerinthus jamaicensis	Twin-spotted sphinx	0.68	—	1	Guelph, Ont.

Notes: Haploid genome sizes (GS) are in picograms and include standard error (SE) where applicable. The number of individuals measured per species (*N*) is also provided.

Ringers saline (1 L distilled H_2O + 7.5 g NaCl + 0.35 g $CaCl_2$ + 0.21 g KCl), a process that is facilitated by the fusion (and often bright colour) of lepidopteran testes. The first method involves squashing the testes in acetic acid and "buzzing" the slide with a vibrating engraving tool, followed by a freeze-flip technique using either dry ice or liquid nitrogen (Rasch et al. 1971; Rasch 1974). This method may free some sperm for measurements, but it is very time consuming. The second method, and the one employed in this study, involves mixing the bundles vigorously in a small vial with a pipette in a 1% solution of Tween 80 detergent before spreading the mixture onto a slide and allowing it to air dry (Fig. 1). This typically frees sufficient sperm for measurements and is considerably less labour intensive than the freeze-flip method.

Feulgen staining and analysis

The Feulgen staining protocol used in the present study was the same as that described by Hardie et al. (2002). This included an overnight fixation in MFA (85% methanol : 10% formalin : 5% glacial acetic acid), a 120-min hydrolysis in 5 mol/L HCl at room temperature, a 120-min stain in fresh Schiff reagent, and a series of bisulfite and distilled water rinses. Slides were allowed to air-dry following staining and were stored in the dark until analysis. Integrated optical densities (IODs) of stained nuclei were examined at 100× using the Bioquant True Color Windows 98 image analysis software package (©1999 R&M Biometrics Inc., Nashville, Tenn.) and an Optronics DEI-750 CE three-chip CCD camera mounted on a Leica DM LS microscope and connected to a BQ6000 frame-grabber board.

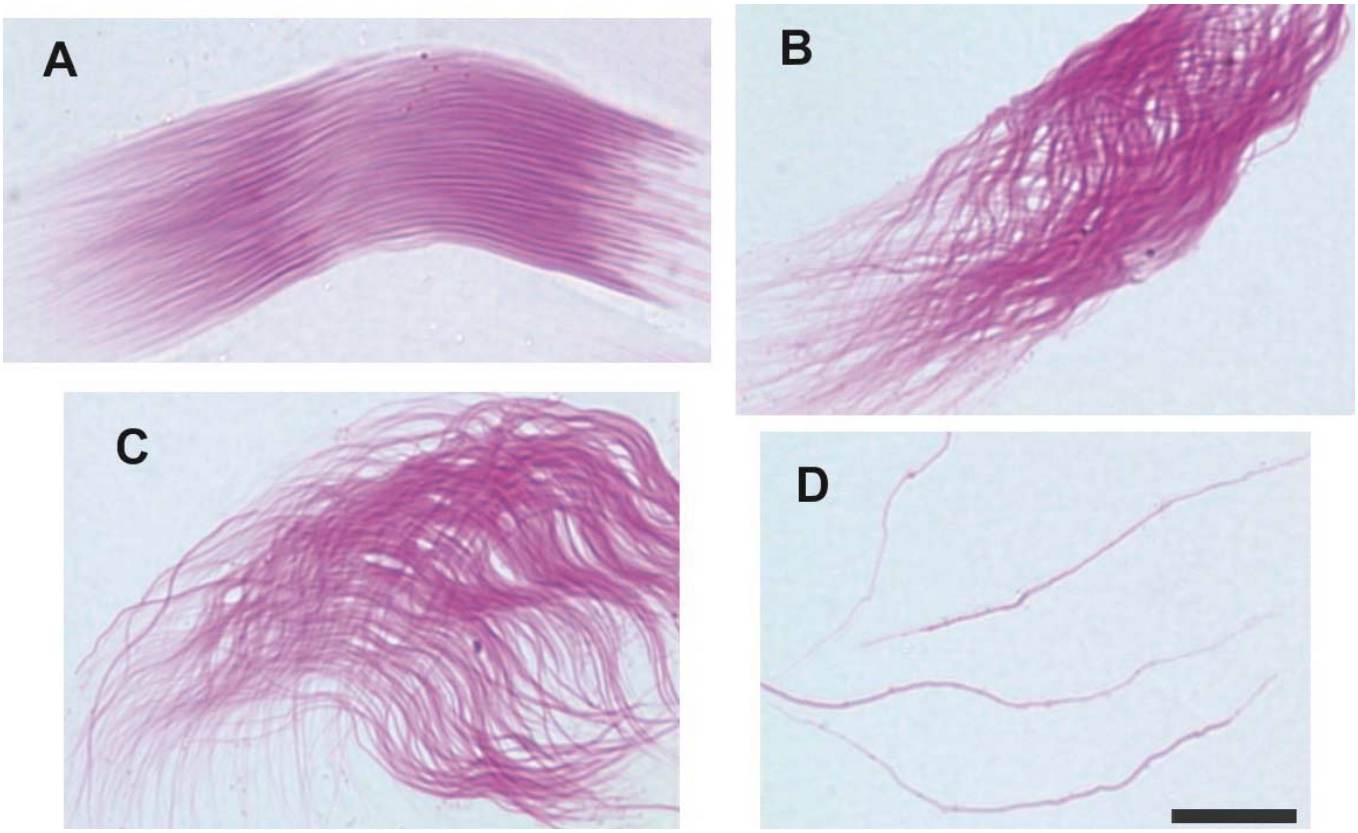
The IODs of moth sperm nuclei were compared against those of *Drosophila melanogaster* (1 C = 0.18 pg) for conversion to absolute DNA contents. Sperm from *Tenebrio molitor* and erythrocyte smears from domestic chicken (*Gallus domesticus*) and rainbow trout (*Oncorhynchus mykiss*) were also included in each staining run as internal checks (Hardie et al. 2002). In many cases only one individual was obtained for a given species, but variation among conspecifics was very low in cases where multiple individuals were available (Table 1).

Results and discussion

Summary of the data set

With the addition of the new values provided in this study, the lepidopteran data set now covers 15 families, with genome sizes ranging from a low of 0.29 pg in the monarch

Fig. 1. Sperm from the moth *Nepytia canosaria* still locked into a bundle (A), partially dispersed (B, C), and dispersed by vigorous pipette mixing in a detergent (Tween 80) (D). Moths display particularly difficult sperm to dissociate from their bundles, and even with detergent mixing it may be somewhat difficult to locate individual undamaged sperm nuclei for measurement. Scale bar = 10 μm.



butterfly, *Danaus plexippus*, to 1.9 pg in the geometrid moth *Euchlaena irraria*. Among the other few butterflies studied, the African satyrid *Bicyclus anynana* has an estimated C-value of 0.49 pg, whereas the tiger swillowtails *Papilio glaucus* and *Papilio canadensis* both have genome sizes of 0.44 pg. The average for all lepidopterans studied to date is 0.66 ± 0.04 pg.

Figure 2 provides a summary of the distribution of variation in genome size among lepidopteran families. No family possesses any unilaterally small or large genome sizes, although groups such as the Notodontidae and Drepanidae may typically have smaller genomes than others like the Arctiidae, Noctuidae, and Sphingidae. Others such as the Lymantriidae and Saturniidae each have at least one representative with relatively large genomes, but as only a single species is known for these families, the generality of this pattern cannot yet be assessed.

In some cases, there appears to be a high level of variation below the family level, suggesting that most variation in lepidopteran genome size does not result from differences among families. However, on closer inspection it becomes apparent that genome size diversity is ordinarily limited among members of a single subfamily. For example, within the Arctiidae, members of the subfamily Arctiinae display only modest variation in C-value, while the clear outliers in this family are members of two different subfamilies (Table 2). Interestingly, the representatives of the arctiid genera *Spilosoma* and *Hyphantria* had almost identical genome sizes, and recent DNA sequencing work has suggested that these may in fact be members of a single genus (P.D.N. Hebert, unpublished data). In like fashion, the other three sets of congeners included in the present study (*Drepana* spp. in the Drepanidae, *Malacosoma* spp. in the Lasiocampidae, and *Papilio* spp. in the Papilionidae) had very similar C-values (Table 1). The Sphingidae is similar, being represented here by four species in the subfamily Sphinginae that vary by only about 0.2 pg from one another, and the Notodontidae also had a low level of variation among species in the family (Table 1). However, the best illustration of subfamily-level constancy comes from the Noctuidae, for which several subfamilies are represented; in each case the largest difference within a given subfamily is about 0.2 pg, whereas the family ranges by more than 1 pg (i.e., over threefold) (Table 2). Generally speaking, such invariance within major groups (subfamilies) implies a relatively gradual process of change in genome size, with C-values evidently under some important constraints that operate differentially among subfamilies. It bears noting, however, that the Geometridae provides an apparent exception to this pattern, with members of the subfamily Ennominae displaying considerable variation in genome size (Table 2).

Although the present survey is very large compared with the previous lepidopteran data set (and indeed, compared with that for most other insect orders), it has included only a tiny fraction of the species in this order. Nevertheless, it is possible to examine some potential phenotypic correlates

Fig. 2. Summary of haploid genome size (C-value) variation in the 15 families of moths and butterflies (order Lepidoptera) studied to date. Families are arranged alphabetically, with moths and butterflies listed separately. Numbers in parentheses indicate the number of species measured per family and error bars represent standard errors. The mean C-value for the Lepidoptera is 0.66 ± 0.04 pg (range 0.29–1.9 pg), based on roughly 60 species that are mostly macromoths.

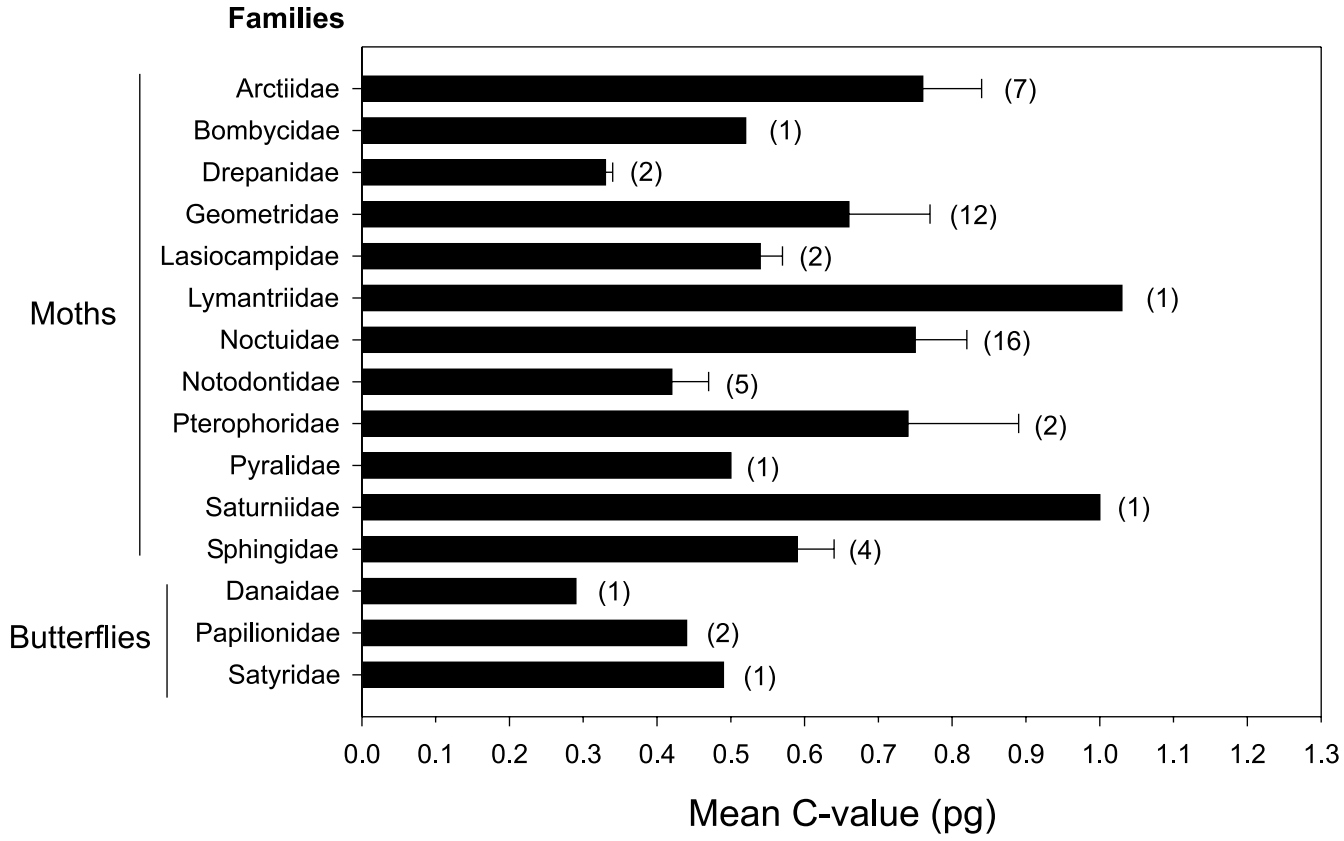


Table 2. Patterns of variation within some families and subfamilies of moths.

Family	Subfamily	Genera
Arctiidae (0.50–1.13)	Arctiinae (0.61–0.98)	*Euchaetes*, *Grammia*, *Halysidota*, *Hyphantria*, *Spilosoma*
	Ctenuchinae (0.50)	*Ctenucha*
	Lithosinae (1.13)	*Hypoprepia*
Geometridae (0.32–1.94)	Ennominae (0.32–1.94)	*Ennomine*, *Ennomos*, *Euchlaena*, *Gueneria*, *Lycia*, *Metanema*, *Nepytia*, *Prochoerodes*, *Semiothisa*, *Xanthotype*
	Sterrhinae (0.46)	*Scopula*
Noctuidae (0.38–1.50)	Acontiinae (0.46)	*Cerma*
	Agaristinae (0.38)	*Eudryas*
	Amphipyrinae (0.54–0.67)	*Condica*, *Macronoctua*, *Macronusta*, *Papaipema*
	Catocalinae (1.50)	*Caenurgina*
	Cucullinae (0.46–0.69)	*Calophasia*, *Cucullia*
	Hadeninae (0.60)	*Lacinipolia*
	Heliothinae (0.41)	*Heliothis*
	Noctuinae (0.57–0.78)	*Noctua*, *Xestia*
	Pantheinae (0.74–0.86)	*Panthea*, *Raphia*

Note: Although there may be considerable variation within families, most subfamilies are remarkably constrained in terms of genome size. C-value ranges (in picograms) are given in parentheses for the family and subfamilies.

that may shed light on the evolution of genome size in moths and butterflies. Other than cell size, the three most common correlates of genome size among animals are body size, metabolic rate, and development. The potential relevance of each of these will be discussed briefly with respect to the Lepidoptera.

Body size

In their recent study of scale versus wing lengths in lepidopterans, Simonsen and Kristensen (2003) considered the possibility that cell/genome size might relate to these morphological parameters. Although body size is correlated positively with genome size in several groups of invertebrates (Gregory et al. 2000), including body (or at least wing) size in some insects (e.g., Ferrari and Rai 1989; Finston et al. 1995; Craddock et al. 2000), these two parameters seem unrelated in lepidopterans based on the data presented here. Notably, the largest moth so far studied (by far), the sphingid *Pachysphinx modesta*, has a below-average C-value of only 0.46 pg. Other relatively large sphingids (though smaller than *P. modesta*) have slightly larger genome sizes (between 0.58 and 0.68 pg), but note that the very small plume moths (family Pterophoridae) have C-values between 0.6 and 0.9 pg. Nevertheless, DNA-content differences in the form of differential endopolyploidization of relevant tissues may still play a role in shaping insect wing-size variation (Craddock et al. 2000; Simonsen and Kristensen 2003), as it does with body size in nematodes (Flemming et al. 2000).

Metabolism

In both mammals and birds (but not in amphibians), there is a significant negative correlation between genome size and mass-corrected metabolic rate (Vinogradov 1995, 1997; Gregory 2002*b*, 2003). Strong-flying birds and bats, in particular, have genomes smaller than the average for their respective classes, suggesting a constraint on genome size imposed by the metabolic demands of powered flight (e.g., Baker et al. 1992; Hughes 1999; Gregory 2002*b*). Interestingly, flying insects possess mass-corrected metabolic rates even higher than those of hummingbirds (Trochilidae) (Sacktor 1976; Suarez et al. 2000). Although comparative metabolic rate data are not available for the lepidopterans studied here, it is notable that the smallest lepidopteran genome yet reported belongs to the monarch butterfly, which is renowned for its extreme seasonal migrations.

Development

In direct contrast to the situation with metabolic rate, amphibians and various invertebrates (but not mammals and birds) display strong negative correlations between genome size and developmental rate (for reviews see Gregory 2002*c*, 2002*d*). More recently, it has been pointed out that developmental complexity, and specifically complex developmental programs involving metamorphosis, may also place constraints on genome size in amphibians and insects (Gregory 2002*c*). Based on a small number of available data, it was suggested that complete metamorphosis places an upper limit of about 2 pg on the C-values of holometabolous insects (Gregory 2002*c*). In this light, perhaps the most important observation in terms of genome sizes in lepidopterans is that all of the nearly 60 species studied so far possess genomes smaller than 2 pg. Lepidopterans are, after all, the classic exemplars of complete metamorphosis among insects.

Areas for future study

The present survey of moth species has revealed some interesting initial patterns, but obviously this must be supplemented with additional data before any strong general conclusions can be drawn regarding the evolution of genome size in lepidopterans. In particular, it would be interesting to examine the possible correlations with body size, metabolism, and developmental rate discussed briefly above using comparative data for these and other species. More broadly, comparisons between groups of moths with diurnal versus nocturnal lifestyles and different relative activity levels could also be very informative. In terms of future sampling, it would be desirable to include more families and more congeners and members of subfamilies, to collect data from more geographically diverse species, and to obtain data for underrepresented taxa like micromoths and butterflies. It is evident that the diversity and familiarity of lepidopterans make them ideal subjects for future work on the evolution of genome size in insects.

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