Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

An exploration of genome size diversity in dragonflies and damselflies (Insecta: Odonata)

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Keywords
body size; chromosome number; C-value; DNA content; image analysis; flight; voltinism.

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Editor: Jean-Nicolas Volff

Received 15 October 2008; revised 16 January 2009; accepted 20 January 2009

doi:10.1111/j.1469-7998.2009.00557.x

Abstract

Like most insect orders, the Odonata (dragonflies and damselflies) remain poorly studied from the perspective of genome size. They exhibit several characteristics that make them desirable targets for analysis in this area, for example a large range in body size, differences in developmental rate, and distinct modes of flight – all of which are related to genome size in at least some animal taxa. The present study provides new genome size estimates and morphometric data for 100 species of odonates, covering about 1/5 of described North American diversity. Significant relationships are reported between genome size and body size (positive in dragonflies, negative in damselflies), and there is also indication that developmental rate and flight are related to genome size in these insects. Genome size is also positively correlated with chromosome number across the order. These findings contribute to an improved understanding of genome size evolution in insects, and raise several interesting questions for future research.

Introduction

The enormous diversity in nuclear genome size among animals (at least 7000-fold) has remained an enigma for more than 50 years. Much has been discovered about the patterns and consequences of genome size variability, although most of this has come from studies of vertebrates and plants (e.g. Bennett & Leitch, 2005; Gregory, 2005). Insects, though hyperdiverse, have not featured prominently in past analyses. Moreover, most data that are available for insects come from only a few orders, while smaller orders have been largely neglected despite their potential to provide important insights.

It is now generally known that genome size is correlated positively with cell size and negatively with cell division rate in several groups (Gregory, 2001). This may result in links between genome size and organism level characteristics such as body size, developmental rate or metabolic rate, depending on the biology of the group. In amphibians, developmental rate and the presence and intensity of metamorphosis appear to be associated with genome size, whereas in mammals and birds the predominant pattern relates to metabolism (reviewed in Gregory, 2005). Such relationships have not been well examined among insects, but genome size has been reported to correlate positively with body size in aphids (Finston, Hebert & Foottit, 1995) and negatively with developmental rate in ladybird beetles and vinegar flies (Gregory, Nedved & Adamowicz, 2003; Gregory & Johnston, 2008). More broadly, there is evidence that orders exhibiting holometabolous development (complete metamorphosis) are usually limited to a genome size of 2 pg or less, whereas various orders with hemimetabolous development (incomplete metamorphosis) exceed this hypothetical threshold by a large extent (Gregory, 2002*a*, 2005). However, limited sampling among orders means that this hypothesis remains in need of much further testing.

Worldwide, about 6000 species of Odonata have been described, most of which are divided into one of two major suborders: Anisoptera (dragonflies, 11 families) and Zygoptera (damselflies, 21 families). A third suborder (Anisozygoptera) is also recognized and includes a single family (Schorr, Lindeboom & Paulson, 2008). Before the present study, only 14 estimates had been reported for the Odonata (Gregory, 2008). Nevertheless, this has been sufficient to indicate at least a five-fold range (from 0.37 pg in *Gomphus flavipes* and *Ophiogomphus cecilia* to 2.16 pg in *Rhionaeschna confusa*).

Though they have received minimal attention in the past, dragonflies and damselflies represent excellent targets for analysis for a variety of reasons. First, as hemimetabolous insects they provide an opportunity to test the hypothetical 2 pg threshold. Second, they exhibit significant diversity in both body size and developmental rate, thereby allowing these characteristics to be evaluated with respect to genome size. Third, dragonflies and damselflies are well known for their flight capabilities, which permits analyses similar to

those that have been carried out in birds. Finally, there has long been interest in chromosomal evolution in this group which could be enlightened through analyses of nuclear DNA amounts.

The present study provided new genome size estimates for 100 species of dragonflies and damselflies from Ontario, Canada and Florida, USA, representing *c.* 1/5 of recorded species diversity in North America (Westfall & May, 1996, Needham, Westfall & May, 2000). These data were explored through comparisons with measurements of body size, life cycle duration, wing parameters, flight strategies and chromosome numbers. The results provide important new information about patterns of genome size diversity in insects, and point out several directions for future research.

Materials and methods

Sources of specimens

Adult dragonflies and damselflies were collected using hand nets around Guelph, Ontario, Canada and in Algonquin Park, Ontario, Canada between June and September 2006 and 2007 and in Tallahassee, Florida and at the Archbold Research Station, Lake Placid, Florida, in May 2007. In total, 427 specimens (mostly males) from 62 dragonfly species and 38 damselfly species were included in this study. All specimens were stored in cellophane envelopes at room temperature in the dark and no additional treatments (e.g. acetone) were used in order to make the specimens available for genetic analyses as part of a separate study.

Genome size estimation

Genome size was estimated using Feulgen image analysis densitometry (FIA) of spermatozoa compared with the same cell type from *Drosophila melanogaster* Oregon R (GS = 0.18 pg). Testes were dissected in Ringer's saline and placed in suspension on a glass slide. Odonate spermatozoa were often found in tight bundles, and were separated mechanically by gently rubbing a pair of dissecting pins against each other (Fig. 1). Dispersed sperm samples were allowed to air dry at room temperature and were stored in the dark until staining in the Feulgen reaction as outlined by Hardie, Gregory & Hebert (2002). In a small number of cases ($n = 7$), sperm could not be obtained and haemocytes were used instead and compared with haemocytes from *Tenebrio molitor* (GS = 0.52 pg). Data measured for four species using both sperm and haemocytes did not differ significantly (paired *t*-test, $P = 0.32$) and were highly correlated ($r = 0.96$).

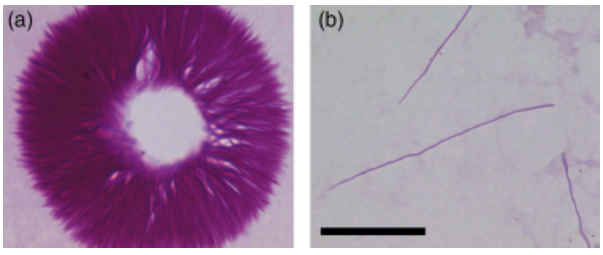

Figure 1 Photomicrographs of Feulgen-stained dragonfly spermatozoa (a) in a bundle, and (b) mechanically dispersed for measurement by computerized image analysis. (a) *Gomphus spicatus* (GS = 0.72 pg), (b) *Aeshna constricta* (GS = 1.76 pg). Note the difference in sperm nucleus size between the two species. Scale bar equals 20μm and applies to both images.

Karyotype data

Karyotypes in this order are composed of holokinetic chromosomes that range in haploid number from $n = 3$ to $n = 15$. However, more than 90% of the species examined to date possess 13–15 chromosomes (Cumming, 1964; Cruden, 1968; Kiauta, 1972). Odonate karyotypes often include microchromosomes (m-chromosomes) and are usually reported for haploid sets as N^{m}, in which N equals the total number of chromosomes and m equals the presence of one microchromosome in the total chromosomal set (Cumming, 1964; Cruden, 1968; Kiauta, 1972). Karyotypes were compiled from the literature for 51 of the species included in the present study (34 dragonflies and 17 damselflies), obtained primarily from the two studies that characterized North American odonates, Cumming (1964) and Cruden (1968) (Table 1). Microchromosomes were ranked at half value (e.g. $12^{m} = 12.5$).

Morphometrics

Specimens were weighed to the nearest 0.001 g at least 6 months after collection. 'Dry weights' *sensu stricto* were not measured as this requires an intensive heat treatment of 60 °C for 24 h (Johnston & Cunjak, 1999), a procedure that might have compromised the utility of the specimens for other genetic analyses. Body size (length in mm) and wing size (length in mm and area in mm^2) were measured for all specimens by photographic image analysis using a Canon 30D Canon, Lake Success, NY, USA digital camera with a 100 mm macro lens and the NIS-Elements BR software 2.30 (Laboratory Imaging, Nikon, Melville, NY, USA 1991–2007). Body size measurements included head, thorax, abdomen and total length, and forewing and hindwing lengths and areas were measured separately. Wing loading (in $mg\,mm^{-2}$) was calculated for each species by dividing body mass by the total surface area of all four wings.

Life cycle duration and nymphal habitat

Temperature-controlled developmental rate data of the type recently analysed for other insects (e.g. Gregory & Johnston, 2008) are not available for odonates. As an alternative, voltinism was considered instead. Whereas the adult stage of odonate species usually lasts between 1 and 3 months, the aquatic nymphal period varies substantially and may last up to 5 years in some species (Corbet, 1962, 1999; Corbet, Suhling & Soendgerath, 2006). Voltinism depends to a significant extent on environmental factors such as rainfall and temperature and exhibits intraspecific variation, but in general odonates have been classified as multivoltine (three

Table 1 Odonate genome size estimates (GS, in pg), standard error (± SE) and number of individuals (*N*) for 62 dragonfly species and 38 damselfly species

Taxonomy	GS (pg)	± SE	*N*	K/R	L
Suborder Anisoptera (Dragonflies)					
Family Aeshnidae					
Aeshna canadensis	2.20	–	1	14/A	3
Aeshna constricta	1.76	0.06	4	–	1
Aeshna eremita	1.85	–	1	–	12
Aeshna tuberculifera	1.78	0.10	2	–	3, 12
Aeshna umbrosa	2.00	–	1	14/A	2
Aeshna verticalis	1.59	–	1	–	11
Anax junius	1.44	–	1	14, 14^{m}/A	6
Basiaeschna janata	1.16	–	1	13/A	14
Epiaeschna heros	1.44	–	1	–	17
Gomphaeschna furcillata	1.20	–	1	–	12
Nasiaeschna pentacantha	1.31	–	1	–	17
Family Cordulegastridae					
Cordulegaster maculata	0.94[a]	–	1	13^{m}/A	12
Family Corduliidae					
Cordulia shurtleffi	1.54[a]	–	1	13/A	11
Dorocordulia libera	0.98	0.03	3	6, 7/A	12
Epitheca canis	1.00	0.02	3	13^{m}/A	17
Epitheca cynosura	1.10	0.06	4	10, 11/A	17
Epitheca princeps	0.98[a]	–	1	–	17
Epitheca spinigera	1.32	–	1	13^{m}/A	12
Neurocordulia yamaskanensis	1.08	–	1	–	12
Somatochlora williamsoni	1.80	–	1	–	12
Somatochlora elongata	2.36[a]	–	1	–	
Family Gomphidae					
Arigomphus pallidus	0.83	–	1	12/B	17
Arigomphus villosipes	0.82	0.03	2	–	1
Dromogomphus spinosus	0.57	–	1	12^{m}/A	14
Gomphus cavillaris	0.71	–	1	–	17
Gomphus (*Gomphurus*) *dilatatus*	0.76	0.05	2	–	17
Gomphus exilis	0.71	0.03	4	12^{m}, 12/A, C	11, 12, 14
Gomphus (*Hylogomphus*) *geminatus*	0.78	–	1	–	17
Gomphus graslinellus	0.73	–	1	–	11
Gomphus minutus	0.75	–	1	–	17
Gomphus spicatus	0.72	–	1	12^{m}/A	11
Hagenius brevistylus	0.93	–	1	–	1
Ophiogomphus rupinsulensis	0.94	0.04	2	12^{m}/A	12
Progomphus obscurus	0.61	0.04	5	12^{m}/A	17
Stylogomphus albistylus	0.72	0.04	2	–	12
Family Libellulidae					
Brachymesia gravida	0.69	–	1	–	17
Celithemis bertha	0.87	–	1	–	17
Celithemis elisa	0.77	0.06	2	13^{m}/A	11
Celithemis eponina	0.91	–	1	–	17
Celithemis ornata	0.54	–	1	13^{m}/D	17
Erythemis simplicicollis	0.56	–	1	13^{m}/A	13
Erythrodiplax minuscula	0.67	–	1	13^{m}/D	17
Ladona julia	0.62	0.03	3	13^{m}/A	11, 14
Ladona deplanata	0.60	0.02	2	–	17
Leucorrhinia glaciallis	0.98	–	1	13^{m}/A	15
Leucorrhinia hudsonica	0.94	–	1	13^{m}, 13/A	12
Leucorrhinia intacta	0.93	–	1	13^{m}, 13/A	11
Leucorrhinia proxima	1.27	–	1	13^{m}/A	16
Libellula incesta	0.74	–	1	13/A	14
Libellula luctuosa	0.87	0.04	2	13/E	4, 5
Libellula pulchella	0.84	0.03	3	13^{m}/A	2
Libellula vibrans	0.95	–	1	13^{m}/A	17
Miathyria marcella	0.41	0.008	2	13, 13^{m}/B, G	18
Perithemis tenera	0.68	0.009	3	13^{m}/B, D	13
Plathemis lydia	0.62	0.05	2	13^{m}/A	2

Table 1. Continued.

Taxonomy	GS (pg)	± SE	*N*	K/R	L
Sympetrum internum	0.78	0.07	2	13^m/A	1, 6
Sympetrum obtrusum	0.82	0.03	3	13^m/A	1, 6
Sympetrum vicinum	0.77	0.006	3	–	5
Tramea carolina	0.67	–	1	13/A	17
Tramea lacerata	0.67[a]	–	1	13/A	1
Family Macromiidae					
Didymops transversa	1.08[a]	–	1	13^m/A	17
Macromiia illionensis georgina	1.07[a]	–	1	–	17
Suborder Zygoptera (Damselflies)					
Family Calopterygidae					
Calopteryx maculata	1.00	0.03	7	13^m/A, B	1, 7, 8
Calopteryx aequabilis	1.11	0.02	4	13^m/A	1, 7
Calopteryx dimidiata	0.94	–	1	13^m/D	17
Hetaerina americana	1.11[a]	–	1	13^m/A, B	7
Family Coenagrionidae					
Amphiagrion saucium	0.89	–	1	–	12
Argia apicalis	0.88	–	1	–	7
Argia bipuctulata	0.94	0.05	2	–	17
Argia fumipennis	0.93	0.02	11	14/D	1, 18
Argia moesta	0.90	0.04	5	–	13, 14, 17
Argia sedulla	0.90	0.02	3	14/B	17
Argia tibialis	0.88	–	1	–	17
Enallagma annexum	1.14	–	1	–	12
Enallagma antennatum	1.35	–	1	–	1, 2
Enallagma basidens	0.94	–	1	–	13
Enallagma boreale	1.26	0.006	2	14/A	15
Enallagma carunculatum	1.00	–	1	14/A	7
Enallagma civile	1.10	0.05	6	14/A	1, 9
Enallagma cyathigerum	1.20	0.02	3	14, 15/A, C, F	1
Enallagma doubledayi	1.15	0.03	6	–	17, 18
Enallagma durum	1.20	–	1	–	17
Enallagma ebrium	1.18	0.02	12	14/A	1, 2; 3
Enallagma exsulans	1.18	0.03	9	–	1; 7
Enallagma geminatum	1.08	–	1	–	12
Enallagma hageni	1.19	0.03	4	–	11, 14
Enallagma signatum	1.28	–	1	–	1
Enallagma vesperum	1.34	–	1	–	14
Ischnura posita	0.96	0.04	7	–	7
Ischnura ramburii	0.88	0.02	3	14^m/D	17, 18
Ischnura verticalis	0.97	0.03	5	14/A	1
Nehalennia integricollis	1.53	0.04	7	–	17
Nehalennia irene	1.80	0.04	3	14/A	13, 14
Family Lestidae					
Lestes congener	0.60	0.03	4	13^m/A	1, 13
Lestes dryas	0.72	0.02	7	13, 13^m/A	1, 2, 12
Lestes eurinus	0.60	–	1	–	12
Lestes forcipatus	0.63	0.01	3	11/A	13
Lestes inaequalis	0.59	–	1	–	12
Lestes rectangularis	0.73	0.04	4	13^m/A	2, 13, 9
Lestes unguiculatus	0.62	0.04	4	–	2, 10

[a]Estimate based on haemocyte samples using haemocytes from *Tenebrio molitor* (GS = 0.52 pg) as the standard.

Karyotype (K) references (R): A) Cruden (1968), B) Cumming (1964), C) Kiauta (1969*a*), D) Kiauta & van Brink (1978), E) Smith (1916), F) van Brink & Kiauta (1964), G) Ferreira, Kiauta & Zaha (1979).

Collection information including location (L), season, and year: 1) Guelph (ON), 2006; 2) Ariss (ON), 2006; 3) Haliburton (ON), 2006; 4) Cambridge (ON), 2006; 5) Muskrat Lake (ON), 2006; 6) Kashagawigamog Lake (ON), 2006; 7) Thames river, Thamesville (ON), 2006; 8) Crowe river, Peterborough (ON), 2006; 9) Terra Cotta (ON), 2006; 10) Katchawanooka Lake (ON), 2006; 11) Guelph (ON), 2007; 12) Algonquin Park (ON), 2007; 13) Hamilton (ON), 2007; 14) Fletcher Lake (ON), 2007; 15) Livingston Lake (ON), 2007; 16) Wollaston Lake (ON), 2007; 17) Tallahassee (FL), 2007; 18) Archbold Research Station, Lake Placid (FL), 2007.

Species names and taxonomic classification follow Schorr *et al.* (2008).

or more generations per year), bivoltine (two generations per year), univoltine (one generation per year), semivoltine (one generation every 2 years) and partivoltine (one generation in more than 2 years) (Corbet *et al*., 2006).

In addition, there is evidence that nymphal habitat is related to the rapidity of development (Corbet *et al*., 2006). Specifically, three types of odonate habitats were recognized by Corbet *et al*. (2006): (1) temporary waters; (2) perennial lentic waters (moving waters including streams and rivers); (3) perennial lotic waters (stationary waters including ponds, wetlands and lakes). Species that develop in temporary waters usually have a generation time of 1 year or less. Perennial lentic waters have similar proportions of species with all voltinism modes, while species living in perennial lotic waters are mainly univoltine, semivoltine or partivoltine (Corbet *et al*., 2006). Thus, species with faster development rates are most often found in temporary waters, whereas species with slower life cycles are most often found in perennial waters.

Voltinism and nymphal habitat were obtained for 36 species for which genome size was also available, taken from the review by Corbet *et al*. (2006). Nymphal habitat was treated as a categorical variable, but voltinism was assessed based on the fastest recorded type for each species and converted to a measure of life cycle duration (years per generation, the inverse of voltinism).

Flight strategies

The dragonfly and damselfly species included in the present study were classified following Corbet (1962), who divided most of the Odonata into 'fliers' and 'perchers'. Fliers are defined as species 'which, when active, remain constantly on the wing,' perchers are 'those which spend most of the active period on a perch from which they make short flights'. All damselflies are perchers, whereas the only perchers among dragonflies are the members of the families Gomphidae and Libellulidae. Fliers include nearly all members of the dragonfly families Aeshnidae, Cordulegastridae, Corduliidae and Macromiidae. A third, much smaller category known as 'gliders' has also been defined, including species which have 'a hyperdevelopment of the anal field of the hindwing;' which 'enables them to glide during sunshine and thus to remain airborne at the expense of minimum activity of the wing muscles.' Almost all gliders are migrant libellulids that also show a percher body type but are most often observed flapping their wings for short periods and taking advantage of wind currents to glide and travel (Corbet, 1962). Only a few gliders have been described to date, and the three included in this study (i.e. *Tramea carolina*, *Tramea lacerata* and *Myathiria marcella*; Corbet, 1962, 1999; May, 1981) did not differ from other dragonfly perchers in terms of genome size and so were included in the latter category.

Statistical analysis

Relationships between genome size and morphometric parameters or life cycle rate were examined using Pearson correlations between species averages. In addition, principal component analyses were conducted on correlations of all available morphological data (body mass, lengths and wing areas). Species-level phylogenetic hypotheses are not yet available for the Odonata, so that phylogenetically independent contrasts (PICs) could not be used. However, it is often the case that uncorrected correlations give similar results to PICs in such analyses (e.g. Ricklefs & Starck, 1996; Gregory & Johnston, 2008). Where appropriate, correlations were corrected for body mass by comparing the regression residuals of the two parameters versus body mass. Relationships between genome size and the categorical parameters for nymphal habitat and flight strategy were examined using ANOVA and *t*-tests. The relationship between genome size and chromosome number was analysed using Spearman rank correlations.

Results

Genome size diversity

Based on the 100 species characterized in this study, odonate genome sizes range about six-fold, from 0.41 pg in *Myathiria marcella* to 2.36 pg in *Somatochlora elongata*, with a mean for the order of 1.01 ± 0.04 pg (Table 1). Means for the two suborders were not significantly different: 1.01 ± 0.05 and 1.02 ± 0.04 pg for dragonflies and damselflies, respectively. In keeping with this, a nested ANOVA showed that none of the variation in the present dataset occurs at the level of suborders within the order; by contrast, 61.1% of the variation can be found among families within the two suborders, 30.8% across genera within families and 8.1% among species within genera.

Family and genus means and ranges for the small number of species studied previously are consistent with the estimates provided in this study, with the exception of the family Gomphidae (albeit for different species from Russia) for which previous estimates were roughly half (0.37–0.40 pg) of those presented here (0.54–0.94 pg). It is not clear whether this represents measurement error or real (perhaps geographic) differences. In any event, estimates from previous studies were not included in the analyses discussed below for the sake of consistency.

Karyotype

Spearman rank correlations between genome size and chromosome number showed a significant positive correlation at the species level ($\rho = 0.41$, $P = 0.002$, $n = 51$). Similar results were obtained within the damselflies ($\rho = 0.49$, $P < 0.05$, $n = 17$) but in dragonflies the relationship was not significant ($\rho = 0.23$, $P = 0.15$, $n = 34$).

Body size

Species measurements provided here for abdomen length, hindwing length and total length are within the ranges reported previously (Westfall & May, 1996, Needham

et al., 2000). In addition to these more commonly reported variables, this study provided mean values for head length, thorax length, forewing length, forewing area and hindwing area (Appendix S1). Not surprisingly, values for the various morphometric parameters were strongly intercorrelated.

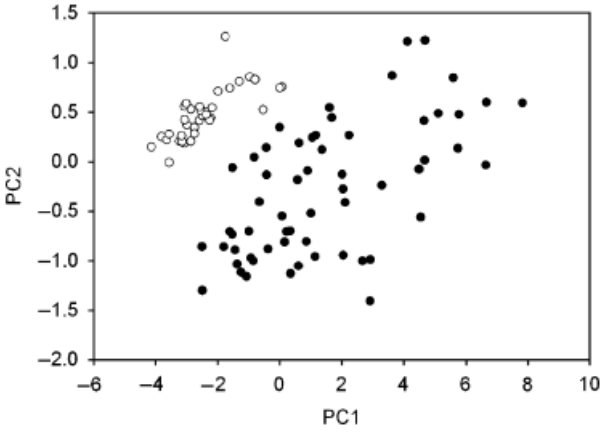

Figure 2 Correlation between principal components PC2 (~5% of variation) and PC1 (~91% of variation) from the combined morphological analysis, showing the separation of dragonflies (●) and damselflies (○).

Principal component analysis provided a single parameter (PC1) that accounted for nearly 91% of the variation in the combined morphometric dataset, with a second component (PC2) covering an addition ~5%. The first five principal components accounted for 99.8% of variation. Plotting PC2 versus PC1 clearly separated the damselflies from dragonflies, indicating significant differences in morphological features between the suborders (Fig. 2).

Genome size and various indicators of body size were significantly correlated, although the relationships differed between the two suborders: in dragonflies these were positive whereas in damselflies they were negative. Figure 3 shows the relationships for body mass, total length and total wing area, and the same significant correlations (positive in dragonflies, negative in damselflies) were found with head and thorax length (all $P<0.02$). Similarly, the single morphological index represented by PC1 was positively correlated with genome size in dragonflies and negatively correlated in damselflies (Fig. 3d).

Life cycle duration and nymphal habitat

Genome size and life cycle duration were positively related in dragonflies ($r = 0.53$, $P<0.01$, $n = 23$) but not in

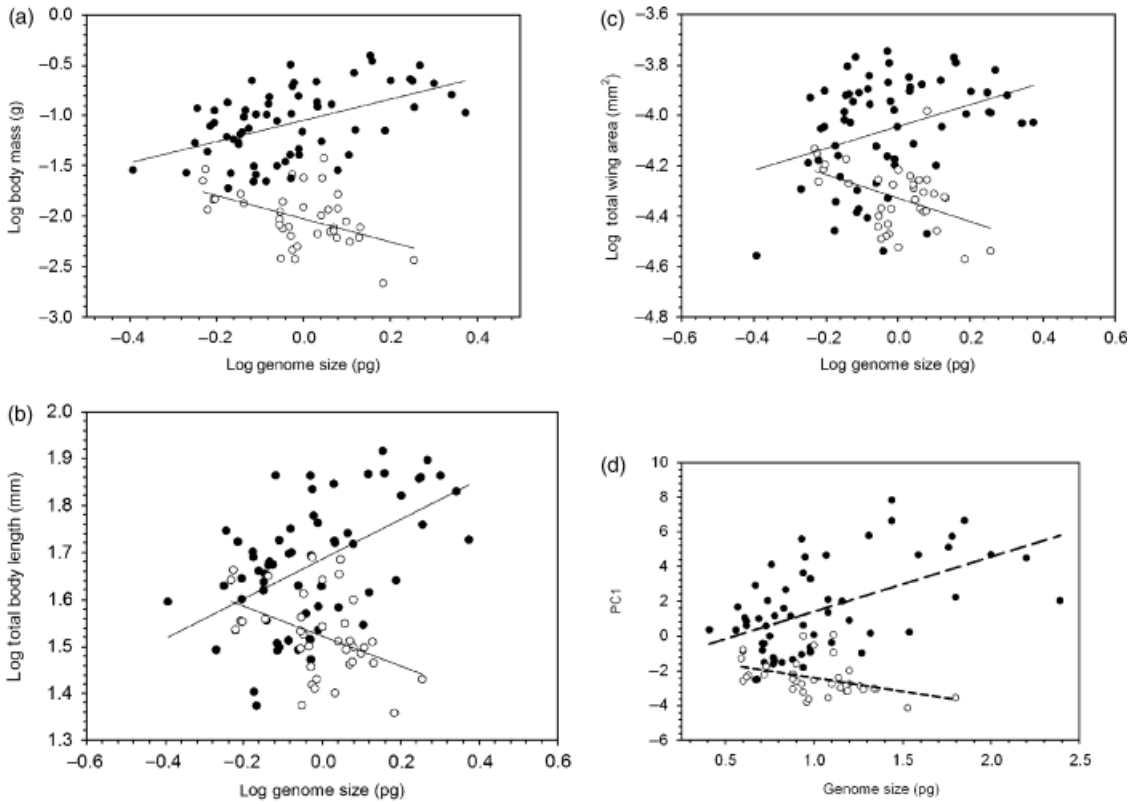

Figure 3 Relationships between genome size and indicators of body size in dragonflies (●) and damselflies (○). (a) Body mass (dragonflies: $r=0.50$, $P<0.0001$, $n=62$; damselflies: $r=-0.48$, $P<0.003$, $n=38$), (b) Total body length (dragonflies: $r=0.53$, $P<0.0001$, $n=62$; damselflies: $r=-0.42$, $P<0.009$, $n=38$), (c) Total wing area (dragonflies: $r=0.49$, $P<0.0001$, $n=62$; damselflies: $r=-0.36$, $P<0.03$, $n=38$), (d) PC1, which accounts for ~91% of the variation in the combined morphological dataset (dragonflies: $r=0.54$, $P<0.0001$, $n=59$; damselflies: $r=-0.41$, $P=0.01$, $n=36$).

damselflies ($P > 0.9$, $n = 14$). In the latter case, nearly all of the relatively small number of species for which information was available were univoltine, such that there was little variation for comparison (but also indicating that genome size can vary substantially among species with similar life cycles).

Only two dragonfly species collected in the present study had nymphal habitats consisting of temporary water bodies. Both had genome sizes around 0.77 pg as compared with an average of *c*. 0.98 pg for species inhabiting perennial lentic or lotic habitats. Statistical comparisons were difficult as a result of the unbalanced representation of the groups, but taken together, species from perennial habitats were significantly larger than the average for the two species from temporary habitats (one sample *t*-test, $P < 0.03$). The situation was similar among damselflies, in which only one species each from temporary and perennial lotic habitats were sampled. However, within this limited sample, the species with a temporary nymphal habitat had a genome size of 0.72 pg, those from perennial lentic habitats averaged 0.88 pg, and the species from a perennial lotic habitat had a genome size of 1.08 pg. As with dragonflies, damselfly species from perennial habitats differed significantly from the value for the species from a temporary habitat (one sample *t*-test, $P < 0.04$).

Wing parameters and flight strategy

As with body mass and length, total wing area was correlated with genome size positively in dragonflies and negatively in damselflies (Fig. 3). Wing loading showed the same pattern (dragonflies: $r = 0.34$, $P = 0.006$, $n = 62$; damselflies $r = -0.43$, $P = 0.008$, $n = 36$), but this disappeared when the correlation was corrected for body mass (all $P > 0.12$). In other words, wing loading appeared to correlate with genome size only insofar as both body mass and wing area are each related to genome size.

There were significant differences among flight strategies, with fliers exhibiting the largest genomes, followed by percher damselflies, and then percher dragonflies (Fig. 4a). Fliers also had significantly larger genomes than all perchers (i.e. both dragonflies and damselflies) considered together (*t*-test, $P < 0.0001$). Although fliers are generally larger than perchers, this pattern is not simply a product of differences in body size as damselflies are much smaller than percher dragonflies (Fig. 4b).

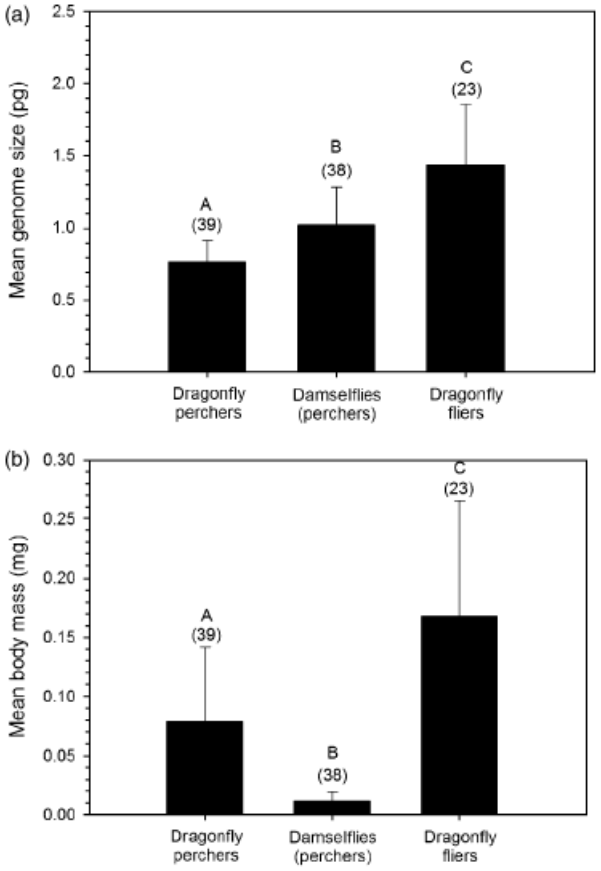

Figure 4 Mean genome size (a) and body mass (b) of dragonflies and damselflies with different flight strategies. Letters indicate significantly different groups (ANOVA and LSD tests), numbers in brackets indicate the number of species per group, and error bars represent standard deviation.

Discussion

Genome size diversity

With the addition of 100 new estimates, the Odonata is now among the best represented insect orders in the genome size dataset (Gregory, 2008). In general, odonate genome sizes can be considered something of an intermediate between those of some holometabolous orders (e.g. Diptera, Hymenoptera) that include some very small genomes, and those of some other hemimetabolous orders (e.g. Orthoptera, Phasmatodea, Hemiptera) with large maximum genome sizes.

Intraspecific variation was minimal in the present study (Table 1), though it must be borne in mind that samples typically were not drawn from widely distributed populations. Moreover, most of the individuals sampled in this study were males, and it is expected that minor differences will be found between males and females in light of the chromosomal sex determination systems in the order (usually XX♀:XO♂, with a few cases of XX♀:XY♂; Ray-Chaudhuri & Dasgupta, 1949; Seshachar & Bagga, 1962; Kiauta, 1969*a*; Perepelov, Bugrov & Warchalowska-Śliwa, 1998). Nevertheless, odonate genomes tend be fairly consistent even at higher taxonomic levels; for example differences among congeners are generally small (Table 1) and there was no mean difference between suborders.

Karyotype evolution

Karyotypic evolution in the Odonata has long been a subject of debate. In particular, the origin of

microchromosomes, which do not segregate in the same manner as other autosomes during meiosis, remains unclear. Early discussions suggested that microchromosomes are the remnants of an autosome in the process of elimination by progressive loss of chromatin (Oguma, 1930). In particular, Oguma (1930) suggested that the ancestral Odonata karyotype consisted of 13 autosomes and a sex chromosome ($n = 13a + X$). From this starting set, an autosome evolved into an m-chromosome ($n = 12a + m + X$) that would eventually be lost ($n = 12a + X$). This process was suggested to have repeated a number of times, resulting in the type number observed in the dragonfly families Petaluriidae ($n = 9^m$) and Gomphidae ($n = 12$ or 12^m). Alternatively, Cumming (1964) proposed that fusions account for inferred reductions in odonate chromosome numbers. Finally, Kiauta (1967) suggested that the type number for all Odonata was $n = 9$ based on the hypothesis that the family Petaluriidae was the most 'basal' extant dragonfly lineage and that any variation in this number was caused by random fusions and fissions. However, this phylogenetic hypothesis is not well supported (M. May, pers. comm., 2008), and in any case the assumption that the chromosomes of modern petaluriids are the same as the ancestral karyotype is potentially problematic as their current karyotype may be of secondary origin, as Kiauta (1969*b*) suggested for the family Gomphidae.

Overall, chromosomal number and genome size correlated positively in the odonates examined in the present study. This suggests that many changes in the karyotypic arrangement of odonate genomes by loss or gain of chromosomes have been concomitant with changes in DNA content, rather than the result of simple fissions or fusions without net changes in DNA content. By way of example, members of the family Gomphidae have fewer chromosomes ($n = 12^m$) and smaller genomes on average (GS = 0.76 ±0.03 pg) than any other dragonfly family, whereas members of the family Aeshnidae have both more chromosomes ($n = 14^m$) and larger genomes (GS = 1.61 ± 0.10 pg). A similar pattern is observed among damselflies in which coenagriionids have the highest chromosome number ($n = 14^m$) and some of the larger genomes in the suborder (1.11 ± 0.04 pg).

That said, some changes in chromosome number in this group do appear to have occurred independently of changes in genome size. For example, Cumming (1964) reported that the dragonfly *Macrothemis hemichlora* contains three chromosomes and not 13 as do other species of the same genus, but their chromosomes seem to contain the same amount of DNA. Conversely, there can be significant changes in genome size without changes in chromosome number, as observed in the genus *Epitheca*, in which *Epitheca cynosura* possesses fewer chromosomes ($n = 10$ or 11) than any of its congeners ($n = 13^m$), but its estimated genome size (GS = 1.10 pg) is larger than that of *Epitheca canis* (GS = 1.00 pg; $n = 13^m$) and smaller than that of *Epitheca spinigera* (GS = 1.32 pg; $n = 13^m$).

It appears that chromosomal rearrangements in odonates have been associated with DNA gain or loss in some cases, but not all, and that other mechanisms are at play in some groups. It is interesting to note that odonate chromosomes appear largely to lack heterochromatin, with C-band patterns in odonates restricted to the telomeres of autosomes (Prasad & Thomas, 1992, Perepelov *et al*., 1998, Perepelov, Bugrov & Warchalowska-Śliwa, 2001). This differs from many other taxa with similar genome size, meaning that odonates may be of particular interest in future analyses of the types and organization of sequences that contribute to genome size diversity independently of gross karyotypic alterations.

Body size

One of the most pronounced patterns observed with regard to odonate genome size diversity is a link with body size. Positive relationships between these parameters have been reported in other arthropods such as aphids (Finston *et al*., 1995) and copepod crustaceans (Gregory, Hebert & Kolasa, 2000), and are particularly likely to occur when cell size, and not just cell number, contributes significantly to body size. What is not entirely clear is why the relationship is positive in dragonflies but negative among damselflies. Most work on odonate growth and body size determination has focused on dragonflies, but it is possible that there are major differences between the two suborders. For example, if body size is affected more by the number and rapidity of cell divisions in damselflies but by cell size in dragonflies, then this could help to explain why genome size relates to body size quite differently in the two groups. In any case, genome size is not proposed as the major determinant of body size, and it is evident that some species with similar genome sizes may differ markedly in body size. Such is the case, for example, in the gomphids *Hagenius brevistylus* (GS = 0.93 pg, total length [TL] = 73.08 mm) versus *Ophiogomphus rupinsulensis* (GS = 0.94 pg, TL = 49.38 mm) or the coenagrionids *Argia moesta* (GS = 0.89 pg, TL = 40.89 mm) versus *Amphiagrion saucium* (GS = 0.90 pg, TL = 23.67 mm).

Nymphal habitat and life cycle duration

Possible patterns relating to development were assessed on two levels in this study: at the level of the order as a whole in terms of hemimetabolous development, and within the order in terms of life cycle duration and nymphal habitat. With regard to the first, it is notable that a few species do exceed the 2 pg threshold hypothesized to be imposed by complete metamorphosis (Gregory, 2002*b*), but that most odonate genomes actually are much smaller than this (typically between 0.5 and 1.5 pg). Of course, metamorphosis is not the only large-scale developmental constraint possible. Most notably, the transition from aquatic nymphs to terrestrial adults involves major morphological changes that may be comparable to metamorphosis in terms of constraints on cell division rate. In this regard, it may be very interesting to compare other hemimetabolous orders with aquatic nymphs including mayflies (Ephemeroptera)

and stoneflies (Plecoptera) with related, strictly terrestrial, hemimetabolous groups.

Within dragonflies, there was a positive correlation between life cycle duration and genome size, meaning that species with larger genomes complete their life cycles more slowly. Similar patterns could not be elucidated in damselflies due to limited sample diversity, but it is notable that in both suborders species inhabiting perennial waters as nymphs seem to have larger genomes than those in temporary waters where development rate is more constrained. Additional study with a more diverse sampling of nymphal habitats would be of interest given these preliminary patterns. It may also be informative to examine species across a latitudinal gradient, as environmental temperature is known to influence voltinism (Corbet *et al*., 2006) (though it bears noting that no obvious patterns are apparent between species collected in Ontario vs. Florida; Table 1). Analyses of flies based on temperature-controlled developmental rate data have shown associations with genome size (Gregory & Johnston, 2008), and similar investigations in odonates would be of great interest if developmental data could be made available for a large sample of species.

Flight

In vertebrates, it has become increasingly apparent that genome size is related in some way to flight. Not only do strong fliers exhibit smaller genomes than weak flying or flightless birds (Hughes, 1999; Gregory, 2005), but genome size is also linked to metabolic rate (Gregory, 2002*a*) and wing loading (Andrews, Mackenzie & Gregory, 2009). Metabolic rate data were not available in the present analysis, and wing loading appeared to correlate with genome size in odonates only through the relationships with body mass and wing area. In general, wing loading seems less important in these insects as they can fly while lifting at least 2.5 times their weight (Marden, 1987) and are often reported flying while missing major portions of their wings. Nevertheless, there is some indication that flight strategy is related to genome size among odonates, with 'fliers' having significantly larger genomes on average than 'perchers' (Fig. 4). The pattern is particularly strong within the dragonflies, suggesting that this is not a taxonomic artefact of including different suborders in the comparison. Indeed, it appears that flight strategy and genome size may have become associated independently more than once in dragonflies (Fig. 5).

A consideration of the family-level phylogenetic relationships among dragonflies sheds further light on the potential link with flight. Perching remains the most common flight strategy and is potentially ancestral. As shown in Fig. 5, this provides two scenarios to consider. In the first, the families Gomphidae and Libellulidae retained the percher strategy, while the flier strategy appeared independently in the dragonfly families Aeshnidae and Cordulegastridae and once before the split of the families Corduliidae and Macromiidae (Fig. 5a). The second (and more parsimonious) scenario involves the independent evolution of a flier strategy once before the split of the flier families while the most recent lineage, the family Libellulidae, regained the ancestral percher strategy (Fig. 5b). In this case, genome size would have both increased along with the appearance of the flier strategy and decreased independently in libellulids which reverted to a perching lifestyle.

On first sight, the relationship with flight strategy reported here appears counterintuitive with respect to the pattern in birds because the odonates that fly most actively have larger genomes than those that spend much of their time perching. However, overall flight efficiency is higher in fliers, which may actually relax some constraints on genome size relative to perchers with more metabolically demanding flight mechanics. On the other hand, it is important to consider that the relationship with flight in birds is thought to arise via constraints on cell size as it pertains to gas exchange (e.g.

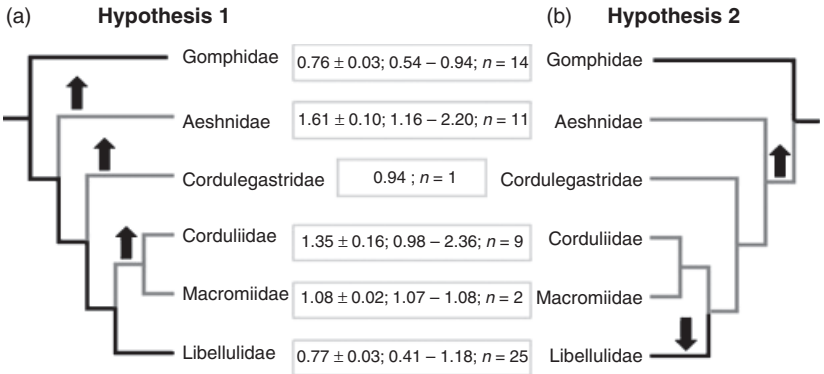

Figure 5 Hypotheses regarding the evolution of genome size (given as mean ± SE and range) and flight strategies among families of dragonflies. Branches representing fliers are denoted in grey and perchers in black. The flier strategy is associated with larger genomes while the percher strategy is associated with small genomes (Fig. 3). (a) Hypothesis 1: Fliers evolved independently three times whereas the percher strategy was retained in the families Gomphidae and Libellulidae. (b) Hypothesis 2: The flier strategy evolved once before the split of the families Aeshnidae, Cordulegastridae, Corduliidae and Macromiidae while the family Libellulidae regained the ancestral percher lifestyle independently. Arrows indicate the direction of genome size change associated with shifts in flight strategy under these hypotheses. Phylogeny adapted from Misof *et al.* (2001) and Ware, May & Kjer (2007).

Hughes, 1999; Gregory, 2002*a*). Insects do not make use of respiratory cells in circulation, and as such any mechanistic link between genome size, cell size and flight in odonates need not relate to metabolism as it seems to in vertebrates.

Some of the major differences between fliers and perchers relate to flight musculature and thermoregulation. Fliers control body temperature and manage heat exchange from their thoracic flight muscles with surrounding air sacs located under the cuticle (May 1976, 1981). They are also generally large but have comparatively small amounts of flight muscle (25% body mass vs. 45% in perchers) which enhances insulation by allowing more space for air sacs (May, 1981, 1991). The flight muscles of fliers can generate enough heat for constant flight even at low ambient temperatures (Sformo & Doak, 2006), and in fact without the ability to divert excess heat to the abdomen (e.g. if the heart is occluded and haemolymph cannot be circulated), they may experience terminal overheating within a few minutes (Heinrich & Casey, 1978). Fliers rarely fly at their highest capacity, and can take advantage of air currents for prolonged flight. Perchers, by contrast, lack large subcuticular air sacs and often fly near their maximum capacity in short bursts (May, 1981, 1991; Rüppell, 1989; Grabow & Rüppell, 1995; Wakeling & Ellington, 1997; Corbet, 1999; Thomas *et al*., 2004). In very general terms, fliers are akin to long-distance walkers, whereas perchers are sprinters.

Concluding remarks

Orders such as the Odonata have traditionally been overlooked in genome size studies, or have been examined only a few species at a time. The present study has provided new genome size estimates for 100 species of dragonflies and damselflies, covering about 1/5 of North American diversity for this order. This clearly demonstrates that broad investigations of genome size diversity in insects are possible with current technologies. In the present study, this has revealed some interesting patterns in genome size diversity, in particular relationships with body size, flight strategy, development and karyotypic evolution. It has also highlighted several avenues for future research, including integrative efforts to elucidate the basis of the positive relationship between genome size and body size in dragonflies and the negative one in damselflies. Additional analyses focusing on developmental rate differences are clearly warranted, as are further tests of hypotheses regarding linkages between genome size and flight in these intriguing insects.

Acknowledgements

We thank Nancy Deyrup, Chris Early, Colin Jones and Michael May for assistance with collections and/or identifications of insects. We also thank Rick Turner for assistance with photography and morphometric analyses, Michael May for helpful comments on an early draft of the paper, and Beren Robinson for statistical advice. This work was supported by a Natural Sciences and Engineering Research Council (NSERC) grant to T.R.G. and a University of Guelph travel scholarship to A.M.A.G.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Odonate body size measurements, including means and standard error for each species for minimum body weight (BDW, in grams) and total length (in mm) for total body (TL), head (HL), thorax (THL), abdomen (AL), forewing (FWL), and hindwing (HWL). Also included are areas (in mm^2) for forewing (FWA), and hindwing (HWA). Species names and taxonomic classification were updated according to Schorr *et al.* (2008).

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