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Genome size correlates with reproductive fitness in seed beetles

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The ultimate cause of genome size (GS) evolution in eukaryotes remains a major and unresolved puzzle in evolutionary biology. Large-scale comparative studies have failed to find consistent correlations between GS and organismal properties, resulting in the ‘C-value paradox’. Current hypotheses for the evolution of GS are based either on the balance between mutational events and drift or on natural selection acting upon standing genetic variation in GS. It is, however, currently very difficult to evaluate the role of selection because within-species studies that relate variation in life-history traits to variation in GS are very rare. Here, we report phylogenetic comparative analyses of GS evolution in seed beetles at two distinct taxonomic scales, which combines replicated estimation of GS with experimental assays of life-history traits and reproductive fitness. GS showed rapid and bidirectional evolution across species, but did not show correlated evolution with any of several indices of the relative importance of genetic drift. Within a single species, GS varied by 4–5% across populations and showed positive correlated evolution with independent estimates of male and female reproductive fitness. Collectively, the phylogenetic pattern of GS diversification across and within species in conjunction with the pattern of correlated evolution between GS and fitness provide novel support for the tenet that natural selection plays a key role in shaping GS evolution.

1. Introduction

The general lack of correspondence between nuclear genome size (hence, GS) and organismal complexity is a classic problem in evolutionary biology [1,2]. Current hypotheses for the evolution of GS all rely on balancing forces which act to expand or to reduce GS. They can be broadly categorized into three non-mutually exclusive classes. First, the ‘junk DNA’ hypothesis recognizes that the selfish intragenomic propagation of transposons and other mobile genetic elements leads to the accumulation of mutations throughout the genome, yielding a one-way ticket to genomic obesity [3]. Such slightly deleterious mutations are then purged by very weak negative natural selection at the individual level [4–6], and the efficacy by which selection can rid the genome of mutationally hazardous DNA increases with increasing effective population size [7]. Second, the ‘selection hypothesis’ suggests that genomic reconfigurations associated with variation in GS has consequences for organismal fitness and that GS may, to a large extent, represent a dynamic balance between positive and negative selection on GS [8]. This could come about in many ways. For example, this hypothesis integrates the adaptive significance of gene duplication [9,10] and recent revaluations of the concept of ‘junk DNA’, suggesting that at least part of what was traditionally considered non-functional DNA may in fact have important effects on phenotypes [2,11–14]. Thirdly, a few other hypotheses do not

involve natural selection on GS. For example, the ‘mutational equilibrium hypothesis’ suggests that GS represents a dynamic balance between DNA gain through large insertions and loss through small deletions, the rates of which are assumed to scale with GS [15]. Similarly, non-random assortative segregation by chromosome size during meiosis may, under some conditions, affect the evolution of GS [16].

Much of what is known about the evolution of GS is based on comparative studies involving widely divergent lineages. Some of these studies have revealed a number of phenotypic correlates of GS, ranging from cell size over physiology to ecologically relevant traits in both animals and plants (see [17–19] for reviews). Yet, broad-scale comparative studies suffer from two limitations. First, because the biology of divergent taxa differs in so many ways, the potential for confounding effects inevitably limits the strength of inferences regarding causation [20,21]. Second, a key question for GS evolution concerns the potential role of natural selection (see above), to which comparative studies involving divergent taxa do not speak directly. Studies at a finer scale, across distinct genotypes within species, would allow direct assessments of selection on GS which would greatly help shed light on the processes that drive GS evolution [6,19].

Although there are several reported examples of intraspecific variation in GS in both animals and plants, most of these studies have been deemed dubious for various reasons [18,19,22] and the degree to which GS size varies within species is somewhat contentious. Yet, a few studies have assessed within-species correlates of GS [23,24]. In *Drosophila melanogaster*, GS varies across populations, and Vieira & Biemont [25] suggested that novel genetic variability induced by the mobilization of transposable elements could allow populations to adapt more successfully to novel environments, but support for this hypothesis is equivocal [26]. Huang *et al.* [27] documented significant variation in GS across inbred lines deriving from a single *D. melanogaster* population (i.e. the DGRP). Although Huang *et al.* [27] failed to find significant covariation between GS and phenotypic traits, Ellis *et al.* [28] documented significant but environment-dependent covariation between GS and several life-history traits across a subset of these inbred lines. Schielzeth *et al.* [29] revealed a negative association between male song attractiveness and GS in the grasshopper *Chorthippus biguttulus*, suggesting that sexual selection may act to reduce GS. In this species, however, variation in the number of supernumerary chromosomes (rather than more common sources of variation in GS) likely caused, or at least contributed to, the observed pattern. Collectively, these studies highlight the fact that within-species efforts to (i) assess variation in GS and (ii) test whether GS is associated with key fitness components are needed to test for and characterize adaptive variation in GS [6,19].

Seed beetles (Coleoptera, Chrysomelidae and Bruchinae) represent a group of ecologically homogeneous granivorous beetles [30]. The larvae infest seeds, typically of legumes, where they complete their development within a single host seed. Although bruchines (some 1350 described species) have a worldwide distribution, most species occur in tropical or subtropical arid or semiarid regions where several species are major pests of legume crops. Bruchines are also karyotypically rather uniform, typically with $n = 8–12$ autosomes and a XY sex-determining system (males being

heterogametic) [31,32]. Here, we use flow cytometry for GS estimation in conjunction with phylogenetic comparative methods and a series of detailed assays of life-history traits and sex-specific reproductive fitness to achieve two goals. First, we conduct a phylogenetic comparative analysis of 12 species in the family to (i) describe the tempo and mode of GS evolution in this clade and to (ii) test for correlated evolution between GS- and species-specific population size and body size at an interspecific scale. Second, we use 18 distinct genotypes of our main model species, *Callosobruchus maculatus*, to (i) quantify and characterize within-species variation in GS and to (ii) ask whether GS shows correlated evolution with life history and sex-specific fitness at this intraspecific scale.

2. Material and methods

(a) Species, populations and rearing

For the interspecific part of this study, we used 12 different seed beetle species (table 1). All species were reared in laboratory climate cabinets at (depending on species-specific optima) 25–30°C, 60–75% RH and a 12D:12L cycle using seeds of their native host plant as medium (*Vigna unguiculata*, *V. angularis*, *V. radiata*, *Phaseolus vulgaris*, *Gleditsia triacanthos* or *Dichrostachys cinerea*).

For the intraspecific part of this study, we used 18 distinct laboratory populations of *C. maculatus*, sampled at different geographical locations (electronic supplementary material, table S2). All populations were reared under standardized conditions on *V. unguiculata* at a population size of 400–500 individuals in 11 stock jars and at 30°C, 60% RH and a 12D:12L cycle in climate cabinets. These populations were all collected as pests in bean storage sites or crop fields and were brought into the laboratory at various points in time (range 1975–2010) and they show different degrees of genetic divergence [33,34], but are fully reproductively compatible: hatching rate of eggs in crosses between populations is invariably very high (more than 95%). We note that *V. unguiculata* is the main natural host for this species and the rearing conditions used (including non-overlapping generations and abiotic conditions) mimic natural conditions [35].

(b) Phylogenies, genome size and assays

We used maximum-likelihood (ML) estimation based on mtDNA sequence data to reconstruct phylogenies, in total 2557 bp from three genes in the interspecific part and 1726 bp from two genes in the intraspecific part. Details on the sequence data and the estimation procedures can be found in the electronic supplementary material.

GS was estimated using flow cytometry. Young adults (aged 1–3 days) ($n = 3–5$ per species and sex for interspecific data; $n = 5–7$ per population and sex for intraspecific data) were prepared for GS estimation. Briefly, the head of a single male or female adult was placed into 1 ml of Galbraith buffer in a 2-ml Kontes Dounce tissue grinder. To this was added the head of a single female *Drosophila virilis*, which served as a GS standard (1C = 328 Mb). Following grounding, filtering and staining, samples were scored for the relative PI fluorescence of diploid nuclei from the heads of the beetle and of the standard using a Partec CyFlow flow cytometer. For further details, we refer to the electronic supplementary material.

We used the body weight of young (1–2 days old) virgin adult males and females of all species ($n = 20$ per species and sex) and populations ($n = 20$ per species and sex) as a measure of body size, and measured male genital morphology (genital spine length) as well as mean egg size per species ($n = 10$ per species)

Table 1. Mean female and male GS in the seed beetles species studied here. GS varies markedly across species ($F_{11,264} = 2179.1$, $p_{\text{rand}} < 0.001$). Males and females differ in GS ($F_{1,224} = 38.2$, $p_{\text{rand}} < 0.001$) and the degree of sexual dimorphism in GS varies across species ($F_{11,224} = 4.1$, $p_{\text{rand}} < 0.001$) (randomization tests).

species	female GS (Mb)	s.e.	n	male GS (Mb)	s.e.	n	karyotype (n) ^a	chromosome length (n, μm) [32]
<i>Callosobruchus analis</i>	958.6	2.9	5	970.8	6.4	5	9 A + X/Y	33.2
<i>C. maculatus</i>	1233.0	2.1	107	1202.4	2.0	98	9 A + X/Y	40.1
<i>C. subinnotatus</i>	1486.1	11.4	4	1388.5	7.6	3	9 A + X/Y	48.9
<i>C. chinensis</i>	757.9	3.8	4	717.6	5.6	3	9 A + X/Y	29.7
<i>C. phaseoli</i>	1114.7	1.8	3	1079.0	3.2	3		
<i>Megabruchidius dorsalis</i>	792.0	8.8	4	766.6	4.1	4		
<i>M. tonkineus</i>	704.5	5.2	4	697.0	3.8	3		
<i>Bruchidius dichrostachydis</i>	1286.3	8.0	4	1245.3	6.1	3		
<i>Decellebruchus atrolineatus</i>	795.8	4.6	3	789.7	1.8	3		
<i>Acanthoscelides obtectus</i>	956.4	11.0	5	949.4	8.8	3	9 A + X/Y	
<i>Zabrotes subfasciatus</i>	743.3	1.3	3	727.0	2.4	3	12 A + X/Y	
<i>Amblycerus robiniae</i>	770.0	4.6	6	764.6	8.7	5		

^aSee the electronic supplementary material.

using a Lumenera[®] Infinity 2–2 digital camera mounted on a Leica[®] MZ8 dissection microscope. Estimates of relative worldwide population size were provided by leading authorities of bruchine biology, naive to the purposes of this study. For *C. maculatus*, we gained population- and sex-specific data on a series of different life-history traits as well as reproductive fitness (table 2) through a number of specific experimental assays. Briefly, male competitive fertilization success was quantified using a standard double mating experiment, based on a sterile male technique protocol. Data on population-specific female lifetime fecundity, offspring production and egg-to-adult viability was attained in a replicated assay ($n = 60$ females per population) where all eggs and hatching offspring were recorded in the laboratory. Development time and growth rate was determined by closely following the development of $n = 96$ beetles from each population. For a fuller description of the methodological procedures, we refer to the electronic supplementary material.

(c) Phylogenetic comparative analyses

(i) Pattern of evolution

Species, as well as populations, are not independent but show a pattern of dependency that derives from shared ancestry. To account for any effects of shared ancestry, we used phylogenetic comparative analyses throughout. The pattern of evolution in GS was characterized by estimating the phylogenetic parameters κ , δ and λ , using ML estimation in BAYESTRAITS v. 2 [36]. Kappa (κ) differentially stretches or compresses individual phylogenetic branch lengths and can be used to test for a punctuational versus gradual mode of trait evolution. Delta (δ) scales overall path lengths in the phylogeny (i.e. the distance from the root to the species). It can detect whether the rate of trait evolution has accelerated or slowed over time as one moves from the root to the tips. Pagel's lambda (λ) is a measure of phylogenetic signal. This key parameter assesses whether the phylogeny correctly predicts the patterns of covariance among tip taxa on

a given trait. In effect, it indicates the extent to which the key assumption underlying the use of comparative methods (that tip taxa are not independent) is true for a given phylogeny and trait. A value of $\lambda = 0$ implies that the phylogenetic correction could be dispensed with altogether. A value of $\lambda = 1$ is consistent with a Brownian motion model of trait evolution.

(ii) Correlated evolution

Our assessment of covariation between GS and life history/fitness within species involved testing for correlated evolution between several sets of variables, which may inflate type I statistical error rates. In order to account for phylogenetic effects in our data without inflating the number of inferential tests, we employed phylogenetic least-squares canonical correlation analyses (PCCA) [37]. This method provides an estimate of the multivariate phylogenetic signal and a likelihood ratio test of the null hypothesis that $\lambda = 0$ (i.e. no phylogenetic signal). More importantly, it provides a single omnibus (Wilk's LLR) test of the overall null hypothesis that there is no multivariate correlated evolution between two sets of variables, by estimating phylogenetically adjusted canonical correlations, thus avoiding the inferential problems that are associated with multiple tests. In our main inferential model, used to test for covariation between GS and phenotypic traits within species, male and female GS were jointly related to all life-history/fitness traits (2 versus 11 traits) in a global PCCA. Given that this model revealed significant correlated evolution, female GS was related to all female- and population-specific life-history/fitness traits (1 versus 8 traits) in a second PCCA model and male GS was related to all male-specific life-history/fitness traits (1 versus 3 traits) in a third. Our analyses were restricted to the first canonical axis and were based on the ML estimate of multivariate λ [37].

To characterize the pattern of correlated evolution between GS and life-history/fitness traits within species, we used

Table 2. The results of phylogenetic least-squares regressions of GS on a variety of life-history traits and reproductive fitness, across species and within *C. maculatus*. SD, sexual dimorphism.

	independent variable	dependent variable	α^a	β	s.e. $_{\beta}$	p	
across species ($n = 12$)	female GS	population size	6.53	-0.05	0.27	0.857	
		female body weight	8.17	-0.29	0.29	0.341	
		egg size	7.69	-0.22	0.30	0.481	
	SD in GS	SD in body weight	15.50	0.14	0.32	0.671	
within species ($n = 13-18$) ^b	female GS	age (years since collection)	15.50	0.01	0.25	0.969	
		egg-to-adult viability	15.50	-0.18	0.34	0.607	
		female offspring production	15.50	0.60	0.29	0.063	
		female fecundity	15.50	0.86	0.30	0.015	
		female body length	15.50	0.02	0.34	0.954	
		female body weight	15.50	-0.30	0.33	0.383	
		female development time	15.50	0.10	0.34	0.774	
		female growth rate (mm d^{-1})	15.50	-0.04	0.34	0.908	
		male GS	male competitive fertilization success	15.50	0.51	0.21	0.033
			male genitalia—dorsal spines	15.50	0.25	0.24	0.320
			male genitalia—ventral spines	15.50	0.52	0.2	0.025

^aML estimate of α (the phylogenetic effect).

^bSee text for omnibus test.

phylogenetic generalized least-squares regression (PGLS) models [36] as implemented in COMPARE v. 4.6b [38]. Here, all traits were standardized to a mean of zero of unit variance prior to PGLS regressions. These models were based on the ML estimate of α , a parameter that is inversely proportional to the strength of the phylogenetic signal [39,40]. For the within-species analyses, these models were not used for hypothesis testing (see above) but to provide estimates of effect size across traits that enable interpretations of multivariate effects. For cross-species analyses, we used PGLS regressions to test and model correlated evolution between GS and indices of effective population size.

3. Results

(a) Pattern of genome size evolution

GS varied substantially across species. The largest genome (*C. subinnotatus*) was more than twice the size of the smallest (*Megabruchidius tonkineus*). Even within the genus *Callosobruchus*, GS varied by a factor of two (table 1). Moreover, species differed significantly in the degree of sexual dimorphism in GS (electronic supplementary material, figure S1). We also found highly significant within-species variation in GS across populations of *C. maculatus* ($F_{17,169} = 5.2$, $p < 0.001$; figure 1). Population-level variation in GS was considerable: the largest and smallest average GS per population differed by some 51.8 Mb in females and 53.5 Mb in males. This corresponds to a difference in GS of 4–5%. The repeatability of mean GS per population, across all replicate individuals, was 0.43 for males and 0.40 for females showing that almost half of the total variance in GS was due to differences between populations.

To describe the pattern of GS evolution, we estimated several parameters that test for tempo, mode and phylogenetic signal in data (see above). Across species within the family,

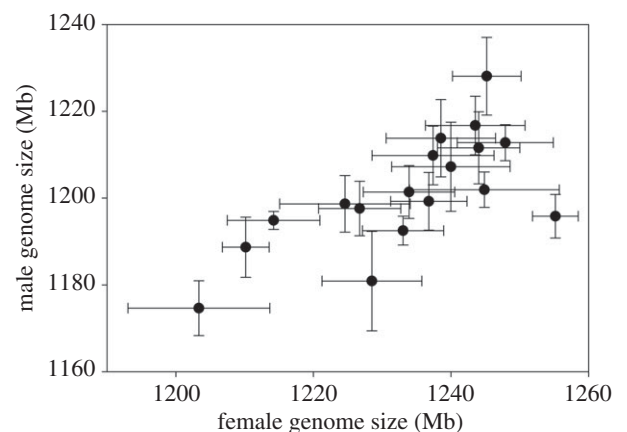


Figure 1. Male and female GS across populations of *C. maculatus*. Error bars represent s.e.m. Although GS varied markedly across populations ($F_{17,169} = 5.2$, $p < 0.001$) and males and females differed in GS ($F_{1,169} = 161.1$, $p < 0.001$), the degree of sexual dimorphism in GS did not differ significantly across populations ($F_{17,169} = 0.9$, $p = 0.576$).

there was no significant phylogenetic signal for either female ($\lambda = 0.06$) or male ($\lambda = 0.05$) GS (figure 2). For both sexes, the ML estimate of Pagel's λ was not significantly different from $\lambda = 0$ ($\chi^2 = 0.04$, $p = 0.84$; $\chi^2 = 0.02$, $p = 0.89$) but was significantly different from a Brownian motion scenario (i.e. $\lambda = 1$) ($\chi^2 = 5.22$, $p = 0.022$; $\chi^2 = 4.30$, $p = 0.038$). The estimate of κ was lower than, but not significantly different from, unity ($\kappa = 0.22$, $p = 0.298$; $\kappa = 0.38$, $p = 0.416$) and that of δ was higher than, but not significantly different from, unity ($\delta = 1.77$, $p = 0.218$; $\delta = 1.65$, $p = 0.294$) for both sexes. We also assessed the pattern of evolution of the relative size of the Y-chromosome to the X-chromosome across species, by superimposing the difference in GS between

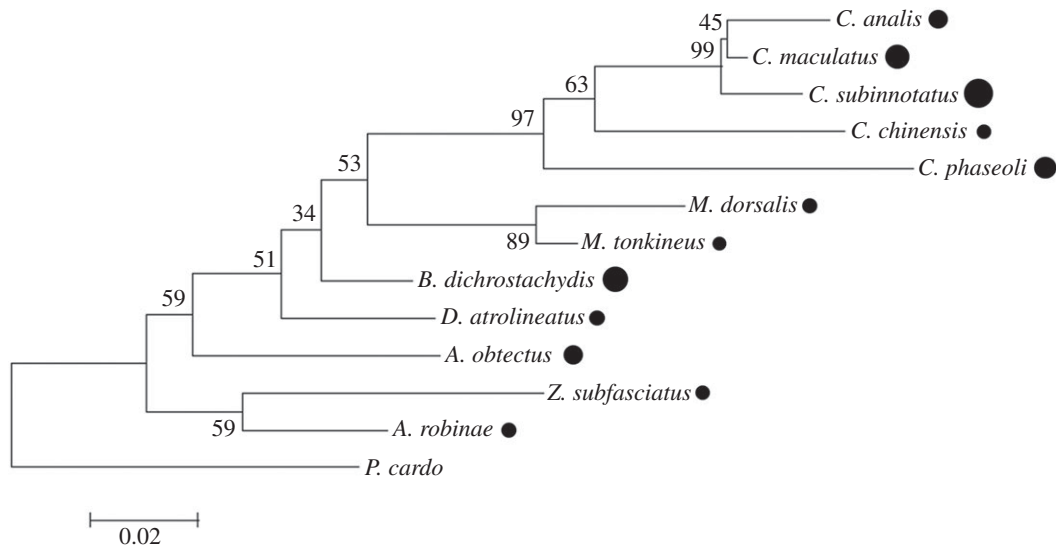


Figure 2. Molecular phylogenetic reconstruction for the 12 species of bruchines used, together with their outgroup. Numbers at nodes represent percentage of trees in which the associated taxa clustered together (based on 2000 bootstrap replicates). The area of black circles by species names is proportional to female GS. Note that there is no significant tendency for more closely related species to be more similar in GS.

females and males upon the phylogeny. We found no significant phylogenetic signal for relative Y-chromosome size. The estimated λ ($\lambda = 0.0$) was not significantly different from $\lambda = 0$ ($\chi^2 = 0.004$, $p = 0.95$) but was highly significantly different from unity ($\lambda = 1.0$; $\chi^2 = 11.94$, $p < 0.001$). Here, the estimate of κ was significantly lower than unity ($\kappa = 0.00$, $p = 0.013$) and that of δ was significantly higher than unity ($\delta = 2.99$, $p = 0.002$).

Across populations of *C. maculatus*, the pattern of GS evolution was similar to that across species (electronic supplementary material, figure S2). Most importantly, there was again no significant phylogenetic signal for either female ($\lambda = 0.21$) or male ($\lambda = 0.47$) GS. For both sexes, the estimate of λ was not significantly different from $\lambda = 0$ ($\chi^2 = 0.14$, $p = 0.70$; $\chi^2 = 3.38$, $p = 0.066$) but was significantly different from a Brownian motion scenario ($\lambda = 1$) ($\chi^2 = 62.86$, $p < 0.001$; $\chi^2 = 120.31$, $p < 0.001$). For both sexes, the estimate of κ was significantly lower than unity ($\kappa = 0.34$, $p < 0.001$; $\kappa = 0.07$, $p < 0.001$) and that of δ was significantly higher than unity ($\delta = 2.99$, $p < 0.001$; $\delta = 2.99$, $p < 0.001$).

In summary, the pattern of GS evolution was largely unrelated to the phylogenetic topology across both scales. GS instead showed rapid evolution with putative cases of both GS expansion and reduction over time. The relatively low values of κ and high values of δ are also consistent with GS being an evolutionary labile trait in this group.

(b) Correlated evolution

Male and female GS, unsurprisingly, showed tightly correlated evolution both across species (PGLS: $\beta' = 1.0$, $s.e._\beta = 0.03$, $p < 0.001$) and within species (PGLS: $\beta' = 1.0$, $s.e._\beta = 0.25$, $p = 0.002$). GS evolution did not correlate with either population size, body size or egg size evolution across species (table 2). Moreover, sexual dimorphism in GS did not show correlated evolution with sexual dimorphism in body size (table 2).

Within *C. maculatus*, phylogenetic least-squares canonical correlation analyses revealed three important insights. First,

there was no significant multivariate phylogenetic signal (i.e. $H_0: \lambda = 0$, $p > 0.396$ in all three PCCA models). Second, GS showed significant concerted evolution with life history and reproductive fitness in our main inferential model ($\chi^2_{22} = 181.2$, $p < 0.001$). This was true also in female-specific ($\chi^2_8 = 19.1$, $p = 0.015$) and male-specific ($\chi^2_3 = 23.1$, $p < 0.001$) PCCA models. Third, inspection of the standardized canonical coefficients in conjunction with the standardized regression coefficients from the phylogenetic least-squares regressions (table 2) showed that this correlated evolution was primarily due to (1) positive covariation between GS and lifetime fecundity ($\beta' = 0.86$)/offspring production ($\beta' = 0.60$) in females and (2) positive covariation between GS and competitive fertilization success ($\beta' = 0.51$)/ventral genital spine length ($\beta' = 0.52$) in males. In conclusion, while GS showed no apparent correlated evolution with any of the general life-history traits (body size, development time and growth rate) across populations, GS did show correlated evolution with independent measures of male and female reproductive fitness. Larger genomes were associated with both higher mass-specific fecundity in females and with elevated competitive fertilization success in males (figure 3).

4. Discussion

The pattern of evolution of GS across and within seed beetle species offers a series of insights into the processes that govern GS evolution. Below, we first discuss the role that genetic drift may play as a major driver of GS evolution. We then probe within-species variation in GS and suggest that the pattern of correlated evolution between GS and reproductive fitness seen here supports a role for natural selection in GS evolution in this clade.

A major and longstanding question regarding the evolution of GS is the relative roles of random events and selection in the modulation and maintenance of GS. Genetic drift alone could indirectly be responsible for GS variation, which should then be related to effective population size

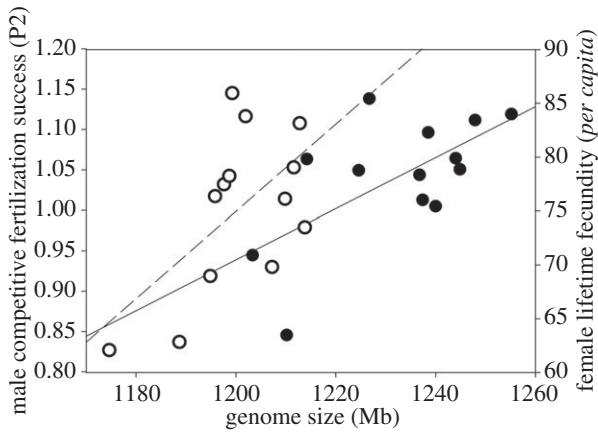


Figure 3. GS is positively related to reproductive fitness in both females (filled circles and solid line; $r = 0.63$, $p = 0.021$; $p_{\text{rand}} = 0.015$) and males (open circles and hatched line; $r = 0.59$, $p = 0.032$; $p_{\text{rand}} = 0.018$) across populations of *C. maculatus* (randomization tests based on 10 000 permutations). The figure shows mass-specific lifetime fecundity for females and competitive fertilization success for males (table 2).

because drift becomes a relatively stronger force when population size is smaller [7,41]. Previous reports of a negative covariation between GS and effective population size [7] have been called into question [21,42,43] and recent studies at a finer taxonomic scale have also failed to find the predicted association [44,45]. Three facets of our results are inconsistent with a major role for genetic drift. First, the evolution of increased body size and propagule size are generally associated with decreased effective population size [46], and Lynch & Conery [7] thus predicted a positive evolutionary relationship between GS and body size. We found no significant correlated evolution between GS and body or propagule size. In fact, the estimated associations were negative rather than positive both across and within species. Our findings are consistent with other recent studies of insects that also failed to find a positive relationship between GS and body size [47,48]. Second, one might predict that the pattern of GS evolution should be ‘punctuational’ if drift has a major effect, as a result of the massive reduction in effective population size that typically accompanies episodes of founder event speciation. However, the pattern of GS evolution in bruchines did not conform to a punctuational scenario. Third, and most importantly, we found no trend towards a correlated evolution between GS and population size across species (table 2). It is unclear precisely how well our measure of relative population size reflects variation in effective population size. Yet, the fact that there are dramatic differences across species that must reflect effective population size (pest status, degree of host specialization, geographical range, etc.; see the electronic supplementary material) suggests that the true relationship between GS and effective population size in this clade is, at most, very weak.

We found no significant phylogenetic signal in GS evolution, which also departed from that expected under a Brownian motion scenario. On a much larger taxonomic scale (across Eukaryotes), GS is known to show a phylogenetic signal and its evolution conforms fairly well to Brownian motion [43,49]. Although the lack of a phylogenetic signal across seed beetle species is consistent with an important role for adaptive processes [50], it is difficult to interpret

in isolation as it may also reflect clade-specific factors other than selection, such as differences in transposable element activity [51]. It is, however, clear that GS in bruchines evolves relatively rapidly and shows bidirectional changes both across (figure 2) and within (SI) species.

In order for selection to mould GS, there must be significant intraspecific variation in GS and that variation must be associated with components of fitness [6,19]. Several previous comparative studies across much more divergent animal taxa have documented positive associations between GS and cell size, body size and development time [19]. We found no such effects in seed beetles, suggesting that aspects of the phenotype other than major organismal differences may be linked to GS at this intraspecific level. Our analyses did reveal pronounced intraspecific variation in GS and we show that this variation is instead positively associated with reproductive fitness in both sexes, as estimated in two independent and sex-specific fitness assays. This implies that larger genomes somehow allow beetles to allocate more resources to reproduction, in both sexes of *C. maculatus*. This interpretation is supported by the fact that GS also showed correlated evolution with an aspect of male genital morphology (ventral spine length), which is known to be both condition dependent and to be associated with increased competitive fertility [52,53]. The fact that GS did not show correlated evolution with time since populations were brought into the laboratory (table 2) suggests that gradual GS-mediated adaptation to the laboratory environment is not a major contributor to the link between GS and fitness. The observed positive link between GS and reproductive fitness could arise for several reasons, which are not mutually exclusive. For example, differences in the amount of non-coding DNA may reflect differences in the ability to regulate and fine-tune gene expression, such that larger genomes may be better able to produce phenotypes in high condition under a wider range of environments [2]. Further, differences in the amount of coding DNA may reflect adaptive gene duplication [9], allowing genotypes with larger genomes to be better buffered against deleterious mutations or otherwise be able to produce more adaptive phenotypes [10,54]. Yet another possibility is that variation in telomere length is positively related to reproductive fitness [55]. Alternatively, populations undergoing frequent laboratory bottlenecks may both be purged of deleterious alleles (due to inbreeding) and show larger GS (due to an increased importance of drift). We note, however, that laboratory populations of *C. maculatus* harbour genetic loads similar in magnitude to both wild populations and other seed beetle species [56,57] and the fact that we found no relationship between years-in-the-laboratory and GS offers no support for this scenario. Disentangling the above possibilities is currently not possible, as the relative contribution of coding and non-coding DNA to the GS variation seen in *C. maculatus* is unknown. Irrespective of the precise molecular causes of the documented association between genotype size and reproductive fitness, however, our results imply that natural selection acts on GS variation in our model system. To this extent, our study is consistent with a few recent studies demonstrating both intraspecific variation in GS and links between GS- and fitness-related traits in insects [28,29]. Our results also suggest that natural selection on GS can at least under some circumstances contain components of positive directional selection. It is, of course, highly

likely that natural selection on GS also contains elements of negative directional selection, perhaps on other components of fitness or under different environmental conditions.

The GS variation we document here is not associated with variation in chromosome number, which varies very little across species (table 1) and is invariable within *C. maculatus* [32]. Hence, the patterns and associations seen cannot be caused by genome duplications or supernumerary chromosomes. Some insight into the nature of GS variation in this group can be gleaned from karyotypic studies. Remarkably, the correlation between our GS estimates and the total physical length of all chromosomes (mitotic metaphase; [32]) across females of four species of *Callosobruchus* was $r = 0.99$ (table 1). Yet, while the ratio of the largest to smallest GS among these congeneric species is 1.96, it is only 1.65 for chromosome length. This clearly shows that larger genomes are more tightly condensed during metaphase and is consistent with the hypothesis that a large part of the difference in GS between species is non-coding heterochromatin.

The relative size of the sex chromosomes varies markedly across seed beetle species (table 1). Theory suggests that genes with sex-specific effects should be enriched on sex chromosomes [58–60], which predicts that larger differences in sex chromosomes would allow greater genetic sex-specificity and, hence, sexual dimorphism [61]. We found no support for this prediction in our data, as sexual dimorphism in GS did not show correlated evolution with overall sexual dimorphism in size. The degree of sex-specific gene expression, to the extent that this is reflected as difference

in overall morphology, thus seems to be dictated by factors unrelated to the relative size of the X and Y chromosomes.

In conclusion, we show that GS varies markedly both between and within seed beetle species and that GS shows rapid and bidirectional evolution. The pattern of evolution of GS is not consistent with a major role for genetic drift in shaping GS and GS did not show correlated evolution with estimates of species-specific relative population size. Within species, GS showed correlated evolution with both male and female reproductive fitness. Collectively, thus, our findings provide novel support for the hypothesis that GS variation results from natural selection in this clade.

Data accessibility. Data are deposited in GenBank (accession nos. KP242017–KP242019) and in the electronic supplementary material.

Authors' contributions. G.A. and J.S.J. conceived this study; G.A., C.H., D.R. and M.T. designed and performed the experiments; G.A., C.E.H. and J.S.J. analysed the data; G.A., A.S., E.I., C.H., D.R., M.T. and J.S.J. wrote the paper.

Competing interests. We declare we have no competing interests.

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