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REVIEW

A meta-analysis of the strength and nature of cytoplasmic genetic effects

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Abstract

Genetic variation in cytoplasmic genomes (i.e. the mitochondrial genome in animals, and the combined mitochondrial and chloroplast genomes in plants) was traditionally assumed to accumulate under a neutral equilibrium model. This view has, however, come under increasing challenge from studies that have experimentally linked cytoplasmic genetic effects to the expression of life history phenotypes. Such results suggest that genetic variance located within the cytoplasm might be of evolutionary importance and potentially involved in shaping population evolutionary trajectories. As a step towards assessing this assertion, here we conduct a formal meta-analytic review to quantitatively assess the extent to which cytoplasmic genetic effects contribute to phenotypic expression across animal and plant kingdoms. We report that cytoplasmic effect sizes are generally moderate in size and associated with variation across a range of factors. Specifically, cytoplasmic effects on morphological traits are generally larger than those on life history or metabolic traits. Cytoplasmic effect sizes estimated at the between-species scale (via interspecies mix-and-matching of cytoplasmic and nuclear genomes) are larger than those at the within-species scale. Furthermore, cytoplasmic effects tied to epistatic interactions with the nuclear genome tend to be stronger than additive cytoplasmic effects, at least when restricting the data set to gonochorous animal species. Our results thus confirm that cytoplasmic genetic variation is commonly tied to phenotypic expression across plants and animals, implicate the cytoplasmic–nuclear interaction as a key unit on which natural selection acts and generally suggest that the genetic variation that lies within the cytoplasm is likely to be entwined in adaptive evolutionary processes.

Introduction

Eukaryotes contain organelles that originate from ancient endosymbiontic associations. These organelles carry genetic information that escapes Mendelian laws of heredity: they are generally uniparentally inherited through the female lineage (Birky, 2001). Mitochondria are the energy-producing compartments of the eukaryote cell, and hence, their existence has presumably been fundamental in facilitating the evolution and maintenance of complex life forms (Koonin, 2010; Lane & Martin, 2010). Mitochondria evolved from the symbiosis of an alpha-proteobacteria with an archaean eukaryote precursor (Dyall *et al.*, 2004). In the green lineage (Chlorophyta and Streptophyta), a second endosymbiosis between a mitochondria-containing eukaryotic cell and a photosynthetic bacterium produced the chloroplast (Gould *et al.*, 2008; Hohmann-Marriott & Blankenship, 2011). The evolution of these endosymbionts into

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cellular organelles involved extreme specialization at the metabolic level, and a co-dependency at the genetic level, accompanying the loss and transfer of a large part of the total gene content originally associated with each endosymbiont, over to the nucleus (Dyall *et al.*, 2004; Kleine *et al.*, 2009). Nevertheless, both organelles have retained a small, but core, collection of the genes necessary for crucial energy-related functions (Gray *et al.*, 1999; Green, 2011).

Sequence polymorphisms in cytoplasmic genomes frequently occur in eukaryotes, but generally the evolutionary processes that have led to the accumulation of these polymorphisms remain elusive (Dowling *et al.*, 2008; Budar & Roux, 2011; Budar & Fujii, 2012; Dowling, 2014). In addition, organellar genomes have adopted extremely different modes of evolution in different lineages, resulting in contrasting genome organizations and types of polymorphisms in plant and animal mitochondria (Lynch *et al.*, 2006). In animals, nucleotide sequence polymorphisms in the mitochondrial DNA (mtDNA) are ubiquitous. mtDNA sequences across species, and between different populations of the same species, are characterized by numerous fixed nucleotide differences (Avise, 1986; Dowling *et al.*, 2008). Mitochondrial allelic variance is also commonly observed to exist within populations and within individuals (Rand *et al.*, 2001; Wolff *et al.*, 2014). These polymorphisms have traditionally been harnessed to facilitate evolutionary phylogenetic and population genetic inferences, based on the assumption that they accumulate under a neutral equilibrium model, and are thus selectively neutral (Galtier *et al.*, 2009). In plants, nucleotide sequence polymorphisms in the chloroplast DNA have been widely used to make similar inferences (Downie & Palmer, 1992; McCauley, 1995; Newton *et al.*, 1999; Provan *et al.*, 2001). In contrast, plant mitochondrial genomes, while generally orders of magnitude larger than those of animals, generally accumulate few point mutations but are highly variable in size, organization and structure, both within and between species (Palmer & Herbon, 1988; Kubo & Mikami, 2007; Darracq *et al.*, 2010; Sloan *et al.*, 2012b).

Over the past two decades, experimental evidence has accumulated, which indicates sizeable levels of phenotype-changing (i.e. functional) genetic polymorphisms exist within cytoplasmic genomes. This evidence is largely comprised of studies showing that distinct cytoplasmic genotypes, which are characterized by divergence at numerous nonsynonymous and synonymous single nucleotide polymorphisms (SNPs), are routinely sensitive to selection (e.g. Ballard & Whitlock, 2004; Dowling *et al.*, 2008; Budar & Fujii, 2012). Furthermore, several studies have suggested that evolutionary trajectories of allelic replacement within the cytoplasm will hinge on trajectories of allelic replacement within the nuclear genome, and vice versa. That is, mitochondrial and nuclear genomes will co-evolve closely, by interacting epistatically to shape the expression of core life history phenotypes (Rand *et al.*, 2004; Dowling *et al.*, 2008; Budar & Fujii, 2012).

In animals, additive effects of mitochondrial haplotypes have been linked to phenotypic expression of fundamental life history traits, such as reproductive success (Smith *et al.*, 2010; Aw *et al.*, 2011; Yee *et al.*, 2013), ageing (Camus *et al.*, 2012), metabolic functioning (Pichaud *et al.*, 2012) and genomewide transcriptomic patterns (Innocenti *et al.*, 2011). Robust evidence for a role of mitochondrial–nuclear (mito-nuclear) coadaptation in the evolutionary dynamics of populations comes from studies showing that disruption of species- or population-specific mito-nuclear gene complexes can result in the reduced expression of key components of life history (Burton & Barreto, 2012; Reinhardt *et al.*, 2013). Mito-nuclear epistasis has been reported to shape the expression of several life history and metabolic traits (Rand *et al.*, 2006; Dowling *et al.*, 2007a,c,d; Clancy, 2008; Ellison & Burton, 2008; Arnqvist *et al.*, 2010). In plants, numerous cases of cyto-nuclear incompatibilities have been observed, following both interspecific hybridization (Levin, 2003) and crosses within species (Bogdanova *et al.*, 2009). These incompatibilities most often lead to albinism or impaired photosynthesis, or to decreased fertility, particularly male sterility (cytoplasmic male sterility; CMS) and abnormalities in flower development (reviewed in Budar *et al.*, 2003; Linke & Borner, 2005; Greiner *et al.*, 2011; Budar & Fujii, 2012). These phenotypes are assumed to result from cyto-nuclear Dobzhanski–Muller epistatic incompatibilities (Greiner & Bock, 2013), except for CMS, which is widely considered as originating from a mito-nuclear genomic conflict (Budar *et al.*, 2003). Although the presence of two organellar genomes makes it difficult to unequivocally attribute the phenotypic modifications to mitochondria or chloroplast, some studies have succeeded in attributing the phenotype alteration to a specific organelle (Belliard *et al.*, 1978, 1979; Galun *et al.*, 1982; Bonnett *et al.*, 1991; Zubko *et al.*, 2002; Bogdanova, 2007).

Thus, a sizeable body of empirical evidence has accumulated over the past two decades, describing cytoplasmic genetic effects on fitness. While these studies, and the emerging patterns, have been the subject of numerous narrative reviews (Blier *et al.*, 2001; Rand *et al.*, 2001, 2004; Ballard & Whitlock, 2004; Ballard & Rand, 2005; Dowling *et al.*, 2008; Budar & Roux, 2011; Greiner *et al.*, 2011; Budar & Fujii, 2012; Ballard & Pichaud, 2013; Greiner & Bock, 2013), they have not been objectively quantified within a meta-analytic framework. Here, we use meta-analysis to evaluate three key evolutionary questions related to the existence of cytoplasmic genetic variance, outlined below.

First, we seek to test whether the strength of cytoplasmic effects is generally larger in plants than in animals. Although it comes with caveats explained

below, we hypothesize that the cytoplasmic effects will be larger in plants for the following reasons. Two cytoplasmic genomes co-segregate within plants (the mtDNA and the chloroplast DNA) and only one in animals (mtDNA). Whereas animal mitochondrial genomes are highly streamlined and lack recombination, their much larger counterparts in plants generally exhibit high recombination rates and are characterized by the presence of introns and large intergenic regions (Palmer, 1990; Knoop, 2004; Kitazaki & Kubo, 2010; Davila *et al.*, 2011). Such factors could conceivably act to increase the efficiency by which the cytoplasmic genomes of plants respond adaptively to selection, and this could increase the amount of phenotype-modifying (i.e. functional) cytoplasmic genetic variation accumulating between divergent populations, relative to animals. The caveat, however, is that plant mitochondrial genomes generally accumulate point mutations at a much lower rate than their animal counterparts (Palmer, 1990), albeit with large variation (e.g. Mower *et al.*, 2007; Sloan *et al.*, 2008, 2012a). This low substitution rate would provide less genetic variation on which selection could act to drive adaptation within the genomes of plants.

Second, as discussed above, it has been suggested that cytoplasmic effects on phenotypes are often manifested via cyto-nuclear allelic interactions, consistent with the requirement for tight cyto-nuclear cooperation (Rand *et al.*, 2004; Dowling *et al.*, 2008; Dowling, 2014). In several reported cases, at least in the insects, the reported additive cytoplasmic genetic effects appear to be smaller in magnitude than the epistatic cyto-nuclear effects (Rand *et al.*, 2006; Dowling *et al.*, 2007a, b,c; Arnqvist *et al.*, 2010). We thus seek to quantitatively test whether the epistatic effect is generally larger than the additive cytoplasmic effect, across kingdoms.

Third, we aim to substantiate a key evolutionary hypothesis (Frank & Hurst, 1996; Gemmell *et al.*, 2004; Innocenti *et al.*, 2011), often called Mother's Curse (Gemmell *et al.*, 2004), which predicts that maternal inheritance of the mitochondrial genome, in animals with separate sexes, will facilitate the accumulation of a set of mtDNA mutations that are male-biased in expression, particularly in regard to their deleterious phenotypic effects. Such male-harming mitochondrial mutations might arise under mutation–selection balance if such mutations exert only slightly deleterious effects in females (Frank & Hurst, 1996), drift (Gemmell *et al.*, 2004), or positive selection if male-harming mutations actually confer positive effects in females (Unckless & Herren, 2009; Innocenti *et al.*, 2011). If this evolutionary process is a ubiquitous process that results in a male-biased mitochondrial mutation load in animals, then mitochondrial genetic effects should generally be larger in males than in females among gonochorous animal species.

Materials and methods

We searched for literature containing relevant information about mitochondrial and cytoplasmic effects using the Internet platform *Web of Science*, as well as by collecting relevant references from review articles. The final *Web of Science* search was conducted on the 5 August 2013, and it contained the search terms for the field 'topic' as follows:

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((cytop* OR cyton* OR mitoch* OR cytot* OR mitot*)
AND (varia* OR effect* OR affect* OR nuclea* OR
interaction) NOT cance* NOT prote* NOT neuro* NOT
phylogeo* NOT bacter*) AND ("reprod* success" OR
fertility OR surviva* OR longevity OR "life span"
OR "chromosome segregation" OR adapta* OR "stress
toleran*" OR fitness OR standard* OR hybrid$ OR
trait$).
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We chose these key words to optimally capture primary literature that reported cytoplasmic effects on our traits of interest (see below), but to avoid capturing studies that did not experimentally partition cytoplasmic from nuclear genetic or environmental effects, such as those focused on phylogenetic inference (i.e. that were devoid of phenotypic data), or studies that harnessed human data. In addition, studies employing cytoplasmic hybrid (cybrid) cell lines were also excluded because our goal was to target phenotypes measurable at the whole organismal level (i.e. life history and morphological traits, but extending to gene expression patterns captured from living individuals) and which were therefore ecologically relevant to organismal fitness. The articles found in the *Web of Science* search, combined with back searches of their reference lists, yielded a final list of 1566 publications. From these, we extracted relevant data from 66 publications (animals = 35, fungi = 2, plants = 29), amounting to a total of 521 effect-size estimates (animals = 326, fungi = 7, plants = 188). Most studies on the list did not experimentally untie cytoplasmic from nuclear genetic effects, and some failed to report the details required to convert the results to standardized effect sizes and were subsequently excluded. The study selection and data collection process is summarized in the electronic supplementary (Fig. S1). The collected and used data did not show any sign of publication bias (see Figs S2–S4).

From the final selection of 66 publications, we collected a range of data including the cytoplasmic effect sizes and sample sizes, as well as kingdom (with three levels: animal, fungi or plant) of the study subject; the underlying genetic architecture (i.e. gene action) associated with the reported cytoplasmic effect, comprising of two levels, additive (i.e. cytoplasmic or mitochondrial) or epistatic (i.e. cyto-nuclear or mito-nuclear interaction); the trait type measured (five levels: gene expres-

sion, immunity-related, life history, metabolic or morphological trait); the experimental design type (two levels: whether the inferences were based on phenotypes scored following F2-backcrosses or full cytoplasmic replacement [i.e. placing the cytoplasm of the maternal lineage alongside a novel nuclear background of the paternal lineage via either introgressive backcrossing over successive generations or via chromosome substitution]); the experimental scale (three levels: whether cytoplasmic replacement was conducted at the intrapopulation, interpopulation or interspecies scale); data type (seven levels: whether the effect sizes were based directly on reported effect sizes or group means and error terms, or via test statistics such as ANOVA *F*-test, odds ratio, *t*-test, chi-square test or *Z*-test); and the sex for animals in which the effect was reported (three levels: male, female or from pooled sexes), if available. We used these additional data as moderators (i.e. fixed effect factors) in our analyses, to determine whether they account for variation in the magnitude of cytoplasmic effect sizes. We also collected additional data on genus and species of the studied organisms (see Table S1).

We calculated standardized effect sizes – Hedges' g, which is weighted by sample size (Hedges, 1981), by converting reported differences in means (and their associated standard deviation), or the test statistics *F*-scores, *t*-scores, *Z*-scores, chi-square-scores or log odds ratios (Del Re, 2013) derived from the selected studies (Fig. 1). When estimating the variance of Hedges' g, levels of replication per study were determined based on the number of genotypes sampled rather than by the total number of individuals sampled per study. Prior to the analyses, we first explored the distribution of sample sizes across each of the fixed factor levels in the data set. We removed factor levels that were characterized by very low sample sizes from the data set, because these would likely yield unreliable results in the subsequent analyses (based on Bayesian MCMCglmms). Specifically, we removed the kingdom level 'fungi' (seven data points based on two studies) and the trait-type levels 'gene expression' (five data points based on two studies) and 'immunity' (12 data points from four studies). Furthermore, we merged the two levels 'intrapopulation' and 'interpopulation' in the 'experimental scale' factor, to increase the replication of this combined level (henceforth this factor compares two levels only: cytoplasmic effect sizes based on 'within-species' cytoplasmic replacement and 'between-species' cytoplasmic replacement). In the factor denoted 'data type', we grouped the effect sizes based on test statistics (*t*-scores, *Z*-scores, chi-square-scores and direct reported effect sizes) into the one level termed 'other statistics', reducing the levels of 'data type' to four (*F*-test, mean, odds ratio and others). This rendered the final data set at 61 studies (animals = 35, plants = 26; two fungi studies excluded and three plant studies excluded via removal of 'gene expression' and 'immunity') with 501 effect-size estimates (animals = 321, plants = 180) for 29 species (animal = 9, plants = 20; Table S1). As far as is known, all 29 species included in the meta-analysis transmit their mtDNA uni-parentally (i.e. maternally). The final raw data are available at Dryad.

We were only interested in the strength, not the direction of the cytoplasmic effects. This is because these effect sizes are based on differences across cytoplasmic genetic variants, and the directionality will hence be arbitrary. Consequently, it was not possible to use standard approaches for meta-analysis. We therefore applied a method previously used by Kingsolver *et al.* (2012), where Gaussian parameter estimates give expected values on a 'folded-over' distribution (Hereford *et al.*, 2004; Morrissey & Hadfield, 2012) given by

$$E(y) = \sigma * \mathrm{sqrt}(2/\pi) * \exp(-|\mu|^2/2\sigma^2) + |\mu| * (1 - 2\Phi * (-|\mu|/\sigma))$$

where $E(y)$ is the expectation on the folded-over distribution, σ the estimated standard deviation, μ the mean and Φ denotes the cumulative distribution function of the standard normal distribution (see electronic supplementary for further details).

Linear mixed models (R-code available on Dryad) were used to estimate the means and variances needed to estimate the expectations (means) of the effect sizes on a folded distribution for each level of each moderator. The variances of the effect sizes were included in all analysis. As such, analyses are obviously dependent on the estimation of specific means and variances for each moderator level; the models were fit with the moderators of interests as fixed effects (i.e. kingdom, gene action, trait type, experimental design type, experimental scale and data type), and by fitting variances (both associated with a model-estimated random effect [Publication ID and the residual component, see Table S2 and Table S3]) specific for each moderator level. The estimation of group specific variances is highly demanding of sample size. Because we were interested in exploring the effect of numerous possible moderators (i.e. kingdom, gene action, trait type, experimental design type, experimental scale and data type), we were not able to fit one model to account for all moderators of interest. Moreover, excluding some of the moderators from the model is likely to introduce biases derived from the effect of the un-modelled moderators. We hence chose an approach where we fitted many models. In each model, we estimated the variances of each category of a moderator (i.e. variances for all moderator levels), but for only two moderators. In addition to the two moderators, per model, for which specific variances were estimated, the mean effect of all other moderators was controlled for in each model, by including these as fixed effects. This was done to minimize the possibility of biases, but will only control for the moderator means on the original Gaussian distribution, and not the variances needed to yield

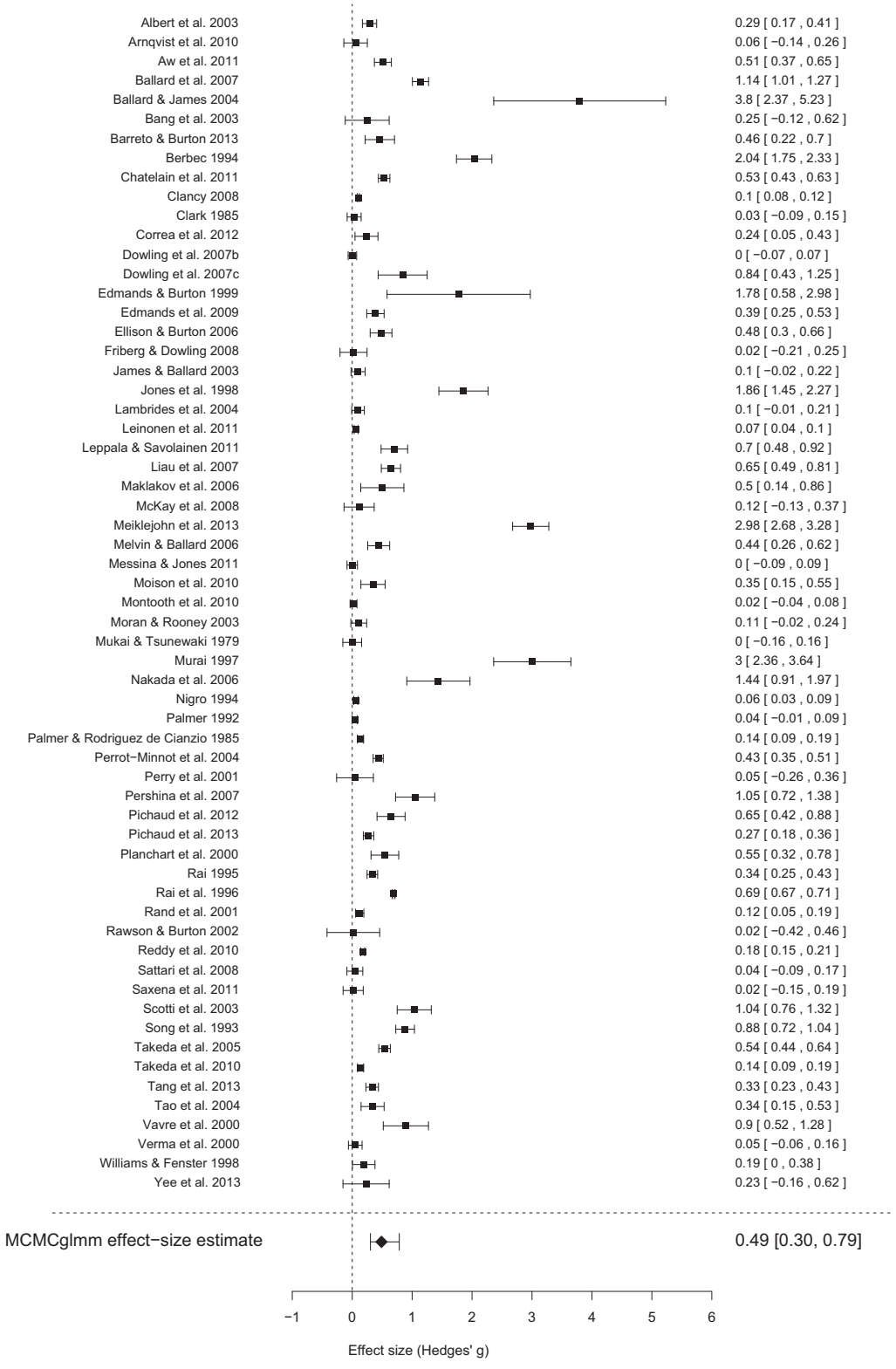

Fig. 1 Averaged absolute effect sizes per publication. Data points based on mean values per study. Squares represent mean effect size, and error bars represent 95% confidence intervals for each publication. Publications are listed on the left side, mean values with lower and upper limit for the 95% CI on the right side.

expectations on the folded-over distribution. Our inferences were thus derived from fitting moderator-specific variances restricted to only two moderators per model. Models for every possible combination of moderator pairs were fitted, such that the effect of each moderator was modelled together with each one of the rest of the moderators.

We were interested in differences across moderator levels while controlling for other variables. In each one of the models described above, we can extract the mean of each moderator level. From each one of the models, and within each moderator, the posterior sample of the mean of one level was set as the base line and the posterior sample of the mean of the other moderator level(s) was subtracted from this base line. As several models were used, this yielded two to five estimates of each difference across moderator levels (gene action = 4; kingdom = 5; experimental scale = 2; experimental design type = 4; trait type = 3; data type = 3). To generalize the estimated effect of a given moderator such that it was not dependent of any specific model in which it was estimated, we pooled the posterior distribution of the differences among moderator levels across all models in which variance estimates for that specific moderator had been extracted. We did this for each moderator (see Fig. S5). If the 95% credibility intervals of a difference do not overlap with 0, this is interpreted as a significant effect. This thus provides an explicit statistical test of the effects of the levels within a moderator (Fig. 1, Fig. S6).

We did not estimate moderator-specific variance for the fixed effect 'data type', because we were not interested in the specific values for this moderator. In addition, we had no *a priori* expectation that data type levels would differ in terms of estimated variances. Initially, genus was included as a random effect in the analyses; however, inclusion of this term resulted in miss-specified (i.e. not executable) models, as a result of the limited taxonomic representation across the data set. However, we also cross-checked our results by including the phylogenetic structure in the analysis (see electronic supplementary, Fig. S7, Table S4). The results with inclusion of phylogeny indicated that our inferences were not confounded by the phylogenetic structure of the included species. Therefore, and because it is not possible to include both phylogeny and our key moderator of interest – kingdom – in the same models, here we present models with kingdom, and excluding phylogeny. All models were run in the MCMCglmm package (Hadfield, 2010) in R (R Development Core Team, 2013), where the parameter estimates were transformed to a folded-over distribution as described above. Flat priors were used for the fixed effects and locally uninformative parameter-expanded priors for the random effects. Both represent little prior knowledge, and the use of different priors returned quantitatively similar results. After a burn-in of 10 000 iterations, we sampled the posterior chain of each one of the models with five hundred ten thousands iterations and a thinning interval of 500, yielding a total posterior sample of 1000. All autocorrelations were in the [−0.1,0.1] interval.

To examine whether cytoplasmic effect sizes in animals consistently differed across each of the sexes, we ran another set of analyses, identical to the approach above, with the exception that we restricted the data set to studies that had reported cytoplasmic effect sizes separately for each sex in gonochorous animal species only (two levels; males = 202 effect sizes, females = 56 effect sizes).

Results

Generally, effect sizes of around 0.2 are considered to be small, effect sizes around 0.5 are moderate, and values above 0.8 represent large effect sizes (Cohen, 1988; Rosenthal, 1996).

General effect sizes

The overall effect size, incorporating all cytoplasmic effect types, was moderate [effect size: 0.4886 (95% CI: 0.3043–0.7854), Fig. 1]. The effect-size estimates for each single moderator level are moderate to strong (see Table 1). The moderators gene action, experimental design type, experimental scale and trait type (particularly morphology) accounted for heterogeneity in effect-size estimates, and we found considerable effect-size differences between moderator levels (Fig. 2, Table 1). Generally, epistatic effects tend to be stronger than additive effects (Fig. 2, Table 1), although this pattern was statistically significant only when focussing on

Table 1 Effect-size estimates for each moderator level ± 95% credibility interval.

Moderator	Level	Effect size	Lower 95% CI	Upper 95% CI
Gene action	Additive	0.7023	0.4447	1.0672
	Epistatic	1.0411	0.6154	1.6347
Kingdom	Animals	0.8025	0.5045	1.1947
	Plants	0.8430	0.4807	1.2541
Trait type	Life history	0.6135	0.4198	0.8657
	Metabolism	0.5530	0.2841	0.9118
	Morphology	1.3075	0.7591	2.1045
Experimental scale	Between species	1.4147	0.8639	2.1675
	Within species	0.4246	0.3261	0.5270
Experimental design type	F2-backcross	0.5427	0.2925	0.8403
	Introgression	0.9735	0.6685	1.3112
Data type	*F*-test	0.8938	0.5599	1.2864
	Mean	0.6925	0.3347	1.1796
	Log odds ratio	0.8184	0.4939	1.2857
	Other	0.4837	0.1836	0.9218

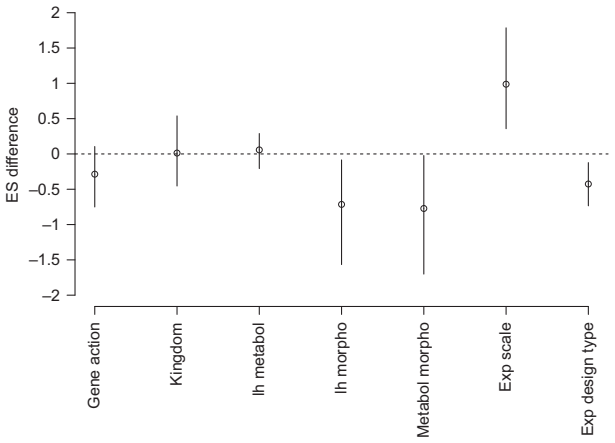

Fig. 2 Posterior distribution of contrasts across moderator levels. Each point represents the posterior probability of a difference between two levels within a moderator. Specifically, 'gene action' represents the difference between additive and epistatic effects; 'kingdom' represents the difference between animals and plants; 'lh metabol' represents the difference between life history traits and metabolic traits; 'lh morpho' represents the difference between life history traits and morphological traits; 'metabol morpho' represents the difference between metabolic traits and morphological traits; 'exp scale' represents the difference between interspecies and intraspecies cytoplasmic substitution; 'exp design type' represents the difference between F2-backcrosses and introgression. See Table 1 for detailed effect sizes. The error bars represent the 95% credibility interval.

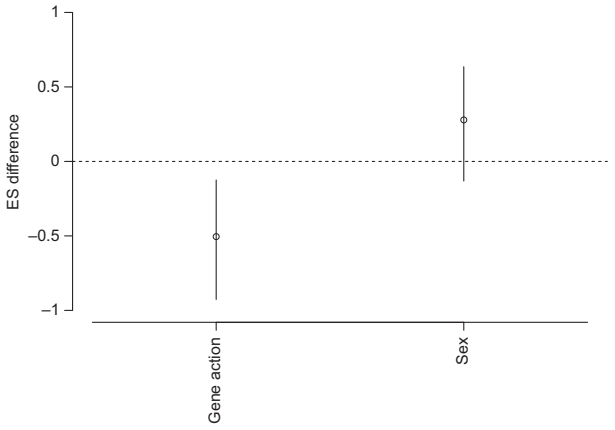

Fig. 3 Posterior distribution of contrasts across moderator levels for gonochorous animals. Each point represents the posterior probability of a difference between two levels within a moderator. Specifically, 'gene action' represents the difference between additive and epistatic effects; 'sex' represents the difference between females and males. The error bars represent the 95% credibility interval.

the sex-specific data from gonochorous animal species (Fig. 3). Cytoplasmic effect sizes of studies that examined the effects of cytoplasmic replacement at the interspecies scale were larger than those that examined effects at the intraspecies scale. Furthermore, we found that the experimental design type, used to examine cytoplasmic effects, was associated with the strength of the effects. Studies employing cytoplasmic replacement techniques reported larger effect sizes than those relying on inferences based on the phenotypes of F2-backcrosses (Figs 2 and S6). Cytoplasmic effects associated with life history and metabolic traits were generally smaller than those associated with morphological traits (Fig. 2, Table 1). Kingdom and data type did not significantly contribute to heterogeneity (Fig. S6). See electronic supplementary for details on random effects (Table S2).

Effects sizes between the sexes in animals

When focussing solely on data obtained from gonochorous animal species in which sex-specific effect sizes were available, the overall cytoplasmic effect size was moderate [effect size: 0.5559 (95% CI: 0.2919–0.9706)] and, as reported above, larger for epistatic than for additive cytoplasmic effects [additive: 0.4293 (95% CI: 0.2235–0.7379]; epistatic: 0.9225 [95% CI: 0.5761–1.3482), Fig. 3]. Effect sizes in females tended to be larger than for males, but this difference was not significant [females: 0.8418 (95% CI: 0.5769–1.1804); males: 0.5809 (95% CI: 0.3045–0.9287), Fig. 3]. See electronic supplementary for details on random effects (Table S3).

Discussion

Our meta-analysis reveals that cytoplasmic genetic effects on the phenotype are widespread and generally of moderate strength. Our results are concordant with the results of recent narrative reviews (e.g. Rand *et al.*, 2004; Dowling *et al.*, 2008; Wolff *et al.*, 2014), which suggested that cytoplasmic genetic effects are often manifested via cyto-nuclear interactions. The finding that cyto-nuclear allelic interactions are observed across taxa, and at least in gonochorous animals are generally larger in magnitude than the additive cytoplasmic genetic effects, is concordant with the premise that cyto-nuclear gene interactions are key to complex life (Rand *et al.*, 2004; Dowling *et al.*, 2008). Eukaryote organellar genomes heavily rely on interactions with nuclear gene products, to ensure optimized gene expression, protein complex assembly, metabolic and physiological functions (Gershoni *et al.*, 2009; Bar-Yaacov *et al.*, 2012).

The cytoplasmic genetic effects we have detected in animals most likely primarily reflect dedicated effects associated with mitochondrial genetic variance. Arthropods often harbour other maternally transmitted cytoplasmic genetic elements, such as *Wolbachia* and *Rickettsia*, and possibly cryptic and unidentified viruses that also exhibit maternal patterns of inheritance (Hurst

& Jiggins, 2005). However, most of the arthropod studies included in our meta-analysis either actively purged their stocks of *Wolbachia* and other bacterial infections through antibiotic treatment or utilized species in which *Wolbachia* spp. are not known symbionts. We therefore assume that *Wolbachia*-type effects do not confound our interpretations. In plants, the extranuclear genetic elements incorporate both mitochondrial and chloroplast genomes, and it is generally impossible to disentangle the relative contributions of each.

Given the plant cytoplasmic genetic environment encompasses two obligate genomes, whereas the animal counterpart contains just one, we predicted that cytoplasmic effect sizes would be larger in plants. This prediction was reinforced by the distinct differences that exist between animal mitochondrial and plant organellar genomes, outlined in the introduction, which would for the most part point to greater adaptive lability of cytoplasmic sequences of plants. However, we did not find any difference between the strength of cytoplasmic effects in plants and animals. The lack of a consistent difference between plant and animal kingdoms might be reconciled by the observation that, unlike the case for most plant species, animal mitochondrial genomes have high rates of SNP accumulation and lack recombination (Lynch *et al.*, 2006), conditions that are highly amenable to the accumulation of deleterious mutations, under a Muller's Rachet-type process (Muller, 1932; Moran, 1996). Thus, although effect sizes across animal and plant kingdoms might converge on similar values, the relevant contribution of adaptive vs. nonadaptive evolutionary processes may differ across kingdoms. That is, a larger proportion of the functional cytoplasmic genetic variance detected in animals might be nonadaptive and consist of moderately deleterious polymorphisms relative to plants (Lynch, 1997; Lynch *et al.*, 2006). Similarly, a higher proportion in plants may be adaptive and shaped directly by selection. Consistent with this suggestion, a role for the cytoplasm in local adaptation has been suggested in several plant studies (Galloway & Fenster, 1999; Sambatti *et al.*, 2008; Iida *et al.*, 2009; Kapralov *et al.*, 2011; Leinonen *et al.*, 2013). In addition, plant mitochondrial genomes carry unconserved open reading frames, mostly created by recombination events, among which some have been identified as sterilizing factors of CMS. These mitochondrial genes can be positively selected by increasing the female fitness of the carrying plants (Lewis, 1941; Shykoff *et al.*, 2003; Dufay & Billard, 2012). Furthermore, a role of ecological conditions in the evolution of CMS genetic factors has been proposed (Caruso *et al.*, 2012). The role of local adaptation in contributing to the genetic architecture of animal mitochondrial genomes remains an outstanding question, with some evidence (Mishmar *et al.*, 2003; Ruiz-Pesini *et al.*, 2004; Balloux *et al.*, 2009), albeit hotly debated (Elson *et al.*, 2004; Kivisild *et al.*, 2006; Sun *et al.*, 2007).

Studies that experimentally 'mixed-and-matched' cytoplasmic and nuclear genomic combinations, placing cytoplasms of one species alongside nuclear backgrounds of a congener species, generally reported larger cytoplasmic effect sizes than those that did the same at a within-species scale. This result is consistent with evolutionary expectations; the greater the evolutionary divergence in organellar and nuclear DNA involved in the crosses, the greater the potential for intergenomic mismatching upon disruption of co-evolved cytonuclear complexes (Levin, 2003; Burton & Barreto, 2012). We also observed lower cytoplasmic effect sizes for studies that based their inferences on experimental designs involving F2-backcrosses rather than on designs utilizing techniques of cytoplasmic replacement. This discrepancy most likely reflects the difference in experimental power and resolution between the approaches. Inferences from F2-backcrosses are confounded by an uncontrolled pool of segregating genetic variance in the nuclear backgrounds, some of which might also exhibit sexual asymmetries in inheritance (e.g. sex chromosomes), and this will hinder estimation of the true cytoplasmic effect size. Cytoplasmic effect sizes were also generally larger when underpinning expression of morphological traits, relative to life history (e.g. ageing, traits related to ageing and reproductive outputs) and metabolic traits. Plausible explanations for why a putative bias exists in the magnitude of cytoplasmic genetic effects for morphological traits remain elusive, given that the direct products of the energy-converting organelles are predicted to directly affect metabolic processes, and variance in the metabolic rate is thought to stand at the root of all life history expression (Dowling *et al.*, 2008; Dowling & Simmons, 2009). The result, however, does suggest that cytoplasmic genetic variance generally does account for substantive variation in the rate of investment into somatic processes throughout life, across taxa. Furthermore, we note that morphological traits are typically associated with high heritability – implying low sensitivity in their expression to environmental variation relative to life history and metabolic traits (Price & Schluter, 1991; Houle, 1992). Low environmental sensitivity will presumably facilitate the detection of underlying cytoplasmic genetic effects in the event that such effects exist.

When focusing only on gonochorous animal species, we did not detect any significant differences between sexes in the magnitude of cytoplasmic (most likely mitochondrial in origin) effect sizes. In fact, if anything, there was a tendency for cytoplasmic effect sizes to be larger in females. The direction of this pattern would appear to be inconsistent with that predicted under the Mother's Curse hypothesis, which posits that the maternal transmission of the mitochondria will render the mtDNA prone to the accumulation of mutations that are male-biased in their associated phenotypic effects (Frank & Hurst, 1996; Gemmell *et al.*, 2004; Innocenti *et al.*,

2011). Under this process, purifying selection should be efficient at eliminating allelic variants in the mtDNA that confer deleterious effects on female function, but will be blind to mutations that harm males while being essentially benign to female function. This should result in the build-up of a male-biased mutation load within the mitochondrial genome, and the footprint of this mutation load should be detectable by observing larger mitochondrial genetic effects underlying male, relative to female, phenotypes (Dowling *et al.*, 2008; Innocenti *et al.*, 2011). The hypothesis has previously been experimentally substantiated in *D. melanogaster* (Innocenti *et al.*, 2011; Camus *et al.*, 2012).

However, we do not believe that the absence of a significant difference in cytoplasmic effect size between the sexes in this study signals the end of the Mother's Curse hypothesis, as a general evolutionary process in animals. A central prediction of this evolutionary hypothesis is that it is primarily male homologues of sexually dimorphic traits that should exhibit male biases in mitochondrial genetic variance (Friberg & Dowling, 2008; Innocenti *et al.*, 2011). This is because, for sexually monomorphic traits, males should be able to salvage the benefits of female-specific adaptation of the mtDNA, given that optimization of the mtDNA-encoded gene products in females should similarly confer optimization of mitochondrial function in males. However, for sexually dimorphic and sex-limited tissues, the intersexual genetic correlation is eroded, and optimization of mitochondrial function in the female homologue of the trait in focus (e.g. the female gamete) may not lead to optimization in the male counterpart (e.g. the male gamete). Therefore, we suggest the most informative tests of the Mother's Curse idea will be those focussed on comparing sex-specific mitochondrial allelic variance underlying sexually dimorphic phenotypes and those that specifically collate male and female data from within the one-and-the-same study. Once an adequate number of such studies exist across taxa, then meta-analytic techniques can be applied to rigorously test the general relevance of Mother's Curse in nature.

To summarize, we report a pervasive moderate effect size associated with cytoplasmic genetic variance, which is general across kingdoms. Whether or not this has implications for the use of cytoplasmic genetic markers, such as the mtDNA, for population genetic and phylogenetic inferences, may in part depend on the nature of the inferences, as well as whether the functional cytoplasmic genetic variance in question has accumulated under natural selection or drift. The evolutionary forces contributing to the accumulation of this variance remain largely elusive (Dowling, 2014). Furthermore, our results quantitatively confirm previous suggestions that the link between the cytoplasmic (including mitochondrial) genotype and phenotype will often be manifested via cyto-nuclear interactions, at least in the animal kingdom (Rand *et al.*, 2004; Dowling *et al.*, 2008; Dowling, 2014). These results are reconcilable with the observation that eukaryotic life's most important functions, in both plants (photosynthesis and mitochondrial energy conversion) and animals (mitochondrial energy conversion), hinge on extensive and integrated interactions between genes that sit in obligate cytoplasmic and nuclear genomes (Budar & Fujii, 2012; Wolff *et al.*, 2014). A complex interplay between selection and genetic drift is likely to shape the genetic variation located in cytoplasmic genomes, as well as the dynamics of the allelic interactions between cytoplasmic and nuclear genomes. These forces should perpetually drive cyto-nuclear co-evolution and ensure that mitochondrial and chloroplast genomes remain as fundamental contributors to population evolutionary processes.

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Supporting information

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

Method S1 Phylogenetic structure and Folded-over distribution.

Figure S1 Flow diagram detailing the number of studies included in each stage of the data acquisition process.

Figure S2 Overall effect sizes and sample sizes.

Figure S3 Effect sizes and sample sizes in gonochorous animals.

Figure S4 Absolute effect-size estimates ordered by publication year.

Figure S5 Flow diagram how effect-size differences between moderator levels were calculated.

Figure S6 Posterior distribution of contrasts across moderator levels.

Figure S7 Posterior distribution of contrasts across moderator levels corrected for phylogeny.

Table S1 List of species with data collected for the formal meta-analysis.

Table S2 Publication variance, residual variance and I^2 estimates.

Table S3 Publication variance, residual variance and I^2 estimates for gonochorous animal species.

Table S4 Effect-size estimates for each moderator level corrected for phylogeny $\pm$ 95% credibility interval.

Data deposited at Dryad: doi:10.5061/dryad.h883s

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