The effect of dietary restriction on reproduction: a meta-analytic perspective

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Abstract

Background

Dietary restriction (DR), a reduction in the amount of food or particular nutrients eaten, is the most consistent environmental manipulation to extend lifespan and protect against age related diseases. Current evolutionary theory explains this effect as a shift in the resolution of the trade-off between lifespan and reproduction. However, recent studies have questioned the role of reproduction in mediating the effect of DR on longevity and no study has quantitatively investigated the effect of DR on reproduction across species.

Results

Here we report a comprehensive comparative meta-analysis of the effect of DR on reproduction. In general, DR reduced reproduction across taxa, but several factors moderated this effect. The effect of DR on reproduction was greater in well-studied model species (yeast, nematode worms, fruit flies and rodents) than non-model species. This mirrors recent results for longevity and, for reproduction, seems to result from a faster rate of decline with decreasing resources in model species. Our results also suggested that not all reproductive traits are affected equally by DR. High and moderate cost reproductive traits suffered a significant reduction with DR, but low cost traits, such as ejaculate production, did not. Although the effect of DR on reproduction was stronger in females than males, this sex difference reduced to near zero when accounting for other co-factors such as the costliness of the reproductive trait. Thus, sex differences in the effect of DR on longevity may be due to a failure to expose males to as complete a range of the costs of reproduction as females.

Conclusions

We suggest that to better understand the generality of the effect of DR, future studies should attempt to address the cause of the apparent model species bias and ensure that individuals are exposed to as many of the costs of reproduction as possible. Furthermore, our meta-analytic approach reveals a general shortage of DR studies that record reproduction, particularly in males, as well as a lack of direct side-by-side comparisons of the effect of DR on males and females.

Key Words

Nutrition – breeding – life history trade-off – meta-analysis – systematic review

Introduction

Dietary restriction (DR), defined as a reduction in food intake without malnutrition [1, 2], has been shown to extend lifespan and protect against age related diseases across a range of studies (see [1, 3] for current reviews). The majority of studies examining DR use one of five laboratory model species: *Saccharomyces cerevisiae* [4], *Caenorhabditis elegans* [5], *Drosophila melanogaster* [6], *Mus musculus* and *Rattus norvegicus* [7], hereafter referred to as "model species" (see [1]). The taxonomic diversity of these model species and the fact that the effect of DR is reproducible in other, less commonly studied taxa (e.g. Primates [8]; arachnids [9]; fish [10]), has been used to suggest that the effect of DR on longevity is underpinned by an evolutionarily conserved mechanism and may thus have application to humans [3]. However, a recent meta-analysis has demonstrated that dietary restriction is nearly twice as effective at extending lifespan in the five model species as it is in non-model species [1]. Such an overarching pattern

77 questions the taxonomic generality of this effect and thus the suggestion of an
78 evolutionarily conserved mechanism.
79 The dominant evolutionary explanation of the effect of DR on longevity is
80 based on the disposable soma theory of ageing [11, 12]. Under DR, it is
81 hypothesised that organisms should reallocate resources away from reproduction to
82 somatic maintenance (and thus survival) in order to increase the chance of surviving
83 the period of resource limitation, and thus reproducing when more favourable
84 conditions return [12]. A key prediction therefore is that increased longevity is a
85 direct consequence of reduced reproduction. This prediction initially appears well
86 supported; both among and within species fecundity is generally negatively
87 correlated with longevity [13] and many studies cite a negative effect of DR on
88 reproduction. However, close inspection reveals that these citations generally involve
89 one of three studies: two using *D. melanogaster* [14, 15], cited 345 and 362 times
90 respectively, (Google Scholar, accessed 07/09/2016), and the third study using rats
91 [16], cited 89 times (Google Scholar, accessed 07/09/2016). More recently, studies
92 have questioned the generality of the longevity-reproduction trade-off underlying the
93 effect of DR, with some data suggesting that longevity and reproduction can be
94 uncoupled [17, 18]. In *D. melanogaster*, for example, significant lifespan extension
95 through DR was achieved in females that were incapable of vitellogenisis or had
96 impaired ovarian activity and could not produce eggs [17]. Furthermore, many
97 studies of DR fail to detect a decrease in reproduction, an increase in longevity or
98 both [19-21]. These exceptions and the fact that a small number of studies using
99 model species (where the DR effect on longevity is known to be greater [1]) are
100 highly cited to support the longevity-reproduction trade-off underlying DR, suggest

101 that an investigation into the generality of the effect of DR on reproduction is
102 warranted.
103 One common observation is sexual dimorphism in the response to DR, with
104 lifespan extension greater in females than in males [22-24]. Although direct
105 comparisons between the sexes within the same study are rare (see below and [22]),
106 the generality of this pattern has been supported by a recent meta-analysis showing
107 a 20% greater lifespan extension under DR in females than males [1]. An intuitive
108 explanation is that females invest more in reproduction than males. However,
109 although this may be true on a per-gamete basis, males invest heavily in
110 reproduction via other avenues e.g. courtship, intra-male competition and territory
111 defence, such that on average the net costs of reproduction must be equal in males
112 and females [25, 26]. The fact that male costs of reproduction are generally not
113 associated with gamete production may mean that males have not been exposed to
114 the full costs of reproduction in current DR studies. In many studies males and
115 females are kept separately and often in isolation (e.g. [21, 23, 27, 28]), and thus
116 males do not experience the costs associated with e.g. courtship and competition.
117 Thus, the sex difference in the effect of DR may be a result of sex differences in the
118 costs of reproduction experienced. If this hypothesis is correct, we would predict a
119 sex difference in the effect of DR on reproductive traits, with DR having more of an
120 effect on higher cost traits. We expect that taking this into account will remove any
121 sex difference in the effect of DR on reproduction.
122 Another area to explore is how reproductive decline changes with increasing
123 levels of DR. The disposable soma theory of DR predicts an initially linear decrease
124 in reproduction with decreasing resources. However, at very low levels of resources
125 survival becomes unlikely and some degree of terminal investment is predicted [12],

resulting in a decrease in the rate of reproductive decline. Recently an alternative to the disposable soma theory of DR has proposed that the response to DR evolved to minimise the loss of reproduction through upregulation of cell recycling mechanisms such as apoptosis and autophagy [29]. We suggest that this theory also predicts a non-linear reproductive decline with increasing DR. However, in this case the decrease in reproduction should be initially shallow, as cell recycling copes with small reductions in resources via recapture of some internal resources; a faster rate of decline should be observed at higher restriction levels. By examining the pattern of reproduction across levels of DR we can test these two hypotheses.

In this study we therefore attempt to address a number of issues surrounding the effect of DR on reproduction using a systematic review and meta-analysis. This method allows us to combine data from a diverse range of species, across a number of different studies. We can then highlight any general trends in the effect of DR on reproduction, whilst controlling for species-specific and study-specific effects. The specific aims of this paper are thus to investigate: (1) the generality of the effect of DR on reproduction; (2) whether, as for longevity, the effect of DR on reproduction is stronger in model than non-model species; (3) whether, as for longevity, there are sex differences in the effect of DR on reproduction; (4) whether these sex differences can be explained by the likely costliness of the reproductive traits investigated; and (5) the shape of reproductive decline with increasing restriction levels. More generally, this study aims to provide a quantitative summary of the current understanding of the effect of DR on reproduction and thus highlight areas where our knowledge is lacking and further research would be valuable.

Materials and Methods

Data collection and effect size extraction

Detailed descriptions of data collection and analysis are given in additional file 1 (dialog S1). Briefly, data were collected through a search of *ISI Web of Science* and *Scopus* using the search strings 'diet* / calor* + restriction + reproduction/fertility/fecundity'. Backward and forward searching was carried out to identify additional papers that were missed in the main database search and the authors' own literature collections on the subject were considered. These searches yielded 1,679 papers (figure 1), of which 26 reported some measure of reproduction in treated (DR) and control females or males and matched the additional selection criteria (see additional file 1, dialog S1 for details). This is perhaps a surprisingly low number of studies given the interest in DR and longevity, highlighting the paucity of studies that also collect data on reproduction. Full details for why studies were rejected are provided in data S3 provided with our data supplement on dryad (doi:10.5061/dryad.3fc02), but a number of studies were rejected as a result of not applying DR consistently across life. It is worth noting that different selection criteria would result in a different selection of studies being included and may affect our results, but we do not think our selection criteria were overly restrictive or would cause any particular bias. The 26 studies used covered 21 species (see additional file 1, figure S1 for phylogenetic tree). From these 26 studies we extracted 205 effect sizes (based on 1096 control and 1132 treatment subjects), expressed as Cohen's *d,* calculated as:

$$d = \frac{\overline{x}_1 - \overline{x}_2}{s}$$

where $\overline{x}_1$ represents the mean value of the reproductive measure for the control group, $\overline{x}_2$ represents the mean for the treatment group and s represents the pooled standard deviation (for s calculation see additional file 1, dialog S1).

Moderators

In meta-analyses, the use of moderators (e.g. the effect of sex) is often required to explain variation in the effect across studies (heterogeneity [30], see additional file 1, dialog S1). Therefore, we extracted and examined the effect of the following moderators: (1) model species or not, (2) sex, (3) degree of restriction, (4) cost of reproductive trait (see below) and (5) type of control feeding (*Ad libitum* or 100% feeding). As a result of the wide variety of reproductive measures taken, we attempted to categorise reproductive traits based on how much of the total cost of reproduction they were likely to represent. Reproductive traits were classified as: low cost, moderate cost or high cost (i.e., on an ordinal scale, see additional file 1 table S1). This measure of cost was graded to take into account species and sex specific costs. For example, in male *D. melanogaster*, ejaculate production was classified as low cost, courtship for a single mating event as medium cost and lifetime courtship investment as high cost. Although subjective, we feel the use of three categories allowed reasonably accurate assignment of traits to a particular category and was necessary to assess how many studies allowed individuals to experience near total reproductive costs. Furthermore, when categorising the cost of trait, we took the study species into consideration, to account for differences in reproductive biology between different species and particularly differences between vertebrate and invertebrate reproductive biology. This also enables cross species comparison, despite the wide variety of reproductive traits being measured.

Statistical Analysis

198 Analysis was carried out in R [31] using the packages *metaphor* [32] and
199 *MCMCglmm* [33] implementing multi-level meta-analysis (MM) and phylogenetic
200 multi-level meta-analytic models (PMM) [34, 35] (see additional file 1, dialog S1 for
201 details). We first ran models without moderators to examine overall patterns and to
202 compare phylogenetic and non-phylogenetic models. We then added single
203 moderators to the models to examine their effects in isolation. Finally, we
204 constructed a full model including all moderators of interest. In the results section,
205 we present mean standardized difference between control and restricted groups,
206 standard errors, and 95% credible intervals (CIs). When comparing phylogenetic
207 models to non-phylogenetic models we present the Akaike information criterion
208 (AIC), which is a model selection index, with the better model having a smaller AIC.
209 Publication bias was examined through visual assessment of the data and through
210 Eggers regression.
211

212 ## Results and discussion

213 **Does DR reduce reproduction universally?**

214 DR on average resulted in a significant reduction in reproduction (mixed-effect
215 meta-analysis, MM: $\beta_{[\text{meta-analytic mean}]} = -0.841$, 95% Confidence Intervals (CI) =[-
216 1.374 to -0.308]). This effect remained robust even when the phylogenetic non-
217 independence of the samples was accounted for (phylogenetic mixed effect meta-
218 analysis, PMM: $\beta_{[\text{meta-analytic mean}]} = -0.841$, CI = [-1.374, -0.308], additional file 1,
219 table S2). However, there was no evidence of a strong phylogenetic signal ($I^2_{[phylogeny]} < 0.001\%$, additional file 1, table S3) in the effect of DR on reproduction,
221 suggesting a consistent pattern across taxa. Although the model including
222 phylogenetic signal was a better fit by AIC score (phylogenetic AIC = 577.33, non-

phylogenetic = 579.86), the improvement was small and was not true for the model including all moderators (see below). To facilitate comparison we present models without phylogenetic signal included from here onwards; results are qualitatively the same for models including phylogenetic signal. Despite the small phylogenetic signal, we observed high heterogeneity amongst studies ($I^2_{[total]}$ = 98.65%, additional file 1, table S3), suggesting that the reduction in reproduction in response to DR was more apparent in certain studies. As stated above, such large heterogeneity (*sensu* [30]) calls for the use of moderators in our models to try to explain variation among studies.

Is there an effect of restriction severity?

As discussed above, an obvious pattern to explore is how reproduction responds to variation in the degree of restriction applied. In general, increasingly severe restrictions appear to increase the lifespan extension achieved by DR, up to the point of malnutrition. However, a linear change in reproduction is not predicted by existing evolutionary theories of DR. We tested these predictions by fitting both a linear and quadratic effect of the degree of restriction. We found a linear negative effect of the degree of restriction (BMM: $\beta_{[Restriction]}$ = -0.0158, CI = [-0.0219, -0.0096], figure 2, additional file 1, table S4), but no significant quadratic effect (MM: $\beta^2_{[Restriction]}$ = -0.884, CI = [-0.925, 2.694], additional file 1, table S4). This result is intriguing as it is counter to the predictions of both current evolutionary theories of DR [12, 29, 36]. One possible explanation for our inability to detect any non-linear pattern is a lack of data at particular restriction levels. Although many of the results analysed here were from studies with reasonably severe dietary restrictions (41 effect sizes, out of 205, with restriction levels greater than 75% of *ad libitum*), there are very few data points

with dietary restriction at *very* low or *very* high levels, particularly in model species (figure 2).

Is there a model species effect?

A recent meta-analysis demonstrated that DR is nearly twice as effective at extending life in model compared to non-model species [1]. We therefore tested whether such a model species effect was also apparent for reproduction. To allow direct comparison, we defined model species as the same five species used in the meta-analysis on lifespan [1] (*i.e.*, *R. norvegicus*, *M. musculus*, *D. melanogaster*, *C. elegans*, *S.cerevisiae*). Our results show that model species suffer a statistically significant reduction in reproduction (MM: $\beta_{[model]} = -2.42$, CI = [-3.41, -1.43], figure 3A, additional file 1, table S5), whereas the reduction in non-model species was lower and marginally non-significant (MM: $\beta_{[non\text{-}model]} = -0.445$, CI = [-0.926, 0.033], figure 3A, additional file 1, table S5). Comparing these effects, DR had a significantly stronger effect on reproduction in model than non-model organisms (MM: $\beta_{[non\text{-}model/model\ difference]} = -1.97$, CI = [-3.07, -0.87], figure 3A, additional file 1, table S5).

In an attempt to disentangle this effect further, we included the interaction between model organism and degree of restriction. This analysis revealed a statistically significant interaction (MM: $\beta_{[restriction * model]} = -0.0415$, CI = [-0.0710, 0.0120], figure 2 & 3A, additional file 1, table S6); the rate of decline of reproduction with increasing DR was steeper in model than non-model species, suggesting that reproduction in model species is more responsive to resource availability than reproduction in non-model species. These results fit well with the findings of Nakagawa *et al.* [1] and with the disposable soma theory of the effect of DR on longevity, if this increased reduction in reproduction results in more resources being available for reallocation to somatic maintenance. However, the obvious question

becomes why do model species have a greater reproductive response to increasing restriction than non-model species?

One possibility is that this is an unintentional effect of selection and subsequent adaptation to the laboratory environment [37]. For example, the laboratory environment is nutrient rich compared to the natural environment and selects for high fecundity but not longevity [38, 39]. Such an environment may inadvertently favour individuals that have greater plasticity in reproduction in response to nutrient availability. If such plasticity is maintained, either because it has no cost under laboratory conditions or because laboratory conditions vary enough to maintain plasticity, populations that have undergone generations of laboratory selection would be predicted to respond more plastically to food availability than populations that had not undergone such selection. On the other hand, natural environments may be predicted to be more variable than laboratory environments, particularly in food availability, and this may be expected to select for increased plasticity in non-model species. Although a small number of studies compare the effectiveness of DR in extending lifespan in laboratory maintained populations versus wild or wild derived populations [37, 38, 40], results are inconsistent. It would therefore be interesting to increase the number of these studies and to use a range of food availabilities (rather than just two) to test whether laboratory populations are more plastic to food availability than wild derived populations. If so, inadvertent laboratory selection for high fecundity in a novel environment may have accounted for this plasticity.

Another possible explanation for the increased reproductive response to nutrient restriction in model species is that researchers can more effectively implement restriction in model species [1]. Model species have been studied in

297 laboratory environments for many generations and thus diets are more likely to be
298 optimised. In non-model species, where we know less about their nutritional
299 requirements, "*ad libitum*" treatments may actually be fed to excess and foods are
300 unlikely to be optimised. Thus when applying DR, the restricted group may be under
301 a much lower restriction levels than expected in non-model species. For example, a
302 75% restriction may actually contain 90% of the nutrients needed. Furthermore, the
303 application of the geometric framework of nutrition to DR studies [41, 42], has
304 provided a growing body of evidence that specific diet composition affect lifespan
305 and reproduction and that this may be as, or even more, important than classical
306 restriction (e.g. [2, 5, 27, 28]). Studies that use the same species may utilize diets
307 with slightly different composition, which would undoubtedly effect results. It stands
308 to reason, however, that model species which are frequently studied, will have better
309 defined nutrient requirements and therefore that there may be less variation in diet
310 composition and more consistent results. Obviously other explanations are possible,
311 but our results and those of Nakagawa et al. [1] highlight the need for more research
312 to investigate the cause of this model organism effect and how it may affect the
313 generality of the conclusions drawn from investigations of DR.

314 **Is there sexual dimorphism?**

315 We next addressed whether there are sex differences in the reproductive
316 response to DR, similar to those observed in the longevity response [1]. Our analysis
317 revealed that females suffer a significant reduction in reproduction under DR (MM:
318 $\beta_{[female]} = -1.05$, CI = [-1.67, -0.43], figure 3A, additional file 1, table S7), but that this
319 reduction is much smaller and statistically non-significant in males (MM: $\beta_{[male]} = -$
320 0.274, CI = -1.291, 0.742, Fig 3A, additional file 1, table S7). However, when
321 comparing the magnitude of the effect between the sexes, we found no statistically

significant difference between males and females (MM: $\beta_{[male / female difference]}$ = 0.776, CI = [-0.414,1.967], figure 3A, additional file 1, table S7). The lack of statistical significance in comparison between the sexes is probably because of a lack of statistical power, with the sample size for males being particularly small, only 42 out of 205 effect sizes. These effect size estimates in males come from seven studies, covering five species, all of which were vertebrates (two bird species, one rodent, one primate and one fish species). The remaining studies were on females and there were no studies that allowed side-by-side comparisons of the effect of DR on males and females of the same species. Thus, studies that allow such direct comparison and generally more studies investigating DR in males would be desirable avenues of future research.

Does the cost of the reproductive trait measured matter?

It seems intuitive that traits which are more costly or encompass a greater proportion of total reproductive investment, such as lifetime egg production, will suffer a greater reduction under DR than low cost traits, such as producing a single ejaculate. We therefore included the estimated costliness of the reproductive trait as a moderator. High and moderate cost reproductive traits were statistically significantly reduced under DR (MM L: $\beta_{[high]}$ = -1.12, CI = [-1.71, -0.54]; $\beta_{[moderate]}$ = -1.05, CI = [-1.62, -0.48], additional file 1, figure S2 and table S8). In contrast, low cost traits suffered a much smaller and statistically non-significant reduction under DR (MM: $\beta_{[low]}$ = -0.244, CI = [-0.861, 0.374], additional file 1, figure S2 and table S8). This result is unsurprising, but has implications for future DR studies. If, as the disposable soma theory of DR suggests, the effect on longevity is due to a decrease in reproduction, future experiments must allow both control and restricted individuals to experience and express high cost reproductive traits. Otherwise, if individuals are

only exposed to a small proportion of the costs of reproduction, the differences between control and restricted individuals are expected to be smaller and more difficult to detect. This may be one explanation for the current sex difference in the effect of DR if females are exposed to more of the costs of reproduction than males (see also below).

This point becomes particularly relevant when examining the current data set in detail. As mentioned above, our search criteria resulted in only 42 effect sizes for males versus 163 for females. Of these 42, only 1 was classed as a high cost reproductive trait (a measure combining all reproductive behaviour into a single score of sexual activity), 18 were moderate cost and the remaining 23 were low cost. The distribution for female traits was: 77 high cost, 69 moderate costs and 17 low cost traits. Given the difference in distribution of the cost categories between males and females (χ^2_{2df} = 51.30, p < 0.001), it is unclear if the above sex differences in the reproductive response to DR are real or simply reflect difference in the costs of traits that have tended to be measured in males and females. To test this we fitted a final, 'full' model, to assess the effect of the inclusion of all moderators considered on the estimated effects.

Putting it all together

When accounting for all of the individual moderators and the interaction between model species and the degree of restriction, the degree of restriction, the cost of the trait and the interaction were all statistically significant predictors of the reduction in reproduction under DR (MM: $\beta_{[Restriction]}$ = -0.357, CI = [-0.520, -0.194]; $\beta_{[cost]}$ = -0.252, CI = [-0.436, -0.067]; $\beta_{[restriction : model]}$ = -1.32, CI = [-2.17, -0.47], figure 3B, additional file 1, table S9). This model had a conditional R^2 value of 78.8% with random effects explaining 33.2% and fixed effects explaining 45.6% of the

variation in effect size between studies [43]. When the interaction between model species and restriction was removed, restriction, model species and cost of trait remained as significant predictors (additional file 1, table S10).

As with the initial models, we also fitted models that accounted for the phylogenetic non-independence of species, with the non-phylogenetic model being the better fit (including interaction, phylogenetic AIC = 530.08, non-phylogenetic AIC = 528.08 (additional file 1, tables S9 and S11); excluding interaction, phylogenetic AIC = 539.22, non-phylogenetic AIC = 537.22 (additional file 1, tables S10 and S12)). This result suggests that the reduction in reproduction observed under DR is robust and phylogenetically conserved ($I^2_{[phylogeny]} < 0.001\%$ additional file 1, table S13), but that the rate of reduction is greater in model species compared to non-model species. Furthermore, the reduction in reproduction was greater when examining more costly traits. Of particular interest when fitting the full model was the effect of including the cost of the trait on the sex difference in the effect of DR. When accounting for all other moderators, the difference between males and females was reduced (MM: $\beta_{[male / female difference]} = -0.151$, CI = [-1.132, 0.830] compared to MM: $\beta_{[male / female difference]} = 0.776$, CI = [-0.414, 1.967] in the model only containing sex, figure 3A and B). This result implies that the supposed sex differences in response to DR are being driven by experimental design, particularly the costs of reproduction experienced by the sexes.

Essential for all meta-analyses is the assessment of potential publication bias, as interpretation of results of meta-analyses assumes minimal publication bias in the literature [44]. Visual assessment of our data showed no obvious sign of publication bias (additional file 1, figure S3). Furthermore, statistical assessment revealed no significant publication bias in our data set once accounting for heterogeneity [35]

(Eggers regression on the 'meta-analytic' residuals; $\beta_{[intercept]} = 0.0780$, S.E. = 0.0778, $p = 0.317$).

Conclusions

Our results represent the first formal meta-analysis of the effect of DR on reproduction, an important issue given some studies suggesting the effect of DR on longevity can be achieved independently of reproduction [17]. Above, we present three main findings that suggest explanations for outstanding issues in this field and avenues for future research. First, DR does lead to a reduction in reproduction but, in line with longevity [1], this effect is stronger in model species. We discuss a number of possible explanations for this phenomenon. However, it is clear more studies are needed as any bias in patterns from model species as a result of laboratory adaptation have far reaching consequences for the role of DR studies in understanding and mitigating ageing and its application to humans [3]. Second, reproduction declines linearly with increasing DR, at odds with both current evolutionary theories of DR [12, 29, 38]. It is possible that our failure to detect a non-linear response of reproduction to DR was due to a lack of data at certain levels of restriction. More work across a broader range of restriction levels is needed to improve our power to detect non-linear effects and thus assess and compare alternative evolutionary hypotheses on DR effects [45, 46].

Finally, although our results support a sex difference in the response of reproduction to DR, they suggest this may be due to males and females being exposed to different levels of reproductive costs in the majority of experiments. An alternative explanation is that the longevity-reproduction trade-off can be uncoupled, with diets that maximize longevity not necessarily minimizing reproduction and that

this effect can be sex specific [2, 28]. Definitive conclusions are difficult to draw because relatively few studies investigate the effect of DR on reproduction in males or allow direct comparison of males and females in the same study using a range of diets (but see [2, 28]). This is presumably because of the difficulty of designing meaningful measures of male reproductive investment that would encompass the majority of the costs. One potential solution is to measure many male reproductive traits and combine them into an overall score of reproductive investment [47]. Even if this is not possible, future DR studies must carefully consider the biology of the study organism and ensure both sexes are exposed to as close to the complete costs of reproduction as possible. For males this will usually include allowing costs such as those incurred while attracting females and direct competition with other males. By doing such experiments, we can start to assess whether sex differences in the response to DR, both in terms of reproduction and longevity, are a real and interesting sexual dimorphism, or an artefact of experimental design.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets and materials analysed during the current study are available in the Dryad repository, doi:10.5061/dryad.3fc02. Temporary access to data and materials: http://datadryad.org/review?doi=doi:10.5061/dryad.3fc02

Competing Interests

The authors declare that they have no competing interests.

Funding

JPM was funded by the Biotechnology and Biological Sciences Research Council (BBSRC) [grant number BB/J01446X/1] through the EASTBIO Doctoral Training Programme. SN was funded by an ARC Future Fellowship (FT130100268). CAW was funded by a Natural Environment Research Council (NERC) post-doctoral research fellowship (NE/I020245/1) and a University of Edinburgh Chancellor's fellowship.

Author Contributions

CAW and JPM conceived and designed the study, with input from SN on the design. Data collection was primarily performed by JPM with input from CAW. JPM and SN led the statistical analysis, but all authors contributed to the final analysis. Phylogenetic tree construction was carried out by ML. JPM wrote the initial draft of the manuscript and all authors contributed to editing the manuscript.

Acknowledgements

We thank the 'Life-History Discussion Group' at the University of Edinburgh, A. B. Phillimore and R. L. Watson for helpful advice, discussion and critical comments on the analysis and interpretation of results.

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Figure Legends

Figure 1. PRISMA flow diagram of data collection. The number of papers identified initially through key word searching is shown in the identification boxes. The number of papers excluded is shown for each stage of screening. Reasons for exclusion are given for papers that made it to final eligibility screening.

Figure 2. The effect of degree of restriction on effect size in model and non-model species. Effect sizes are Cohen's *d*, the standardised mean difference in reproduction between the control and restricted groups (see methods and additional file 1, dialog S1). Model species are represented by squares and the dashed line. Non-model species are represented by circles and solid line. Model species suffer a greater rate of decline in reproduction with increasing degree of restriction. Point sizes indicate the variance in the estimate of the effect size. Details of statistics are given in the main text.

609 Figure 3. Forest plots showing effect sizes (Cohen's *d*, standardised mean difference
610 in reproduction between the control and restricted groups (see methods and
611 additional file 1, dialog S1)) of key moderators for the effect of dietary restriction (DR)
612 on reproduction. Each point represents the Cohen's *d* value with the 95% credible
613 intervals (CIs). Panel A represents the outputs from univariate models, with each
614 moderator fitted individually. Each moderator subgroup (e.g. model or non-model
615 species) is represented by a single point. Contrasts represent the difference between
616 effect sizes of the subgroups (e.g. the difference between model (M) and non-model
617 (N) species). Restriction:Model, represents the interaction between degree of
618 restriction (%) and model or non-model species. Panel B shows the output from our
619 full model accounting for all moderators, with each point representing the effect size
620 for that moderator.
621

622 ## Additional Materials

623 Further information is provided in Additional File 1.doc, which contains more detailed
624 methods, supplementary figures and supplementary tables.

Dialog S1

Collecting studies on dietary restriction (DR) and reproduction.

The data for the meta-analysis were collected through a search of ISI Web of Science and Scopus during December 2013 by J. P. Moatt using the search string 'diet*/calor* + restriction + reproduction/ fertility/fecundity'. Backward and forward searching was carried out to identify additional papers that were missed in the main database search, as well the authors' own literature collections on the subject were considered. Authors of interest were contacted in attempt to obtain unpublished data for inclusion in the analysis. However, no unpublished data matching the selection criteria were found. Grey literature and non-English language papers were also considered during selection. Of the 1,679 unique papers the search returned, papers were selected which had applied DR and reported some measure of reproduction, for treated (DR) and control females or males (usually presented as a means and standard errors). Papers were included if they met the following criteria:

1. Papers must be original empirical data using real animals, not reviews or computer simulations.
2. Animals must not be mutant or transgenic.
3. Degree of dietary restriction must be explicitly stated.
4. Intermittent feeding is allowed, as long as fasting period does not exceed the equivalent of every other day feeding. Feeding days must not allow compensatory gorging.
5. Information on the control groups intake must be given, and be either *ad libitum* or 100%.

6. Restriction must have been initiated prior to copulation and must remain constant throughout the course of the experiment.
7. There were no other confounding cofactors, such as resveratrol or pathogen treatment.

Additionally, we excluded studies where only measures of reproductive hormone levels were reported or information necessary for calculating effect sizes was missing (e.g. sample sizes, variances). Screening was carried out by J. P. Moatt between January and June 2014. Although the screening was carried out alone, discussion over the inclusion of a number of papers took place between C. A. Walling and J. P. Moatt.

Extracting effect size

In the majority of papers, reproductive data was presented in the main text as mean and standard error as well as sample sizes. In studies where this was not the case, authors were contacted in an attempt to obtain the relevant data. Effects sizes were then calculated using an effect size calculator [1]. Effect sizes are the standardised mean difference (SMD) Cohen's *d*, a measure of the difference in reproduction between the control and restricted groups, standardised by the pooled standard deviation estimates from the two groups.

$$d = \frac{\overline{x}_1 - \overline{x}_2}{s}$$

X_1 = mean for control group

X_2 = mean for treatment group

s = pooled standard deviation. Calculated as below:

$$s = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

n_1 = sample size of control group

n_2 = sample size of treatment group

s_1 = standard deviation of control group

s_2 = standard deviation of treatment group

Extracting Moderators (DR associated variables)

Methods sections from each paper were examined and any relevant moderators were extracted and recorded as follows:

- Model Species: 1 = yes, 0 = no, model species counted as the same five model species as in Nakagawa *et. al.* [2]: yeast (*Sacchromyces cerevisiae*), nematode (*Caenorhabditis elegans*), fruit fly (*Drosophila melanodaster*), mouse (*Mus musculus*) and rat (*Rattus norvegicus*).
- Strain name/type: unique strain names for a particular species (note that unique names are given for WT or the same strain names for different species).
- Sex: sex of the group *d* was extracted for (M = male, F = female).
- Food schedule: feeding regime used (D = daily, W = Weekly).
- Type of restriction being used: CNM = Calorie and nutrient manipulation, these were papers that included a number of diets of varied composition. However, these studies were only included if each diet was provided at multiple restriction levels, including a control level; FC = food concentration, where lower concentrations of the same food medium were

used in treatment relative to control group; FS = feeding schedule, where restriction was implemented through a feeding schedule, as less frequent feeding than in the control group, e.g. every other day feeding vs. every day feeding; FW = food weight, where the same food was given in smaller quantities in treatment relative to control group.

- Feeding regime of control: 0 = 100% feeding, where individuals were given a set quantity and this was counted as fully fed; 1 = *ad libitum* where unrestricted access to food was allowed.
- Units of control and treatment group nutrition levels (when given): e.g., J/day/individual.
- Calories in control diet (when information provided): caloric density of the food.
- Costliness of the reproductive trait: A categorical measure that describes the degree to which the reproductive trait measured reflects the total cost of reproduction in the species used: 1 = low cost – trait represents a relatively small fraction of the total cost of reproduction in that species, 2 = moderate cost, trait represents a moderate fraction of the total cost of reproduction in that species, 3 = high cost, trait represents the majority of the cost of reproduction in that species. This measure accounted for differences between species and sexes within species. For example, in *D. melanogaster*, ejaculate production is classed as low cost, courtship for a single mating event represents a medium cost and lifetime courtship investment is high cost, as courtship is thought to be one of the most costly aspects of reproduction for male *D. melanogaster* [3]. For females,

daily egg production represents a medium cost, whereas lifetime egg production is high cost, see Table S1.

- Reproductive measure examined: e.g., lifetime egg production, number of sperm.
- Units of the reproductive trait measured (where necessary): e.g., mass of eggs produced in g.
- The value of the reproductive trait being measured for the control group.
- Standard deviation of the mean for control group.
- Number of control individuals.
- Caloric value of restricted diet (when given).
- Restriction level, represented as a percentage decrease from control group: e.g. 40% restriction means treatment group give 60% of control diet.
- The value of the reproductive trait being measured for the restricted group.
- Standard deviation of the mean for restricted group.
- Number of restricted individuals.

Any other information considered relevant or important was noted. For complete records see Data S1 and for the detailed description of all the columns in the data table see Dialog S2.

Constructing phylogenetic tree

A topological (without branch lengths) phylogenetic tree was constructed for the subset of species included in this study using the Interactive Tree of Life (http://itol.embl.de/index.shtml). Polytomies among insect orders were resolved using information obtained from Trautwein *et al.* [4].

General meta-analytic techniques

For the main analyses we used mixed effects meta-analysis (MM) or phylogenetic mixed effects meta-analysis (PMM) implemented in the *metaphor* package [5], version 1.9-3, and *MCMCglmm* package [6] for R (R core team (2014)). As model results we present mean standardized difference between control and restricted groups, standard errors, and 95% credible intervals (CIs). When comparing phylogenetic models to non-phylogenetic models we present the Akaike information criterion AIC, which is a model selection index, with the better model having the smaller AIC. The *R* scripts for all analyses are available as supplementary materials with this article.

Main meta-analytic models (Model 1 and 2)

Models 1 and 2 (Table S2) were simple models only fitting the effect size as a response variable, with the intercept as the fixed factor and the following random factors; study ID, animal (species ID), group ID (identifies cases where multiple types of reproduction traits were reported for the same groups of individuals) and effect size ID. These were to account for the main sources of non-independence between our measures. Model 1 only differed from Model 2 in that it accounted for phylogenetic variance.

Heterogeneity

A meta-analysis will inevitably bring together studies that differ in design and set up, particularly in reference to treatments, exposures and outcomes explored, this is referred to as heterogeneity [7]. We must account for heterogeneity to explain the differences observed between the studies included in a meta-analysis. Here, we used an extended version of I^2 [7] as our heterogeneity statistic, which is described

in Nakagawa and Santos [8]. This multi-level model extension of I^2 enables us to obtain heterogeneity due to each level or random factor.

Meta-analytic models with moderators (Models 3-11)

Our main question was to see whether investment in reproduction was decreased under DR. However, we also explored variables we thought may be important predictors of variation in the effect of DR on reproduction, known as moderators. We added each moderator separately to the main meta-analytical model (Model 2) to assess the effect of individual moderators (Models 3-7). These moderators included: (a) whether the control group was fed a specific pre-defined amount or concentration of food (100%) or were allowed *ad libitum* access to food (only included in full models 8 - 11), (b) whether the species was one of the five model species or not (Table S4, Model 3), (c) which sex was being studied (Table S5,Model 4), (d) the linear and quadratic effect of degree of restriction (Table S6, Model 5), (e) the relative cost of the reproductive trait being studied (low, moderate and high, Table S1 for trait classification, Table S7 for model output, Model 6). We also fitted the interaction between model/non-model species and degree of restriction (Table S8, Model 7). We then created a number of full models where all moderators were fitted at the same time (Tables S9-S13,Models 8 - 11). Models 8 and 9 included all moderators and the interaction between model/non-model species and degree of restriction. Models 10 and 11 included all moderators but excluded the interaction between model/non-model species and degree of restriction. Models 9 and 11 are models which account for the phylogenetic variance.

Publication Bias

Publication bias is the favouring of statistically significant results during publication, regardless of the underlying effect size. We used two typical ways of assessing publication bias: (1) visual inspection via a funnel plot and (2) Eggers regression, which assess bias through a regression method [9]. However, these methods assume that effect sizes are independent of each other. We therefore used meta-analytic residuals (sampling error and residuals) from our full model for Egger regression to fulfil this assumption. [8].

Supplementary figures

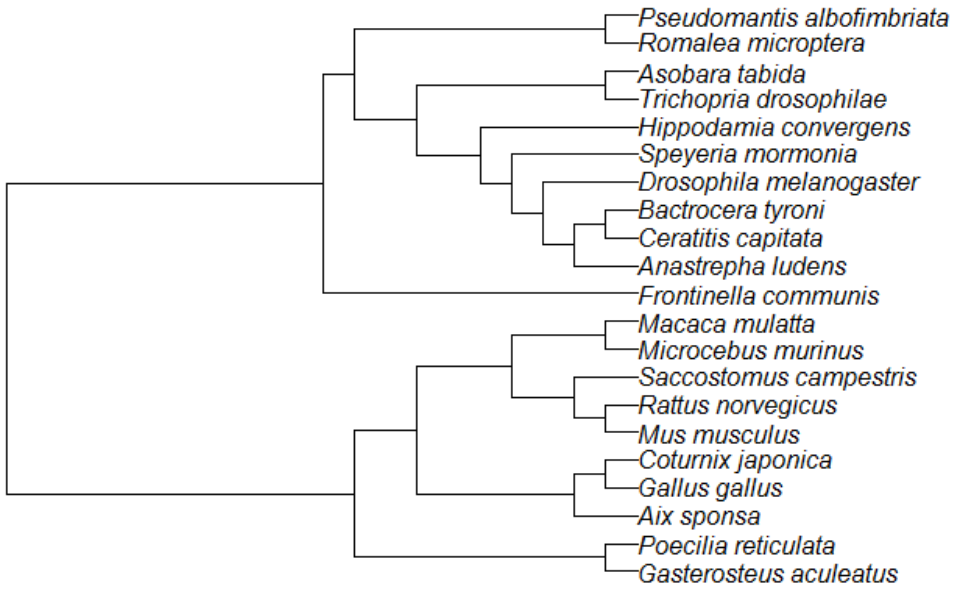

Figure S1. Phylogenetic tree of the 21 species used in the meta-analysis.

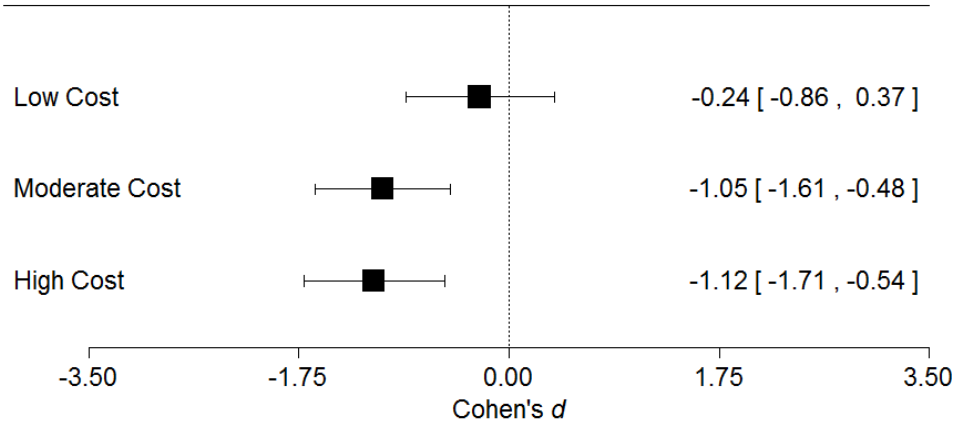

Figure S2. Forest plot showing effect sizes (Cohen's *d*) for the effect of dietary restriction (DR) on reproduction, for different levels of cost of reproductive trait included as a moderator. Each point represents the Cohen's *d* value for that moderator with the 95% credible intervals (CIs). High and moderate cost traits undergo a significant reduction under DR, however low cost traits do not.

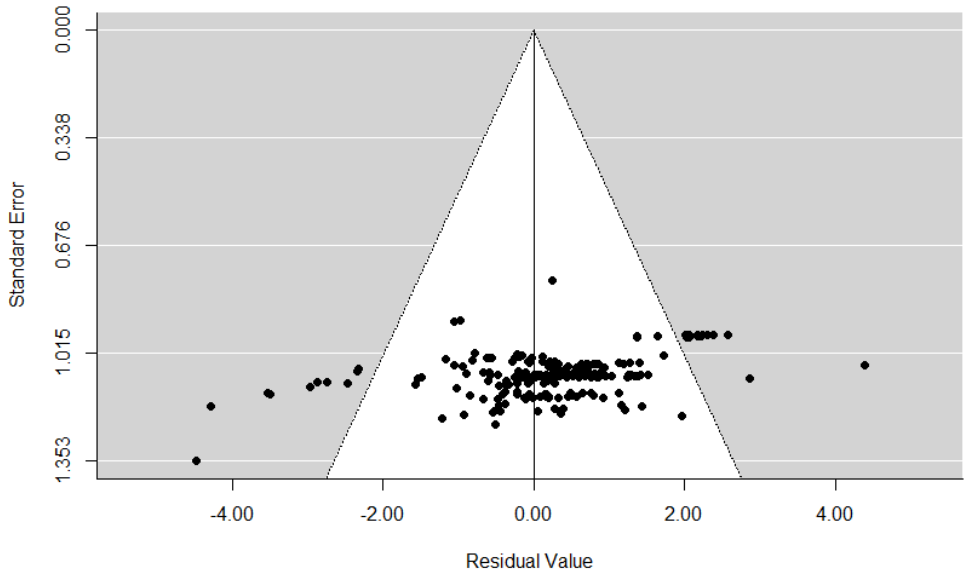

Figure S3. Funnel plot to allow visualisation of potential publication bias in our data set. The X axis represents the residual values from the non-phylogenetic mixed effects model containing all moderators and the interaction of restriction and model species, the Y axis represents the standard error. Publication bias indicated if data points clustered towards zero residual values as standard error decreases. Visual inspection suggests this is not the case.

Supplementary Tables

Table S1 List of reproductive traits and the cost category they were assigned.

Low Cost (n=40)	**Medium Cost** (n=87)	**High Cost** (n=78)
Number of eggs fertilised (measured when only males under DR)	Testes weight, lifetime investment in sperm production	Number of females pregnant at least once in lifetime, lifetime investment in reproduction
Proportion of fertile eggs that hatch (measured when only males under DR)	Daily fecundity, high cost but not lifetime investment	Total fecundity, lifetime investment in egg production.
Pair formation when both sexes under DR, measured as proportion of birds that successfully pair	Size of 1st egg clutch, similar to above, high cost but not lifetime investment.	Reproductive effort, lifetime measure
All sperm / ejaculate composition, e.g. sperm length, ejaculate volume, proportion of live sperm etc	Date of 1st egg production, age of sexual maturity	Lifetime clutch production
Time per clutch, time to lay eggs	Gestation length, assuming more significant cost to female than litter growth/weight	Number of females reproducing during breeding season.
Mating-oviposition interval, not measuring number of eggs produced or matured in this time	Male courtship of females, known to be costly but only one reproductive behaviour measured	Sexual activity, measuring full range of male precopulatory behaviour
Foetal growth (g per day)	Egg load, females were unmated, killed and dissected.	
	Eggs counted midway through life	
Litter body mass at birth	Reproductive success for single breeding season, not lifetime reproductive success	
Egg mass, investment in single egg	Litter size, combination of egg number and provisioning of foetus	
	Number of clutches/eggs for part of life, not lifetime investment in eggs	
	Reproductive period (days), measure of single reproductive season	
	Oviposition days for single breeding season	
	Reproductive success, single breeding season	

Table S2 Comparing phylogenetic mixed effect model (PMM, Model 1) and non-phylogenetic mixed effect model (MM, Model 2) estimates of the effect of DR on reproduction. AIC taken from ML models.

	Effect size	SE	Lower CI	Upper CI	AIC
PMM	-0.841	0.272	-1.374	-0.308	577.33
MM	-0.841	0.272	-1.374	-0.308	579.86

Table S3 Table of heterogeneity statistics (I^2 values) for Models 1 and 2.

	Model 1	Model 2
Total Heterogeneity	98.65	98.65
Variance due to Phylogeny	0.0000667	NA
Variance due to Study	74.83	74.83
Variance due to Group	3.91	3.91
Residuals against sampling error	19.91	19.91

Table S4 Estimated effect sizes from the non-phylogenetic mixed effect model with the linear and quadratic effect of restriction as moderators (Model 5)

	Effect size	SE	Lower CI	Upper CI
Restriction	-0.016	0.003	-0.022	-0.010
Restriction^2	0.884	0.923	-0.925	2.694

Table S5 Estimated effect sizes from the non-phylogenetic mixed effect model with model/non-model species fitted as a moderator (Model 3).

	Effect size	SE	Lower CI	Upper CI
Model	-2.416	0.506	-3.406	-1.425
Non-model	-0.447	0.245	-0.926	0.033
Contrast	-1.969	0.562	-3.070	-0.868

Table S6 Estimated effect sizes from the non-phylogenetic mixed effect model with the interaction between model species and restriction fitted as moderators (Model 7)

	Effect size	SE	Lower CI	Upper CI
Restriction	-0.013	0.003	-0.020	-0.007
Model	0.769	1.035	-1.261	2.798
Restricition:Model	-0.042	0.015	-0.071	-0.012

Table S7 Estimated effect sizes from the non-phylogenetic mixed effect model with sex as a moderator (Model 4)

	Effect size	SE	Lower CI	Upper CI
Female	-1.051	0.316	-1.671	-0.431
Male	-0.274	0.519	-1.291	0.742
Contrast	0.776	0.608	-0.414	1.967

Table S7 Estimated effect sizes from the non-phylogenetic mixed effect model with cost of trait fitted as a moderator (Model 6)

	Effect size	SE	Lower CI	Upper CI
Low Cost	-0.244	0.315	-0.861	0.374
Moderate Cost	-1.050	0.288	-1.615	-0.484
High Cost	-1.124	0.298	-1.708	-0.539

Table S9 Estimated effect sizes from the non-phylogenetic mixed effect model with all moderators fitted, including the interaction between restriction and model species (Model 8). AIC taken from ML models.

	Effect size	SE	Lower CI	Upper CI
Year	0.034	0.018	-0.001	0.067
Ad Lib feeding	-0.173	0.434	-1.024	0.678
Restriction	-0.357	0.083	-0.520	-0.194
Model species	-1.074	0.625	-2.298	0.150
Male	-0.151	0.501	-1.132	0.830
Scaled cost	-0.252	0.094	-0.436	-0.067
Restricition:Model	-1.317	0.435	-2.169	-0.465

AIC = 528.08

Table S10 Estimated effect sizes from the non-phylogenetic mixed effect model with all moderators fitted, omitting the interaction between restriction and model species (Model 10). AIC taken from ML models.

	Effect size	SE	Lower CI	Upper CI
Year	0.014	0.019	-0.024	0.051
Ad Lib feeding	0.295	0.470	-0.627	1.217
Restriction	-0.390	0.084	-0.554	-0.226
Model species	-1.634	0.685	-2.977	-0.291
Male	-0.148	0.569	-1.264	-0.069
Scaled cost	-0.257	0.096	-0.446	-0.054

AIC = 537.22

Table S11 Estimated effect sizes from the phylogenetic mixed effect model with all moderators fitted, including the interaction between restriction and model species (Model 9). AIC taken from ML models.

	Effect size	SE	Lower CI	Upper CI
Year	0.034	0.018	-0.001	0.070
Ad Lib feeding	-0.173	0.434	-1.024	0.679
Restriction	-0.357	0.083	-0.520	-0.194
Model species	-1.074	0.625	-2.298	0.150
Male	-0.151	0.501	-1.133	0.830
Scaled cost	-0.252	0.094	-0.436	-0.067
Restricition:Model	-1.317	0.435	-2.169	-0.465

AIC = 530.08

Table S12 Estimated effect sizes from the phylogenetic mixed effect model with all moderators fitted, omitting the interaction between restriction and model species (Model 11). AIC taken from ML models.

	Effect size	SE	Lower CI	Upper CI
Year	0.014	0.019	-0.024	0.051
Ad Lib feeding	0.295	0.470	-0.627	1.217
Restriction	-0.390	0.084	-0.554	-0.226
Model species	-1.634	0.685	-2.977	-0.291
Male	-0.148	0.569	-1.264	0.968
Scaled cost	-0.257	0.096	-0.446	-0.069

AIC = 539.22

Table S13 Table of heterogeneity statistics (I^2 values) for Models 8 and 9.

	Model 8	Model 9
Total Heterogeneity	97.54	97.58
Variance due to Phylogeny	NA	0.00002
Variance due to Study	59.54	59.54
Variance due to Group	0.00006	0.00
Residuals against sampling error	38.04	38.03

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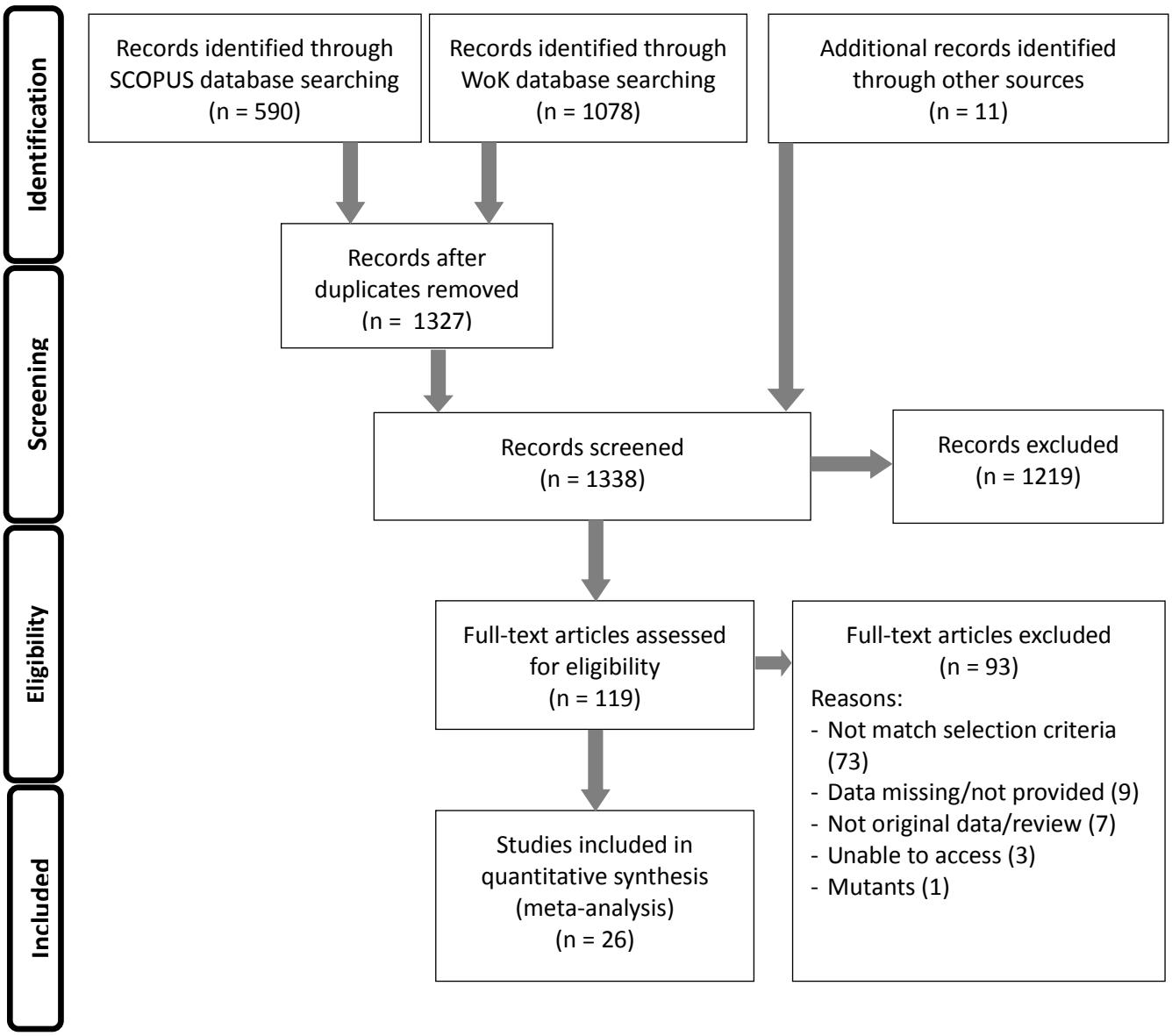
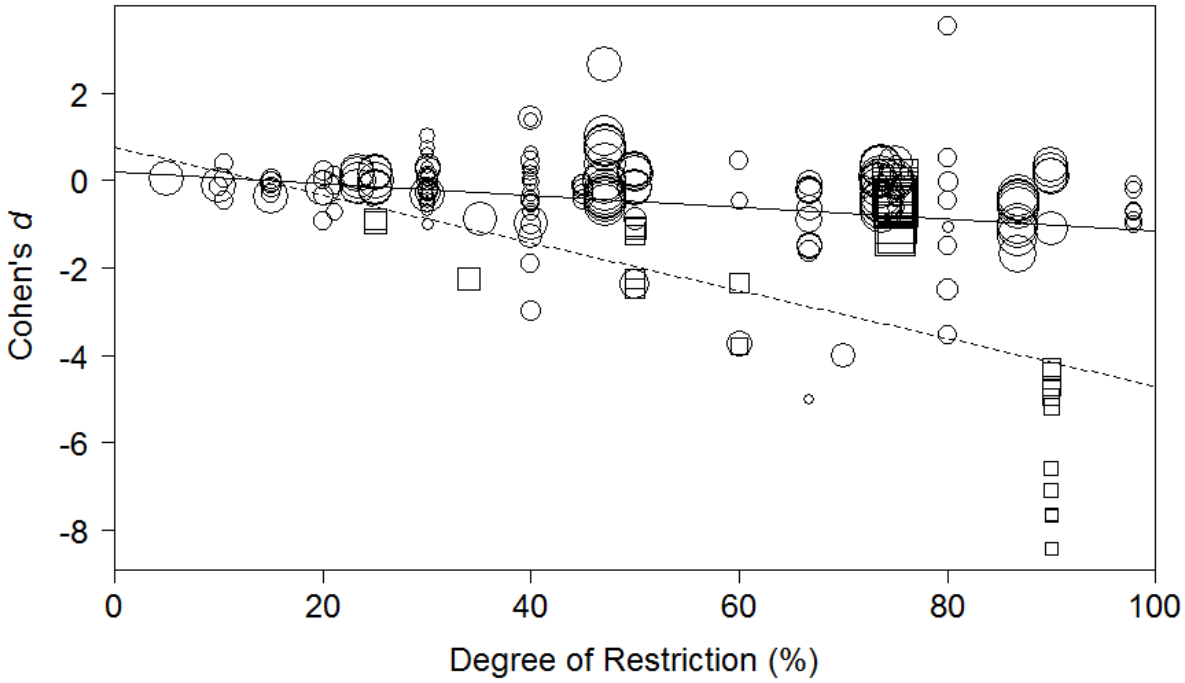
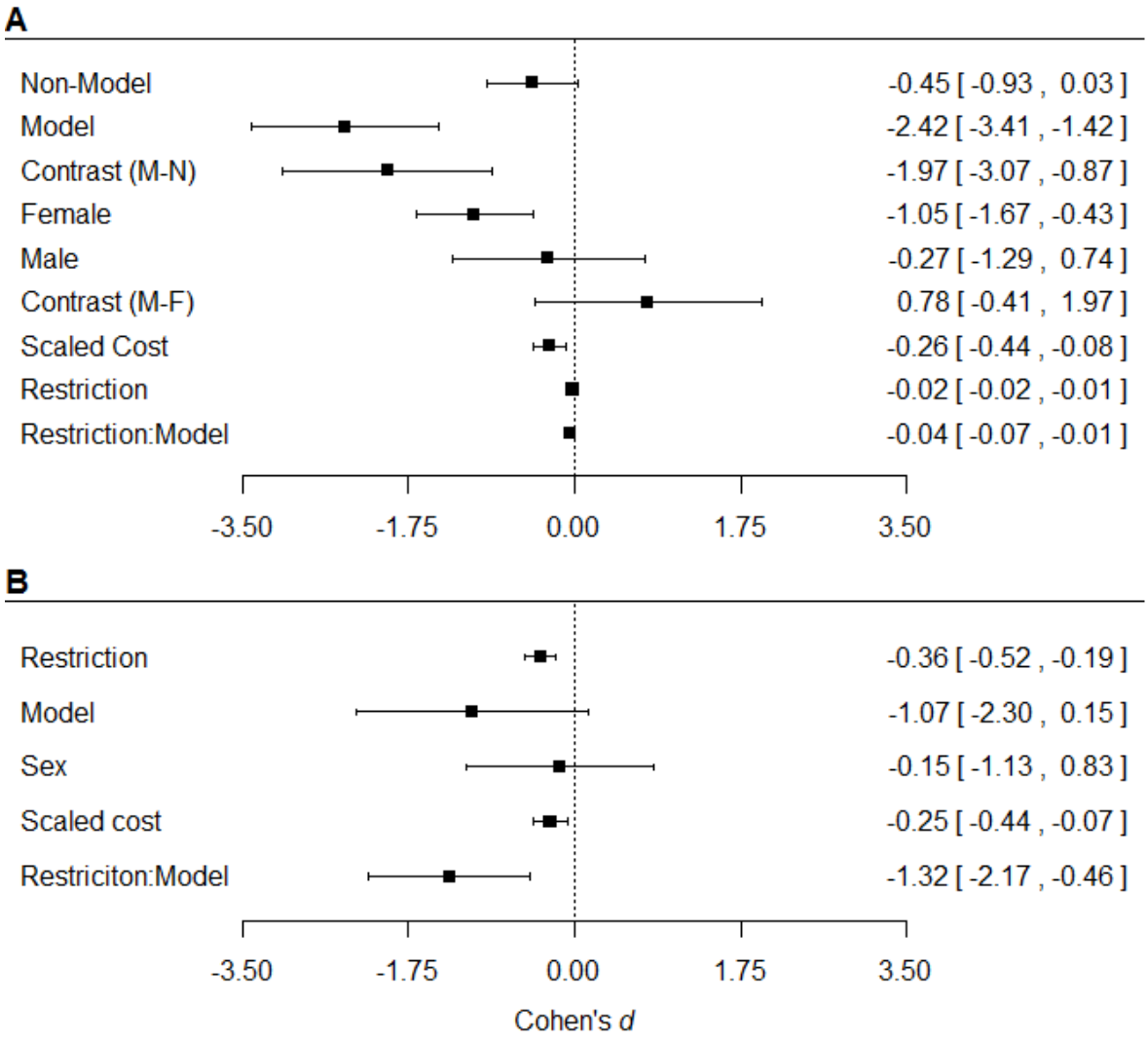

Identification
Screening
Eligibility
Included
Records identified through SCOPUS database searching (n = 590)
Records identified through WoK database searching (n = 1078)
Additional records identified through other sources (n = 11)
Records after duplicates removed (n = 1327)
Records screened (n = 1338)
Records excluded (n = 1219)
Full-text articles assessed for eligibility (n = 119)
Full-text articles excluded (n = 93)
Reasons:
- Not match selection criteria (73)
- Data missing/not provided (9)
- Not original data/review (7)
- Unable to access (3)
- Mutants (1)
Studies included in quantitative synthesis (meta-analysis) (n = 26)


Cohen's d
2
0
-2
-4
-6
-8
0
20
40
60
80
100
Degree of Restriction (%)


A
Non-Model
Model
Contrast (M-N)
Female
Male
Contrast (M-F)
Scaled Cost
Restriction
Restriction:Model
-0.45 [-0.93 , 0.03]
-2.42 [-3.41 , -1.42]
-1.97 [-3.07 , -0.87]
-1.05 [-1.67 , -0.43]
-0.27 [-1.29 , 0.74]
0.78 [-0.41 , 1.97]
-0.26 [-0.44 , -0.08]
-0.02 [-0.02 , -0.01]
-0.04 [-0.07 , -0.01]
-3.50
-1.75
0.00
1.75
3.50
B
Restriction
Model
Sex
Scaled cost
Restriciton:Model
-0.36 [-0.52 , -0.19]
-1.07 [-2.30 , 0.15]
-0.15 [-1.13 , 0.83]
-0.25 [-0.44 , -0.07]
-1.32 [-2.17 , -0.46]
-3.50
-1.75
0.00
1.75
3.50
Cohen's d