Hadfield

General underlying mechanism

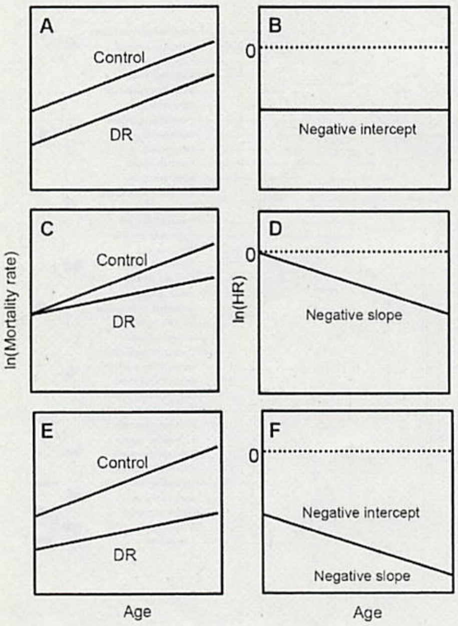
{biological generality}

meta-mean

biological に重要か?

2013-02-07

Fig. S5



Modern MA (1976)

"father of modern MA"

Gene Glass psychotherapy effective?

Social → Medical → Biological

Hunt (1997) journalist

- overcomes statistical power problem
- a bigger picture & biological generality, stat significance
- File drawer problem (non-sig results) → bias correction

prior distribution?

unpublished prob?

effect size.

- phylogenetic relatedness

PMM Lynch 1991 Evol.

$Y_j \sim N(y_j, V_j)$

$y_i = \mu + PHY_{i[j]} + SPP_{i[j]} + STU_j$

$PHY \sim N(0, \sigma^2_{PHY} R_{SPP})$ — distance matrix

MCMCglmm ← multi-trait

author-effect? author-bias?

Dietary Restriction The less they eat, the longer they live

- not caloric restriction, protein restriction / model-animal vs non-model

系統 selection — artifact?

→ No phylogenetic signal?, No sex or model ssp. effects?

public bias OR (model spc bias)

pass way X (negative DR)

longevity — Cal, Protein — ln HR

Protein が重要と考えられている

Example

- Griffin & West (2003, Science) kin selection ← relatedness
- Vitamin C 抗酸化??

- time-lag effect

時間とともに effect が下がる!

ミリカンとか?

MA for Students

3 benefits of being meta-analyst — Stat. training, Reading many papers

MA + narrative

"Mini" meta-analysis

Hybrid review

"Local" meta-analysis —— 説明変数の HBM?

Data rich & theory poor

論文のえらび方

Medical な分野 プロトコル がある

prisma protocol

最初は小さいところから.

species がかぶってるので Ecology は

Fig. S1

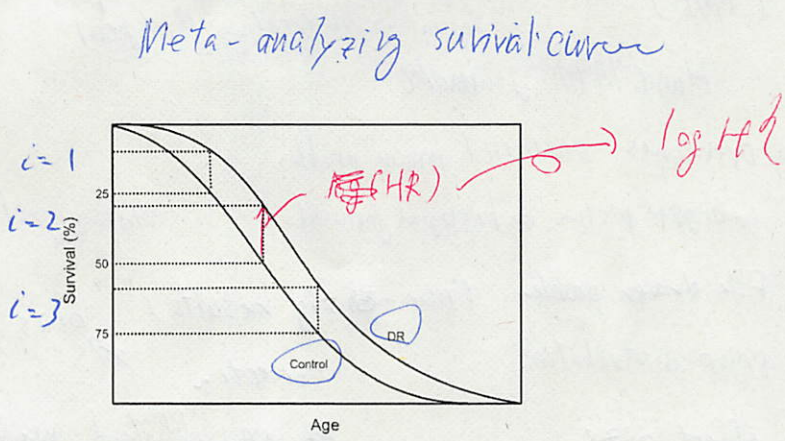


Fig. S2

Fig. S3

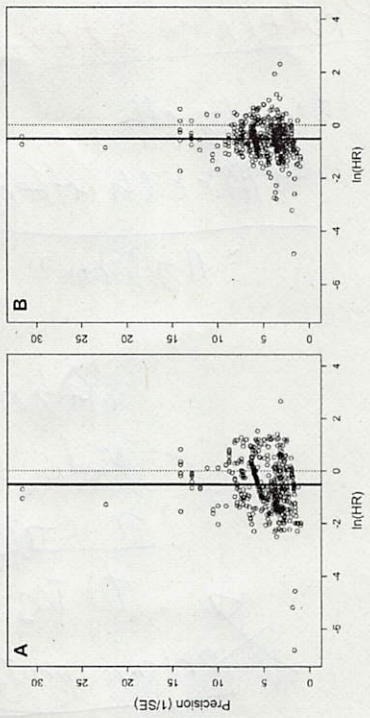
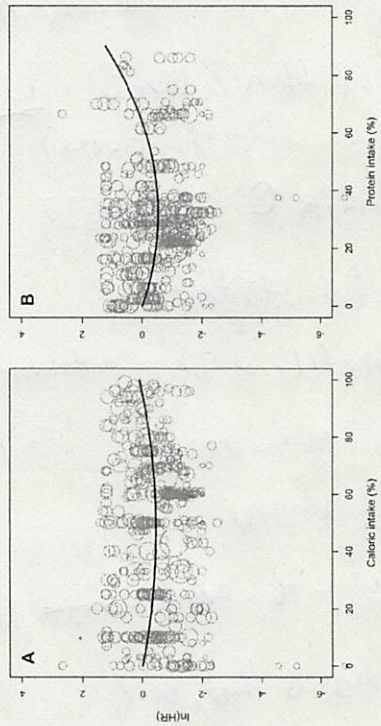


Fig. S4



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Comparative and meta-analytic insights into life extension via dietary restriction

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Summary

Dietary restriction (DR) extends the lifespan of a wide range of species, although the universality of this effect has never been quantitatively examined. Here, we report the first comprehensive comparative meta-analysis of DR across studies and species. Overall, DR significantly increased lifespan, but this effect is modulated by several factors. In general, DR has less effect in extending lifespan in males and also in non-model organisms. Surprisingly, the proportion of protein intake was more important for life extension via DR than the degree of caloric restriction. Furthermore, we show that reduction in both age-dependent and age-independent mortality rates drives life extension by DR among the well-studied laboratory model species (yeast, nematode worms, fruit flies and rodents). Our results suggest that convergent adaptation to laboratory conditions better explains the observed DR–longevity relationship than evolutionary conservation although alternative explanations are possible.

Key words: age-dependent mortality; age-independent mortality; caloric restriction; comparative analysis; evolutionary conservation; evolutionary convergence; meta-analysis; protein restriction.

Introduction

It is generally accepted that dietary restriction (DR, a reduction in food intake without malnutrition) has health benefits such as prolonging lifespan and protection from various diseases (diabetes, cancer and cardiovascular disease; Fontana *et al.*, 2010; Partridge, 2010; Piper *et al.*, 2011). Such benefits have been demonstrated in a wide diversity of species, across several animal phyla (Mair & Dillin, 2008). Much of the evidence concerning DR comes from five laboratory model species (Le Bourg, 2010): yeast (*Sacchromyces cerevisiae*), the nematode (*Caenorhabditis elegans*), the fruit fly (*Drosophila melanogaster*), the mouse (*Mus musculus*) and the rat (*Rattus norvegicus*), in which the effects of DR were first reported in 1935 (McCay *et al.*, 1935; hereafter, we will refer to these five species as the 'model species'). The phylogenetic diversity represented by these five species has underpinned a widely held belief in an evolutionarily conserved mechanism that mediates the relationship between DR and longevity (Le Bourg & Rattan, 2006; Mair & Dillin, 2008; Le Bourg, 2010). A recent report on the effect of DR on lifespan in long-lived rhesus monkeys (*Macaca mulatta*; Colman *et al.*, 2009) reinforces this view, raising the hope that DR has the same beneficial effects for our own species (Fontana *et al.*, 2010; Partridge, 2010). Nevertheless, the beneficial effects of DR do not appear to be universal: studies of houseflies and several species of rotifers (Kirk, 2001; Cooper *et al.*, 2004), for example, failed to detect any life-extending effect of DR. Such studies call into question the possibility of any evolutionarily conserved mechanism.

Evolutionary explanations for DR effects on lifespan invoke a life history trade-off. Among and within species, fecundity negatively correlates with longevity, the so-called cost of reproduction (Williams, 1966). Dietary restriction results in increased longevity, but also in decreased fecundity, at least in laboratory animals (Partridge *et al.*, 2005a). In the wild, many organisms encounter periods of starvation during which they should devote energy to somatic maintenance and repair, prolonging lifespan to survive until a nutritionally richer period when they can afford to reproduce (Kirkwood & Shanley, 2005). Findings on sex differences in the effects of DR support this cost-of-reproduction view. In *Drosophila* and several strains of mice, the life-prolonging effect is much more pronounced in females than in males (Partridge *et al.*, 2005a). Such a sex difference is expected, because males are generally thought to invest less into reproduction.

Studies of single-gene mutations in the model species are now elucidating some of the molecular pathways of DR effects (Bartke, 2011). Mutations that prolong lifespan are usually involved in slowing down nutrient-signalling pathways. For example, down-regulation of the target of rapamycin (TOR) pathway extends lifespan in yeast, *C. elegans*, drosophila and mice (McCormick *et al.*, 2011). Also, reduced activity in the insulin/insulin-like growth factor signalling (IIS) pathway leads to longer lifespan in *C. elegans*, *Drosophila* and mice (Kenyon, 2011). Dietary restriction is thought to influence one or more of these nutrient-signalling pathways, although how exactly DR acts on these pathways is still poorly understood (Fontana *et al.*, 2010; Partridge, 2010). The mutation studies have been viewed as strong evidence against the DR effect arising from convergent adaptation and strong support for its evolutionary conservation (Mair & Dillin, 2008). Nevertheless, the unequivocal demonstration of the life extension by DR in the five model species does not prove the universality or conservation of the DR–longevity relationship (Le Bourg & Rattan, 2006; Le Bourg, 2010). For many generations, these model species have lived under laboratory conditions, which usually provide constant food supply, are free from pathogens, parasites and predators and select for fecundity and appetite but against longevity (Miller *et al.*, 2002; Austad & Kristan, 2003). Thus, some researchers have controversially speculated that the effect of DR could be a laboratory artefact, for example, by alleviating the detrimental consequences of overfeeding (Le Bourg, 2010).

A second debate concerning DR studies is whether restriction of caloric intake *per se* can extend longevity (Masoro, 2006). DR is often referred to as 'caloric restriction' because a reduction in calories was believed to be the key factor prolonging an organism's lifespan. A series of recent studies, however, suggests that the balance between macronutrients (the ratio between proteins and carbohydrate/fat) is more important than caloric restriction (Simpson & Raubenheimer, 2009). In several insect species (crickets and drosophila), fixed-calorie diets containing lower ratios of protein to carbohydrates ('protein restriction') extended longevity (e.g. Mair *et al.*, 2005; Lee *et al.*, 2008; Maklakov *et al.*, 2008; Fanson *et al.*,

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2009). Furthermore, a study on drosophila suggests that adequate ratios of amino acids within protein intake are key for lifespan extension via DR (Grandison *et al.*, 2009), but there is limited experimental evidence as to whether such clear effects of protein restriction apply to mammalian species. Some researchers argue that caloric restriction is crucial for the mammalian life-extending effect of DR (Masoro, 2006), although there is indirect support for the effect of protein restriction in humans (Fontana *et al.*, 2008). It seems that both caloric and protein restriction may play a role in producing life extension by DR.

Here, we attempt to resolve these two debates by conducting a comprehensive and comparative meta-analysis on a wealth of published studies, investigating the relationship between DR and survival. Importantly, the comparative meta-analytic approach enabled us to combine a wide variety of species from a great number of studies and to extract general trend from what appears to be contradictory results while controlling for species-specific and study-specific effects (Hadfield & Nakagawa, 2010). Our main aims are the followings: (i) to determine the universality of DR effects on longevity between sexes and among species, especially focusing on the model and non-model species, and (ii) to quantify the importance of both caloric restriction and protein restriction in the effect of DR on longevity. Additionally, in the model species, we investigate whether DR affects either age-independent or age-dependent mortality rate or both (Partridge *et al.*, 2005b; Phelan & Rose, 2005). An overall objective of this study is to quantitatively synthesize the current state of knowledge on this important topic for the first time, and thus, to present an overview of the empirical evidence.

Results and discussion

Universality of life-extending effect of DR

We located 145 studies investigating the relationship between DR and longevity in 36 species, which matched our selection criteria (see Experimental procedures). We extracted 529 effect sizes from these studies (Data S1); the effect size measure used is the natural logarithms of hazard ratio, ln(HR) (Table S1). In short, a set of three ln(HR) values were extracted from each pair of survival curves (consisting of the control group and the DR group) at three relative time intervals (during which 0–25%, 25–50% and 50–75% of the control group died), and the overall estimates from these three values constituted effect size values as ln(HR) (Fig. S1; Parmar *et al.*, 1998; Williamson *et al.*, 2002). Negative ln(HR) values mean that individuals in DR groups were less likely to die at a given point on average than ones in the control groups.

Overall, DR reduced the risk of death by 60% (Bayesian mixed-effects meta-analysis, BMM; Hadfield, 2010; Hadfield & Nakagawa, 2010; $\beta_{[\text{meta-analytic mean}]} = -0.434$, 95% credible interval (CI) = −0.704 to −0.171; Table S2). This effect remains robust even when phylogenetic non-independence among 36 species was accounted for (Bayesian phylogenetic mixed-effects meta-analysis, BPMM: $\beta_{[\text{meta-analytic mean}]} = -0.515$, CI = −0.953 to −0.093; Figs 1 and S2, Table S1 and Data S2). We observed moderate to high heterogeneity (Higgins & Thompson, 2002; BPMM: $I^2 = 53.73$, CI = 41.15–66.00; Table S2; hereafter, results only from BPMM are presented, see Table S2–S6 for equivalent results from BMM); that is, the life-extending effect of DR is more apparent in certain species and/or studies.

In meta-analysis, significant heterogeneity calls for moderators (e.g. the effect of sex), which may explain such heterogeneity (Higgins & Thompson, 2002). Thus, we tested the controversial suggestion that the life-prolonging effect of DR is only true for the model species, along with a less contentious idea that DR has more influence on females than on males. We found that the life-extending effect of DR was 20% smaller for male organisms than for females and also that DR was nearly twice as effective in prolonging lifespan in the model species than in the non-model species (BPMM: $\beta_{[\text{female/male difference}]} = 0.218$, CI = 0.038–0.411 and $\beta_{[\text{non-model/model difference}]} = -0.666$, CI = −1.121 to −0.222; Fig. 2A,B and Table S3). Although the significant sex effect is more or less expected from previous work (Partridge *et al.*, 2005a), our finding is, to our knowledge, the first quantitative proof for the generality of the sex effect in the DR–longevity relationship. The housefly study, where DR convincingly failed to induce life extension (Cooper *et al.*, 2004), has often been cited as evidence against the universality of the DR–longevity relationship (Le Bourg, 2010). Nevertheless, we point out that all houseflies in this experiment were males, so that the negative result could be predicted from our meta-analytic result, and it is not conclusive evidence against a life-extending effect by DR in this species. In contrast to the sex effect, the significant and clear model species effect we discovered is unexpected and fascinating. This finding supports the idea that the life-extending effect of DR is related to living in peculiar laboratory conditions for many generations (Miller *et al.*, 2002; Austad & Kristan, 2003; more discussion later).

Importantly, the validity of estimates from meta-analysis relies on the assumption that there is negligible publication bias in a particular research topic (Egger *et al.*, 1997). Inspection of funnel plot symmetries of our data revealed no obvious signs of publication bias in our data set (Figs 2C–H and S3; for the results of a regression approach (Egger *et al.*, 1997), consistent with the absence of publication bias, see Table S4 and Dialog S1). Therefore, our estimates are likely to be reliable.

Caloric restriction or protein restriction?

We now build upon the above analyses to investigate the relative importance of caloric and protein restrictions. It is noted that, on the one hand, the variable caloric intake (%) represents the relative percentage of caloric intake for the DR group in relation to the control group where caloric intake was 100%. One the other hand, the variable protein intake (%) is the percentage of total food energy coming from protein in relation to the other macronutrients, namely carbohydrate and fat for both groups (see Dialog S1).

We found significant quadratic effects of both caloric and protein intake on the risk of death, with the life-extending effect of caloric intake peaking around 50% and that of protein intake about 30% (BPMM: $\beta^2_{[\text{caloric intake}]} = 1.785$, CI = 0.664–2.907; $\beta_{[\text{caloric intake}]} = -1.702$, CI = −2.815 to −0.651; $\beta^2_{[\text{protein intake}]} = 5.352$, CI = 3.219–7.358; and $\beta_{[\text{protein intake}]} = -3.088$, CI = −4.440 to −1.389; Figs 3and S4 and Table S5). Our results indicate that the effect of protein intake is larger than that of caloric intake, illustrated in Fig. 3A,B. This result is remarkable because, while most studies included in our data set explicitly changed the caloric intake between control and DR groups, very few studies deliberately manipulated protein intake (Data S1 and Dialog S2). Nonetheless, different studies covered a wide range of protein intake (0% up to approximately 90%). The contours of the DR effect on longevity in Fig. 3C show the importance of the balance between caloric intake and protein intake for DR to be effective.

This bivariate action of DR may explain many equivocal results in the literature, where researchers usually focused on caloric intake rather than protein intake for their interpretation. For example, animals that were food-restricted by 50% would not necessarily show the benefit of DR, if they were also fed a high-protein diet. Our results strongly support a recently proposed protein restriction hypothesis (Simpson & Raubenheimer, 2007,

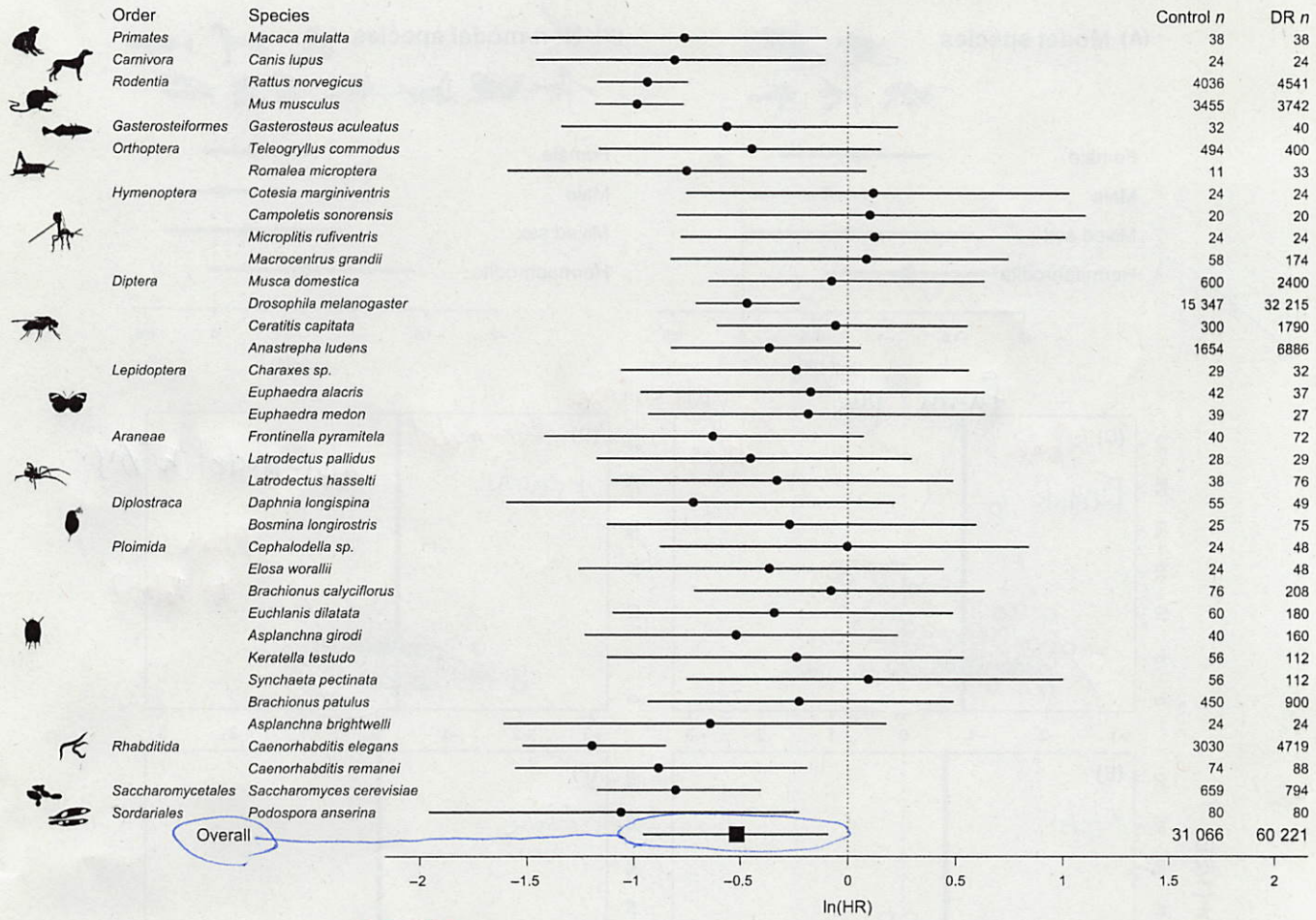

Fig. 1 A forest plot of effect size, logarithm of hazard ratio [ln(HR)], estimates for the relationship between dietary restriction (DR) and survival. An overall meta-analytic posterior mean and posterior means for 36 species (as random factors; see Dialog S1 and Fig. S1) with 95% credible intervals (CIs) are shown (Table S2). Control *n* and DR *n* represent the numbers of independent animals (or colony numbers for *Sacchromyces cerevisiae* and *Podospora anserina*) used (in 145 studies). The overall mean supports the life-extending effect of DR, and also, for the model species, this effect is consistently supported (95% CIs are not touching or crossing zero).

2009) with an implication that this phenomenon may be general across the animal kingdom.

Notably, when we used alternative values based on actual food consumption as a measurement of caloric restriction, where available (32% of all the data points; Model 14 in Table S6), the life-extending effect of caloric intake disappeared but that of protein intake remained virtually unchanged (BPMM: $\beta^2_{[\text{caloric intake}]} = -0.087$, CI = −0.441 to 0.227; $\beta_{[\text{caloric intake}]} = -0.059$, CI = −0.580 to 0.431; $\beta^2_{[\text{protein intake}]} = 5.119$, CI = 2.937–7.264; and $\beta_{[\text{protein intake}]} = -2.976$, CI = −4.467 to −1.386; Table S6). Although this particular result is difficult to explain and reconcile, caloric restriction may not be the main determinant for the life-extending effect of DR, as indicated in a series of insect studies (e.g. Mair *et al.*, 2005; Lee *et al.*, 2008; Maklakov *et al.*, 2008; Fanson *et al.*, 2009).

How are aging trajectories changed?

We next examined the five model species in which the effect of DR was apparent to reveal how DR exerted life-extending effects. There are two ways that mortality rates can be reduced: (i) they are reduced by a constant fraction across the lifespan of an organism (i.e. a change in the age-independent mortality rate, also known as initial mortality rate) and (ii) the rate at which mortality rates increase across the lifespan is reduced (i.e. a change in age-dependent mortality rate; Partridge *et al.*, 2005b; Phelan & Rose, 2005). In our data set, the two mechanisms of life extension can be identified and distinguished by meta-analytically estimating intercepts and slopes of ln(HR) values over the three relative time intervals (0–25%, 25–50% and 50–75% of the control group being dead, as described in Dialog S1 and Fig. S5). Our data set for the model species included 290 estimates for both intercepts and slopes from 105 studies (Data S3). Statistically speaking, we should observe a negative intercept with a zero slope if only age-independent mortality change is at work, whereas a significantly negative slope with a zero intercept if only age-dependent mortality is occurring. If both types of mortality change occur, a negative intercept and negative slope should be observed.

Overall, both age-dependent and age-independent mechanisms contributed to the DR life-extending effects (BMM: $\beta_{[\text{intercept}]} = -0.516$, CI = −0.686 to −0.354; $\beta_{[\text{slope}]} = -0.181$, CI = −0.252 to −0.120; Fig. 4, Table S7). Previous studies have claimed that in drosophila, DR reduces the age-independent mortality rate but not the age-dependent mortality rate, whereas for rodents, much of the life extension by DR stems from decreasing the age-dependent mortality rate (Partridge *et al.*, 2005b; Phelan & Rose, 2005). However, our meta-analytic results show that the

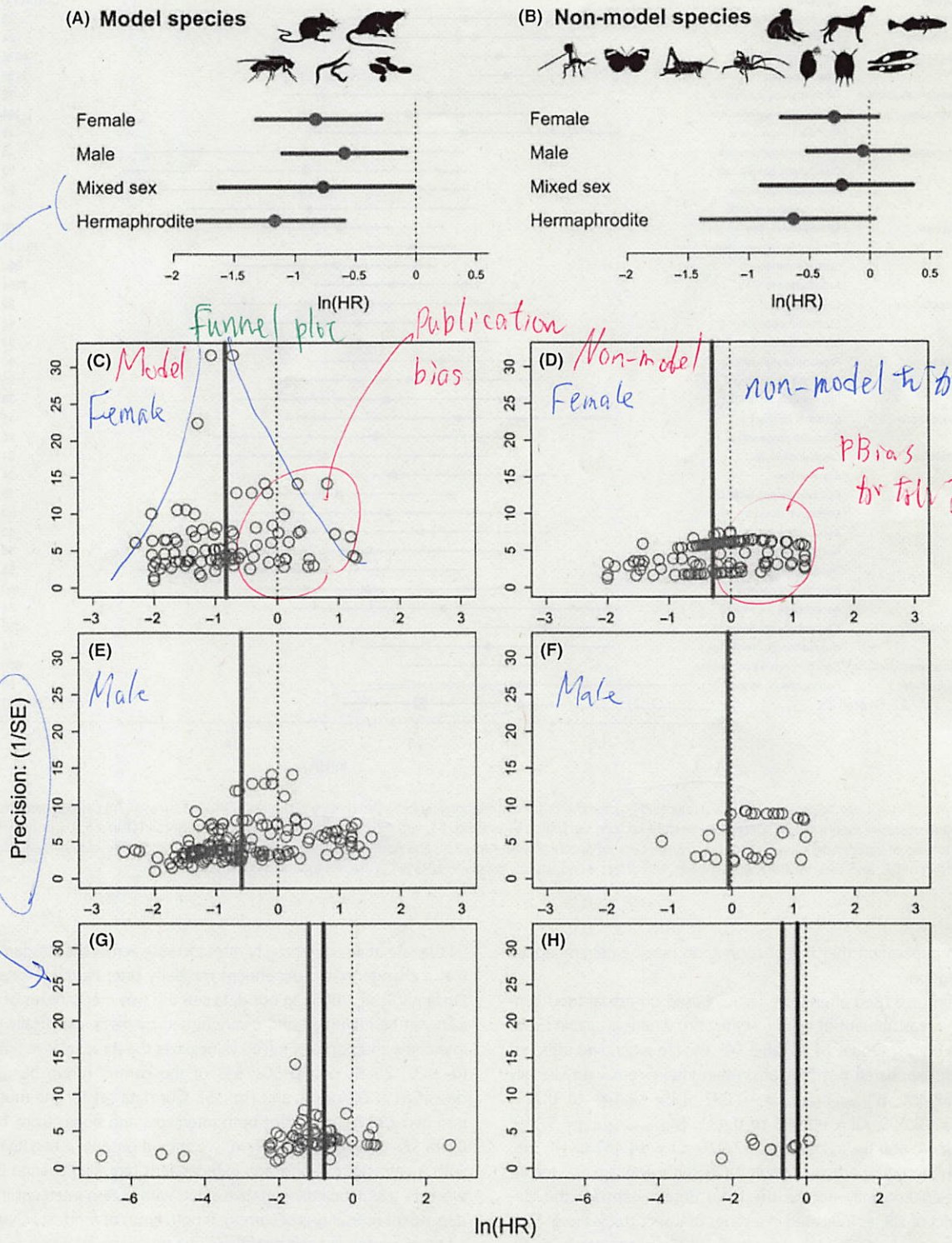

Fig. 2 The effects of sex and species status on the relationship between dietary restriction (DR) and survival. (A, B) The model species consistently support the relationship between DR and longevity [i.e. negative ln(HR)] in all types of sex categories (red: females, blue: males, purple: mixed sexes or males and females, and green: hermaphroditic organisms), whereas the non-model species consistently fail to support this relationship. Also, males responded less to DR than females. Data shown are posterior means and 95% credible intervals (Table S3). (C to H) Funnel plots with posterior means (solid lines) with different sex types and species statuses (model species: C, E and G and non-model species: D, F and H). No obvious signs of publication bias can be seen.

DR effects generally result from the dual actions of age-dependent and age-independent mortality changes. How DR brings about these two effects simultaneously will be an important future question.

Peculiarly, yeast seemed to be the only species where we did not find convincing evidence for age-dependent mortality change (BMM; $\beta_{[intercept]} = -0.695$, CI = −1.263 to −0.144; $\beta_{[slope]} = -0.006$,

Dialog S1

Collecting studies on dietary restriction (DR)

Of over 2000 studies this search yielded, papers were selected which contained a graphical survival curve, or in the case of some older studies, gave the complete dataset from which a survival curve could be constructed using *MS Excel* and *R* (R Development Core Team 2011). Papers were also excluded from analysis based on a number of methodological criteria:

1. Studies must be the original empirical data using real animals, not reviews or computer simulations,
2. Animals must not be transgenic or mutant (e.g., Ames dwarf mice, *indy*-mutant drosophila, *eat*-mutant *Caenorhabditis elegans*),
3. The degree of calorie restriction must be made explicit (e.g., not a study of different oils in food without information on quantities of the oils and food),
4. Animals were not fasted for a long period (with exception of adult *C. elegans*), although every other day feeding and a special case of weekly feeding in spiders was allowed,
5. Information on control group fed either *ad libitum* or 100% of the food allowance must be included,
6. Dietary (caloric) restriction must be constant throughout life after initiation of the diet and the animals must die naturally. Those studies with intermittent periods of restriction were excluded, as were those that sacrificed animals and only provided biomarker data,
7. Animals were not treated with any other confounding factors, such as the resveratrol drug, irradiation or injection with pathogenes.

Also, comparisons from survival curves between the control and treatment groups were only obtained between groups of the same sex and strain. These criteria limited the number of studies to 145, comprised of 36 species, with 529 comparisons between treatment groups and their controls. It is important to note that all of these studies restricted calories to certain degrees (Fig. 3A and Fig. S4A). *EndNote X3* was used to manage the papers and a *File Maker Pro* database to collect the records.

Extracting effect size (survival data)

Data on survival was collected from the studies by determining the time when 25% (LT25), 50% (LT50), and 75% (LT75) of the controls were dead and the percentage of the individuals from the relevant treatment groups that were dead at these times (Fig. S1 and Data S1). *Free Ruler* (http://www.pascal.com) was used on PDF files, while for studies available only in hardcopy survival curves were measured by hand.

We calculated the natural logarithm of hazard ratio, ln(HR) (Table S1) and its standard error for each pair of a treatment group and its control, using the equations below ():

$$\ln(\mathrm{HR})_i = \frac{d_{E,i} - e_i}{w_i}, \quad (1)$$

$$\mathrm{var}(\ln(\mathrm{HR})_i) = \frac{1}{w_i}, \quad (2)$$

$$e_i = (d_{E,i} + d_{C,i})\frac{n_{E,i}}{n_{E,i} + n_{C,i}}, \quad (3)$$

$$w_i = (d_{E,i} + d_{C,i})\frac{n_{E,i} n_{C,i}}{(n_{E,i} + n_{C,i})^2}, \quad (4)$$

where $n_{E,i}$ and $n_{C,i}$ are the number at risk during the interval $[t_{i-1}, t_i)$ for the experimental (DR) group and the control group, respectively, $d_{E,i}$ and $d_{C,i}$ are the number of events (deaths) in the interval $[t_{i-1}, t_i)$ for the experimental (DR) group and the control group, respectively, and time points are denoted by i ($i = 1,\ldots, p$; in our

case, $p = 3$ at LT25, LT50 and LT75).

The above calculations were performed over three time intervals: LT0-LT25, LT25-LT50, LT50-LT75. The summary statistic, ln(HR), and its variance over these three intervals for each pair of survival curves (denoted as *j*, below) are ():

$$\ln(\mathrm{HR})_j = \frac{\sum_{i=1}^{3} w_i \ln(\mathrm{HR})_i}{\sum_{i=1}^{3} w_i}, \quad (5)$$

$$\mathrm{var}(\ln(\mathrm{HR})_j) = \frac{1}{\sum_{i=1}^{3} w_i}. \quad (6)$$

The main reason for choosing these three points (LT25, LT50 and LT75) was to make most of published data, specifically: (i) more detailed quantifications (e.g., every 10%) were difficult for many papers due to the quality of survival curve figures. (although such quantifications could lead to more accurate estimates of ln(HR); Williamson *et al.* 2002), (ii) by using the time of death for control animals, it was possible to compare ln(HR) across species with different lifespans, and (iii) a large proportion of papers lacked data on survival after 75% of control were dead. We note that 66 out of 529 pairs of survival curves lacked the interval LT50-LT75. In such cases, the overall ln(HR) was calculated from the two intervals (LT0-LT25 and LT25-LT50); we used 529 effect sizes for analysis (apart from analysis on age trajectories; see below). We repeated our main analyses, using a subset of the data, which included three intervals ($N = 463$), and found our qualitative conclusions remain the same (the results are not shown). It is also noted that this unique way of extracting ln(HR) values from pairs of survival curves opens up opportunities for many meta-analytic studies within and across species in the gerontology literature (see Table 1).

Extracting moderators (variables associated with DR)

Experimental designs and treatment factors were extracted from the method sections of each paper and recorded as follows (note that abbreviations in parentheses represent how we recorded categories within variables in our dataset; see Dialog S2):

- Strain name/type (e.g., WT = wild type).
- Sex categories (M = males, F = females, N = none/hermaphrodite or MF = males and females).
- Reproductive status (0 = virgin, 1 = reproducing).
- Food schedule (D = daily, EOD = every other day or W = weekly).
- Type of dietary restriction (FW= food weight, where exactly the same food was given in smaller quantities without the above consideration with or without microelement supplementation; BW = body weight, where periodic adjustments were made in order to keep the treatment group at a prescribed percentage body weight less than that of the controls (either by averages or pair-feeding), it is very similar to food weight; FC = food concentration, where treatments were offered a food medium of a certain dilution of the control amount; FD = feeding day, where both groups were fed *ad libitum* but the treatment groups were only allowed access to the food on certain days; NM = nutrient content manipulation; CNM = caloric and nutrient manipulation).
- Food intake measured and reported or not (0 = no, 1 = yes).
- Feeding regime of the control (0 = 100%, 1 = *ad libitum*).
- Caloric values of the control group diet and that of the treatment group diet