Sex and Death: What Is the Connection?

Review

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A cost of reproduction, where lifespan and fecundity are negatively correlated, is of widespread occurrence. Mutations in insulin/IGF signaling (IIS) pathways and dietary restriction (DR) can extend lifespan in model organisms but do not always reduce fecundity, suggesting that the link between lifespan and fecundity is not inevitable. Understanding the molecular basis of the cost of reproduction will be informed by elucidation of the mechanisms by which DR and IIS affect these two traits.

Introduction

A hypothetical organism, the Darwinian Demon (Law, 1979), starts reproduction at birth and continues to produce copious numbers of offspring throughout its long lifespan. Natural selection favors this simultaneous maximization of fecundity and longevity because it maximizes the genetic contribution to the next generation. However, in the real world these two traits often show an inverse relationship with each other. Among both mammals and birds, long lifespans are associated with low fecundity (Holmes et al., 2001; Read and Harvey, 1989). Both experimental manipulations and natural genetic variation often have opposite effects on fecundity and lifespan (e.g., Reznick et al., 2000). The frequent occurrence of this "cost of reproduction" (Williams, 1966) suggests that its mechanisms may be conserved during evolution. However, its basis in molecular physiology remains unresolved.

One view is that the cost of reproduction is driven by conflicting requirements for resources (de Jong, 1993; Kirkwood and Holliday, 1979; van Noordwijk and de Jong, 1986; Williams, 1966). Costly processes such as reproduction, somatic maintenance and repair, growth, and movement compete for resources and it is impossible to maximize allocation to them all, so trade-offs must be made. Resources that are diverted into reproduction cannot also be used for somatic repair and maintenance, and reproduction will hence shorten lifespan. An alternative view is that reproduction itself, or

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the processes enabling it, directly inflict somatic damage (Barnes and Partridge, 2003). Under either hypothesis, there would be a causal, inverse relationship between reproductive rate and lifespan (Figure 1).

Natural genetic variation between species and individuals often involves many genes. These quantitative genetic effects impede the identification and manipulation of the genes responsible for differences in lifespan and fecundity. A powerful alternative approach has come from analysis of single-gene mutations and experimental interventions in model organisms, particularly the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and laboratory rodents, the mouse *Mus musculus* and the rat *Rattus norwegicus*.

Mutations in single genes can extend lifespan in model organisms, and mutations in orthologous genes can do so in different organisms (Kenyon, 2001; Partridge and Gems, 2002; Tatar et al., 2003). For instance, mutations in genes encoding components of the insulin/IGF-like signaling (IIS) pathway, originally shown to extend lifespan in *C. elegans* (Friedman and Johnson, 1988; Kenyon et al., 1993; Klass, 1983), have been shown also extend lifespan in the fruit fly *Drosophila* (Clancy et al., 2001; Tatar et al., 2001) and the mouse (Bluher et al., 2003; Holzenberger et al., 2003). In addition, a number of mutations that disrupt the growth hormone axis and lead to alterations in IIS pathway function also extend lifespan in mice (Brown-Borg et al., 1996; Coschigano et al., 2000; Flurkey et al., 2001, 2002; Liang et al., 2003).

An environmental intervention, dietary restriction (DR), also extends lifespan in many organisms, including yeast *Saccharomyces cerevisiae* (Jiang et al., 2000; Lin et al., 2000), *C. elegans* (Houthoofd et al., 2003; Johnson et al., 1990; Klass, 1977; Lakowski and Hekimi, 1998), *Drosophila* (Chapman and Partridge, 1996; Chippindale et al., 1993; Partridge et al., 1987), rodents (Weindruch and Walford, 1988), and possibly primates (Lane et al., 2004; Mattison et al., 2003). DR is also associated with health benefits in humans (Fontana et al., 2004). Excessive reduction in food intake can also shorten lifespan through starvation, and we here consider only lifespan-extension by reduced nutrition. The mechanisms by which DR increases longevity are not fully identified in any organism. The widespread extension of lifespan by reduced nutrient intake is suggestive of evolutionary conservation of mechanisms but could also result from convergent evolution.

As well as extending lifespan, reduced IIS and DR can both impair, delay, or abolish fecundity in *Drosophila*, *C. elegans*, and the rat and mouse (Bruning et al., 2000; Burks et al., 2000; Chandrashekar and Bartke, 2003; Chapman and Partridge, 1996; Chippindale et al., 1993; Clancy et al., 2001; Gems et al., 1998; Giannakou et al., 2004; Holehan and Merry, 1985b; Kenyon et al., 1993; Klass, 1977; Partridge et al., 1987; Tatar et al., 2001; Tissenbaum and Ruvkun, 1998; Weindruch and Walford, 1988). Reduced fecundity in response to DR has been suggested to be an evolved mechanism for maximizing long-term reproductive success during per-

A   Re-allocation of resources under reduced IIS or DR

Normal   **Reduced IIS or DR**

Resources ⟶ Reproduction   Resources ⇢ Reproduction

Repair and ⇢ Lifespan   Repair and ⟶ Lifespan
maintenance   maintenance

B   Reproduction inflicts damage

Normal   **Reduced IIS or DR**

Resources ⟶ Reproduction   Resources ⇢ Reproduction

                   ⊣   ⊣

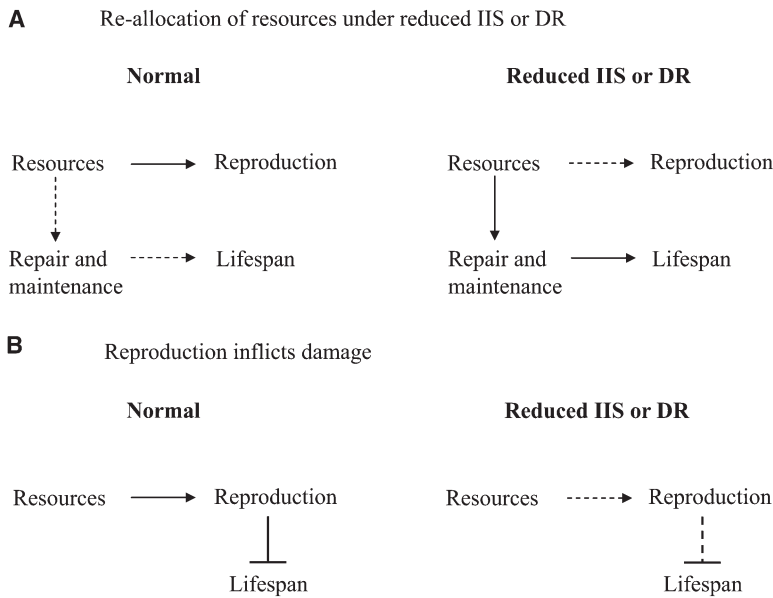   Lifespan   Lifespan

Figure 1. Re-allocation of Resources and Generation of Damage by Reproduction Could Generate a Trade-off between Fecundity and Lifespan. These two processes are not mutually exclusive.

(A) Reduced IIS and DR cause re-allocation of resources from reproduction to repair and maintenance and hence increase lifespan.

(B) Reduced IIS and DR cause resources to be withdrawn from reproduction, which lowers generation of damage and hence increases lifespan.

iods of food shortage. When prospects for reproduction are poor and offspring survival will be low, long-term reproductive success may be best ensured by reducing reproductive effort and hence increasing survival until return of the food supply (reviewed in Shanley and Kirkwood, 2000), implying that reduction in fecundity is necessary for extension of lifespan by DR.

DR and reduced IIS are potential candidates for discovering the physiological basis of the cost of reproduction. Increased lifespan could occur by readjustment of a trade-off with reproduction, by either of the mechanisms illustrated in Figure 1. If this idea is correct, there should be an incompatibility between high fecundity and long life, and an increase in one should therefore invariably be accompanied by a decrease in the other. We review the evidence for this idea by considering the effects of IIS and DR on lifespan and fecundity in *C. elegans*, *Drosophila*, the mouse, and the rat, and we discuss the relationship between the effects of IIS and DR and the negative correlation between lifespan and fecundity that is seen during evolution.

The Effects of Insulin/IGF-like Signaling and Dietary Restriction on Lifespan and Fecundity in *C. elegans*, *Drosophila*, and Rodents
C. elegans
Insulin/IGF-like Signaling. Mutations in genes encoding components of the IIS pathway (see Figure 2A) can extend lifespan and delay or reduce fecundity (e.g., Friedman and Johnson, 1988; Hertweck et al., 2004; Kenyon et al., 1993; Kimura et al., 1997). Several approaches have been used to determine if the fecundity defects seen in IIS mutant *C. elegans* are causal in the extension of lifespan. All IIS mutations that extend adult lifespan are temperature sensitive. At the restrictive temperature they cause the developing worm to arrest as a dauer larva, and they therefore postpone reproduction. At the permissive temperature the mutants bypass the dauer stage and become long-lived adults, in some

cases with a developmental delay that in turn delays in the onset of reproduction. The longevity of the original long-lived mutant in *age-1(hx546)*, encoding the catalytic subunit of the PI3 kinase, was at first attributed to reduced fecundity (Friedman and Johnson, 1988) but was subsequently attributed to a mutation in another gene, and the *age-1* mutant itself has normal fecundity (Johnson et al., 1993). Moreover, some mutants in *daf-2*, encoding the IIS receptor, have also been reported to extend lifespan without a reduction in fecundity (Gems et al., 1998; Kenyon et al., 1993), suggesting that lifespan and fecundity are not locked in an obligate trade-off.

The strongest test of the relationship between fecundity and lifespan in *C. elegans* in IIS mutants has involved reductions of IIS at different times during development and adulthood (Dillin et al., 2002). Using double-stranded RNA interference (RNAi), it was demonstrated that lowered activity of *daf-2* in the adult worm is sufficient to produce the full extension of adult lifespan and that reduction of expression in the preadult period is without effect on this trait. In contrast, the effects of IIS on fecundity were apparently exerted entirely in the pre-adult period. The timing of the effects of IIS on fecundity and lifespan are therefore different, and the fact that IIS applied to the adult can extend lifespan with no loss of fecundity challenges the idea that there is a trade-off between the two traits (Dillin et al., 2002). It remains unclear whether the effects of disrupted IIS (mutation or RNAi) on fertility in *C. elegans* reflect a role of IIS in the control of fertility in wild-type animals, or whether it is merely a consequence of disrupted development. Recently, Jenkins et al. (2004) reanalyzed the data in Dillin et al. (2002) and found a slight reduction in early fertility in animals treated at the onset of adulthood. However, the Dillin et al. study showed clearly that *daf-2* RNAi later in adulthood increased lifespan without reducing fecundity.

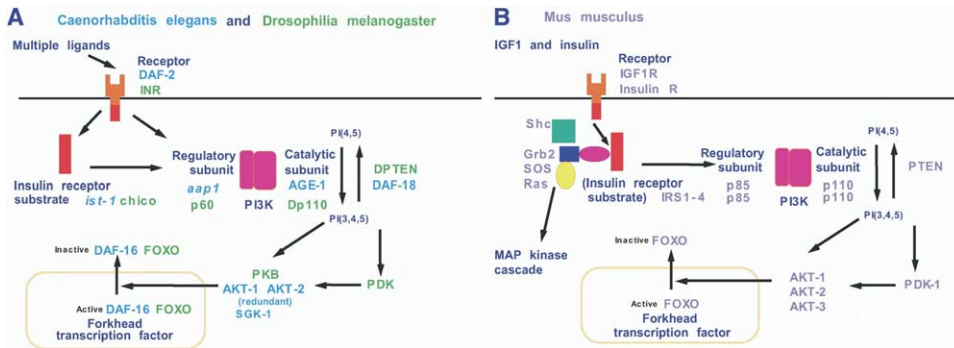Direct manipulation of reproduction in *C. elegans* has

Figure 2. Simplified Outline of Insulin/IGF1 Pathways

(A) *C. elegans* and *Drosophila*.
(B) The mouse.

been informative about the role of reduced fecundity in extension of lifespan by reduced IIS. Laser ablation of the two germline precursor cells of the gonad in hatchling worms abolishes reproduction and extends adult lifespan (Hsin and Kenyon, 1999), as do mutations that reduce germline proliferation (Arantes-Oliveira et al., 2002). That germline removal extends lifespan suggests that the presence of the germ cells may be costly. However, ablation of the whole gonad, which contains both somatic and germline cells, has no effect on lifespan, even though this manipulation also removes the germ cells and prevent any reproduction. Thus, rather than exerting a cost, the germline seems to produce a signal that shortens lifespan while the somatic gonad produces an opposing signal of equal strength that lengthens it (Hsin and Kenyon, 1999). The extension of lifespan by ablation of the germline depends upon the forkhead transcription factor *daf-16*, an essential effector of IIS, suggesting that the germline inhibits *daf-16* activity. Another gene, *daf-12*, which encodes an orphan nuclear hormone receptor (Antebi et al., 2000) is also necessary for the extension of lifespan by germline ablation (Hsin and Kenyon, 1999). These findings suggest that germline removal extends lifespan through a signaling event, rather than a reduced cost of reproduction.

In conclusion, three findings imply that readjustment of a trade-off between fecundity and lifespan does not underlie the extension of lifespan by reduced IIS in *C. elegans*: (1) Some long-lived IIS mutants have normal fecundity. (2) Reduced IIS in the adult can extend lifespan without reducing fecundity. (3) Whole gonad ablation does not extend lifespan while germline ablation does.

Dietary Restriction. Data on the effects of DR on fecundity and lifespan in *C. elegans* are limited (see Walker et al., 2005 for a review). DR has been applied in three ways. Dilution of the *E. coli* bacteria in the food medium resulted in an increase in lifespan to a peak at intermediate bacterial concentration (Johnson et al., 1990; Klass, 1977). Over the range of bacterial dilution where lifespan increased, fecundity of the worms declined (Klass, 1977) (Figure 3). *eat* mutations, which interfere with the pumping of the pharynx of the worm

during feeding, also extend lifespan, to an extent that correlates with the reduction in feeding rate, but fecundity data for *eat* mutants were not reported (Lakowski
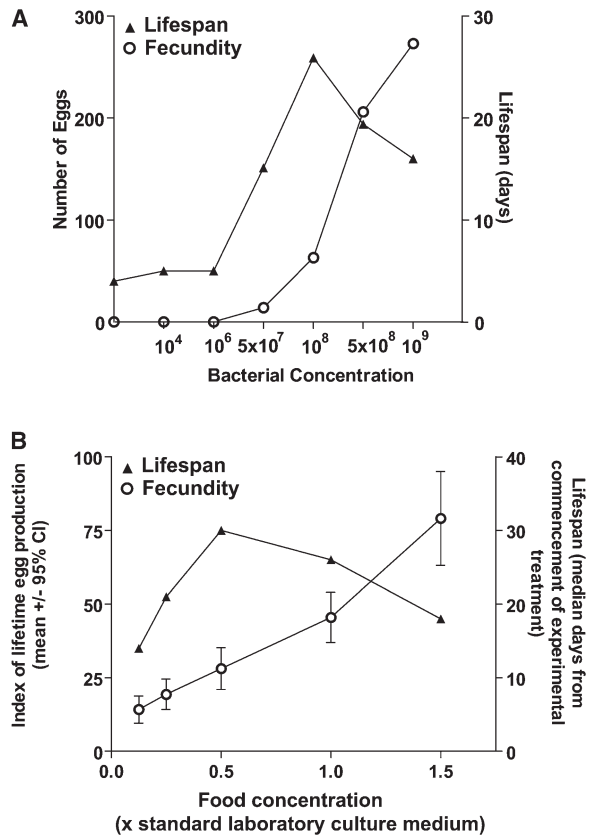

Figure 3. Effects of Food Dilution on Lifespan and Fecundity in *C. elegans* and *Drosophila*

(A) *C. elegans*. Drawn from data in Klass (1977). Note that data derived from bacterial levels of greater than 10^9 have been omitted.
(B) *Drosophila*, re-drawn from data in Chapman and Partridge (1996).
Dietary restriction occurs to the right of the peak in lifespan, starvation to the left.

and Hekimi, 1998). Culture in axenic medium also greatly increases the lifespan of the worms and dramatically decreases their fecundity (Vanfleteren and Braeckman, 1999). However, these three interventions do not have identical effects on stress resistance and metabolism (Houthoofd et al., 2002a, 2002b), and they may therefore operate through somewhat different mechanisms. These systems for DR in *C. elegans* require further definition, but the results to date do not pose any inconsistencies with the idea that DR readjusts a trade-off between fecundity and lifespan.

Drosophila

Insulin/IGF-like Signaling. Mutation of the genes encoding the single IIS receptor (Tatar et al., 2001) (see Figure 2A) and the insulin receptor substrate (Clancy et al., 2001), ablation of neurosecretory cells producing insulin-like ligands (Broughton et al., 2005), and overexpression of the forkhead transcription factor dFOXO in the fat body (Giannakou et al., 2004) and the head fat body (Hwangbo et al., 2004) all increase lifespan by up to 85%. Reduced IIS can also abolish or reduce female fecundity. Females transheterozygous for two different mutations in the IIS receptor (Tatar et al., 2001) or null for the receptor substrate (Bohni et al., 1999; Drummond-Barbosa and Spradling, 2001) are sterile. Females with ablation of insulin-producing cells (Broughton et al., 2005) or overexpressing dFOXO in the fat body show reduced fecundity (Giannakou et al., 2004). However, long-lived females overexpressing dFOXO in head fat body showed normal fecundity (Hwangbo et al., 2004). Thus, not all perturbations to IIS that extend lifespan also reduce fecundity, paralleling the situation in *C. elegans*.

Overexpression of dFOXO confined to the adult fly both extends lifespan (Giannakou et al., 2004; Hwangbo et al., 2004) and can reduce female fecundity (Giannakou et al., 2004). IIS therefore operates during adulthood to modulate both lifespan and fecundity, unlike the situation in *C. elegans*. The effects of genetic manipulation of IIS in the pre-adult period have not been reported. Investigation of the role of sterility in the extended lifespan of females homozygous for a null mutation in *chico*, encoding the insulin receptor substrate, produced negative results. When *chico* was combined with *ovo^{D1}*, which blocks oogenesis at stage 4 and can itself produce a small extension of lifespan, *ovo^{D1}* females, despite their sterility, did not live as long as either fertile *chico^1* heterozyotes or sterile *chico^1* homozygotes (Clancy et al., 2001). If *chico^1* extends lifespan by inducing sterility, then it either acts before stage 4 in oogenesis or in parallel pathways affecting fecundity.

Mutations in the IIS receptor and in *chico* induce female sterility mainly through a failure of vitellogenesis (Tatar et al., 2001; Drummond-Barbosa and Spradling, 2001). Female sterility of one mutant in the IIS receptor was associated with reduced production of juvenile hormone by the *corpora allata*. Vitellogenesis was restored by application of the juvenile hormone mimetic methoprene (Tatar et al., 2001). However, females sterile due to a different mutation in the IIS receptor or to a mutation of *chico* showed minimal or no restoration of vitellogenesis with methoprene (Tatar et al., 2001; Richard et al., 2005). Sterility due to *chico* mutation is not attributable to the systemic environment in mutant

females. Wild-type ovaries implanted into *chico* females underwent normal vitellogenesis, although the ovaries of the host failed to mature. *chico* ovaries implanted into wild-type females failed to undergo vitellogenesis, while the host ovaries did so normally. The sterility of *chico* females therefore cannot be attributed to systemic factors, including those emanating from the ovary (Richard et al., 2005). *chico* females did not show lowered production of juvenile hormone from their *corpora allata*, ruling this out as an explanation of either their sterility or their increased lifespan (Richard et al., 2005). Reduced signaling by juvenile hormone therefore does not play a consistent role in female sterility due to reduced IIS.

The sterility of mutant IIS females is similar to the effect of deficiency in signaling by the steroid hormone 20-hydroxy-ecdysone. Reduced production of ecdysone by the ovary has been reported in females mutant for the IIS receptor (Tu et al., 2002), and mutations in the ecdysone signaling pathway can extend lifespan, albeit without a reduction in fecundity (Simon et al., 2003). In the mosquito *Aedes aegypti*, insulin induces secretion of ecdysone from the ovaries (Graf et al., 1997). Ecdysone signaling could therefore mediate the effects of IIS on fecundity and/or lifespan. However, no lowering of ecdysone synthesis was found in sterile *chico* females, suggesting that this does not play a consistent role in the reduced fecundity of IIS mutant *Drosophila* (Richard et al., 2005).

The main finding to challenge the idea of a readjustment of a trade-off between fecundity and lifespan by IIS is that, as in *C. elegans*, some IIS mutations that extend lifespan do not also lower fecundity.

Dietary Restriction. DR in *Drosophila* (see Partridge et al., 2005, for a review) can be applied by reducing live yeast in the food or by coordinate dilution of all components. By either method, lifespan is extended and female fecundity is reduced (Chapman and Partridge, 1996; Chippindale et al., 1993; Partridge et al., 1987). Food restriction during the pre-adult, growth period does not extend adult lifespan (e.g., Tu and Tatar, 2003). When the food medium is diluted, lifespan peaks at intermediate food concentrations, while female fecundity increases with food concentration across this range, as in *C. elegans* (Figure 3). Daily and lifetime fecundity both increase with food concentration, suggesting that, in both organisms, DR does not merely rescue the effects of toxic overfeeding. The daily fecundity and the length of the remaining reproductive period are increased relative to controls in females returned to full feeding after DR (Partridge et al., 1987). Sterility induced by X irradiation of late pupae or by the mutation *ovo^{D1}* did not reduce the response of lifespan to DR (Mair et al., 2004). Therefore, if DR extends lifespan by readjusting a trade-off with fecundity, then the costly aspects of reproduction either occur earlier than stage 4 in oogenesis or involve parallel pathways.

The responses of both fecundity and mortality rate to DR are acute. When DR females are re-fed or DR is imposed, the flies within hours change their rate of oogenesis and egg-laying (Chapman et al., 1994; Drummond-Barbosa and Spradling, 2001; Partridge et al., 1987). Similarly, the death rate of flies switched from

DR to control feeding or vice versa rapidly (within 48 hr) switches to levels characteristic of flies kept throughout adulthood in the new feeding regime (Mair et al., 2003). The temporal responses of survival and fecundity to changes in nutrition are therefore similar, suggesting that they may share common control mechanisms.

In yeast, Sir2, which encodes a protein deacetylase, was suggested to mediate increase in replicative lifespan by reduced glucose concentration in the food medium (DR) (Lin et al., 2000). However, subsequent studies showed that Sir2-null strains of yeast can respond normally to DR (Jiang et al., 2002; Kaeberlein et al., 2004). Overexpression of the Sir2 ortholog *dSir2* in *Drosophila* extends lifespan, which is not further extended by DR (Rogina and Helfand, 2004). *Drosophila* null for *dSir2* do not extend lifespan in response to DR (Rogina and Helfand, 2004). Two drugs that activate Sir2-like proteins (STACs, SirTuin activating compounds) extend lifespan and increase female fecundity (Wood et al. 2004), but not in flies that are null for *dSir2*, and no further increase in lifespan was seen in DR flies fed STACs (Wood et al., 2004). These findings suggest that *dSir2* may mediate the response of lifespan to DR in *Drosophila*. However, extension of lifespan by STACs was associated with decreased mortality before day 40 (Wood et al., 2004), whereas DR decreases mortality throughout life (Mair et al., 2003), pointing to nonidentical mechanisms. The finding that STACs, which may mimic aspects of DR, can increase both lifespan and fecundity challenges the idea that DR adjusts a trade-off between fecundity and lifespan.

Mutations in *Indy* have been shown, when heterozygous, to extend lifespan in female and male *Drosophila* (Rogina et al., 2000). *Indy* encodes a protein with sequence homology to mammalian sodium dicarboxylate cotransporters, which import Krebs cycle intermediates into cells. *Indy* is expressed in the midgut and the fat body, the fly functional equivalent of mammalian liver and white adipose tissue. *Indy* mutations may create a metabolic state similar to that found in DR (Rogina et al., 2000), although this remains to be directly demonstrated. *Indy* mutations extend lifespan by lowering the rate at which mortality increases with age (Marden et al., 2003), while DR produces a coordinate lowering of mortality rates at all ages (Mair et al., 2003), suggesting that mechanisms are not identical. On standard food, *Indy* flies were more fecund than controls, challenging the idea that lifespan was extended by re-adjustment of a trade-off with fecundity. However, *Indy* flies show lower fecundity than controls on a low-concentration diet (Marden et al., 2003), suggesting the possibility of a conditional trade-off.

The findings that mutation of *Indy* and feeding of flies with STACs can increase both fecundity and lifespan are the main challenges to the idea that DR extends lifespan in *Drosophila* by re-adjustment of a trade-off with fecundity. The data from DR itself are consistent with this idea.

Rodents

Comparison of the longevity of virgin and mated male and female rats has not, on the whole, revealed differences in lifespan and pathology, if late obstetric causes of mortality and the side effects of intensive breeding regimes are avoided (Takeshita et al., 1996), suggesting

that reproduction per se does not shorten lifespan in rats. However, the observation that DR extends lifespan and alters reproductive function and the recent development of genetically manipulated mice that recapitulate IIS mutations in *C. elegans* and *Drosophila* has energized this field of study.

Insulin/IGF-like Signaling. In mice, mutations that modulate IIS pathways (see Figure 2B) increase lifespan and impair reproductive function (Table 1). Ames and Snell dwarf mice have similar underlying genetic abnormalities (Barger et al., 2003; Liang et al., 2003) with defective development of anterior pituitary cells that produce growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH). *Little* mice harbor a mutation in the GH-releasing hormone receptor and have reduced GH and PRL levels (Eicher and Beamer, 1980). All three strains display reduced IGF1 and insulin and increased mean and maximum lifespan (Brown-Borg et al., 1996; Flurkey et al., 2002; Flurkey et al., 2001). Female Ames and Snell mice display infertility, which is ameliorated by prolactin treatment. Male mice, when maintained on a pure inbred background, are infertile but on a heterogeneous genetic background are fertile (Bartke and Brown-Borg, 2004). Female *little* mice have delayed sexual maturation (Flurkey et al., 2001). These findings suggest a link between reduced reproductive function and increased lifespan through modulation of IIS, but confounding endocrinopathies complicate interpretation. Data from Vergara et al. (2004) suggest that, at least in male Snell mice, restoration of fertility by treatment with thyroxine and GH does not abolish the increased lifespan.

Gene targeting in mice has revealed a role of several IIS components in reproductive function and lifespan. Mice lines have been generated in which ligands (insulin, IGF1, and IGF2), receptors (IR, IGF1R, GHR), and immediate downstream signaling molecules (IRS proteins and other adaptor molecules including p66Shc) have been deleted. Assessment of longevity and reproductive function is not complete in all mutants (Table 1).

IGF1-null mice are infertile dwarfs (Baker et al., 1993), but their lifespan has not been reported. Mice lacking both allelic copies of insulin or with global deletion of either the insulin receptor or the IGF1R die in the early postnatal period (Accili et al., 1996; Duvillie et al., 2002; Liu et al., 1993). In contrast, female mice with heterozygote deletion of IGF1R on a 129Sv background display a 26% increase in mean lifespan (Holzenberger et al., 2003). IGF1R heterozygote mice have mild growth retardation but elevated IGF1 levels and are resistant to oxidative stress. Up to 13 months of age, pregnancy rates, live births per mother, mating behavior, and oestrous cycle length were similar in mutant and control mice with no apparent differences in cycle length or the timing of onset of puberty. However, in common with other mouse studies, the complete reproductive lifespan and lifetime fertility rates with maximum numbers of pregnancies and live births were not measured. It was unclear whether mothers were permitted to lactate and wean their litters.

Deletion of the insulin receptor (IR) has been achieved in a range of tissues. Mice with neuronal deletion (NIRKO mice) display mild impairments of fertility with reduced pregnancy and live birth rates in female ani-

Table 1. Long-Lived Rodents and Their Reproductive Phenotypes

Summary of Reproductive Characteristics of Rodents with Increased Lifespan through DR or Altered IIS

Rodent Model	Mean Lifespan Extension (% increase)		Reproductive Phenotype		References
	Males	Females	Males	Females	
CFY Sprague Dawley rats under DR (to achieve 50% body weight of controls)	36	36	Delayed sexual maturation and reproductive senescence. Reduced lifetime breeding performance.	Delayed sexual maturation and reproductive senescence. Reduced lifetime breeding performance.	Merry and Holehan (1979), Merry and Holehan (1981), Holehan and Merry (1985a, 1985b) and reviewed in Weindruch and Walford (1988)
Mice under 25%–50% DR (e.g., C57Bl/6, strain A, C3B10RF1)	20–65	20–65	Less sensitive but reduced fertility reported in C3H mice.	Acyclic: Delayed reproductive senescence on return to ad libitum feeding.	Reviewed in Weindruch and Walford (1988)
Mice under graded DR from 15%–45% (Quackenbush mouse strain)	ND	ND	ND	Graded reduction in fertility with increasing DR.	Zamiri (1978)
Ames dwarf mice	49	68	Variable: sterile to subfertile depending upon genetic background.	Sterile but may be reversed by prolactin treatment.	Brown-Borg et al. (1996) and reviewed in Bartke and Brown-Borg (2004)
Snell dwarf mice	26	42	Variable: sterile to subfertile depending upon genetic background.	Sterile but may be reversed by prolactin treatment.	Flurkey et al. (2002) and reviewed in Bartke and Brown-Borg (2004)
Lit/lit mice	23	25	Delayed sexual maturation and defective sexual behavior.	Delayed sexual maturation.	Flurkey et al. (2001) and reviewed in Chandrashekar and Bartke (2003)
GHR-null mice	55	38	Delay of pregnancy, reduced litter size when male and female null mice intercrossed. Female null mice fertile but deficits in follicular function and sexual maturation.		Coschigano et al. (2000), Zaczek et al. (2002) and reviewed in Chandrashekar and Bartke (2003)
p66Shc-null mice	30 (sex not indicated)		ND	ND	Migliaccio et al. (1999)
IGF1 receptor heterozygote-null mice	33 (in females only)		ND	Normal	Holzenberger et al. (2003)
FIRKO mice	18 (mixed sex)		Normal	Normal	Bluher et al. (2003)

ND: not determined.

mals (Bruning et al., 2000). Since disruption of IIS pathways in neuronal tissue extends lifespan in *C. elegans* (Libina et al., 2003; Wolkow et al., 2000), it would be interesting to determine lifespan in NIRKO mice, but no data have yet been reported. However, the presence of insulin resistance and neurogenerative disease suggests that these mice may not be long lived (Bruning et al., 2000; Schubert et al., 2004).

Deletion of IR in adipose tissue (to produce FIRKO mice) results in reduced body mass, decreased adiposity, and reduced levels of insulin but normal IGF1 levels. FIRKO mice are resistant to obesity and age-related deterioration in insulin sensitivity and glucose tolerance, resembling in part DR mice, and display an 18% increase in mean lifespan in both sexes; maximum lifespan has not been reported (Bluher et al., 2003). Fertility in these mice is apparently normal (C. Ronald Kahn, personal communication).

Growth hormone receptor (GHR) null mice have an approximately 40% increase in mean lifespan and increased maximum lifespan (Bartke and Brown-Borg, 2004; Coschigano et al., 2000). Matings between wild-type male and GHR-null females have revealed defects in a number of reproductive parameters (Zaczek et al., 2002).

Deletion of IIS components such as insulin receptor substrate proteins (IRS) and p66Shc has also been achieved in mice. Mice lacking IRS1 are viable dwarfs and display mild insulin resistance (Araki et al., 1994), but data on reproductive function or lifespan have not been reported. Deletion of IRS2 produces female infertility due to defects in the hypothalamo-pituitary gonadal axis. Lifespan has not been reported for females but males are short lived due to the development of type 2 diabetes (Burks et al., 2000; Withers et al., 1998). Female mice with deletion of IRS4 have mild reproductive phenotypes, with reduced numbers of live births (Fantin et al., 2000); lifespan data are not available. In contrast, mice lacking p66Shc display an approximately 20% increase in mean lifespan and are stress resistant (Migliaccio et al., 1999). No information is available on reproductive function in these mice.

In general, systemic or neuronal disruption of IIS impairs fertility. Furthermore, a more limited number of IIS

mutations have been shown to extend mean lifespan. Taken together, these observations suggest that reproductive performance and lifespan could be linked, perhaps through common IIS mechanisms. However, the reports of normal reproductive function in long-lived IGF1R heterozygote and FIRKO mice and the maintenance of the increased lifespan with restoration of fertility in male Snell dwarves challenge the concept of trade-offs between fecundity and longevity.

Dietary Restriction and Caloric Supplementation in Rodents. DR extends lifespan in mice and rats and affects reproductive function (Weindruch and Walford, 1988). Merry and Holehan have documented the effects of DR from weaning (at a level to produce an approximately 40% extension in mean lifespan) on the onset of puberty in male and female SD rats (Holehan and Merry, 1985a; Merry and Holehan, 1979). All female rats on ad libitum feeding were mature by 45 days of age, whereas only about 50% of DR females were mature by 160 days. In male rats, DR appears to have less severe effects upon maturation and, while DR may cause hypoandrogenism, spermatogenesis is largely preserved and puberty is mildly delayed (Merry and Holehan, 1981). DR in rats therefore delays reproductive maturity in both sexes, with females being significantly more affected.

Elegant work by Merry and Holehan comprehensively documented lifetime breeding performance in female and male rats under DR (Holehan and Merry, 1985b; Merry and Holehan, 1981). Males sired significantly reduced numbers of litters at most ages tested. In females, litter size and the number of offspring produced during reproductive lifespan were reduced, although DR rats were able to continue to breed at later ages. In these studies, females were ad libitum fed during lactation, omitting any effect of DR on lactation, a major energetic cost of mammalian reproduction. In rats subjected to DR and then returned to ad libitum feeding, as in *Drosophila*, reproduction continues at greater chronological ages than the age of last reproduction in permanently ad libitum fed animals (Weindruch and Walford, 1988). However, such rats produce only a limited number of litters, and the rate of decline in litter size was identical to that seen in ad libitum fed controls (Holehan and Merry, 1985b). DR therefore delays reproductive senescence but does not alter its rate once it commences. These studies demonstrate that DR extends the reproductive lifespan and delays reproductive senescence in female rats, but at the cost of reduced numbers of live offspring during the reproductive lifespan. Reproductive costs are, in general, less severe in male rats.

DR at levels greater than 25% in general renders female mice anovulatory and sterile (reviewed in Weindruch and Walford, 1988). Lower levels of DR, in contrast, do not have such an impact upon fertility but do delay sexual maturation. Zamiri examined the effects of three levels of food intake, 85%, 70%, and 55% of ad libitum levels, in the outbred Quackenbush mouse strain (Zamiri, 1978). DR had no effect on ovulation rate over three litters, but the 55% restriction lowered implantation rates. There was a graded effect of DR on late embryonic survival, litter size, and birth weight, with DR 55% causing an approximately 40% reduction in littering rate. Effects on lifespan were not measured and only three reproductive cycles were examined, but this study demonstrates a fall in fertility rates with increasing DR. DR has been reported to have less effect upon male reproductive function (reviewed in Weindruch and Walford, 1988). The reasons for differences between rats and mice in the severity of the effects of DR upon reproduction are not clear, but one possibility is that the large rat strains used in longevity studies retain more body fat than mice while under DR. The effects of DR in female mice therefore point toward a trade-off between reproduction and lifespan extension.

A distinct approach to the role of caloric intake in reproductive function has been dietary supplementation in wild populations of rodents. Such studies are based on the assumption that wild mammals in general have a restricted food supply (reviewed in Boutin, 1990). Of five murine population studies reported, three found an increase in the length of the breeding season and four found an increase in the proportion of females breeding. In contrast, a study of the Australian house mouse did not find increased reproduction in the supplemented areas, possibly because the mice were limited by water supply (Ylonen et al., 2003). These data are not conclusive, but they suggest that food limitation in the wild can limit reproductive function. However, these studies did not attempt any measurement of effects on lifespan.

Germline ablation increases longevity in *C. elegans* (Hsin and Kenyon, 1999). In rodents, ovarian transplantation has been investigated, a procedure that transfers not only germ cells but also hormone-producing cells. Transplantation of ovaries from young mice into old, ovariectomized CBA mice increased mean lifespan compared to nontransplanted controls but did not alter maximum lifespan (Cargill et al., 2003). Although this result is intriguing, there was no control sham operation of animals of the same age (11 months) as the experimental animals.

DR in rodents in general reduces lifetime fertility rates in both sexes, with a graded reduction in fertility with increasing levels of DR. Furthermore, DR delays the onset of reproductive competence but, once a rodent is switched back to ad libitum feeding, fertility returns even at ages beyond which reproductive senescence has normally occurred. These findings suggest that this is a potential mechanism to restore fertility when energy supplies are restored. However, even in this situation, total reproductive output is lower than in ad libitum fed animals.

Monkeys and Humans

DR has yet to be demonstrated to extend lifespan in primates, but it modifies several biomarkers that are also altered in long-lived, DR rodents (Roth et al., 2004). Sexual maturation is delayed in prepubescent rhesus monkeys subjected to DR in early life. In contrast, reproductive parameters are unaltered in female animals under 30% DR for 6 years (Mattison et al., 2003). These findings contrast with the rodent data, but the level of DR used is less than that in most rodent studies. Naturally occurring DR in human populations has been complicated by under-nutrition and co-morbidities such as infection (Roberts et al., 2001). However, chronic en-

ergy shortage delays sexual maturation in both sexes, and women who develop under states of reduced energy availability have decreased ovarian function (Ellison, 2003; Ellison et al., 1993). Lactating women have more prolonged post-partum amenorrhoea under low-calorie conditions and fecundity is reduced (Lunn et al., 1984; Roberts et al., 1982; Worthman et al., 1993). Under extremely reduced calorie intake and reduced body fat mass (e.g., in anorexia nervosa and highly trained athletes) amenorrhoea occurs. Ovarian function is hence sensitive to energy balance and energy flux (Ellison, 2003). Male reproductive function (sperm count and viability) is largely unaffected by energetic status (Abbas and Basalamah, 1986; Ellison, 2003). Studies of lifespan in humans who have undergone castration have given some tantalizing but contradictory data with Nieschlag reporting no effect upon lifespan in castrati (Nieschlag et al., 1993) while Hamilton and Mestler report increased mean lifespan in castrated mentally retarded prisoners (Hamilton and Mestler, 1969).

Discussion

In general, where DR extends lifespan, fecundity is adversely affected, in *C. elegans*, *Drosophila*, and rodents. The only discordant data come from *Indy* mutants and STACs in *Drosophila*, which have been suggested to mimic the effects of DR and which increase both lifespan and fecundity (Rogina et al., 2000; Wood et al., 2004). However, the effects on fecundity and lifespan of DR itself are consistent with the idea that there is an evolved mechanism for increasing survival and optimizing offspring production in the face of periodic food shortage. DR can increase and/or prolong fecundity after a return to full feeding in *Drosophila* and rats. Determining the role of reduced fecundity during DR in the extension of lifespan and increase in subsequent fecundity will require direct manipulation of the pathways by which DR reduces fecundity, which will become possible only with a much deeper understanding of the physiological mechanisms at work.

Three lines of evidence suggest that lowered fecundity may not provide a mechanism for extension of lifespan by reduced IIS. First, not all IIS mutations that increase lifespan reduce fecundity in *C. elegans*, *Drosophila*, and mouse. Second, reduced IIS that is confined to the adult can extend lifespan without loss of fecundity in *C. elegans*. Third, ablation of the whole gonad does not extend lifespan in *C. elegans*, while removal of the germline does so. Even one genuine exception to the rule that lifespan can be extended only by reducing fecundity implies that there can be no obligate trade-off between them. As for DR, full resolution of this issue will require direct manipulation of the pathways by which reduced IIS reduces fecundity. But, if there is no obligate trade-off, then why do lifespan and fecundity change in opposite directions to one another so routinely during evolution? Several wider considerations suggest that a real trade-off may be masked by the circumstances of experimental studies.

Nature and the Laboratory Environment

Nature is in general a more exacting place and resources are less available than in the nutrient-rich conditions in laboratory cultures. The cost of reproduction is most evident under conditions of resource shortage and can be absent or even negative if acquisition of resources is increased (de Jong and van Noordwijk, 1992). This may explain why long-lived *Indy* mutant *Drosophila* showed lowered fecundity only on a decreased calorie diet (Marden et al., 2003). In the benign and nutrient-rich laboratory environment animals are presumably operating at some distance from the limits set by ultimate physiological constraints, and the finding that some mutations or drug treatments can increase lifespan with no reduction in fecundity may indicate that resource acquisition is greater than in nature. The fact that these mutations can extend lifespan without a reduction in fecundity under at least some circumstances is important. However, it will be essential to determine how these mutations behave in a variety of environments.

In *C. elegans*, long-lived IIS mutants that have normal fecundity are none the less at a disadvantage under normal circumstances because they are dauer constitutive and suffer from delayed reproduction. Lengthened development time in nature is likely to be accompanied by an increased death rate during development, while a delay in the age of first breeding is disadvantageous for an organism such as *C. elegans* that probably does most of its breeding in expanding populations (Hodgkin and Barnes, 1991). Any developmental delay and even a small reduction in early fecundity can have a substantial cost to fitness. Such small differences may explain why IIS mutant worms are at a disadvantage when experimentally competed against wild-types (Jenkins et al., 2004; Walker et al., 2000).
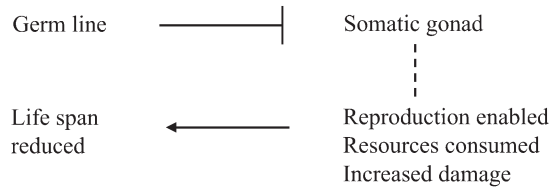
Measuring Fecundity

If full reproductive activity is not allowed to occur during measurement of fecundity, then important effects could be missed. In studies of rodents it is not usually possible to allow females to reproduce continuously, in order to measure number of litters and litter size over the lifetime. In addition, in mammals generally, the greatest nutritional drain on females occurs during lactation. Most studies have either supplemented feeding in lactating rats or not reported whether pups were removed, thereby reducing the cost of reproduction. It is clear, however, that intensive breeding regimes can have adverse effects upon lifespan but mainly due to the induction of obstetric pathologies and without clear impact upon somatic aging.
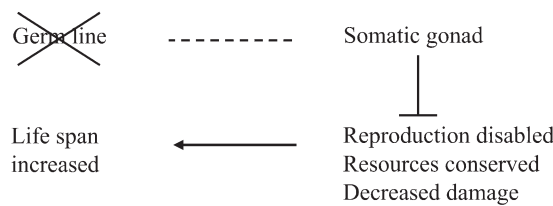
There may also be some issues with the appropriate way to measure fecundity in *C. elegans*. "Fecundity" usually refers to the brood size of self-fertilizing hermaphrodites. *C. elegans* hermaphrodites are protandrous, producing first sperm and then eggs; hence, the brood size of self-fertilizing animals is limited by the number of self-sperm produced (optimally, around 300). If mated with males, hermaphrodites can produce over 1,400 progeny over an extended reproductive period. The effect of IIS on reproduction in hermaphrodites with unlimited sperm has not been reported. In nature, *C. elegans* may encounter a variable food supply, with reproduction occurring opportunistically at times when food is available. Under these conditions, the worms regularly "bag," during which the developing eggs hatch and the hatchlings devour the parent from within. The total number of eggs hatched will be lower than if bag-

A   Intact gonad

Germ line	⊢ Somatic gonad
	⋮
Life span reduced	← Reproduction enabled Resources consumed Increased damage

B   Germ line ablated

✕ Germ line	┄ ┄ ┄ ┄ Somatic gonad
	⊥
Life span increased	← Reproduction disabled Resources conserved Decreased damage

C   Gonad ablated

✕ Germ line	┄ ┄ ┄ ┄ ✕ Somatic gonad
	⋮
Life span reduced	← Reproduction enabled Resources consumed Increased damage
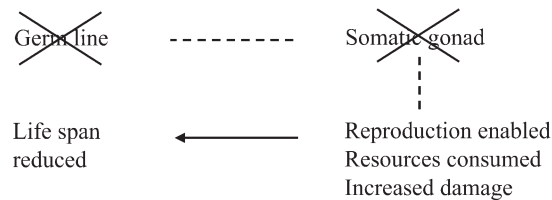
Figure 4. A Model for Reproductive Signaling in which Putative Signals from the Germline and the Somatic Gonad Coordinate Reproductive Activity in the Gonad and Elsewhere in the Worm within the Overall Context of a Cost of Reproduction

(A) With the gonad intact, the germline suppresses signaling by the somatic gonad, costly reproductive activity outside the gonad is enabled, and lifespan is shortened by the associated consumption of resources or generation of damage.
(B) With the germline ablated, signaling by the somatic gonad is no longer suppressed, reproductive activity outside the gonad is hence disabled, costs are not incurred, and lifespan is extended.
(C) When the entire gonad is removed, no signal is emitted by the somatic gonad, costly reproductive activity outside the gonad is enabled, and lifespan is shortened by the associated consumption of resources or generation of damage.

ging does not occur, but the success of the offspring in reproducing may be improved because of better nutrition during development. Thus, *C. elegans* may facultatively undergo reproductive death and represent a semelparous organism. Bagging is under neuroendocrine control: retention of eggs in the absence of food may be suppressed by ectopic serotonin. In addition, bagging is common among free-living nematodes and in some species occurs constitutively (McCulloch and Gems, 2003).

Mutants May Not Produce a Normal Reallocation of Resources
The finding that lifespan is not extended by removal of the whole gonad in *C. elegans* could imply that exten-

sion of lifespan by ablation of the germline alone cannot be accounted for in terms of resource re-allocation (Hsin and Kenyon, 1999). However, ablation of the whole gonad could fail to remove the cost that is abolished by germline removal, as is true for any intervention that reduces fecundity but does not extend lifespan. Processes that contribute to reproduction are not confined to the gonad, and whole gonad removal may fail to send the appropriate signals elsewhere in the worm to cease costly activities if, for instance, the somatic gonad signals to tissues elsewhere in the worm that are involved in enabling reproduction (Figure 4).

Laboratory Species and Strains
Laboratory strains can have genetic peculiarities that complicate interpretation. Both *Drosophila* and mice show genetic adaptation to laboratory culture. *Drosophila* that are introduced to the laboratory for the first time from the wild undergo a rapid reduction in lifespan and increase in fecundity (Sgrò and Partridge, 2000), and established laboratory strains are shorter lived than newly collected ones (Linnen et al., 2001). Laboratory mice are selected for enhanced reproductive rate (Miller et al., 2002), and they also eat more (Austad and Kristan, 2003) and do not live as long (Miller et al., 2002). DR and mutations in IIS could therefore extend lifespan more in these animals than in their wild counterparts, if deleterious effects on lifespan of laboratory adaptation are ameliorated. In support of this idea, a comparative survey revealed that the degree of lifespan extension seen with overexpression of the *Drosophila* Cu/Zn superoxide dismutase was greatest in strains of flies where the controls were shortest lived (Orr and Sohal, 2003).

Control strain lifespan may also be an issue in mice. For example, for the IGF1R heterozygote mice (on a 129Sv background) (Holzenberger et al., 2003), FIRKO mice (on a hybrid 129Sv × C57Bl/6 background) (Bluher et al., 2003), and p66Shc-null mice (Migliaccio et al., 1999), the mean lifespan of the control groups was short compared to published values and the extension of lifespan was modest compared to that seen in pituitary dwarves and under DR (Barger et al., 2003). The rescue of deleterious laboratory-strain characteristics could extend to a positive effect on fecundity. It is therefore important that experiments are conducted with robust, long-lived strains of animals.

Conclusions
Dietary restriction extends lifespan and reduces fecundity in model organisms, findings that are consistent with the idea that there is a trade-off between these two traits. Some data from the effects of IIS appear to challenge the idea that lifespan is extended by re-adjustment of a trade-off with reproduction, but caution is required in accepting this conclusion. Benign laboratory environments, the way that fecundity is measured, and genetic adaptation to laboratory culture could all mask a cost of reproduction. Better understanding of the molecular mechanisms by which altered IIS and DR modulate fecundity will allow definitive resolution of the issue. Assessment of these interventions in robust strains of animals under a variety of environmental con-

ditions, including stress and competition, will be essential to put this information in an appropriate context.

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