Published in "Experimental Gerontology 46(5): 369–375, 2011"
which should be cited to refer to this work.

Survival costs of reproduction in *Drosophila*

Thomas Flatt *

Institute of Population Genetics, Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210 Vienna, Austria

Reproduction shortens lifespan in practically all organisms examined so far, but the underlying mechanisms remain largely unknown to date. Here I review what evolutionary and molecular biologists have learned about such "costs of reproduction" in the fruit fly (*Drosophila melanogaster*) since Maynard Smith's (1958) seminal discovery that sterile mutants in *D. subobscura* live substantially longer than fertile wildtype flies. Together with observations from the nematode worm (*Caenorhabditis elegans*) and other organisms, the data from *Drosophila* suggest that there are at least four general principles that underlie trade-offs between reproduction and lifespan: (1) trade-offs between survival and reproduction are widespread; (2) the relationship between increased lifespan and decreased fecundity can be uncoupled under certain conditions; (3) while survival costs of reproduction might not necessarily be due to competitive resource allocation, we lack robust alternative explanations for their occurrence; and (4) physiological trade-offs between reproduction and longevity do not always translate into evolutionary genetic trade-offs. I conclude that – despite much recent progress – our current understanding of the proximate basis of survival costs of reproduction remains very limited; much future work on the genetics and physiology of such trade-offs will be required to uncover their mechanistic basis.

1. Introduction

In 1958, the evolutionary geneticist John Maynard Smith discovered that sterile *grandchildless* ("ovariless") mutants in the fruit fly *Drosophila subobscura* live substantially longer than fertile wildtype flies (Maynard Smith, 1958). While many previous observations had already suggested that reproductive processes can shorten lifespan, Maynard Smith's study was one of the first experimental manipulations to demonstrate the existence of a trade-off between reproduction and survival, the so-called "cost of reproduction" (Williams, 1966). As far as we know today, reproduction tends to shorten lifespan in most organisms (e.g., Reznick, 1985; Bell and Koufopanou, 1986; Barnes and Partridge, 2003; Flatt and Promislow, 2007; Harshman and Zera, 2007; Tatar, 2010; Kenyon, 2010).

Here I discuss four general principles that have emerged from evolutionary, physiological, and genetic studies of trade-offs between survival and reproduction since Maynard Smith's (1958) landmark paper: (1) survival costs of reproduction are widespread, both at the physiological and genetic level; (2) although commonly observed, trade-offs between survival and reproduction can be uncoupled under certain conditions; (3) although costs of reproduction might not necessarily be caused by competitive resource allocation, we lack robust alternative models to explain why such trade-offs exist; and (4) survival costs of reproduction that are manifest at the physiological level do not necessarily imply the existence of a trade-off at the evolutionary genetic level.

My discussion focuses on recent observations on the mechanisms underlying survival costs of reproduction made in the fruit fly *D. melanogaster*, but I am also frequently drawing parallels to findings in the nematode worm (*Caenorhabditis elegans*) and in other organisms. I am mostly concentrating on females since most of the work on costs of reproduction in *Drosophila* has been carried out in this sex (since it is often much easier to measure female fecundity than male reproductive output); to date, sex differences in terms of lifespan and survival costs of reproduction remain unfortunately poorly understood (e.g., Burger and Promislow, 2004; Magwere et al., 2004). Moreover, while I am mainly discussing physiological costs associated with egg (or sperm) production, it should be mentioned that many different aspects of

* Tel.: +43 1 25077 4334; fax: +43 1 25077 4390.
E-mail address: thomas.flatt@vetmeduni.ac.at.

reproduction can – at least in principle – generate survival costs: searching for mates, courtship, mating, responses to the opposite sex, competition between mating rivals, and searching for oviposition sites, etc. Although it is interesting to ask how such behavioral aspects of reproduction and intersexual interactions might affect lifespan, a discussion of such costs is beyond the scope of this paper.

2. Survival costs of reproduction are widespread

"Costs of reproduction" (Williams, 1966) are a particular kind of so-called "trade-offs" between life history traits (Stearns, 1992; Roff, 1992); a trade-off exists when an increase in one life history trait (e.g., a reproductive trait) that improves fitness ("reproductive success") is coupled to a decrease in another life history trait (e.g., survivorship) that reduces fitness. Such trade-offs between survival and reproduction can have at least two different sources (e.g., Bell and Koufopanou, 1986): on the one hand, fecundity or fertility might reduce survival because of the costly production of gametes, and on the other hand, survivorship might be decreased due to the elevated mortality risk associated with courtship and mating behavior (or with some other behavioral aspect of reproduction or intersexual interaction).

At the evolutionary genetic level, survival costs of reproduction are thought to arise from alleles with opposite effects on reproduction versus survival (e.g., Williams, 1957; Rose, 1991; Leroi et al., 2005; Flatt and Promislow, 2007; Flatt and Schmidt, 2009). Because natural selection is weak at advanced ages and cannot oppose deleterious late-life effects, Williams (1957) postulated that aging might evolve because selection favors pleiotropic alleles with positive effects on reproduction early in life that outweigh negative effects late in life ("antagonistic pleiotropy" or "trade-off" theory of aging). Such genetically based trade-offs are evolutionarily important since they can constrain the simultaneous evolutionary optimization of correlated traits, such as survival and reproduction, with respect to fitness (Stearns, 1989, 1992; Roff, 1992).

At the physiological level, trade-offs between survival and reproduction are thought to be caused by competitive allocation of limited resources into reproduction versus somatic maintenance and survival (e.g., Kirkwood, 1977; Holliday, 1989; Stearns, 1989, 1992; Rose and Bradley, 1998; Shanley and Kirkwood, 2000; Zera and Harshman, 2001; Flatt and Schmidt, 2009). Consequently, Williams' (1957) model for the evolution of aging can be reformulated in terms of physiology (e.g., Kirkwood, 1977): aging might evolve because natural selection favors alleles that increase the competitive allocation of energetic resources into reproduction at the expense of investment into maintenance, repair, and survival ("disposable soma" theory of aging).

How common are such survival costs of reproduction? Numerous observations and experiments have established that reproduction can reduce adult survivorship in a wide range of organisms (see reviews and discussion in Reznick, 1985; Bell and Koufopanou, 1986; Finch, 1990; Stearns, 1992; Roff, 1992; Rose and Bradley, 1998; Reznick et al., 2000; Leroi, 2001; Barnes and Partridge, 2003; Leroi et al., 2005; Kenyon, 2005; Partridge et al., 2005; Williams, 2005; Flatt and Promislow, 2007; Harshman and Zera, 2007; Flatt et al., 2008; Toivonen and Partridge, 2009; Flatt and Schmidt, 2009; Tatar, 2010, and references therein).

In *Drosophila*, evolutionary biologists have performed several artificial selection and experimental evolution experiments that demonstrate the existence of survival costs of reproduction and that are consistent with Williams' (1957) concept of antagonistic pleiotropy (e.g., see Stearns and Partridge, 2001 for a review). For example, direct artificial selection for extended lifespan increases adult survival but decreases early reproduction (Zwaan et al., 1995), and selection for reproduction at old ages increases lifespan but reduces early fecundity (e.g., Rose, 1984; Luckinbill et al., 1984; Arking, 1987; Partridge et al., 1999).

Additional evidence for a trade-off between reproduction and lifespan comes from manipulative experiments that eliminate reproduction, for instance by removing or destroying gonads (cf. Leroi, 2001). In now classical work, Maynard Smith (1958) showed that sterile offspring of the *grandchildless* mutant in *D. subobscura* live longer than fertile controls, and Lamb (1963) reported that irradiated *D. subobscura* females are long-lived. More recently, Sgro and Partridge (1999) combined these two approaches, irradiation and sterile mutants, to examine the cost of reproduction in *D. melanogaster* lines selected for breeding at old age ("old" lines) versus young age ("young" lines). Relative to "young" lines, flies from "old" lines show extended lifespan and lowered early fecundity, suggesting that the lifespan extension observed in the "old" lines comes at a cost in terms of reproduction. By manipulating these lines, Sgro and Partridge (1999) found that both irradiation and crossing the lines with a dominant female sterile mutation, ovo^{D1}, abolishes the difference in lifespan between long-lived "old" and "young" control flies. Again, this result suggests that reproduction is costly and that eliminating reproduction extends lifespan (but see Leroi, 2001 for a critique).

Other manipulations have also found a negative relationship between decreased reproduction and long life. Dietary restriction (DR), for example, promotes lifespan but reduces fecundity in fruit flies (e.g., Min et al., 2007; Skorupa et al., 2008; also see review in Tatar, 2007, and Section 4 below), and longevity in *Drosophila* can also be increased when restricting mating opportunities or removing oviposition substrate (e.g., Partridge et al., 1987; Fowler and Partridge, 1989; Chapman et al., 1995).

Most recently, molecular geneticists interested in aging have examined survival costs of reproduction by analyzing fecundity and fertility in long-lived mutants or transgenic lines (e.g., Leroi, 2001; Tatar et al., 2003; Partridge et al., 2005; Kenyon, 2005; Leroi et al., 2005; Flatt and Promislow, 2007; Harshman and Zera, 2007; Flatt et al., 2008; Toivonen and Partridge, 2009; Flatt and Schmidt, 2009; Tatar, 2010, and references therein). Most notably perhaps, various mutations in the insulin-like/IGF-1 signaling (IIS) pathway have been found to extend lifespan but reduce fecundity or fertility in *C. elegans*, *Drosophila*, and the mouse (for detailed reviews see Partridge et al., 2005 and Partridge et al., in this issue). For example, mutations in the *Drosophila insulin-like receptor* gene (*dInR*) can cause lifespan extension up to 85%, but mutant females are infertile with non-vitellogenic ovaries (Tatar et al., 2001). Interestingly, these flies have reduced production of juvenile hormone (JH) in their corpora allata, the glands synthesizing JH, and application of a JH analog is sufficient to restore egg development and fecundity and to reduce life expectancy to the level seen in wildtype flies (Tatar et al., 2001; also see Section 5 below).

Plenty of additional examples of long-lived mutants with reduced reproduction could be given (cf. Leroi, 2001; Partridge et al., 2005; Kenyon, 2005; Leroi et al., 2005; Tatar, 2010); taken together, many of these cases support the existence of alleles or mutations with antagonistic effects on reproduction and lifespan, as had been postulated by Williams (1957) (for a recent discussion also see Flatt and Promislow, 2007; Flatt and Schmidt, 2009). Yet, as we shall see below, the opposing effects of reduced IIS on reproduction and lifespan can often be decoupled, and there exist in fact many long-lived mutants that do not seem to pay a cost of reproduction.

3. Longevity–reproduction trade-offs can sometimes be uncoupled

Despite the frequent observation of trade-offs between reproduction and lifespan among many organisms, evolutionary and molecular biologists have documented an increasing number of cases where lifespan can be increased without any apparent costs in terms of reduced reproduction. For instance, numerous long-lived mutants in model organisms do not display reduced fecundity or fertility; here I briefly discuss a few examples from worms and flies that shall

illustrate this major point (also see discussion and additional examples in Leroi, 2001; Barnes and Partridge, 2003; Partridge et al., 2005; Kenyon, 2005; Harshman and Zera, 2007; Tatar, 2010).

In *C. elegans*, for instance, mutants of *age-1* encoding the PI3 kinase gene involved in IIS are long-lived without loss of fecundity (Johnson et al., 1993), and certain long-lived mutants of the insulin/IGF-1 receptor encoded by *daf-2* do not display reduced reproduction (Gems et al., 1998; Kenyon et al., 1993). Remarkably, silencing *daf-2* with RNAi in pre-adult stages extends lifespan but decreases fertility, while RNAi in the adult promotes longevity without impairing reproduction, suggesting that while *daf-2* can pleiotropically regulate both reproduction and lifespan, the regulatory connection between these traits can be uncoupled (Dillin et al., 2002). Similarly, and contrary to the expectation of a resource allocation trade-off (see Section 4 below), various methods to inhibit reproduction in *C. elegans* do not make worms live longer: gonad ablation does not increase lifespan (Kenyon et al., 1993), and several mutants that fail to produce sperm or oocytes have normal lifespan despite being sterile (e.g., Arantes-Oliveira et al., 2002; also see Leroi, 2001; Tatar, 2002, 2010; Barnes and Partridge, 2003).

Work in *Drosophila* also provides many examples of such an uncoupling between survival and reproduction (cf. Partridge et al., 2005; Tatar, 2010). Overexpression of the forkhead transcription factor *dFOXO* downstream of IIS in pericerebral head fat body tissue extends lifespan but does not reduce fecundity (Hwangbo et al., 2004), whereas overexpression in abdominal fat body promotes longevity but impairs fecundity (Giannakou et al., 2004). Similarly, lifespan and reproduction seem to be uncoupled in long-lived mutants of the insulin receptor substrate *chico*: while both heterozygous and homozygous mutant females ($chico^1/+$; $chico^1/chico^1$) are long-lived and have decreased fecundity (Clancy et al., 2001; Tu et al., 2002), reduced reproduction does not account for their increased lifespan since the dominant female sterile mutation ovo^{D1} does not abolish the longevity effects of the $chico^1$ mutation (Clancy et al., 2001). Consistent with the view that reduced reproduction might not be sufficient to extend lifespan, female-sterile mutants of *egalitarian* produce eggs with 16 rather than 15 nurse cells, with egg chambers degenerating before they acquire yolk, yet are not long-lived (Flatt et al., 2008). Intriguingly, in some cases lifespan extension is even associated with increased reproductive output: mutations in *Indy*, a gene that encodes a transporter protein for Krebs cycle intermediates, are long-lived but more fecund than wildtype control flies (Marden et al., 2003), and females with a mutation in the *ecdysone receptor* (*EcR*) show increased lifespan as well as greater age-specific fecundity and fertility relative to control flies (Simon et al., 2003).

These few examples from flies and worms thus clearly illustrate that survival costs of reproduction, common as they may be sometimes, are far from being inevitable or obligatory: at least under certain conditions, increased lifespan can be uncoupled from decreased fecundity. In fact, taken at face value, these findings seem to challenge the very existence of trade-offs between survival and reproduction. So how can we reconcile the notion that costs of reproduction are common when at the same time lifespan and reproduction can be so readily uncoupled? Although we cannot yet fully answer this question, several experiments indicate that trade-offs might be far more dynamic, flexible, and context-dependent than is widely assumed.

While it is true that some long-lived laboratory mutants do not exhibit reduced reproduction or any impairment of other measurable fitness traits, trade-offs are often discovered when such mutants are exposed to other environmental conditions. For example, long-lived *C. elegans age-1* mutants do not have decreased reproduction, but have lower fitness than wildtype worms when they are nutritionally stressed (Walker et al., 2000), and certain long-lived *daf-2* mutants do not display any obvious fitness costs, yet lose out when competed against wildtype worms (Jenkins et al., 2004). Moreover, while long-lived *Drosophila Indy* mutants produce more eggs than control flies under normal food conditions, mutant females exhibit lowered fecundity on a decreased calorie diet, suggesting that *Indy* mediates a conditional trade-off between survival and reproduction (Marden et al., 2003).

Trade-offs between lifespan and reproduction might thus not always be found for at least three reasons. First, increased lifespan must not necessarily trade off with fecundity or fertility, but might trade off with other fitness components instead (also see Williams, 1957; Stearns, 1992, and discussion in Flatt and Schmidt, 2009). While fecundity and fertility are clearly major fitness traits, it must be recognized that fitness components other than egg number can also be very important (e.g., offspring quality and survival), both in terms of determining fitness and of being involved in trade-offs. Second, the expression of the longevity–reproduction trade-off can be highly plastic, i.e. contingent upon the particular environmental context (also cf. Stearns, 1989). Third, mutational analysis of survival costs of reproduction might disturb or even destroy the normal relationship between survival and reproduction through unintended pleiotropic effects, for example by disrupting physiology (e.g., Harshman and Zera, 2007). However, it is also important to keep in mind that the uncoupling of survival and reproduction in laboratory mutants over a single generation is not subject to the same evolutionary selection pressures as the relationship between these traits in a natural population of wildtype individuals.

4. Costs of reproduction are not necessarily caused by competitive resource allocation

Since reproduction is thought to be energetically costly, and because dietary restriction (DR) promotes longevity at the expense of fecundity in *Drosophila* and many other organisms (e.g., Partridge et al., 1987, 2005; Chapman and Partridge, 1996; Min et al., 2007; Tatar, 2007; Lee et al., 2008; Mair and Dillin, 2008; Skorupa et al., 2008), it has been suggested that the longevity–reproduction trade-off might represent an energetic resource allocation trade-off. Under this intuitively plausible scenario, investment into reproduction is favored at the expense of survival under good food conditions, whereas under food limitation resources are thought to be diverted away from reproduction to support somatic maintenance and survival until conditions for reproduction have improved again (e.g., Kirkwood, 1977; Holliday, 1989; Rose and Bradley, 1998; Shanley and Kirkwood, 2000; Zera and Harshman, 2001; Flatt and Schmidt, 2009). Such competitive resource allocation and reallocation are the predominant physiological mechanism put forward to explain the existence of life history trade-offs in general (e.g., van Noordwijk and de Jong, 1986; Stearns, 1989, 1992; Houle, 1991; de Jong and van Noordwijk, 1992; Roff, 1992; Worley et al., 2003), an idea that goes back to Etienne Geoffroy Saint Hilaire's 1818 "Loi de Balancement" (see Leroi, 2001). However, despite the frequent claim that trade-offs ought to be resource-based, and some evidence supporting this notion (e.g., see Boggs, 2009 for a recent review), mechanisms that are independent of energetic investment might also be able to account for the existence of trade-offs (also see Tatar, 2011-this issue).

One alternative is the "direct constraints" or "direct reproductive costs" model of trade-offs (Tatar and Carey, 1995; Tatar, 2001), which posits that reproductive processes or structures do not act as energetic resource sinks that limit investment into somatic maintenance and survival, but instead cause damage, impair or inhibit processes of somatic maintenance and survival (also see Leroi, 2001; Barnes and Partridge, 2003; Harshman and Zera, 2007; O'Brien et al., 2008). For example, the increased physiological activity necessary for enabling reproduction might require high levels of oxidative metabolism, and the resulting accumulation of reactive oxygen species might impair survival (Tatar and Carey, 1995; Tatar, 2001; O'Brien et al., 2008). While not providing a direct proof, several experiments are consistent

with this model: many long-lived fly and worm mutants with reduced reproduction are resistant to oxidative stress (see reviews in Kenyon, 2005; Partridge et al., 2005) and, conversely, increased reproduction in flies elevates susceptibility to oxidative stress (e.g., Salmon et al., 2001). In other cases, bodily maintenance, defense, or repair functions might be downregulated because their activation would interfere with optimal reproductive performance (e.g., Tatar and Carey, 1995; Tatar, 2001; Harshman and Zera, 2007). In support of this view, transgenic flies that overexpress the heat shock protein Hsp70, a chaperone that protects against heat stress, live longer yet show reduced egg hatchability (Silbermann and Tatar, 2000). Indeed, several lines of recent evidence now suggest that the trade-off between reproduction and lifespan might not necessarily be caused by competition of survival functions and reproductive processes for limited resources.

One major observation challenging the notion of a resource-based trade-off is that laser ablation of the entire gonad in *C. elegans* does not extend lifespan, suggesting that the gonad might not be an energetic sink that withholds resources that could be used to support longevity (Kenyon et al., 1993). Moreover, complete sterilization by laser ablation of fertility-impaired and long-lived *daf-2* mutants does not further increase their already long lifespan (Kenyon et al., 1993). Thus, if the gonad is an energetic sink that limits longevity, sterilizing both genotypes should eliminate potential differences in reproductive investment between them, yet lifespan is still extended in the sterilized mutant relative to the sterilized control. But if eliminating reproduction in these worms does not affect their lifespan, does this mean that the reproductive system does not influence aging at all? Not necessarily: a number of experiments in both *C. elegans* and *Drosophila* suggests that the apparent negative relationship between reproduction and lifespan might be due to the effects of molecular signals emanating from the gonad that repress longevity (e.g., see reviews in Leroi, 2001; Tatar, 2002; Barnes and Partridge, 2003; Harshman and Zera, 2007; Kenyon, 2010).

Intriguingly, laser ablation of the precursor cells that give rise to the entire gonad (i.e., germline and somatic gonad precursor cells) has no effect on lifespan, whereas specifically ablating germline precursor cells extends lifespan by about 60% (Hsin and Kenyon, 1999), and these effects can also be recapitulated using reproductive mutants (Arantes-Oliveira et al., 2002). Since removing the whole reproductive system does not affect lifespan, the animals do not live longer simply because they fail to produce energetically costly progeny; in fact, lifespan can be extended when germ cells are genetically ablated even when adults were already investing into egg production (Arantes-Oliveira et al., 2002).

Germline ablation also extends lifespan in other nematodes (Patel et al., 2002), and effects similar to those seen in worms have also been observed in *Drosophila*. The failure to form primordial germ cells in *grandchildless*-like mutants (*tudor*, *germ cell-less*, *oskar*) of *D. melanogaster* increases lifespan either only slightly or not at all (Barnes et al., 2006; Flatt et al., 2008; Shen et al., 2009), but in *D. subobscura grandchildless* mutant females that lack a primordial germline are long-lived (Maynard Smith, 1958), and genetic ablation of germline stem cells in the late 3rd instar or the adult can extend lifespan in *D. melanogaster* (Flatt et al., 2008).

The results on germline ablation in worms and flies suggest a model whereby molecular signals emanating from the germline promote aging, whereas the somatic gonad is the source of opposing signals that repress aging (e.g., Hsin and Kenyon, 1999; Leroi, 2001; Arantes-Oliveira et al., 2002; Tatar, 2002; Barnes and Partridge, 2003; Flatt et al., 2008; Yamawaki et al., 2008, 2010; Greer et al., 2010; Suh and Vijg, 2010; Kenyon, 2010). Thus, under this model, the negative relationship between survival and reproduction is explained by signaling mechanisms independent of direct competition for limiting nutrients.

While these compelling observations should clearly lead us to reconsider the classical trade-off concept (cf. Leroi, 2001), they do not completely rule out competitive resource allocation as a proximate explanation (e.g., Barnes and Partridge, 2003; Harshman and Zera, 2007; Flatt and Schmidt, 2009). Reproductive processes might induce energetic investments into peripheral, non-gonadal tissues that continue even after the gonad has been removed, and curtailing reproduction by gonadectomy or other forms of sterilization might thus not eliminate all costs of reproduction (Barnes and Partridge, 2003). In addition, as suggested further above, lifespan extension by reduced (but not completely abolished) reproductive effort might actually require gonad-produced hormonal signals that are unable to act when the entire gonad is removed via gonadectomy or laser ablation (e.g., Hsin and Kenyon, 1999; Barnes and Partridge, 2003; Harshman and Zera, 2007; Flatt et al., 2008; Yamawaki et al., 2008). But even if the somatic gonad remains intact, the effects of germline ablation on lifespan do not necessarily exclude the possibility of a resource-allocation trade-off. For example, conditions of low reproduction (e.g., reduced proliferation of germline stem cells due to poor environmental conditions — see Drummond-Barbosa and Spradling, 2001; LaFever and Drummond-Barbosa, 2005; Hsu et al., 2008; e.g., mimicked by artificial germline removal in laboratory experiments) might activate the production of endocrine signals in the somatic gonad that communicate levels of energetic demand to peripheral tissues (also see Narbonne and Roy, 2006).

Despite these caveats, experiments looking at the metabolism of the fly provide suggestive evidence that speaks against a resource-basis for survival costs of reproduction.

Djawdan et al. (1996) examined metabolic aspects underlying the trade-off between survival and reproduction by investigating flies that exhibit increased longevity following laboratory selection for postponed reproduction relative to unselected control flies. The authors were unable to detect any difference in energy content and adult metabolic rate between short- and long-lived flies. Furthermore, long-lived flies with reduced early fecundity showed higher accumulation of lipids and carbohydrates than short-lived flies with high early fecundity, yet resources accumulated by the long-lived flies had a lower energy content than the additional eggs produced by the short-lived flies. This result indicates that even if there is a trade-off in energy allocation between fecundity and metabolic storage it is far from being quantitatively exact, casting doubt on a resource based explanation for the observed trade-off (Djawdan et al., 1996).

O'Brien et al. (2008) went a step further and directly measured resource investment into somatic tissues versus reproduction upon dietary manipulation by labeling nitrogen and carbon in the dietary yeast with stable isotopes and mass spectrometry (also see the discussion by Tatar, this issue). Long-lived flies subject to DR showed a greater ratio of investment to somatic tissue relative to allocation to eggs, yet contrary to the expectation from the classic resource allocation trade-off model short-lived flies on a full diet exhibited greater net somatic acquisition and allocation of dietary carbon, nitrogen and essential amino acids. The longevity induced by DR can therefore not be explained by greater absolute somatic investment, and the authors suggest that DR might extend lifespan through somatic investment relative to damage that is incurred from reproduction independent of investment (O'Brien et al., 2008). If this is the case, the observed trade-off between reproduction and lifespan would also be a resource-allocation trade-off, albeit one that is indirect, with high resource levels driving increased reproduction which in turn inflicts direct costs, for example by causing somatic damage (e.g., Tatar and Carey, 1995; Tatar, 2001; also see Tatar, this issue).

In several instances, however, reproduction can be decoupled from lifespan in ways that are difficult to reconcile with either the resource allocation or the direct constraints model (see Section 3 above, and Tatar, this issue). Flies made sterile by mutation in the gene *oo18 RNA-binding protein* (*orb*) do not increase lifespan when provided with yeast (Good and Tatar, 2001), suggesting that the response of

fecundity and survival in response to yeast is uncoupled in these flies. Similarly, adult wildtype flies that are yeast-deprived as 3rd instar larvae exhibit decreased ovariole number and fecundity, yet are not long-lived (Tu and Tatar, 2003). Conversely, DR can promote longevity independent of reduced reproduction, as is evidenced by experiments showing that DR increases lifespan in a *Drosophila* mutant with oogenic arrest, ovo^{D1}, and in gonadectomized *C. elegans* (Mair et al., 2004; Crawford et al., 2007).

Another striking example of the uncoupling between reproduction and lifespan is provided by elegant work from Grandison et al. (2009) (cf. Flatt, 2009 for a discussion). While DR promotes longevity at the expense of fecundity, the authors managed to revert these phenotypes by adding back all essential amino acids to the DR diet: upon add-back, lifespan was decreased and egg production increased as much as observed under full feeding. Remarkably, when manipulating each amino acid singly, Grandison et al. (2009) found that supplementing DR food with methionine alone was sufficient to increase fecundity as much as full feeding without any reduction of longevity. Thus, full feeding seems to simultaneously promote fecundity and decrease lifespan via different nutrients; both survival and reproduction can apparently be maximized without any trade-off between them when dietary components are finely balanced (cf. Lee et al., 2008; Skorupa et al., 2008; Flatt, 2009).

Perhaps consistent with the interpretation of Grandison et al. (2009), lifespan and reproduction exhibit different dietary optima (i.e. different protein (P) to carbohydrate (C) ratios, P:C) (Lee et al., 2008). While these distinct nutritional optima provide support for the existence of a trade-off, the observed pattern can neither be readily explained by the resource allocation nor the direct constraints model: egg production first rises slowly until reaching a ratio of P:C = 1:2 and then falls, whereas lifespan decreases steeply and continuously as the P:C ratio rises. This means that for a range of nutritional conditions intake of specific amounts of protein relative to carbohydrate maximizes egg production at the expense of longevity, yet beyond this range further protein ingestion reduces both egg production and lifespan (Lee et al., 2008; also see Skorupa et al., 2008 and the discussion in Tatar, this issue). As these recent studies suggest, part of the difficulty in clearly defining the proximate basis of the trade-off between reproduction and lifespan might lie in the non-linear, context-dependent, and flexible nature of the relationship between these traits.

Although the notion of a resource allocation trade-off is clearly a gross oversimplification, diet, metabolism, reproduction, and longevity are nonetheless intricately connected, in ways that are not yet well understood (e.g., see discussion in Flatt, 2009; Flatt and Schmidt, 2009). For example, while DR can extend lifespan in worms whose gonad has been ablated, it fails to do so in *C. elegans* with an intact somatic gonad but lacking germ cells (Crawford et al., 2007). Moreover, germline ablation in this nematode promotes longevity by modulating fat storage and metabolism (Wang et al., 2008; O'Rourke et al., 2009). While the underlying connections await future discovery, it is likely that organisms, regularly exposed to unpredictably changing environments, have evolved regulatory fine-tuning mechanisms that coordinate metabolic physiology in a way that maximizes fitness given prevailing environmental and physiological constraints.

A potentially very interesting but little explored alternative explanation for reproductive costs has been provided by Bell and Koufopanou (1986): organisms with a fixed number of mitoses might have a small, finite and non-renewable pool of primary germ cells, so that any early increase in expenditure of germ cells is necessarily coupled to decreased expenditure later in life. Thus, if the organism dies at some random point after the last egg has been laid, then any increase in early fecundity will be coupled with decreased longevity. Such "reproductive determinism" (Bell and Koufopanou, 1986) can be seen as an example of a resource allocation trade-off, with the limiting resource being the germ cells themselves; however, whether such a pattern provides a robust explanation for trade-offs in organisms with a fixed number of mitoses – such as nematodes and rotifers – remains presently unclear (cf. Bell and Koufopanou, 1986).

5. Physiological costs of reproduction do not always imply evolutionary trade-offs

Life history trade-offs such as the one between survival and reproduction can manifest themselves at both the physiological, individual level and the evolutionary, population level (e.g., Stearns, 1989, 1992), and this can have important consequences for whether we are able to detect the existence of a trade-off between survival and reproduction in the first place (Flatt and Kawecki, 2007).

A trade-off between reproduction and survival exists at the physiological or individual level when individuals with higher reproductive effort have a shorter lifespan, or when individuals with increased lifespan have reduced reproductive output (also see Section 2 and Stearns, 1989, 1992). Physiological trade-offs might either be caused by competition among two (or more) functional processes for limited resources within a single individual, or by antagonistic signaling processes independent of resource allocation; they can be readily established through phenotypic manipulation experiments or when examining mutant phenotypes (see Sections 2 and 4; also see Tatar and Carey, 1995; Barnes and Partridge, 2003; Harshman and Zera, 2007).

In contrast, an evolutionary genetic trade-off occurs at the population level when an evolutionary change in a trait that increases fitness is linked to an evolutionary change in another trait that decreases fitness (Stearns, 1989, 1992). This is the case, for example, when the evolution of higher reproductive effort is accompanied by reduced lifespan as a correlated response to selection, or when the evolution of increased longevity is correlated with the evolution of reduced reproduction. In such cases, the trade-off is due to a negative genetic correlation between survival and reproduction among individuals in the population, so that some genotypes have reduced fecundity but increased lifespan, whereas others have reduced lifespan but increased fecundity. Evolutionary genetic trade-offs can be established using quantitative genetic breeding designs or by using artificial selection experiments (e.g., Stearns, 1992). However, the relationship between physiological and evolutionary trade-offs is not one-to-one, and physiological trade-offs might not translate into trade-offs at the population level.

When the expression of a physiological trade-off is genetically variable among individuals in the population, it can contribute to an evolutionary trade-off and thus to the response to selection (Stearns, 1989, 1992). However, when the physiological (intra-individual) trade-off is genetically fixed within the population, all individuals exhibit a qualitatively identical physiological relationship between survival and reproduction, and the physiological trade-off does not contribute to the evolutionary trade-off. Thus, while evolutionary genetic trade-offs are ultimately based in physiological trade-offs at the individual level, the opposite is not necessarily true, and the absence of an evolutionary trade-off does not imply the absence of a survival cost of reproduction at the physiological level.

A recent study by Flatt and Kawecki (2007) illustrates the importance of distinguishing between physiological and evolutionary trade-offs with regard to survival and reproduction. The authors found that exposing wildtype flies to an exogenous synthetic analog of juvenile hormone (JH) increases egg production but reduces adult survival, suggesting that JH is a physiological mediator of the trade-off between survival and reproduction, as had been postulated previously (Tatar and Yin, 2001; Tatar et al., 2001; Flatt et al., 2005). However, in flies selected to become resistant to the JH analog (JHa) treatment with JHa did not reduce lifespan as much as in wildtype control flies and did not increase egg production (Flatt and Kawecki, 2007).

Together, these results suggest that JH has antagonistic physiological effects on survival and reproduction, but that selection for reduced JH sensitivity affects lifespan independent of reproduction. Thus, at the population level, evolutionary changes in JH signaling do apparently not mediate the evolutionary trade-off between survival and reproduction (Flatt and Kawecki, 2007).

Whether such an uncoupling between physiological and evolutionary trade-offs occurs frequently is unknown, but the fact that genetically variable wildtype populations of *D. melanogaster* respond in almost all cases to selection for increased lifespan by evolving reduced early fecundity suggests that at least evolutionary survival costs of reproduction are common (e.g., see Stearns and Partridge, 2001 for a review), and one would expect such trade-offs at the population level also to be rooted in physiological trade-offs at the individual level.

6. Summary and conclusions

Here I have reviewed what has recently been learned about survival costs of reproduction by focusing on findings in the fruit fly, *D. melanogaster*, and by drawing parallels to another genetic model system of aging, the nematode *C. elegans*. It has become clear now that trade-offs between survival and reproduction are extremely widespread: for example, many long-lived fly mutants exhibit reduced fecundity and fertility, and practically all selection experiments for increased lifespan in *Drosophila* have found that the evolution of longevity is coupled to decreased early reproduction. Many of these findings are consistent with Williams' (1957) antagonistic pleiotropy or "trade-off" model for the evolution of aging. However, despite this frequent occurrence of a negative relationship between adult survival and reproduction in flies and many other organisms, we are beginning to see an increasing number of examples where the trade-off is uncoupled or "broken" or where its expression is context-dependent. Despite some tentative evidence to the contrary, many of these observations are clearly at odds with the classical but simplistic notion of a competitive resource allocation trade-off between reproduction and survivorship. A more recent and now quite popular alternative view is that longevity–reproduction trade-offs might in fact be due to pleiotropic regulatory signaling processes that are independent of energy investment. However, even this model, which is based on the notion of direct reproductive costs, cannot easily explain all current data. That the relationship between reproduction and lifespan is complicated is also underscored by the realization that physiological trade-offs between reproduction and survival do not necessarily translate into evolutionary genetic trade-offs at the population level, a fact that has important implications for our understanding of the evolution of aging. Thus, although we have made a lot of progress in unraveling the nature of survival costs of reproduction, we are still far away from fully understanding the mechanisms whereby reproduction and lifespan is either strongly coupled or not.

Acknowledgements

I thank the editor of this special issue, Blanka Rogina, for her invitation to write this review; Marc Tatar for stimulating discussions about the proximate basis of survival costs of reproduction; and two anonymous reviewers for helpful comments on a previous version of this paper. I apologize to those colleagues whose work I could not cite due to space limitations. My work was supported by a grant from the Austrian Science Foundation (FWF) to TF (P21498-B11).

References

Arantes-Oliveira, N., Apfeld, J., Dillin, A., Kenyon, C., 2002. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. Science 295, 502–505.
Arking, R., 1987. Successful selection for increased longevity in *Drosophila*: analysis of the survival data and presentation of a hypothesis on the genetic regulation of longevity. Exp. Gerontol. 22, 199–220.
Barnes, A.I., Partridge, L., 2003. Costing reproduction. Anim. Behav. 66, 199–204.
Barnes, A.I., Boone, J.M., Jacobson, J., Partridge, L., Chapman, T., 2006. No extension of lifespan by ablation of germ line in *Drosophila*. Proc. Roy. Soc. London B 273, 939–947.
Bell, G., Koufopanou, V., 1986. The cost of reproduction. Oxf. Surv. Evol. Biol. 3, 83–131.
Boggs, C.L., 2009. Understanding insect life histories and senescence through a resource allocation lens. Funct. Ecol. 23, 27–37.
Burger, J.M., Promislow, D.E., 2004. Sex-specific effects of interventions that extend fly life span. Sci. Aging Knowl. Environ. pe30, doi:10.1126/sageke.2004.28.pe30.
Chapman, T., Partridge, L., 1996. Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter with males. Proc. Roy. Soc. London B 263, 755–759.
Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., Partridge, L., 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. Nature 373, 241–244.
Clancy, D.J., Gems, D., Harshman, L.G., Oldham, S., Stocker, H., Hafen, E., Leevers, S.J., Partridge, L., 2001. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science 292, 104–106.
Crawford, D., Libina, N., Kenyon, C., 2007. *Caenorhabditis elegans* integrates food and reproductive signals in lifespan determination. Aging Cell 6, 715–721.
de Jong, G., Van Noordwijk, A.J., 1992. Acquisition and allocation of resources — genetic (co)variances, selection, and life histories. Am. Nat. 139, 749–770.
Dillin, A., Crawford, D.K., Kenyon, C., 2002. Timing requirements for insulin/IGF-1 signaling in *C. elegans*. Science 298, 830–834.
Djawdan, M., Sugiyama, T.T., Schlaeger, L.K., Bradley, T.J., Rose, M.R., 1996. Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. Physiol. Zool. 69, 1176–1195.
Drummond-Barbosa, D., Spradling, A.C., 2001. Stem cells and their progeny respond to nutritional changes during *Drosophila* oogenesis. Dev. Biol. 231, 265–278.
Finch, C.E., 1990. Longevity, senescence, and the genome. The University of Chicago Press, Chicago and London.
Flatt, T., 2009. Ageing: diet and longevity in the balance. Nature 462, 989–990.
Flatt, T., Kawecki, T.J., 2007. Juvenile hormone as a regulator of the trade-off between reproduction and life span in *Drosophila melanogaster*. Evolution 61, 1980–1991.
Flatt, T., Promislow, D.E.L., 2007. Still pondering an age-old question. Science 318, 1255–1256.
Flatt, T., Schmidt, P.S., 2009. Integrating evolutionary and molecular genetics of aging. Biochim. Biophys. Acta 1790, 951–962.
Flatt, T., Tu, M.P., Tatar, M., 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. BioEssays 27, 999–1010.
Flatt, T., Min, K.J., D'Alterio, C., Villa-Cuesta, E., Cumbers, J., Lehmann, R., Jones, D.L., Tatar, M., 2008. *Drosophila* germ-line modulation of insulin signaling and lifespan. Proc. Natl Acad. Sci. USA 105, 6368–6373.
Fowler, K., Partridge, L., 1989. A cost of mating in female fruitflies. Nature 338, 760–761.
Gems, D., Dutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.L., Larsen, P.L., Riddle, D.L., 1998. Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. Genetics 150, 129–155.
Giannakou, M.E., Goss, M., Junger, M.A., Hafen, E., Leevers, S.J., Partridge, L., 2004. Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. Science 305, 361.
Good, T.P., Tatar, M., 2001. Age-specific mortality and reproduction to adult dietary restriction in *Drosophila melanogaster*. J. Insect Physiol. 47, 1467–1473.
Grandison, R.C., Piper, M.D., Partridge, L., 2009. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. Nature 462, 1061–1064.
Greer, E.L., Maures, T.J., Hauswirth, A.G., Green, E.M., Leeman, D.S., Maro, G.S., Han, S., Banko, M.R., Gozani, O., Brunet, A., 2010. Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in *C. elegans*. Nature 466, 383–387.
Harshman, L.G., Zera, A.J., 2007. The cost of reproduction: the devil in the details. Trends Ecol. Evol. 22, 80–86.
Holliday, R., 1989. Food, reproduction and longevity: is the extended lifespan of calorie-restricted animals an evolutionary adaptation? BioEssays 10, 125–127.
Houle, D., 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution 45, 630–648.
Hsin, H., Kenyon, C., 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature 399, 362–366.
Hsu, H.J., LaFever, L., Drummond-Barbosa, D., 2008. Diet controls normal and tumorous germline stem cells via insulin-dependent and -independent mechanisms in *Drosophila*. Dev. Biol. 313, 700–712.
Hwangbo, D.S., Gersham, B., Tu, M.-P., Palmer, M., Tatar, M., 2004. *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature 429, 562–566.
Jenkins, N.L., McColl, G., Lithgow, G.J., 2004. Fitness cost of extended lifespan in *Caenorhabditis elegans*. Proc. Roy. Soc. London B 271, 2523–2526.
Johnson, T.E., Tedesco, P.M., Lithgow, G.J., 1993. Comparing mutants, selective breeding, and transgenics in the dissection of aging processes of *Caenorhabditis elegans*. Genetica 91, 65–77.
Kenyon, C., 2005. The plasticity of aging: insights from long-lived mutants. Cell 120, 449–460.
Kenyon, C., 2010. A pathway that links reproductive status to lifespan in *Caenorhabditis elegans*. Ann. NY Acad. Sci. 1204, 156–162.
Kenyon, C., Chang, J., Gensch, E., Rudner, A., Tabtiang, R., 1993. A *C. elegans* mutant that lives twice as long as wild type. Nature 366, 461–464.
Kirkwood, T.B.L., 1977. Evolution of ageing. Nature 270, 301–304.
LaFever, L., Drummond-Barbosa, D., 2005. Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. Science 309, 1071–1073.

Lamb, M.J., 1963. The effects of radiation on the longevity of female *Drosophila subobscura*. J. Insect Physiol. 10, 487–497.
Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N., Raubenheimer, D., 2008. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. Proc. Natl Acad. Sci. USA 105, 2498–2503.
Leroi, A., 2001. Molecular signals versus the Loi de Balancement. Trends Ecol. Evol. 16, 24–29.
Leroi, A.M., Bartke, A., Benedictis, G.D., Franceschi, C., Gartner, A., Gonos, E., Feder, M.E., Kivisild, T., Lee, S., Kartal-Ozer, N., et al., 2005. What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech. Ageing Dev. 126, 421–429.
Luckinbill, L.S., Arking, R., Clare, M.J., Cirocco, W.C., Buck, S.A., 1984. Selection for delayed senescence in *Drosophila melanogaster*. Evolution 38, 996–1003.
Magwere, T., Chapman, T., Partridge, L., 2004. Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster*. J. Gerontol.: Biol. Sci. 59A, 3–9.
Mair, W., Dillin, A., 2008. Aging and survival: the genetics of life span extension by dietary restriction. Ann. Rev. Biochem. 77, 727–754.
Mair, W., Sgro, C.M., Johnson, A.P., Chapman, T., Partridge, L., 2004. Lifespan extension by dietary restriction in female *Drosophila melanogaster* is not caused by a reduction in vitellogenesis or ovarian activity. Exp. Gerontol. 39, 1011–1019.
Marden, J.H., Rogina, B., Montooth, K.L., Helfand, S.L., 2003. Conditional tradeoffs between aging and organismal performance of *Indy* long-lived mutant flies. Proc. Natl Acad. Sci. USA 100, 3369–3373.
Maynard Smith, J., 1958. The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura*. J. Exp. Biol. 35, 832–842.
Min, K.J., Flatt, T., Kulaots, I., Tatar, M., 2007. Counting calories in *Drosophila* diet restriction. Exp. Gerontol. 42, 247–251.
Narbonne, P., Roy, R., 2006. Regulation of germline stem cell proliferation downstream of nutrient sensing. Cell Div. 1, 29.
O'Brien, D.M., Min, K.-J., Larsen, T., Tatar, M., 2008. Use of stable isotopes to examine how dietary restriction extends *Drosophila* lifespan. Curr. Biol. 18, R155.
O'Rourke, E.J., Soukas, A.A., Carr, C.E., Ruvkun, G., 2009. *C. elegans* major fats are stored in vesicles distinct from lysosome-related organelles. Cell Metab. 10, 430–435.
Partridge, L., Green, A., Fowler, K., 1987. Effects of egg-production and of exposure to males on female survival in *Drosophila melanogaster*. J. Insect Physiol. 33, 745.
Partridge, L., Prowse, N., Pignatelli, P., 1999. Another set of responses and correlated responses to selection on age at reproduction *in Drosophila melanogaster*. Proc. Roy. Soc. London. B 266, 255–261.
Partridge, L., Gems, D., Withers, D.J., 2005. Sex and death: what is the connection? Cell 120, 461–472.
Patel, M.N., Knight, C.G., Karageorgi, C., Leroi, A.M., 2002. Evolution of germ-line signals that regulate growth and aging in nematodes. Proc. Natl Acad. Sci. USA 99, 769–774.
Reznick, D., 1985. Costs of reproduction — an evaluation of the empirical-evidence. Oikos 44, 257–267.
Reznick, D., Nunney, L., Tessier, A., 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol. Evol. 15, 421–425.
Roff, D.A., 1992. The evolution of life histories. Theory and Analysis. Chapman and Hall, New York.
Rose, M.R., 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38, 1004–1010.
Rose, M.R., 1991. Evolutionary biology of aging. Oxford University Press, New York and Oxford.
Rose, M.R., Bradley, T.J., 1998. Evolutionary physiology of the cost of reproduction. Oikos 83, 443–451.
Salmon, A.B., Marx, D.B., Harshman, L.G., 2001. A cost of reproduction *in Drosophila melanogaster*: stress susceptibility. Evolution 55, 1600–1608.
Sgro, C.M., Partridge, L., 1999. A delayed wave of death from reproduction in *Drosophila*. Science 286, 2521–2524.
Shanley, D.P., Kirkwood, T.B.L., 2000. Calorie restriction and aging: a life-history analysis. Evolution 54, 740–750.
Shen, J., Ford, D., Landis, G., Tower, J., 2009. Identifying sexual differentiation genes that affect *Drosophila* life span. BMC Geriatr. 9, 56.
Silbermann, R., Tatar, M., 2000. Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. Evolution 54, 2038–2045.
Simon, A.F., Shih, C., Mack, A., Benzer, S., 2003. Steroid control of longevity in *Drosophila melanogaster*. Science 299, 1407–1410.
Skorupa, D.A., Dervisefendic, A., Zwiener, J., Pletcher, S.D., 2008. Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. Aging Cell 7, 478–490.
Stearns, S.C., 1989. Trade-offs in life-history evolution. Funct. Ecol. 3, 259–268.
Stearns, S.C., 1992. The evolution of life histories. Oxford University Press, Oxford.
Stearns, S.C., Partridge, L., 2001. The genetics of aging in *Drosophila*, In: Masoro, E., Austad, S. (Eds.), Handbook of the Biology of Aging, 5th ed. Academic Press, pp. 353–368.
Suh, Y., Vijg, J., 2010. The long and short of fertility and longevity. Cell Metab. 12, 209–210.
Tatar, M., 2001. Senescence. In: Fox, C.W., Roff, D.A., Fairbairn, D.J. (Eds.), Evolutionary Ecology — Concepts and Case Studies. Oxford University Press, Oxford, pp. 128–141.
Tatar, M., 2002. Germ-line stem cells call the shots. Trends Ecol. Evol. 17, 297–298.
Tatar, M., 2007. Diet restriction in *Drosophila melanogaster*. Design and analysis. Interdiscip. Top. Gerontol. 35, 115–136.
Tatar, M., 2010. Reproductive aging in invertebrate genetic models. Ann. N.Y. Acad. Sci. 1204, 149–155.
Tatar, M., 2011. The plate half full: Status of research on the mechanisms of dietary restriction in *Drosophila melanogaster*. Exp. Gerontol. 46, 363–368 (this issue).
Tatar, M., Carey, J.R., 1995. Nutrition mediates reproductive trade-offs with age-specific mortality in the beetle *Callosobruchus maculatus*. Ecology 76, 2066–2073.
Tatar, M., Yin, C.-M., 2001. Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. Exp. Gerontol. 36, 723–738.
Tatar, M., Kopelman, A., Epstein, D., Tu, M.-P., Yin, C.-M., Garofalo, R.S., 2001. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science 292, 107–110.
Tatar, M., Bartke, A., Antebi, A., 2003. The endocrine regulation of aging by insulin-like signals. Science 299, 1346–1351.
Toivonen, J.M., Partridge, L., 2009. Endocrine regulation of aging and reproduction in *Drosophila*. Mol. Cell. Endocrinol. 299, 39–50.
Tu, M.P., Tatar, M., 2003. Juvenile diet restriction and the aging and reproduction of adult *Drosophila melanogaster*. Aging Cell 2, 327–333.
Tu, M.P., Epstein, D., Tatar, M., 2002. The demography of slow aging in male and female *Drosophila* mutant for the insulin-receptor substrate homologue *chico*. Aging Cell 1, 75–80.
van Noordwijk, A., de Jong, G., 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. Am. Nat. 128, 137–142.
Walker, D.W., McColl, G., Jenkins, N.L., Harris, J., Lithgow, G.J., 2000. Evolution of lifespan in *C. elegans*. Nature 405, 296–297.
Wang, M.C., O'Rourke, E.J., Ruvkun, G., 2008. Fat metabolism links germline stem cells and longevity in *C. elegans*. Science 322, 957–960.
Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11, 398–411.
Williams, G.C., 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am. Nat. 100, 687–690.
Williams, T.D., 2005. Mechanisms underlying the costs of egg production. Bioscience 55, 39–48.
Worley, A.C., Houle, D., Barrett, S.C.H., 2003. Consequences of hierarchical allocation for the evolution of life-history traits. Am. Nat. 161, 153–167.
Yamawaki, T.M., Arantes-Oliveira, N., Berman, J.R., Zhang, P., Kenyon, C., 2008. Distinct activities of the germline and somatic reproductive tissues in the regulation of *Caenorhabditis elegans*' longevity. Genetics 178, 513–526.
Yamawaki, T.M., Berman, J.R., Suchanek-Kavipurapu, M., McCormick, M., Maria Gaglia, M., Lee, S.-J., Kenyon, C., 2010. The somatic reproductive tissues of *C. elegans* promote longevity through steroid hormone signaling. PLoS Biol. 8, e1000468.
Zera, A.J., Harshman, L.G., 2001. The physiology of life history trade-offs in animals. Ann. Rev. Ecol. Syst. 32, 95–126.
Zwaan, B., Bijlsma, R., Hoekstra, R.F., 1995. Direct selection on life span in *Drosophila melanogaster*. Evolution 49, 649–659.