Germline stem cell maintenance as a proximate mechanism of life-history trade-offs?

Drosophila selected for prolonged fecundity have a slower rate of germline stem cell loss

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We suggest that the commonly observed trade-offs between early- and late-life reproduction may be mediated by genetic variation in germline stem cell maintenance. Stem cell biology provides a natural framework and experimental methods for understanding the mechanistic basis of life-history evolution. At the same time, natural variation in life-history strategies can serve as a powerful tool for identifying the genes and molecular pathways involved in the maintenance of stem cells in aging adults. We illustrate the connections between life-history and stem cells with examples drawn primarily from *Drosophila melanogaster* and *Caenorhabditis elegans*, and suggest a number of testable hypotheses and avenues for future investigation that can be addressed with existing models and tools.

Keywords:
fecundity; germline stem cells; life-history evolution; reproductive trade-offs

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Abbreviations:
AFR, age at first reproduction; **ALR,** age at last reproduction; **DILP,** *Drosophila* insulin-like peptide (a family of insect hormones); **GSC,** germline stem cell; **JH,** juvenile hormone; **TGF-β,** transforming growth factor beta (a family of signaling molecules).

Introduction

Trade-offs have played a central role in the study of life-history evolution and antagonistic pleiotropy [1–2]. Through pleiotropy, genetic linkage, and developmental and physiological constraints, many traits cannot evolve independently, so that an increase in one fitness component is accompanied by a decrease in another. A classic example of a life-history trade-off is the general pattern of negative genetic correlation between early- and late-age reproduction, where increased reproduction early in life can lead to lower late-age fertility as well as reduced lifespan [3–4]. For example, in *D. melanogaster* strains selected for late-life reproduction increases in lifespan are accompanied by declines in early-age fecundity, while selection for early reproduction is correlated with a reduction in lifespan [5–8]. In the most general sense, these trade-offs can be thought of as a result of competition among different organismal functions for a limited pool of physiological resources [9], although recent evidence suggests that the trade-offs between reproduction and longevity may be based on reciprocal signaling between the soma and the germline, rather than on direct competition for nutrients [10–11].

Artificial selection experiments in *Drosophila* have shown that alternative life-history strategies have a genetic

basis [4, 5] – but what are the proximate molecular-genetic and physiological mechanisms of life-history trade-offs? Here, we argue that the trade-off between early and late reproduction may be mediated by genetic variation in germline stem cell maintenance. Given our extensive and rapidly growing knowledge of stem cell biology in *Drosophila* and other model organisms [12–16], this hypothesis suggests a number of avenues for future investigation based on testable molecular mechanisms. By drawing connections between stem cell biology and reproductive trade-offs, we show how recent advances in molecular genetics can put a mechanistic underpinning under long-standing evolutionary questions.

Model system

In *Drosophila*, as in other iteroparous animals (see Box for glossary of terms), continued reproduction depends on the maintenance of germline stem cells (GSCs). In the female ovary, the GSCs are contained in a morphologically defined niche, where they are attached to the somatic cap cells. Each ovariole has a single niche that usually holds 2-3 GSCs at the time of eclosion. GSC maintenance in the ovary requires a physical attachment to the cap cells and continuous signaling from the somatic niche, as well as endogenous germline factors [12–14]. A GSC can divide asymmetrically to produce another GSC, which stays in the niche, and a daughter cystoblast, which then develops into a mature egg chamber as it progresses down the ovariole. Alternatively, symmetric GSC division can produce either two cystoblasts or two GSCs [13, 14].

Females of *D. melanogaster* kept under optimal conditions typically become sterile before death [17]. Daily female fecundity in most strains peaks 7–10 days after eclosion, and declines thereafter [18]. Since oogenesis in *D. melanogaster* takes 8–10 days from stem cell division to the formation of a mature egg [19], this observation suggests that the loss of GSCs begins immediately after eclosion and proceeds throughout adult life. This prediction is confirmed by direct histological observations and clonal analysis. Approximately half of GSCs in the female ovary are lost between 13 and 29 days after eclosion, and the half-life of an individual GSC has been estimated at 4.6 weeks [12, 16]. A similar depletion of the GSC pool with age is observed in the male testis [20]. The loss of GSCs is primarily due to premature differentiation rather than cell death [13, 16].

Despite the limited life span of individual stem cells, *Drosophila* females may produce eggs for over 2 months, suggesting that the lost GSCs can be replaced [21]. Histological observations show that the rate of GSC loss is significantly slower than it would be in the absence of stem cell replacement [13]. The niche not only maintains GSCs in their undifferentiated state and stimulates their division, but can also induce de-differentiation of early cystoblasts back into functional stem cells [13, 22]. Lost GSCs may be replaced either by the progeny of neighboring GSCs, or by de-differentiated cystoblasts. Failure of this replacement, as well as a declining rate of GSC division and accelerated loss through differentiation, appear to contribute to aging-induced sterility [12, 20, 23, 24].

Hypothesis

The finite number of GSCs and the limited rate of their division suggest a proximate mechanism for the trade-off between early and late reproduction. Short-term fecundity can be increased at the expense of future fecundity by increasing the proportion of GSC divisions that produce two differentiating cystoblasts – essentially, by sacrificing the chances of future reproduction. In contrast, increasing the number of de-differentiating cystoblasts and the proportion of GSC divisions that produce two GSC daughter cells can increase future reproduction at the expense of immediate fecundity. This mechanism follows the classical ''Y'' model of life-history trade-offs, where a limited resource is allocated towards two competing organismal functions in a mutually exclusive way [9]. In this case, however, undifferentiated stem cells rather than nutrients or energy play the role of a limited resource (Fig. 1).

Under this model, the co-existence of alternative life-history strategies reflects the segregation of natural genetic variation affecting GSC behavior. GSC maintenance, division, and differentiation are controlled by a variety of molecular pathways (see below). ''Short-term alleles'', which maximize the production of differentiating cystoblasts, and ''long-term alleles'', which maximize the maintenance of undifferentiated stem cells, can be present in any of the pathway components.

Both types of alleles will be antagonistically pleiotropic [2] due to the fundamental limitation of the GSC pool. Since different life-history strategies can be favored under different environmental conditions, genetic variation for stem cell behavior can be maintained in natural populations by fluctuating selection as well as by mutation-selection balance. In the laboratory, selection for early fecundity will lead to the fixation of alleles that favor cystoblast production, leading to rapid depletion of the GSC pool and the loss of potential for late-life reproduction. Conversely, selection for late-life reproduction will fix the alleles that favor maximum GSC maintenance at the expense of early fecundity.

Preliminary study

To test this hypothesis, we examined *D. melanogaster* strains that were artificially selected for early or late reproduction [5] (S-strains and L-strains, respectively). Selection was performed by retaining either the earliest or the latest cohorts of progeny produced by the previous generation.

Along with noting longer and shorter lifespans in the L- and S-strains, respectively, Luckinbill and Clare [5] observed that the S-strain fecundity was up to five-fold higher than in the L-strains during days 2–6 after eclosion, but the L-strains were still producing progeny late in life. We have replicated these measurements more than 20 years later, and confirmed that reproduction peaks in the first week of adult life but ceases completely after five weeks in an early reproducing S-strain. In contrast, a late-reproducing L-strain remains fertile for 70–90 days and actually has higher fecundity in weeks 5–7 than in weeks 1–3 (Fig. 2A). Consistent with earlier findings [5], the lifetime reproductive output is also considerably higher in the L- than in the S strain.

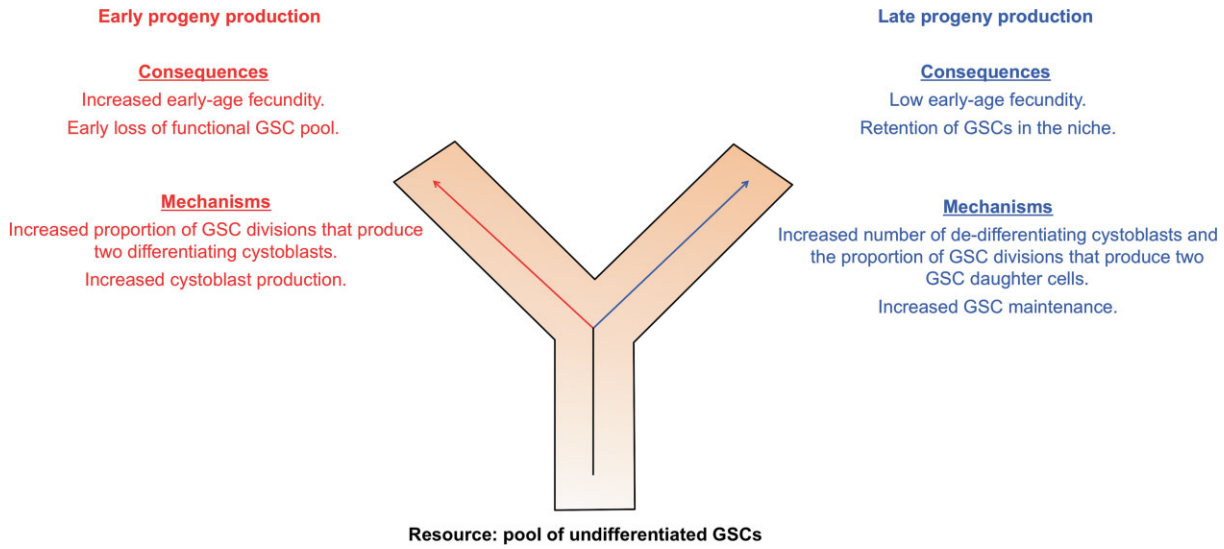

Figure 1. A model of reproductive life-history trade-offs involving GSC maintenance. The left branch represents mechanisms and consequences associated with early-age fecundity, whereas the right branch represents late-age fecundity.

We measured the rate of GSC loss in the S- and L-strains throughout the adult life by staining the ovaries with antibodies against Vasa and Hts [25]. To separate intrinsic reproductive senescence from the effects of mating and the influence of male seminal proteins [26], virgin females were collected soon after eclosion and maintained in small batches on rich media. Randomly chosen females were dissected at various time points between 2 and 60 days after eclosion. For each time point and strain, we counted the average number of GSCs per ovariole in 12–46 females, using one ovary per female. We found that both strains start with approximately the same number of GSCs, but the rate of GSC loss is much faster in the S- than in the L-strain (Fig. 2B).

At two days post-eclosion, the L- and S-strains had on average 2.2 + 0.16 and 1.9 + 0.37 GSCs per ovariole, respectively. However, by day 14 the S-strain had an average of 0.6 + 0.5 GSCs per ovariole whereas the L-strain still had 1.94 + 0.23. These results may explain an earlier observation that the production of mature oocytes is on average delayed in strains selected for late-life reproduction [27]. Interestingly, both strains show an increase in the number of GSCs after an initial phase of GSC loss (Fig. 2C). This increase is particularly strong in the L-strain, suggesting that late-life fertility depends on the replenishment as well as efficient maintenance of stem cells. In the future, it will be important to replicate these measurements in other early- and late-reproducing strains derived from different starting populations, and to test for possible interactions between genotype and nutritional regime in determining the rate of GSC loss.

What is the molecular nature of genetic variation in GSC maintenance?

Our thought experiment suggests that GSC function can follow the Y-model of resource allocation. A small preliminary study confirms the existence of genetic variation that can mediate the trade-off between rapid GSC differentiation (early reproduction) and strong GSC maintenance (late reproduction). The biology of GSCs is studied better in *D. melanogaster* than in any other model, allowing us to speculate about the cellular and molecular mechanisms underlying this variation. GSC behavior and egg production are controlled by both local and systemic mechanisms. The first set of mechanisms is deployed directly in the somatic niche and involves local cell-cell interactions including paracrine signaling, physical attachment between cells, and the function of endogenous germline factors [13, 14]. The second group of factors involves the interactions between diet and hormonal signaling [11, 28, 29]. Recently, both types of GSC-maintaining mechanisms have been found to decline naturally with age, suggesting that reduction in some of these signals may contribute to the loss of fecundity through aging [15, 20, 21, 23, 24]. In the following sections, we outline candidate local and systemic mechanisms (Fig. 3), and discuss how genetic variation in these mechanisms could lead to reproductive life-history trade-offs.

Local molecular mechanisms

TGF-β signaling

The Dpp signaling pathway plays the central role in GSC maintenance. Dpp, a member of the TGF-β family of morphogens, is expressed in the cap cells in the somatic niche, and mutant GSCs that lack Dpp receptors or transcriptional effectors undergo premature differentiation [13, 16]. A second TGF-β homolog, Gbb, also contributes to GSC maintenance [23, 24]. On the opposite side, the *bag-of-marbles* (*bam*) gene acts cell-autonomously in the GSCs to promote their differentiation [30]. Dpp signaling acts, at least in part, by preventing *bam* expression in the GSCs that are in direct physical contact with the niche [25] (Fig. 3).

The activity of Dpp and Gbb signals in wild-type *Drosophila* females declines

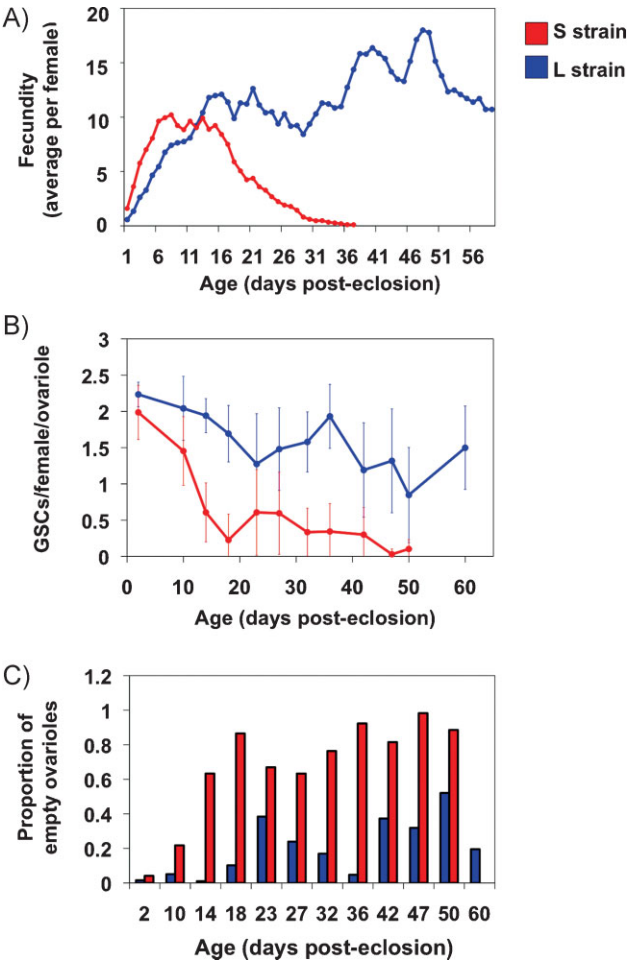

Figure 2. Loss of germline stem cells in early-reproducing (red) and late-reproducing (blue) strains. The strains used were L1 and Sa of Luckinbill and Clare [5]. **A**: Average number of eggs laid per female per day (three-day moving average). **B**: Average number of GSCs per ovariole, measured in 12–46 females per time point. **C**: Proportion of ovarioles with zero GSCs, averaged across the same 12–46 females at each time point. The proportion of empty ovarioles in the L1 strain declines between 23 and 36 days post-eclosion, showing robust replenishment of GSCs.

with age [23, 24]. Genetic experiments show that females that either express reduced levels of Dpp or Gbb ligands in somatic niche cells, or lack components of TGF-β signal transduction in the germline, exhibit a greatly reduced half-life of individual GSCs and accelerated GSC loss [16, 23]. Even more strikingly, removal of the TGF-β antagonist *Dad* in the germline extends GSC half-life, suggesting that TGF-β signaling may be a rate-limiting component of adult stem cell maintenance [16]. Interestingly, continuous high levels of Dpp lead to a rapid loss of GSCs and cap cells and subsequent loss of fecundity [23, 24] whereas increased Gbb activity in the aged niche prolongs GSC and cap cell persistence [23]. These observations suggest that finely tuned activities of the TGF-β ligands in the niche play a key role in setting the balance between GSC maintenance and differentiation. Mutations that affect either the expression or the response to these signals can tilt this balance in different directions, leading to life-history strategies that increase either early or late-life reproduction. For example, the S-strains [5] may have increase levels of Dpp signaling early in life (resulting in increased early proliferation and premature loss of GSCs), while the L-strains may have moderate levels of Dpp signaling or increased levels of Gbb later in life or both.

Physical attachment of GSCs to the somatic niche

Since TGF-β signaling from the cap cells has only short-range effects, GSC maintenance requires physical contact between the GSCs and the niche [13]. This attachment is mediated by the E-cadherin *shotgun* (*shg*) and the β–catenin homologue *armadillo* (*arm*); mutations in either gene lead to GSC loss [31]. The differentiation promoting genes *bam* and *benign germ cell neoplasia* (*bgcn*) act in part by downregulating Shg accumulation at the GSC-niche junction. Conversely, GSCs expressing higher levels of *shg* out-compete other cells for niche occupancy [32]. The *Rab11* GTPase, which regulates the assembly of GSC-niche junction components, and the germline-specific gap junction component *zero population growth* (*zpg*) are also necessary for GSC maintenance [33, 34].

The levels of *shg* in the niche-GSC junctions decline with age [23]. Partial loss of *shg* function accelerates GSC loss, while increased expression of *shg* in GSCs extends their maintenance and function [23]. One would predict, for example, that *shg* alleles that result in stronger attachment between GSCs and the niche would promote late-life reproduction at the expense of early fecundity, while alleles that result in weaker attachment would favor the opposite life-history strategy. *arm*, *rab11*, *zpg*, and other genes involved in GSC-niche attachment may behave in a similar fashion.

The role of endogenous germline factors

Endogenous germline factors required for GSC maintenance and renewal include Piwi, which functions in GSCs to regulate RNA-mediated gene silencing and transposon suppression [35]. Mutations in the *Drosophila piwi* gene or the related genes in mice and zebrafish [36, 37] lead to GSC loss. Other components of the endogenous RNAi pathway, such as *Dicer-1* and *Argonaute-1* (*Ago-1*), are also required cell-autonomously for GSC maintenance: *Dicer-1* mutant GSCs have delayed cell divisions [38], while *Ago-1* mutant GSCs are rapidly lost [39]. Finally, expression of the antioxidant enzyme *Sod* in GSCs declines during aging, and overexpression of *Sod* in either

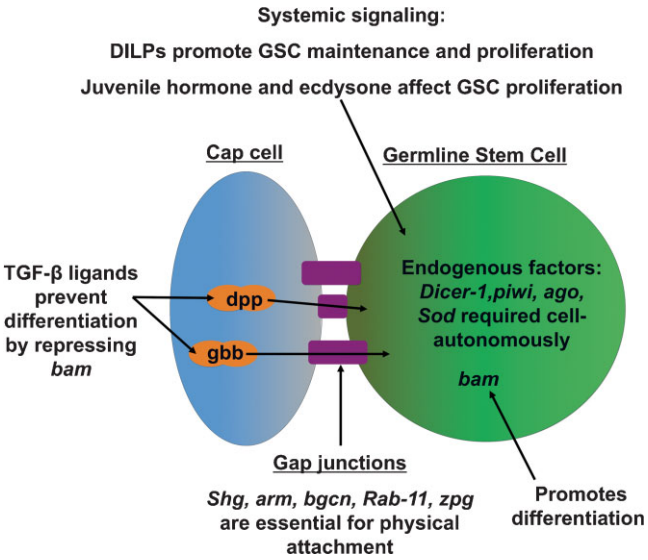

Figure 3. A simplified diagram of molecular pathways contributing to GSC maintenance and differentiation in *Drosophila melanogaster* females.

GSCs or the niche cells results in prolonged GSC maintenance and function [23]. These phenotypes suggest that naturally occurring alleles that reduce *piwi*, *Ago-1*, or *Sod* activity in the germline may tilt the balance between GSC maintenance and differentiation toward early-life reproduction, while alleles with stronger activity would favor late-life fecundity.

As the preceding discussion shows, a wide range of molecular pathways could potentially contribute to natural genetic variation in the rate of GSC loss, and thus to the evolution of trade-offs between early and late-life reproduction. Moreover, it is important to realize that our knowledge of GSC aging is derived from the analysis of candidate genes that have major qualitative phenotypes in young adults. It is conceivable that different pathways act specifically to prolong stem cell maintenance in aging adults. This notion is supported by the observation that different Dicer proteins and microRNAs are required at different stages in GSC development and maintenance [40]. Whatever the mechanisms, the fundamental logic of stem cell maintenance is that the more GSCs differentiate early in life, the smaller pool is kept for late-life reproduction, and *vice versa*. Thus, the number of different loci and mutations that can contribute to the trade-off between early and late reproduction by affecting stem cell behavior can be quite large, consistent with the notion that most life-history traits are controlled by many genes of small effect [1].

Systemic mechanisms

Physiological, and especially hormonal mechanisms of trade-offs involving longevity and reproduction have been studied extensively in recent years [9, 11, 28, 41–43]. In parallel, much progress has been made in understanding how diet and endocrine signaling affect GSC behaviour in *D. melanogaster* and *C. elegans* [29, 44, 45]. Together, these traditionally separate research directions suggest a number of ways in which stem cell maintenance could mediate life-history trade-offs.

Insulin signaling

Quite sensibly, GSC proliferation is affected by dietary conditions. In both *D. melanogaster* and *C. elegans*, this effect is mediated in part by insulin-like peptide signaling [44, 45]. In *Drosophila*, insulin-like peptides (DILPs) upregulate GSC division in well-fed females by directly controlling the GSC cell cycle through PI3K, dFOXO, and additional unknown mediators [45]. However, DILPs promote GSC maintenance as well as proliferation. In females that are fed a nutrient-poor diet or are mutant for the *Drosophila* insulin receptor *dinr* or *chico*, a key component of insulin signal transduction, GSC loss is accelerated [29]; conversely, overexpression of DILPs in the somatic niche in aged females increases late-life GSC retention [29]. DILPs promote GSC maintenance both directly by increasing E-cadherin expression in the GSCs, and indirectly by controlling the number of cap cells *via* Notch signaling [29]. Natural genetic variation in the downstream targets of the insulin pathway may provide yet another potential mechanism for the trade-off between immediate and delayed reproduction. Dietary restriction and nutrient imbalance reduce both early and late-life fecundity [45, 46], suggesting that higher DILP signaling is associated with both higher GSC retention and higher GSC proliferation – in other words, it is more likely to affect the overall reproductive output than the balance between early and late reproduction. On the other hand, variation in the sensitivity or response to insulin signaling in particular cell types may bias GSCs toward greater retention or differentiation under the same environmental conditions.

Other hormonal signals

Other insect hormones play important roles in the trade-offs between longevity and reproduction. For example, *Drosophila* adults that were treated with juvenile hormone (JH) as larvae show increased early life fecundity and reduced lifespan [43].

Experimental evidence suggests that GSCs respond to JH [28], and that JH production is in turn affected by insulin signaling [28, 47]. Similarly, ecdysone signaling is also affected by insulin and nutritional state [48] and is required for GSC proliferation [49]. Furthermore, flies genetically ablated for the JH-secreting corpus allatum have greatly reduced fecundity and increased lifespan, suggesting that variation in hormone production could indeed affect GSC-mediated life-history trade-offs [50]. As more is understood about the mechanisms of hormone action in the gonad, new systemic determinants of GSC maintenance and differentiation will likely emerge.

Does GSC activity modulate lifespan?

The germline not only listens to its somatic environment, but also talks

back. For example, Notch signaling from GSCs to somatic niche cells stimulates their proliferation and the production of TGF-β ligands, which signal back to the GSCs [51]. This feedback loop underscores the intimate association between the germline and the soma in the ovarian stem cell niche. Systemic signals from the germline to the soma may be equally important. Gonad ablation experiments suggest that signals from the germline may contribute to systemic aging of the organism, while the somatic gonad tissue may produce counteracting longevity-promoting signals [10]. In both *C. elegans* and *D. melanogaster*, removing the germline increases lifespan and DILP levels, providing evidence for a feedback loop between GSC proliferation and insulin production [11, 52]. Thus, the rate of GSC loss may partly shape hormonal changes in the aging organism, leading to complex feedback effects that may in turn influence the balance between short-term and long-term fecundity.

Future directions

The hypothesis linking stem cell maintenance to reproductive trade-offs is amenable to experimental testing. The most unbiased approach would be to map and identify the quantitative trait loci (QTL) controlling the balance between early and late reproduction in natural populations. A paucity of QTL studies focusing on reproductive trade-offs reflects the challenge of quantifying age-dependent phenotypes with a strong environmental component [53–55]. Inexpensive high-throughput genome sequencing should alleviate this problem by allowing genome-wide scans for allele frequency differences between pools of early- and late-reproducing individuals or strains [54]. In a complementary approach, analysis of candidate gene expression in early- and late-reproducing strains may offer direct insights into the cellular basis of natural variation in the rate of stem cell loss. For example, does the expression of *bam*, *shg*, *gbb*, and other candidate genes differ systematically between genotypes displaying early *versus* late fecundity?

In searching for the proximate causes of the trade-off between early and late reproduction, it is important to keep in mind that this trade-off is far from the only factor affecting late-life fecundity. Improved nutrition can increase the total lifetime reproductive output, affecting early and late fecundity in approximately equal measure [46]. Conversely, many loss-of-function mutations can curtail both early and late reproduction (see above), or reduce one without any compensatory increase in the other [16, 56]. Thus, if early and late fecundity are examined in isolation, genetic variation in the trade-off between GSC maintenance and expenditure will be only one of the many factors influencing these parameters. Future experiments will need to be carefully designed to target the balance between early and late gamete production, rather than total lifetime fecundity or late-life fecundity as such.

How general is the GSC trade-off hypothesis?

Most of the evidence for reproductive trade-offs in *Drosophila* and *C. elegans* comes from genetic and artificial selection studies in the laboratory. Ultimately, however, analyzing variation in stem cell behavior in natural populations will be necessary in order to understand the evolutionary forces maintaining this variation. A recent study found a life-history cline in the North American populations of *D. melanogaster*, where diapausing genotypes have greater lifespan and stress resistance while the non-diapausing genotypes are shorter lived but have higher reproductive output [57]. Another possible model is provided by the naturally occurring *abnormal abdomen* genotype in *D. mercatorum*, which results in increased early fecundity and shortened lifespan.

This phenotype may be adaptive during arid conditions when the life expectancy of adult flies is greatly decreased [58, 59]. Comparing the dynamics of GSC maintenance in different genotypes in such populations will help elucidate the role of GSC trade-offs in life-history evolution in nature.

On a broader taxonomic scale, we expect the GSC trade-off model to apply primarily to animals that invest a major fraction of physiological resources into gamete production, and whose reproductive output at any given time is limited by the number of gametes produced. *Drosophila* and *C. elegans* are classical examples of this strategy, along with most other insects and nematodes. Among vertebrates, populations of Trinidad guppies, where natural selection in response to predation results in different reproductive schedules and fecundities [60], may provide a promising model. Similarly, threespine stickleback populations have undergone adaptation to different lifestyles. Anadromous sticklebacks, which spend most of their lives in the sea but migrate to freshwater habitats to reproduce, begin reproduction later in life but have higher initial fecundity than resident freshwater populations [61]. Our growing understanding of GSC biology in fish [62–64] should make it possible to test whether genetic variation in GSC maintenance contributes to reproductive trade-offs in these and other populations.

The GSC trade-off model is less likely to apply to animals that invest in their offspring predominantly through mechanisms other than gamete production. A number of studies have addressed the relationship between longevity and reproduction in long-lived vertebrate species [65–68]. In swans, a positive genetic correlation between age at first reproduction (AFR) and age at last reproduction (ALR) is observed, despite the fact that selection acts to decrease the former and increase the latter [65]. This may indicate the existence of a physiological constraint linking early and late reproduction, although the mechanism of this constraint and its influence on the *number* of progeny produced early and late in life is unknown. In humans, the relationship between early- and late-life female fertility is of course complicated by cultural factors. In one of the best studied pre-industrial populations, there was a positive phenotypic correlation between AFR and ALR, *i.e.* women who started to reproduce early also ceased reproduction young [66].

However, the genetic correlation between AFR and ALR in the same population was weakly negative and non-significant, perhaps because AFR showed very little additive genetic variance [67]. Although the molecular pathways that control reproductive

Box

Glossary

Iteroparous: A mode of reproduction describing organisms that reproduce multiple times in a lifetime [1].
Germline stem cell (GSC): A stem cell that divides asymmetrically multiple times to produce daughter cells that eventually develop into gametes [12].
GSC niche: The somatic gonad cells that are in direct contact with the GSCs. This microenvironment is essential for GSC maintenance [13, 14].
GSC symmetric division: A relatively rare mode of GSC division that produces functionally equivalent cells. If the GSC niche is full, symmetric division can result in the differentiation of both daughter cells. If the niche has vacant space, it can result in two GSCs [13, 14].
GSC maintaining mechanisms: Molecular pathways that promote the retention of functional GSCs in the niche. **Endogenous** mechanisms act in the GSCs themselves, **local** mechanisms involve genes acting in the somatic niche cells, and **systemic** mechanisms involve signals originating from outside the gonad [14, 15].
Antagonistic pleiotropy: The situation where an allele that enhances one trait is deleterious to another. For example, alleles that increase early-life fecundity may decrease late-life fecundity or lifespan [2].
Short term alleles: Hypothesized alleles that maximize the production of differentiating GSC daughter cells, resulting in higher fecundity early in life but leading to the loss of GCSs and premature sterility.
Long term alleles: Hypothesized alleles that promote GSC maintenance at the expense of decreased gamete production early in life.
Mutation-selection balance: The equilibrium between the rate at which deleterious mutations arise in the population and the rate at which they are eliminated by natural selection [1].
Reproductive aging (reproductive senescence): Gradual decline in gonad quantity and quality caused by intrinsic cellular processes [56, 69].
Juvenile hormone: A key insect hormone that regulates development, reproduction, and other aspects of insect physiology [28, 43].
Abnormal abdomen (aa): A genetic syndrome segregating in Hawaiian populations of *Drosophila mercatorum*. aa flies have increased early-age fecundity and reduced lifespan relative to wild type [58].

aging may be conserved between vertebrates and invertebrates [69], genetic variation in reproductive strategies may well affect different aspects of reproduction in animals that experience different selective pressures.

Conclusions

The molecular basis of trade-offs between early- and late-life reproduction remains unknown. We suggest, however, that genetic variation in the balance between germline stem cell maintenance and differentiation provides a likely proximate mechanism. Our growing knowledge of stem cell biology in *Drosophila*, *C. elegans*, and other animals suggests specific molecular mechanisms that could contribute to this variation, as well as a way to test their contribution to life-history trade-offs. By integrating evolutionary and molecular-genetic approaches, we may finally identify the genes, regulatory pathways, and perhaps even specific alleles that contribute to life-history evolution in nature.

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