CHAPTER 11

Mechanisms underlying reproductive trade-offs: Costs of reproduction

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11.1 Introduction

The extraordinary variation in reproductive life histories is often overlooked. We tend to be more familiar with traits studied under the umbrella of sexual selection, such as astonishing plumage patterns and weaponry, than we are with the equally striking variability in how often, when, and over what period organisms reproduce. Understanding how this diversity of reproductive life histories evolves is a key challenge in evolutionary biology.

Fisher (1930) was the first to formulate mathematically the important tenets of life histories. He suggested that individuals of a certain age would have a "reproductive value," defined as the mean amount of expected future reproductive success for individuals of that age and sex in a population. Natural selection will act to maximize the reproductive value of an organism at each age by balancing growth, maintenance, and reproduction (e.g., Fisher 1930, Charlesworth 1980), and theory shows that the fitness of a particular life history is strongly linked to *r*, the intrinsic rate of increase for a population or the "Malthusian parameter" (Charlesworth 1980, Lande 1982). However, an "ideal" life history (e.g., one that maximizes both reproductive rate and survival) is constrained by reproductive trade-offs or "costs of reproduction" (a negative relationship between reproductive activity and future reproduction or lifespan; Williams 1966b). There can be ecological trade-offs in which elevated reproductive activity renders organisms more vulnerable to predation or parasitism, but also intrinsic trade-offs, which limit the reproductive output that can be achieved at each age because of competition between life history traits for a share of a finite resource pool (Van Noordwijk and De Jong 1986). Elucidating the nature of these costs of reproduction is central to understanding the diversity of life histories (Charlesworth 1980, Stearns 1992).

In this chapter, we first set the scene by defining some of the key life history traits that are observed to trade-off with one another and to result in costs of reproduction. Included is a broad but brief summary of reproductive trade-offs and how to measure them, to illustrate the full breadth over which reproductive costs can occur (for in-depth reviews on this topic, see Stearns 1989, 1992, Roff 1992, 2007b). The main focus of this review then describes recent advances in elucidating the mechanisms that underlie the physiological and evolutionary costs of reproduction. We then highlight the importance of deriving an understanding of reproductive costs in a fitness-based framework. Finally, some gaps that remain in our mechanistic understanding of reproductive costs are identified, and we discuss new, and potentially fruitful, avenues for investigation.

11.2 Key life history traits and costs of reproduction

Key life history traits (e.g., Stearns 1992) include:

- size at birth
- growth rate
- age and size at sexual maturity
- number, size, and sex ratio of offspring produced
- reproductive schedule and age-specific reproductive investment
- age-specific mortality
- lifespan.

Over 40 different trade-offs between these life history traits have been identified (Stearns 1989), including those between:

- current reproductive rate and survival
- current and future reproductive rate
- the number and size of offspring

The "cost of reproduction" describes the trade-offs in the first two of these major categories. The majority of mechanistic research has focused more narrowly on the trade-offs between reproduction and lifespan, or "survival costs of reproduction." We define lifespan as a potentially important life history trait, as it is significantly influenced by survival probability. However, it is important to use a fitness-based framework in order to correctly assess the information emerging from mechanistic studies of lifespan in long-lived model organisms (see Section 11.5). For example, extended post-reproductive lifespan may confer limited fitness benefits. However, the processes involved in extending lifespan, identified via the study of long-lived mutants, may also be significant modulators of life history traits throughout life.

We focus on defining and describing the *intrinsic* costs of reproduction that arise because of trade-offs between reproductive rate and future survival or lifespan, and between current and future reproduction. However, there is also a growing literature on the *ecological* costs of reproduction, where elevated reproductive activity alters the susceptibility of organisms to extrinsic threats such as increased predation, disease, or parasitism (Sheldon and Verhulst 1996).

11.3 Intrinsic costs of reproduction: Trade-offs between reproductive activity and survival or future reproductive rate

Reproductive trade-offs fall into two categories: physiological and evolutionary (Stearns 1989, 1992; Flatt and Schmidt 2009). To date, the overwhelming research effort has focused on investigating the mechanisms underlying physiological trade-offs. For a summary of methods used to measure physiological and evolutionary costs of reproduction, see Boxes 11–1 and 11–2.

Box 11–1 Methods for measuring costs of reproduction

1. Measuring physiological costs of reproduction

(i) Phenotypic manipulations

Phenotypic manipulations can demonstrate how the physiological costs of reproduction are manifested in real organisms and give some indication of the magnitude of the cost. For example, manipulating the reproductive rates of organisms assigned randomly to groups in similar environments can successfully reveal the costs of reproduction in decreased future survival and fertility (Reznick 1985, Bell and Koufopanou 1986, Partridge and Harvey 1988). This technique has been increasingly employed in measurements of the responses of organisms to different diets (e.g., Chapman and Partridge 1996, Skorupa *et al.* 2008, Grandison *et al.* 2009; see review by Partridge *et al.* 2005a). Such studies can reveal how elevated reproductive rates lead to shortened lifespan, and how extended longevity is often associated with lowered age-specific fertility (for further discussion see below). The extent of reproductive costs will often depend upon the environment (Reznick 1985, Fricke *et al.* 2009), which highlights a great need for studies employing a much broader range of environmental conditions (Cornwallis and Uller 2010). For example, in many cases trade-offs are only seen under stressful conditions (e.g. Stearns 1989, Marden *et al.* 2003). Field studies on birds have also revealed the costs of reproduction using phenotypic manipulations of brood size (e.g. Gustafsson and Sutherland 1988).

(ii) Genetic manipulations

In genetic manipulations reproductive trade-offs are identified through direct manipulation of the genetic pathways that are predicted to be involved. The idea is to manipulate components of trade-offs by using loss of function or over-expression mutants and study their phenotypic effects on reproductive rate or survival. This has been an enormous growth area for research over the last 10–15 years, as genes that are important in determining

trade-offs between reproduction and longevity have been identified (see main text). Within this category fall the many kinds of experiments that manipulate components of reproductive pathways, for example germ line removal (Maynard Smith 1958, Barnes *et al.* 2006, Flatt *et al.* 2008b), nutrient sensing pathways (Libert *et al.* 2007, Partridge *et al.* 2005a), and genetic manipulations of heat shock chaperone genes (Tatar 1999, Silbermann and Tatar 2000). A particularly powerful approach is to combine phenotypic and genetic manipulations to test the effects of, for example, the dietary components involved in trade-offs along with the genes that respond to those components.

(iii) Phenotypic correlations

Physiological costs of reproduction can also be measured by testing for phenotypic correlations, where fertility and survival are measure in organisms allowed to reproduce at their normal rate. The limitations are that it can be difficult to determine the causal relationships involved because any observed correlation may be caused by a common correlation with another uncontrolled factor (Reznick *et al.* 2000). However, this approach has been used successfully to gather evidence for costs of reproduction in human populations (e.g., Westendorp and Kirkwood 1998, Helle *et al.* 2002; see also Chapter 10). The value of correlative techniques can be substantially increased by combining it with approaches (i) and (ii) above to demonstrate causal links between the life history traits predicted to show trade-offs.

2. Measuring evolutionary costs of reproduction

(i) Genetic correlations and correlated responses to selection

The measurement of genetic correlations between life history variables can give strong evidence for evolutionary costs of reproduction (Rose and Charlesworth 1981, Lande 1982, Reznick 1985) and can be derived directly from artificial selection, experimental evolution, or from breeding experiments (Falconer 1981). Genetic correlations between life history variables can indicate the presence and extent of antagonistic pleiotropy. A negative genetic correlation between early fecundity and longevity would imply that, on average, mutations that increase fecundity also decrease longevity (Reznick 1985). Correlated responses to artificial selection are measured in artificial selection or experimental evolution. For example, Rose and Charlesworth (1981) reported a decrease in the early fecundity of lines of *D. melanogaster* that were selected for late-age reproduction by using older adults as parents in successive generations. Reduced fecundity is also commonly observed in response to selection for increased lifespan (e.g., Zwaan 1999).

Selection experiments (and inbred lines) can also be combined with QTL analysis for a useful way of determining genes with major effects on life history traits (e.g., Leips and Mackay 2000). Such techniques have been used to determine genes that affect longevity (e.g., Lai *et al.* 2007), and there is, in principle, no reason why such methods could not be used to detect genes with major effects upon life history trade-offs. Evolutionary trade-offs can also come from the characterization of isogenic lines for evolved differences in the shape of trade-offs. These approaches have so far been underused in the study of reproductive trade-offs and represent a potentially useful avenue for future work.

There are various caveats about the measurement of genetic correlations from artificial selection and breeding experiments: (i) even in the laboratory, the measurement of quantitative traits can be imprecise (Falconer 1981), (ii) genetic variation in the rate of reproduction is generally much lower than can be produced by phenotypic manipulations, hence the power to detect genetic correlations may be lower than for phenotypic correlations, (iii) genetic correlations may not remain constant over time or during artificial selection. Indeed, in some selection experiments the sign of the genetic correlation between two fitness-related traits changed from positive to negative (e.g., Archer *et al.* 2003). It is also important to consider whether trade-offs that evolve under benign and constant environments in the laboratory will reflect those seen under natural conditions. An advantage is to combine evolutionary manipulations and measurements of genetic correlations with the physiological techniques described in 1 above. Concordance between the results of studies using these varied techniques allows powerful inferences to be made.

(ii) Population and species comparisons

Comparative data can indicate broad-scale evidence for costs of reproduction. For example, within several different groups of animals, there are negative correlations between high reproductive output and repeated breeding, giving evidence for a trade-off between current and future reproductive output (e.g., in several species of triclads, mites, many species of lizards, and birds; Roff 1992, Stearns 1992). Caveats include the influence of gene–environment interactions, which may confound when comparing the reproductive rates of different populations or species in

continues

Box 11-1 (*continued*)

environments to which they are unequally adapted. Another potential drawback is the impact of ecological variables and population dynamics (e.g., Gustafsson and Sutherland 1988). Correlated life history traits may also be independent adaptations to different environments and their association need not, therefore, imply a constraint or trade-off. Combining the results of comparative data with experimental studies is an advantage, if it is possible, because, as mentioned earlier, it allows the causal relationships to be elucidated with greater confidence.

Box 11-2 New directions in measuring physiological and evolutionary costs: Genomic approaches

Genomic approaches can measure both physiological and evolutionary trade-offs and there has been a rapid increase in their deployment (e.g., Bochdanvovits and De Jong 2004). Included in this category are the determination of expression profiles by microarray or deep sequencing technology, sequencing of candidate genes involved in determining lifespan across different populations (Schmidt *et al.* 2000), detecting signatures of selection in candidate genes, and sequencing entire genomes. A promising approach is the full genomic characterization of lines selected for reproductive trade-offs, to test for evolved differences in gene sequences and the shape of such relationships. These methods cut across phenotypic and evolutionary trade-offs because they can measure the downstream responses to phenotypic and genetic manipulations to selection for different reproductive strategies (e.g., McElwee *et al.* 2007). They can also measure the expression or sequence of genes or genomes across populations or species. Genomic, and also the currently underutilized proteomic, analyses are set to provide an increasingly important global and tissue-specific signature of the impact of reproductive costs.

11.3.1 Physiological costs of reproduction

Physiological trade-offs occur within individuals and can represent plastic responses to resource levels (Stearns 1992). For example, an organism experiencing an overabundance of resources might elevate current reproductive rate at the expense of future survival or reproduction. Conversely, it might be beneficial to conserve energy reserves if resources are scarce, and increase current and future survival probabilities until resource levels increase. Organisms are faced with these allocation "decisions" because current and future reproductive rates, along with survival, cannot all be maximized. Plastic responses allow organisms to adjust reproductive rates to prevailing conditions. The result is that current reproductive rate may trade-off with future reproductive rate and survival. Even though this kind of trade-off is contrasted with evolutionary trade-offs (see below), the plasticity is itself, of course, an evolved strategy.

There is evidence that the physiological costs of reproduction can be influenced by hormones and/or by nutrient-sensing pathways, and this evidence is discussed in section 11.4. However, the nature of physiological costs will depend upon resource acquisition modes. So-called "income breeders" are those organisms that maintain no energy reserves. Their current reproductive rate depends entirely on current food intake and such organisms have "direct costing" of reproduction (Sibly and Calow 1986). For income breeders, trade-offs between different life history traits will depend largely on current foraging strategies rather than physiological trade-offs *per se*. On the other hand, "capital breeders" are those able to stockpile energy reserves, either during development or through times of glut. Capital breeders exhibit "absorption costing" (Sibly and Calow 1986), i.e., where costs can be buffered. Reproductive rate can therefore be maintained at a level that is not directly linked to the prevailing conditions. In this case, decisions concerning which

resource allocation strategy to adopt are more complex. Many species will fall somewhere between income and capital breeders. To date, the majority of theoretical and empirical investigation has been into systems that exhibit capital breeding. Interestingly, even in organisms such as *D. melanogaster* that can maintain some energy reserves, reproductive activity is tightly linked to external resource levels. This can be seen in the remarkably tight temporal correlation between mortality rate and reproductive schedule in *D. melanogaster* switched between good- and poor-quality diets (Mair *et al.* 2003).

11.3.2 Evolutionary costs of reproduction

Evolutionary trade-offs are revealed by the existence of fixed life history strategies that differ between individuals. For example, artificial selection for high early reproductive rate often results in a correlated response in shortened lifespan, and *vice versa* (e.g., Rose and Charlesworth 1981, Partridge and Fowler 1992, Sgrò and Partridge 1999). These observations reveal survival costs of reproduction because individuals cannot normally show high reproductive rate *and* long lifespan. Such data provide important evidence for antagonistic pleiotropy (Williams 1957). Mechanisms underlying evolutionary costs of reproduction have been revealed by testing the effects of abolishing egg production in lines artificially selected for early and late age reproduction (Sgrò and Partridge 1999), and by assessing hormone titers in wing polymorphic crickets (Harshman and Zera 2007; Chapter 24 and Sections 11.3.5 and 11.4 below). Nevertheless, there is relatively little mechanistic work in this area so far. This is an important oversight because it is not yet clear whether physiological and evolutionary trade-offs occur via the same underlying mechanisms. It would be interesting to know, for example, whether individuals selected for early- or late-age reproduction retain equal capacity to express physiological trade-offs; that is, whether the effects underlying these different kinds of trade-offs are additive. It would also be useful to know whether nutrient signaling evolves during artificial selection for early- and late-age reproduction.

A further type of evolutionary trade-off is found in the broad-scale differences between reproductive output and fecundity that occur between species, as identified in comparative analyses (Partridge and Gems 2006). Such patterns must be products of selection for different reproductive strategies, but very little mechanistic work has yet been conducted. It would greatly illuminate the study of reproductive costs to test for differences in the expression or sequence of genes influencing trade-offs in different populations or species. The study of evolutionary gerontology or "Evo-Gero" (Partridge and Gems 2006) is therefore a promising new field. One of the few examples of this kind of mechanistic work to date concerns the lifespan extending *Methuselah* gene in *D. melanogaster* (Lin *et al.* 1998), which shows an intraspecific geographic cline in sequence variation (Schmidt *et al.* 2000).

11.3.3 Mechanisms underlying reproductive costs

Current reproduction could trade off with future reproduction or with lifespan, either because it diverts resources away from somatic maintenance or because it causes damage. Both explanations predict a causal and negative relationship between reproductive rate and future reproduction/survival. Long-lived animals often show increased resistance to heat and other stresses (see Section 11.7 and review by Partridge *et al.* 2005a). However, increasing evidence from studies of model organisms suggests that reproductive trade-offs arise because of links between resource acquisition (diet and nutrients), metabolism, lifespan, and reproduction (Flatt 2009). The main lines of evidence for this conclusion are as follows (adapted from Flatt and Schmidt 2009):

- The existence and extent of trade-offs between reproduction and lifespan depend upon nutrient levels (e.g., Chapman and Partridge 1996, Marden *et al.* 2003).
- The increased lifespan seen in *D. melanogaster* lacking a germ line is accompanied by reduced levels of glucose and trehalose (Flatt *et al.* 2008b), suggesting that the germ line is intimately involved in nutrient signaling.

- The increased lifespan seen in *C. elegans* mutants without a germ line depends upon the presence of a downstream component (Daf-16/FOXO) of the insulin/IGF-like signaling (IIS) pathway (Arantes-Oliveira *et al.* 2002), showing that the extension of lifespan is dependent on intact nutrient sensing pathways.
- The removal of germ cells in *C. elegans* leads to fat deposition throughout the body and modulation of the extent of fat deposition can itself alter lifespan (Wang *et al.* 2008). This again suggests a link between the germ line and nutrient metabolism.
- *C. elegans* that lack germ cells (but not the gonad itself) show increased lifespan that cannot be further extended by dietary restriction (DR). Hence the removal of the germ line and the effects of DR appear to be non-additive and may represent part of the same mechanism (Crawford *et al.* 2007).

Although the evidence for a role of nutrients in mediating reproductive costs is increasing, the details of the links between all these processes are not yet well understood (Flatt 2009). The majority of the mechanistic data come from intraspecific phenotypic or genetic manipulations resulting in lifespan extension. These data therefore help us to understand the trade-off between reproduction and lifespan. There are, however, few mechanistic data on the mechanisms underlying trade-offs between current and future reproductive rate, or on mechanisms underlying evolutionary trade-offs in general.

In the following sections we outline some of the known mechanisms that influence the trade-off between current reproductive rate and lifespan: nutrients and nutrient sensing, hormones, immunity, and damage repair mechanisms.

11.3.4 Nutrients, nutrient sensing, and costs of reproduction between reproductive rate and lifespan

Nutrient signaling can influence the extent and shape of reproductive trade-offs (Fig. 11–1) and may allow individuals to exhibit plastic trade-offs by shifting resources from reproduction to somatic maintenance. Whether these trade-offs are mediated by organisms physically shifting resources from one process to another or by molecular signaling is not yet known (see Section 11.5). It is now well established that DR leads to extended longevity in yeast, flies, worms, rodents, and perhaps primates, as well as resulting in beneficial effects on health in humans (reviewed in Partridge *et al.* 2005b). These phenotypic manipulations are sometimes associated with decreased age-specific reproductive output (e.g., Chapman and Partridge 1996, Toivonen and Partridge 2009), which implies that how nutrients are sensed and used may underlie the trade-off between reproductive rate and survival. Consistent with this, there are many studies in which genetic manipulations have been used to alter expression in components of nutrient sensing pathways such as in the IIS and target of rapamycin (TOR) pathways. These manipulations usually result in increased lifespan and are sometimes (although not always, Grandison *et al.* 2009), associated with decreased age-specific reproduction (Clancy *et al.* 2001, reviewed in Toivonen and Partridge 2009).

Great strides have recently been made in identifying the specific nutrients that influence reproductive trade-offs (Skorupa *et al.* 2008). An emerging theme is that such trade-offs are not simply founded upon variation in resource levels (e.g., calories) but by the balance of nutrients available (Mair *et al.* 2005, Grandison *et al.* 2009). Studies in *D. melanogaster* (Lee *et al.* 2008b) and in field crickets *Teleogryllus commodus* (Maklakov *et al.* 2008b) reveal that lifespan and reproductive rate can be maximized by different diets. Components of the diet such as casein, in *D. melanogaster* (Min and Tatar 2006), and essential amino acids such as methionine, in *D. melanogaster* and rodents (Miller *et al.* 2005, Zid *et al.* 2009), alter lifespan. In a series of ingenious dietary component add-back experiments, methionine has been identified as the amino acid that promotes longer life in *D. melanogaster* (Grandison *et al.* 2009). What is intriguing is that the addition of methionine to the diet was able to restore egg production in long-lived flies on an otherwise restricted diet. This finding apparently indicates that the trade-off between reproductive rate and lifespan can be abolished if dietary nutrients are finely tuned (Grandison *et al.* 2009, see Section 11.5). The known roles of the two main nutrient signaling pathways in influencing trade-offs between reproductive rate and lifespan are outlined below. Much remains to be discovered about the

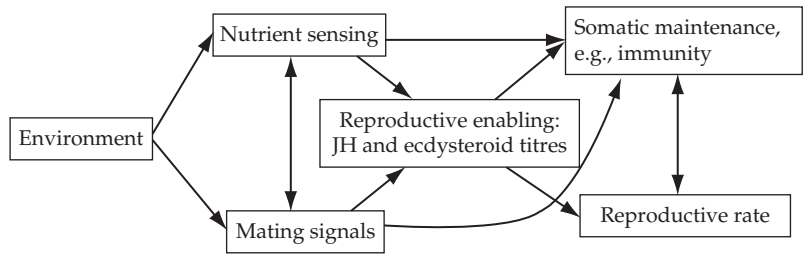

Figure 11-1 Potential links between the major players that determine reproductive costs. Nutrient sensing pathways detect nutrient levels/condition determined by the local environment and may determine the extent of reproductive activation (enabling) that can occur, or may have only direct effects on somatic maintenance. The quality or quantity of mating signals (and responses to them) may depend on the environment directly or indirectly, via nutrient sensing. Mating signals can determine the extent of reproduction, but may also have direct effects on somatic maintenance that bypass reproductive enabling. Information on the level of reproduction is integrated and inputs into the level of reproduction and somatic maintenance. Reproductive hormones may themselves directly suppress somatic processes such as the immune system. The challenge is to determine the existence and relative importance of these links.

exact role of these pathways in mediating the costs of reproduction more generally.

11.3.4.1 Insulin signaling

Elegant experiments employing single gene mutations in the nutrient sensing IIS pathway have shown that reduced insulin signaling can lead to extended longevity (e.g., by manipulation of *chico*, *InR*, *Lnk* and *dilp* 2,3, and 5; Clancy *et al.* 2001, Tatar *et al.* 2001b, Ikeya *et al.* 2002, 2009, Broughton *et al.* 2005, Grönke *et al.* 2010, Slack *et al.* 2010). This effect is sometimes also associated with reduced fertility or female sterility (e.g., Clancy *et al.* 2001, Tatar *et al.* 2001b) and the effects of insulin signaling appear to be evolutionarily conserved (Partridge *et al.* 2005a). These findings support the general idea of a trade-off between longevity and reproductive rate that is mediated at least in part by insulin signaling. However, not all of the genes in the insulin pathway whose manipulation leads to extended longevity also lead to reduced fecundity. For example, *chico* heterozygotes and certain long-lived flies with altered dFOXO signaling do not have impaired fertility (reviewed in Toivonen and Partridge 2009). Nevertheless, reproductive trade-offs may sometimes be apparent only under stressful conditions such as food limitation, so may be overlooked. For example, the fertility of *Indy* long-lived mutants appears normal under food abundance, but is reduced under food limitation (Marden *et al.* 2003). This is not a *Drosophila*-specific phenomenon. There are similar inconsistencies in *C. elegans* (e.g., for the *Daf*-2 gene) and in mice, where long life is associated with decreased fertility in some but not all strains that are long-lived as a result of manipulations to insulin signaling (Toivonen and Partridge 2009). It seems that there is no obligate relationship between increased lifespan and decreased fertility mediated by insulin signaling, and reasons for these discrepancies are discussed below (see Chapter 13).

Insulin signaling may influence reproductive costs because this pathway conveys information about the total resource budget. Alternatively, it may be the case that insulin signaling genes themselves alter reproductive hormone levels. For example, mutations in the *Drosophila* insulin receptor (*DInR*) are associated with both reduced levels of ecdysteroid release from ovaries *in vitro* and with reduced juvenile hormone (JH) biosynthesis (Tatar *et al.* 2001b). The latter effect was seen *in vitro* using the *chico* mutation in some experiments but not others (Richard *et al.* 2005).

11.3.4.2 TOR signaling

The target of rapamycin (TOR) nutrient sensing pathway is responsive to amino acid levels and also interacts with insulin signaling. As for IIS, mutations in members of the TOR pathway also extend lifespan in *Drosophila* (Kapahi *et al.* 2004) and in yeast. It is not yet known whether lifespan extension via TOR signaling also reduces fertility, so it is not yet clear the extent to which TOR influences fer-

tility and survival trade-offs. The impact of the cross-talk between the IIS and TOR pathways on reproductive rate is also not yet known (Flatt 2009).

11.3.5 The presence of a germ line and costs of reproduction between reproductive rate and lifespan

Evidence for the importance of the germ line in mediating trade-offs between reproduction and lifespan comes from classic studies in which extended lifespan was seen in animals that lay no, or reduced numbers of, eggs (Maynard Smith 1958). The underlying hypothesis is that in intact animals, nutrients are signaled to the germ line to match reproductive rate to nutrient levels. In the absence of the germ line, resources are not allocated to reproduction and stay in longevity assurance, leading to longer lifespan.

Early studies showed increased lifespan in *grandchildless* females of *Drosophila subobscura* that lacked a germ line (e.g., Maynard Smith 1958). Sterilization of females by low doses of X-irradiation can also produce extended lifespan (e.g., in *Ceratitis capitata*; Chapman *et al.* 1998). These results are consistent with the idea that some aspect of reproductive activity leading to egg production is costly. Similar results have been obtained from studies of germ line ablation in *C. elegans* (e.g., Leroi 2001). Elevated rates of egg-production and exposure to males cause a drop in *D. melanogaster* female lifespan (Partridge *et al.* 1987); these effects could be due to the costs of egg production or costs of mating. However, elevated egg production by itself can lead to decreased lifespan in both phenotypic manipulations and in selection experiments (e.g., Partridge *et al.* 1987, Sgrò and Partridge 1999). The latter study provides one of the few pieces of mechanistic evidence for an evolutionary trade-off between current reproductive rate (egg production) and lifespan. The ovo^{D1} mutation conferring sterility on females was crossed into lines of flies selected for early- and late-age reproduction. The subsequent patterns of mortality in egg laying and non-laying females suggested that reproduction (egg laying) caused a delayed wave of increased mortality. This suggests that similar pathways can operate in both physiological and evolutionary trade-offs.

Recent studies have highlighted that it is the developmental stage at which the germ line is inactivated that is important. For example, early ablation of the germ line during development leads to no effect on lifespan (Barnes *et al.* 2006), possibly because of increased signaling of the somatic gonad that can proliferate in the absence of the germ line. However, later-acting germ line silencing had the predicted effect of increasing lifespan (Flatt *et al.* 2008b). Germline removal extended lifespan in both sexes. However, the nature of any trade-off in males is not yet known. Germline-lacking males will court and mate at levels similar to those of control males (e.g., Chapman *et al.* 1993), but the investment of such males in courtship and mating, and their reproductive hormone titers, have not yet been tested.

11.4 Reproductive hormones as mediators of trade-offs between reproductive rate and lifespan

Hormones have disparate effects that underlie many different life history traits and therefore they have long been thought to play a central role in mediating life history trade-offs (Harshman and Zera 2007). In insects, activation of reproduction is influenced by the balance of JH and ecdysteroid signaling (Nijhout 1994). For example, in *D. melanogaster* females, reproduction is controlled by the actions of 20-hydroxyecdysone (20E) and JH. The balance between 20E and JH determines whether oocytes undergo vitellogenesis (the uptake of yolk proteins, stimulated by JH) or apoptosis (stimulated by 20E) (Soller *et al.* 1999). In males, JH is essential for the formation and function of the accessory glands, which synthesize much of the non-sperm part of the ejaculate. 20E is also important; it is synthesized in the male prothoracic gland and regulates spermatogenesis and accessory gland development.

Evidence for the involvement of hormones in life history trade-offs between reproduction and dispersal comes from selection experiments in wing-polymorphic crickets. Elevated early reproduction is linked with high JH and ecdysteroid titres in short-wing morphs. This elevated early reproductive rate is associated with a low dispersal ability

(i.e., the presence of short wings). Consistent with this, the application of a JH mimic to long-winged morphs of *Gryllus firmus* produces changes in lipid metabolism, ovarian growth, and flight muscle to levels more characteristic of short-winged morphs (Harshman and Zera 2007). The role of JH on lifespan and in mediating trade-offs has been investigated in *Drosophila* (Flatt and Kawecki 2007), and there appears to be no obligate trade-off with fecundity. Similarly, reductions in signaling by the ecdysteroid pathway (achieved via mutations to ecdysone receptor, *EcR*) cause an increase in lifespan with no apparent decrease in fecundity (Simon *et al.* 2003). Further support for a role of JH in influencing lifespan comes from studies of diapausing insects. Elevated JH is associated with reduced lifespan in the Monarch butterfly, and levels of JH are also low in the diapause stage (in which aging is reduced) of several invertebrate species (reviewed by Flatt *et al.* 2005). However, it is not yet clear whether these associations covary with reproductive costs. JH is essential for vitellogenesis, which can itself be costly (e.g., Partridge *et al.* 1987, Sgrò and Partridge 1999), and mating also causes a significant increase in JH levels in females of many insects.

11.5 Male seminal fluid proteins as mediators of trade-offs between reproduction and lifespan in females

There are considerable costs for females in mating itself (Fowler and Partridge 1989). Using transgenic males in which a population of seminal fluid producing cells is genetically ablated (Chapman *et al.* 1995) shows that the cost of mating in female *D. melanogaster* is explained by the transfer of seminal fluid proteins. Furthermore, there is a dose–response effect of seminal fluid proteins on lifespan and lifetime reproductive success. Whether there is one seminal fluid protein or many that are responsible for this cost of mating remains an open question. There are four seminal fluid proteins that are toxic when ectopically over-expressed (Mueller *et al.* 2007). In addition, there are associations between sequence variation at two seminal fluid protein loci and the differences in lifespan between virgin and singly mated *D. melanogaster* females (Fiumera *et al.* 2006).

One of the candidate costly seminal fluid proteins is the sex peptide (SP; Wigby and Chapman 2005, Fricke *et al.* 2010). The potential involvement of SP in mating costs is intriguing because it also activates systems known to be costly. For example, likely candidates for SP-mediated mating costs in female *D. melanogaster* are JH, the immune system, and/or nutrient intake. SP causes the release of JH-BIII from the corpora allata, which stimulates vitellogenesis and hence oocyte progression in the ovary (Soller *et al.* 1999). High levels of JH could decrease lifespan directly and are already found to be negatively associated with length of life in other insects (Flatt *et al.* 2005). JH might also be costly because it increases vitellogenesis, i.e., egg production, which has itself been shown to be costly (e.g., Sgrò and Partridge 1999). JH could also incur costs because it suppresses the immune system, as shown in *Tenebrio molitor* (Rolff and Siva-Jothy 2002). Alternatively, mating costs in females may result from effects of increased SP on immunity, independent of JH levels. Although SP could be costly via its effects on female feeding rate, behavioral observations have correlated feeding with the ability to lay eggs and not with female survival *per se* (Barnes *et al.* 2008). What is now needed are measurements of the potential reproductive costs following manipulations of immune and hormonal pathways, independent of the receipt of SP and the presence or absence of a germ line.

11.6 The immune system as a mediator of costs between current reproductive rate and survival

The idea that maintaining the immune system, mounting an effective immune response, or evolving an immune response may be costly and may be traded off against other life history components, such as reproduction, has gained increasing credence (reviewed in Lawniczak *et al.* 2007). Much work has been done in birds and has focused on males and their responses to testosterone. In *D. melanogaster*, constitutive expression of antimicrobial peptides via the Toll (Tl) pathway results in female sterility because Tl, as well as being a switch to upregulate immunity, is also involved in dorso–ventral pattern formation in eggs. This suggests

that there are likely to be costs of antibacterial immune defense over and above those of antibacterial peptide production itself (reviewed in Lawniczak *et al.* 2007). This resembles a "design" (rather than "allocation") trade-off, i.e., where genes are selected in one context because of evolutionary history but come to fulfill other new functions, sometimes with lowered efficiency.

Reproductive activity in *D. melanogaster* males is also traded off against the ability to clear bacteria following an experimental injection (McKean and Nunney 2001). The potential trade-offs between immunity and sexually selected traits in males became of interest because of the idea that hormone-dependent traits are "honest" signals of male quality under the immunocompetence handicap hypothesis (Sheldon and Verhulst 1996, Lawniczak *et al.* 2007). Mating-induced declines in immune function have been shown in damselflies and *D. melanogaster* males and immunity-related genes show significant changes in expression following mating in *D. melanogaster* females (reviewed in Lawniczak *et al.* 2007). Taken together, the data suggest that mating costs could be incurred because mating suppresses immunity, which then leads to a decline in fitness. This could be because of allocation trade-offs, limited resource pools, or a type of "design" trade-off as mentioned above (e.g., perhaps males suppress female immunity because high immunity otherwise impairs fertilization).

Despite the hypothesis that trade-offs between reproduction and the immune system are likely to be mediated by hormones (Flatt *et al.* 2008a), there are relatively few experimental data so far on the mechanistic links between JH and the immune system (Flatt *et al.* 2005, see Chapter 13). In the mealworm beetle, *Tenebrio molitor*, mating decreases the activity of phenoloxidase (PO), a major humoral effector system, in both sexes (Rolff and Siva-Jothy 2002). Furthermore, the downregulation of PO is mediated by JH. Experimental injections of JH into male *T. molitor* also increase the attractiveness of pheromones produced by males, whilst simultaneously suppressing immune function (affecting both PO activity and ability to encapsulate non-self). Hence, the costs of mating could arise because mating produces JH, which suppresses immunity, leading to a decline in fitness. A caveat is that JH titer was not measured, and accurate measurement of JH titers *in vivo* remains an empirical hurdle in the study of insect endocrinology. However, new techniques based on mass spectrometry are emerging. In summary, it is not yet clear whether the links between immunity and reproductive rate occur because of resource trade-offs or because elevated reproductive rate leads to increased damage, perhaps leading to disease susceptibility.

11.7 Damage as a mediator of trade-offs between current reproductive rate and survival

The possibility that trade-offs between reproduction and survival are influenced by resource allocation is described above, but it is also possible that such trade-offs occur because of increased levels of damage. Such damage could occur because reproductive processes cause direct damage to the soma or alternatively suppress repair or protection mechanisms (Salmon *et al.* 2001, Wang *et al.* 2001). Consistent with the idea of direct damage to the soma are the positive associations between the level of reproductive activity and the level of reactive oxygen species (ROS; e.g., Dowling and Simmons 2009). Many long-lived strains of *D. melanogaster* and *C. elegans* are also resistant to heat stress and to oxidative stress (reviewed in Partridge *et al.* 2005a). Female *D. melanogaster* that overexpress a heat shock chaperone (Hsp70, which protects proteins from the effects of misfolding at high temperatures) extends lifespan but reduces egg hatchability (Silbermann and Tatar 2000). This is consistent with the idea that protection mechanisms such as Hsp70 expression are costly and can trade-off with life history traits. Further evidence for this view comes from the links between elevated reproductive rate and decreased immunity (see Chapter 23). Data on whether trade-offs between reproduction and immune function result from resource allocation decisions may help to resolve whether damage is manifested directly because of the effects of elevated reproduction, or indirectly via suppression of protection mechanisms. It will be interesting to discover how often elevated damage (e.g., ROS) features in pathways that mediate reproductive costs.

11.8 Resource allocation: Allocation versus adaptive signaling

A common theme of much of the experimental work described above is the lack of consistency with which extended lifespan leads to decreased fertility, especially in studies that test the effects of single gene mutations. This observation is important because it challenges the view that "Y" resource allocation models are an appropriate framework in which to study trade-offs. Such models refer to the situation where a set pool of resources are allocated to reproduction or somatic maintenance but not both (Van Noordwijk and De Jong 1986). In this final section we explore these ideas and possible explanations for these discrepancies (Flatt and Promislow 2007).

There are three main lines of evidence to suggest that the literal application of "Y" models may not fully explain the proximate mechanisms underlying the relationships between life history traits. Firstly, the elimination of reproduction does not necessarily extend life span. In *C. elegans* ablation of the whole gonad, genetic sterilization or chemical inhibition of egg production all fail to extend lifespan (Hsin and Kenyon 1999, Arantes-Oliveira *et al.* 2002, reviewed in Barnes and Partridge 2003). Also, *daf*-2 gene mutants of *C. elegans* have a significantly increased longevity over the wild type, but ablation of the wild-type germ line does not extend lifespan more than in *daf*-2 mutants. This suggests that *daf*-2 does not extend lifespan simply through re-allocating resources from reproductive processes (Leroi 2001). Secondly, although mutations that extend lifespan generally cause reduced fecundity, some apparently also increase fecundity (e.g., *daf*-2 in worms, *EcR* in flies), or have no effect on fecundity (see Barnes and Partridge 2003). Recent findings using *D. melanogaster* suggest that, given a carefully calibrated balance of dietary components, trade-offs between reproduction and lifespan can be avoided (Grandison *et al.* 2009). Thirdly, there are marked sex differences in the response of males and females to interventions that increase longevity. A mutation in the IGF-1 receptor in the mouse is found to extend female but not male lifespan. Similarly, lifespan extension in both *chico* and *EcR* mutant females is much greater than seen in males (Clancy *et al.* 2001, Simon *et al.* 2003). Such sex differences require an explanation.

A proximate explanation for this challenge to traditional life history theory is that trade-offs are mediated by molecular signals (Leroi 2001). This hypothesis is derived from observations in *C. elegans,* where ablation of the gonad fails to extend lifespan, but ablation of the germ line alone (prior to proliferation) causes lifespan to double (Hsin and Kenyon 1999). This may indicate that reproductive ability *per se* is not the key to determining longevity. Instead, what is important is the presence or absence of specific reproductive tissues. A model compatible with these findings is that the proliferating germ line produces a signal that down-regulates life span, which is counter-balanced by an equal and opposite signal from the somatic gonad (Fig. 11-2, adapted from Barnes and Partridge 2003). Ablation of the whole gonad leaves longevity unaffected (because both signals are removed) but ablation of the germ line eliminates the negative signal, extending lifespan. The putative molecular signals represent arbitrary connections between life history traits and may be independent of resource availability. Importantly, the hormonal effects of such gonad ablations in the worm are now being elucidated (Gerisch *et al.* 2007, see Chapter 22). An alternative view is that resource allocation is important in the evolution of life histories, but that life history traits do not trade-off via a literal apportioning of resources (Fig. 11-2). For example, as described above, the use of nutrients in reproduction itself might generate damage, rather than simply diverting resources from somatic protection (i.e., longevity; Barnes and Partridge 2003). An interesting empirical avenue is to track the movement of resources between different tissues. Studies that track the passage of amino and fatty acids between tissues in the wing dimorphic cricket *Gryllus firmus* provide evidence that the literal diversion of resources can occur. Long-winged morphs divert more resources to the production of triglycerides used for flight and less to ovarian protein than the short-winged morph (reviewed in Harshman and Zera 2007).

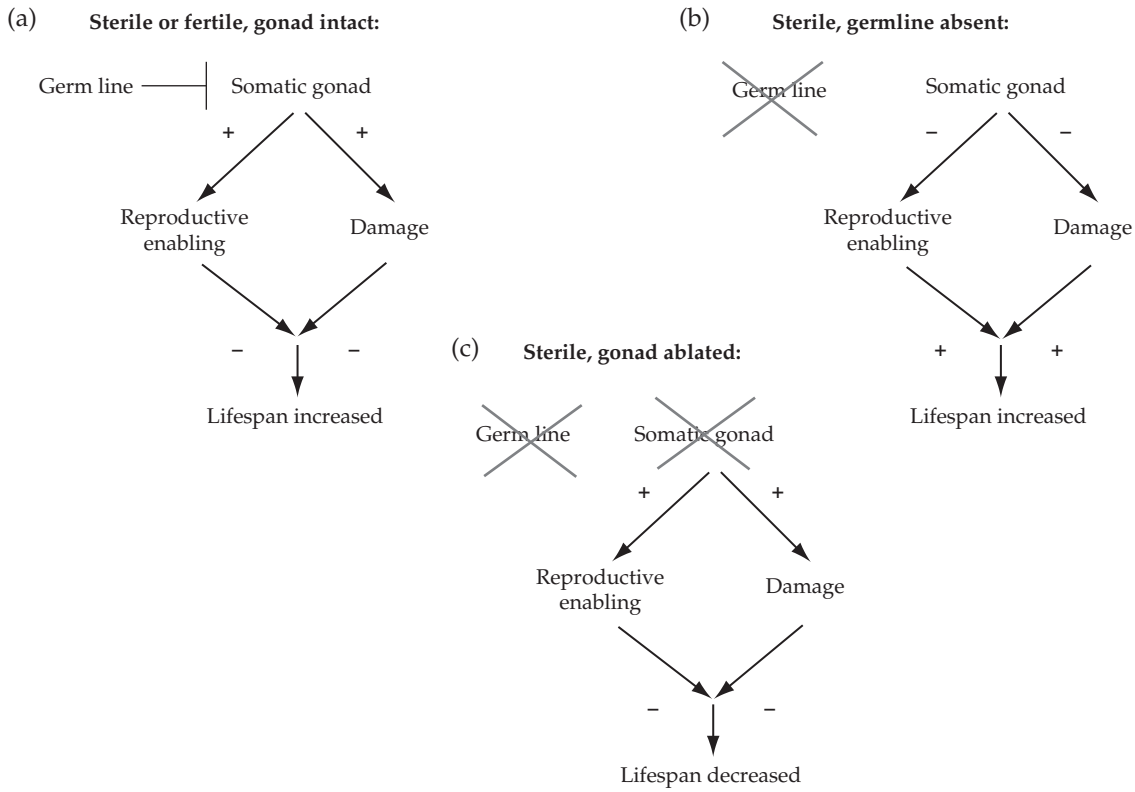

Figure 11-2 A model for adaptive reproductive signaling. Here, signals from the germ line and somatic gonad co-ordinate reproduction and life span adaptively. An intact germ line (a) causes allocation to reproduction even if the organism is otherwise sterile. In (b), the absence of a germ line allows signaling from the somatic gonad to cause investment in somatic processes. Whole gonad ablations (c) knock out the sources of all signals, mimicking a default state where investment in reproduction is high at the cost of lifespan. Reproduction and longevity can be uncoupled by manipulations to germ line or somatic gonad signaling, but the connections between the traits are adaptive because in an unmanipulated state, reproductive state is matched to local environmental conditions. Longevity and reproduction can be unlinked because costs of reproduction may still be maintained in non-reproductive individuals by the allocation of resources to reproductive function where this allocation is not blocked by sterilization. Adapted from Barnes and Partridge (2003).

The apparent absence of trade-offs between reproduction and survival could also arise because of the methodology employed. For example, gonad ablation is an extreme manipulation, so perhaps it is not surprising that resources are not shifted from reproduction to maintenance under these conditions. In addition, mutational analysis of the cost of reproduction may not be an appropriate method for confirming the absence of trade-offs, because mutations of large effect (such as those in the insulin signaling pathway) could have pleiotropic effects that obscure the normal relationships between different life history traits (Harshman and Zera 2007). The existence and shape of many trade-offs observed in the laboratory may also be significantly altered under stressful conditions and in situations where there are interactions with other ecological costs.

11.9 Costs of reproduction in a fitness-based framework

Central to studies of costs of reproduction should be an accurate quantitative assessment of key components of life history in terms of fitness—the sum of the products of individual age-specific survival probabilities times age-specific reproductive output (Charlesworth 1980). As described above, many phenotypic and genetic manipulations can extend longevity, but do so by rescheduling reproductive rate. Therefore it is necessary to be

cautious and avoid viewing all manipulations that increase longevity as beneficial in fitness terms. This issue is acute, particularly in the experimental study of life history, because it is essential to assess the extent to which we can learn about fitness and fitness trade-offs from long-lived mutants. To assess fitness accurately, consideration is needed of the effects of manipulations on both survival and the timing of reproduction (i.e., to measure the effect of manipulations on current versus future or residual reproductive output; see Fig. 11-3 for a useful way to present such data). However, fitness can be defined in several ways (Endler 1986, Clutton-Brock 1988) and the strategy that maximizes fitness may be different for decreasing, stable, or expanding populations.

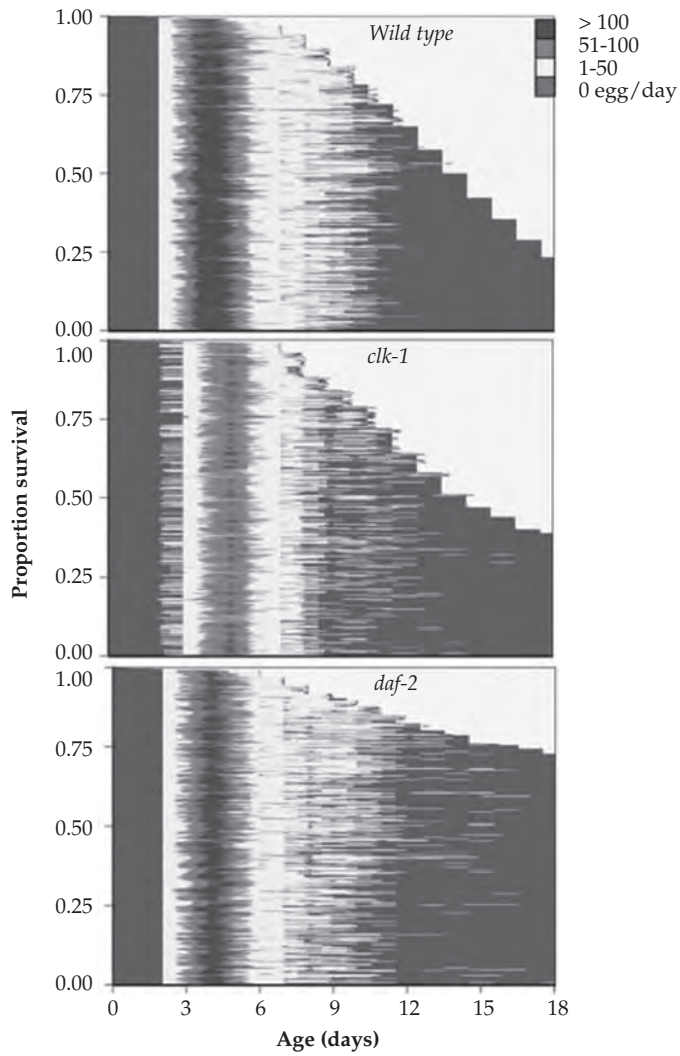

Figure 11-3 Event history diagrams. Individual event histories can help determine when in the lifetime of an organism the most important reproductive events occur, with respect to fitness. Here, the event history diagrams depict individual life histories of *C. elegans* wild type, *clk-1* and *daf-2* strains. Each row represents a single individual in increasing order of lifespan (for $n = 1000$, 800, and 800 individuals, respectively). The degree of shading depicts the amount of daily egg production. Despite increased lifespan for both *clk-1* and *daf-2* strains, a high level of early reproduction in the wild type confers a greater intrinsic rate of increase. Reproduced, with permission, from Chen *et al.* (2007).

A frequently adopted approach is to correlate changes in life history traits with lifetime reproductive success (LRS). However, a drawback to the use of LRS is that it fails to account for differences in the individual value of offspring (Brommer *et al.* 2002). In addition to genotypic and phenotypic variation in the quality of offspring, there is strong selection on the timing of offspring production from a purely demographic standpoint. Two individuals with identical LRS and lifespan can have different fitness due to the way that they partition current to future reproduction. Fitness may be maximized at intermediate levels of LRS due to a trade-off between offspring production and the timing of offspring production (Brommer *et al.* 2002). The costs of reproduction may also be age-dependent so that the trade-off might only be detected at some ages (or across some age classes), but not at others. The use of rate-sensitive measures of fitness may well be an advantage in this context (for a review see Metcalf and Pavard 2007). The important message is that it is not enough to consider effects of, for example, single gene mutations on lifespan or LRS alone to make robust conclusions about ultimate costs. To do this, findings should be placed in a fitness-based framework to investigate trade-offs between current and future reproduction (residual reproductive value).

11.10 New directions

In this final section we discuss a few of the promising, and perhaps underutilized, avenues for future research, which together will generate a richer and deeper mechanistic understanding of reproductive costs.

11.10.1 Mechanistic data are incomplete

The majority of mechanistic studies to date have used phenotypic or single gene manipulations to examine phenotypic responses, and most of these have examined the relationship between reproductive rate and lifespan. There are few mechanistic data on the reproductive trade-offs between current and future reproduction. This leaves open the opportunity for a much broader investigation of the mechanisms underlying the responses in selected lines, and of the extent to which they overlap with the responses seen upon phenotypic manipulations, as well as of the differences between populations and species. A stronger integration of evolutionary and molecular genetics of life history trade-offs is also necessary and we look forward to the comparative study of mechanisms and trade-offs that is now possible with the advent of new genomic technologies (Box 11-2).

11.10.2 The evolution and conditional economics of reproductive costs

As has been noted by many, current trade-offs are not necessarily indicative of the existence or level of costs that existed earlier in evolutionary time (Stearns 1992, Rowe and Day 2006). However, using experimental evolution and model systems with short generation times it should be possible to track the evolutionary trajectory of life history costs. For example, female costs of reproduction can indeed evolve even over relatively modest evolutionary timescales (Linklater *et al.* 2007). The evolution and mating economy of costs of reproduction is of crucial importance in understanding how mating systems are shaped by selection (Fricke *et al.* 2009). The role of the environment in determining the magnitude of costs of reproduction has long been realized, but tests of the effects of systematic manipulations of the local environment on the magnitude of reproductive costs are scant (Cornwallis and Uller 2010, Fricke *et al.* 2010). Manipulations of the local environment can include resource levels, but also disease levels and access to the opposite sex (Kokko and Rankin 2006). Another potentially promising area is the analysis of comparative demography, specifically the idea that there may be predictable differences between species in the shape of the cost of reproduction with age. Related to the evolution of reproductive trade-offs is also whether their effects are reversible (e.g., Mair *et al.* 2003) and whether they alter the rate of aging itself as well as lifespan (e.g., Priest *et al.* 2008). In addition, trans-generational reproductive trade-offs may have significant effects on inclusive fitness, but as yet there are no mechanistic data to probe the potentially important mechanisms involved.

11.10.3 Integration of life history data from social species

There is a gap in integrating information from the study of life histories from social and non-social species (see Chapter 20). There is a rich source of novel mechanistic data emerging from the study of social species, and there are several ways in which the study of life history and reproductive costs under sociality can provide new insights. Three examples are briefly discussed here.

11.10.3.1 Novel insights into soma (growth) and reproductive trade-offs

Social colonies often have an initial growth phase in which only workers are produced, followed by a reproductive phase in which sexuals (new queens and males) emerge. There may also be an orphan stage in which the colony continues after the death of the queen, with workers sometimes producing males. There is therefore a clear and easily measurable resource allocation to soma (growth of worker numbers) and reproduction (sexuals). This offers new and experimentally tractable ways in which to investigate trade-offs above the level of the individual, using principles that pertain at the individual level in non-social species (Bourke and Franks 1995).

11.10.3.2 Reproductive conflicts

A fundamental difference between eusocial and non-social species is the division between germ line and soma (Bourke and Franks 1995). In eusocial species, the germ line resides in the reproducing individuals (queens and males), with most of the soma in the sterile workers. Because of asymmetries in relatedness between sexuals and workers in a social colony, the interests of the germ line and soma are not perfectly aligned as they are within non-social reproducing individuals. This opens up novel sources of conflict between germ line and soma, and new opportunities for the expression of reproductive costs.

11.10.3.3 Maximizing reproductive rate and longevity

If ever there was a "Darwinian Demon" that could reproduce at high rate over a long lifespan, then it is likely to be found within social insect queens, some species of which can live and reproduce for decades (Keller and Genoud 1997). The extreme longevity of queens over that of workers is achieved in individuals that bear identical genotypes. This indicates, therefore, that differential gene expression can, at a stroke, produce startlingly different life histories through phenotypic plasticity. The gene expression differences that underlie the queen–worker divide are now being characterized (e.g., Keller and Jemielity 2006). There is much to gain from the study of the mechanisms by which genes can be turned on and off to produce such extremes in lifespan and reproductive output (Gräff *et al.* 2007), and in searching for parallels in non-social systems.

11.11 Summary

1. The costs of reproduction are a superlative example of a life history phenomenon amenable to mechanistic analysis at all levels and great progress has been made in understanding the underlying physiological and genetic basis of such costs.
2. It is important to consider physiological and evolutionary trade-offs in the study of reproductive costs and to determine whether there are common underlying mechanisms.
3. Our brief review of the current state of knowledge of the mechanisms underlying reproductive costs highlights that several pathways are emerging as important. These include nutrients and nutrient sensing mechanisms, reproductive hormones, immunity, and reproductive signals from males. However, we still lack knowledge of how, and in which order, these pathways are connected.
4. The application of new genomic technologies will yield new insights into the genomic signature of reproductive costs both within and between individuals and populations, and between species.
5. It is important to measure reproductive costs in a fitness-based framework. A wider application of such approaches may resolve some of the inconsistencies in the effects of manipulations of pathways implicated in causing reproductive costs.
6. Several existing lines of research are underutilized. Benefit will be derived from a full understanding of the conditional economics of reproductive costs, as this tells us about the magnitude of reproductive costs, the evolution of the mechanisms by which they occur, and also the evolutionary dynamics which shape them. In addition, the effective integration of

data from the study of life histories in social and non-social systems will reap great rewards.

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