Published in "Journal of Evolutionary Biology 20(5): 1655-1664, 2007"
which should be cited to refer to this work.

Evolutionary biology of starvation resistance: what we have learned from *Drosophila*

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Abstract

Most animals face periods of food shortage and are thus expected to evolve adaptations enhancing starvation resistance (SR). Most of our knowledge of the genetic and physiological bases of those adaptations, their evolutionary correlates and trade-offs, and patterns of within- and among-population variation, comes from studies on *Drosophila*. In this review, we attempt to synthesize the various facets of evolutionary biology of SR emerging from those studies. Heritable variation for SR is ubiquitous in *Drosophila* populations, allowing for large responses to experimental selection. Individual flies can also inducibly increase their SR in response to mild nutritional stress (dietary restriction). Both the evolutionary change and the physiological plasticity involve increased accumulation of lipids, changes in carbohydrate and lipid metabolism and reduction in reproduction. They are also typically associated with greater resistance to desiccation and oxidative stress, and with prolonged development and lifespan. These responses are increasingly seen as facets of a shift of the physiology towards a 'survival mode', which helps the animal to survive hard times. The last decade has seen a great progress in revealing the molecular bases of induced responses to starvation, and the first genes contributing to genetic variation in SR have been identified. In contrast, little progress has been made in understanding the ecological significance of SR in *Drosophila*; in particular it remains unclear to what extent geographical variation in SR reflect differences in natural selection acting on this trait rather than correlated responses to selection on other traits. *Drosophila* offers a unique opportunity for an integrated study of the manifold aspects of adaptation to nutritional stress. Given that at least some major molecular mechanisms of response to nutritional stress seem common to animals, the insights from *Drosophila* are likely to apply more generally than just to dipterans or insects.

Introduction

Stress can be defined as any environmental factor that acts to reduce the fitness of an organism. Thus, almost by definition, environmental stress is one of the most important sources of natural selection, as certified by many specific adaptations evolved to alleviate the consequences of stress (reviewed e.g. in Hoffman & Parsons, 1991; Randall *et al.*, 1997). One of the most ubiquitous causes of stress, at least for animals, is shortage or suboptimal quality of food. Many species must cope with periodical malnutrition or starvation, and even those for which food may seem abundant (e.g. herbivorous insects) may be limited by availability of specific nutrients and the need to cope with toxic secondary chemicals (White, 1993). Because of the central role of energy for organisms, improved resistance to starvation is likely to involve changes at different levels of the phenotype, from intracellular signalling to life-history patterns.

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Much what we know about the evolutionary biology of acute SR, in particular about its genetic and molecular aspects, comes from studies of *Drosophila*, in particular those involving experimental evolution, genetic analysis and dissection of plastic responses to nutritional stress. In addition to the usual advantages of fruit flies as the study system, they are not affected by the ethical and legal concerns associated with imposing acute starvation on vertebrates. Furthermore, the molecular mechanisms of physiological response to nutritional stress seem broadly conserved throughout the animal kingdom (Tatar *et al.*, 2003; Partridge *et al.*, 2005; Arsham & Neufeld, 2006). Thus, insights about the genetics and physiology of adaptation to starvation gained from *Drosophila* may apply more generally than only to dipterans or insects.

Almost a decade ago, Hoffmann & Harshman (1999) reviewed the data on genetic variation in starvation (and desiccation) resistance and correlated life-history patterns, within and among populations of *Drosophila*, as well as between species. In this paper, we review the progress in our understanding of the evolutionary biology of SR gained in the last decade. We take a broad perspective, aiming to integrate insights from field studies, selection experiments, genetic analyses as well as physiological and molecular studies. After a brief description of the methodology, and summarizing the evidence for genetic variation and phenotypic plasticity of SR we devote the central part of the paper to the advances made since Hoffmann & Harshman (1999) in the understanding of the mechanisms of SR, and its relationship with resistance to other stressors and with life-history traits. Subsequently we review the still very limited list of genes thought to contribute to heritable variation in SR, and discuss the ecological and evolutionary significance of SR. Most studies we cite below were carried out on *D. melanogaster*, this species is implied unless we specify otherwise.

Measurement of starvation resistance

Virtually all studies of SR in *Drosophila* have focused on acute starvation, i.e. complete food deprivation of adult flies. Because flies are much more sensitive to dehydration (desiccation), water is provided as water-saturated plugs, stripes of filter paper, or non-nutritional agarose. This can be done on groups of flies; they do not obtain nutrition from carcasses of other flies (Huey *et al.*, 2004). Starvation resistance is usually quantified as the time until death under those conditions. Depending on strain, selection history, previous nutrition and sex, the average time to death under starvation ranges widely between about 20 h (males of sensitive strains previously fed on a rich medium) to more than 200 h (females of selected strains previously maintained under caloric restriction; e.g. Chippindale *et al.*, 1993; Harshman & Schmid, 1998; Harshman *et al.*, 1999a; Baldal *et al.*, 2005; Harbison *et al.*, 2005). Experimental selection for SR typically involves depriving adult flies of food until 50–90% individuals have died; the next generation is then bred from the survivors (Chippindale *et al.*, 1996; Djawdan *et al.*, 1997; Harshman & Schmid, 1998; Harshman *et al.*, 1999a; Bubliy & Loeschcke, 2005; Hoffmann *et al.*, 2005a).

An obvious alternative to acute starvation would be chronic dietary restriction or malnutrition. Yet, even though the effects of dietary (caloric) restriction in *Drosophila* have been intensively studied in the context of ageing (Partridge *et al.*, 2005), the genetic basis and phenotypic correlates of adaptation to dietary restriction have apparently been rather neglected (for an exception see Bochdanovits & de Jong, 2003). In contrast, adaptation to larval crowding was studied relatively often (Guo *et al.*, 1991; Joshi & Mueller, 1996; Sokolowski *et al.*, 1997; Joshi *et al.*, 1998; Borash & Ho, 2001; Sanders *et al.*, 2005). However, food shortage is only one and possibly not the most important aspect of larval crowding (Borash & Ho, 2001). The physiological and life-history adaptations to larval crowding and its ecological significance are likely to be substantially different to those of adult SR; in this review we concentrate on the latter.

Genetic variation for starvation resistance

Most *D. melanogaster* populations seem to harbour ample genetic variation for SR, as evidenced by the rapid and large responses to laboratory selection (up to several-fold increase in time to death) usually observed for this trait (studies reviewed in Hoffmann & Harshman, 1999; also, e.g. Archer *et al.*, 2003; Hoffmann *et al.*, 2005a). An evolutionary change in SR is also often observed as a correlated response to selection on other traits (see below). Additional evidence for genetic variation in SR comes from variation among isofemale lines established from the same population (Hoffmann *et al.*, 2001 in *D. melanogaster*; Hallas *et al.*, 2002 in *D. serrata*). There is also evidence for genetically based differences among populations; we discuss them in a later section of the paper.

Plasticity of starvation resistance

Greater SR requires physiological changes which are likely to trade-off with other fitness-related traits (see the following sections). Thus, natural selection should favour genotypes capable of shifting their physiology towards greater SR in response to cues heralding a period of starvation, such as crowding or declining food quality or quantity. In other words, SR is expected to show some degree of adaptive phenotypic plasticity. In line with this prediction, reducing the amount of nutrition, in particular protein (yeast), offered to adult flies (caloric restriction) increases their SR, with up to twofold difference between females previously fed *ad libitum* yeast and those given no yeast (Chippindale *et al.*, 1993; Leroi *et al.*, 1994; Kapahi *et al.*, 2004; Piper *et al.*, 2005; Burger *et al.*,

2007). However, prolonged exposure to dietary restriction has been reported to reduce SR (Burger *et al.*, 2007). Zwaan *et al.* (1991) reported that SR of adult flies also increases with the larval density they experienced, but a recent study (Baldal *et al.*, 2005) failed to replicate that result. Increased resistance to starvation and some other types of stress forms a part of the diapause phenotype, induced by low temperature and short photoperiod (Tatar & Yin, 2001; Schmidt *et al.*, 2005b). Finally, Harshman *et al.* (1999a) demonstrated heritable variation for the inducibility of SR. They selected flies for survival on decomposing lemon (an unsuitable resource) as the only source of food. The selection lines not only became more resistant to starvation, but also evolved a novel induced response: an increase in their SR was triggered by prior exposure to lemon; this plastic response was absent in the unselected controls (Harshman *et al.*, 1999a). In other words, the selected lines evolved the ability to use lemon compounds as a cue indicating nutritional stress and upregulate their SR in its anticipation. All these results indicate that SR is considerably phenotypically plastic and can be enhanced in response to environmental cues indicating the likelihood of food shortage. It is an open question to what extent these plastic responses are mediated by the same physiological and molecular mechanisms as those responsible for genetic variation in SR.

Physiology of starvation resistance

From the viewpoint of the energy budget, one can imagine three main ways to increase SR: (1) sequestering greater energy reserves (Fig. 1a); (2) reducing the rate at which the reserves are used under starvation conditions (Fig. 1b); (3) lowering the minimal level of body energy content which allows survival (Fig. 1c).

An increase in energy reserves (Fig. 1a), in particular in lipid stores, seems to be a common mechanism underlying experimental evolution of greater SR in *Drosophila* (studies reviewed in Hoffmann & Harshman, 1999; Borash & Ho, 2001; Hoffmann *et al.*, 2005a). Harshman *et al.* (1999a) observed an increase in the activity of enzymes associated with lipid biogenesis. Lipid content and SR also usually change in parallel in lines selected for lifespan (Service, 1987; Zwaan *et al.*, 1995; Vermeulen *et al.*, 2006). Starvation-resistant flies may carry greater lipid reserves already at eclosion, even if selection for SR has been imposed 2 weeks after eclosion (Chippindale *et al.*, 1996). Thus, changes in lipid metabolism underlying SR already occur during larval stage. However, those starvation-resistant lines continue to increase their lipid reserves during early adulthood (Chippindale *et al.*, 1996), so greater reserves at eclosion are not the whole story. An increase in lipid reserves is also induced by protein-poor adult diet (Simmons & Bradley, 1997; Piper *et al.*, 2005); this response presumably mediates at least part of the induced response of SR to caloric restriction. Nonetheless, the relationship between lipid reserves and SR is not universal. For example, Hoffmann *et al.* (2001) did not find any correlation between lipid content and SR among isofemale strains derived from wild populations, either within or across populations, whereas Baldal *et al.* (2005) observed that raising larvae under crowding conditions increases the adult fat content without improving SR. In summary, storing more reserves is a common adaptation to starvation in laboratory experiments, but higher lipid content does not automatically lead to greater SR.

Evidence for greater efficiency of using metabolic reserves as an adaptation to starvation (Fig. 1b) is more equivocal. One candidate parameter would be metabolic rate. In one study (Djawdan *et al.*, 1997), lines selected for SR had a lower metabolic rate per unit body mass (both in the presence and absence of food), but that was because they contained more metabolically inert lipid and carbohydrate stores. Their metabolically active tissues did not have a lower mass-specific metabolic rate (Djawdan *et al.*, 1997). Another set of starvation-resistant lines had a lower mass-specific metabolic rate than unselected controls, but the same metabolic rate per individual: they were 21% heavier due to greater lipid and carbohydrate stores (Harshman *et al.*, 1999a).

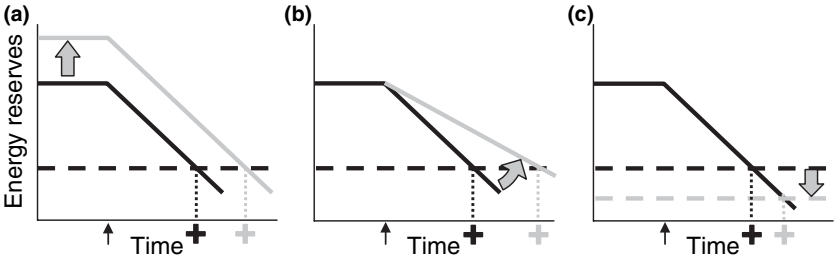

Fig. 1 Three models of enhancing starvation resistance. Upon onset of starvation (thin arrow), the animal must satisfy its metabolic needs by using up its energy reserves (solid line). Death (cross) occurs when the reserves are depleted below a threshold at which irreversible damage to vital organs occurs (dashed line). Starvation resistance could be enhanced by (a) maintaining more reserves, (b) by reducing the rate at which the resources are used up, and (c) by making vital organs more tolerant to low energy levels.

However, reduction of the metabolic rate seems to be part of the plastic response to starvation (Djawdan *et al.*, 1997; Marron *et al.*, 2003) and, at least in some cases, to caloric restriction (Simmons & Bradley, 1997, but see Partridge *et al.*, 2005). Lower metabolic rate is also a part of the diapause phenotype (Tatar *et al.*, 2001), also characterized by greater resistance to starvation (Schmidt *et al.*, 2005b). To summarize, rather than a constitutive reduction in the metabolic rate, adaptation to starvation seems to involve a plastic switch to a more frugal use of energy reserves when food becomes limited.

Lines selected for SR have also been reported to show lower locomotor activity (Hoffmann & Parsons, 1993; Williams *et al.*, 2004), which is one way to reduce energy expenditure. However, when deprived of food, unselected flies become much more active (Knoppien *et al.*, 2000). This does not need to be a contradiction. Greater mobility may be the best way to find scarce food under natural conditions, but this option is excluded in laboratory selection experiments. This example points to the more general problem of the relevance of laboratory selection experiments as a model of selection in nature.

Finally, SR might also be improved if the threshold amount of resources required for survival (minimal irreducible amounts; Briegel *et al.*, 2001) was lowered (Fig. 1c). This hypothesis could be addressed by comparing the lipid, protein or glycogen content at death between SR-selected lines and their controls. To our knowledge, this has not been done.

General vs. specific stress resistance

The literature reviewed by Hoffmann & Harshman (1999) pointed to a robust (although not universal; e.g. Djawdan *et al.*, 1998) association between starvation and desiccation resistance, presumably reflecting the positive effect of increased carbohydrate stores on both. Recent studies confirmed this evolutionary relationship (e.g. Bubliy & Loeschcke, 2005; Hoffmann *et al.*, 2005a). There is also some evidence for a positive genetic correlation between SR and resistance to oxidative stress (Harshman *et al.*, 1999a); we discuss the probable functional significance of this correlation in the next section.

Some other forms of stress resistance have been occasionally reported to be genetically correlated with SR, but the data are inconsistent. For example, both an increase (Borash & Ho, 2001) and a decline (Sanders *et al.*, 2005) in SR was observed as a correlated response to selection under larval crowding. Plastic responses of SR to larval crowding are also inconsistent among experiments (see above). These results suggest that there are alternative ways of adapting to a high larval density (Joshi *et al.*, 1998; Borash *et al.*, 2000), only some of them leading to higher SR of the adults. Hoffmann *et al.* (2005a) observed a negative genetic correlation between starvation and cold resistance in lines selected for either resistance trait; they suggested that the relative proportions of phospholipids and triglycerides in the membrane may underlie this apparent trade-off. However, in another experiment SR increased as a correlated response to selection for cold shock resistance (Bubliy & Loeschcke, 2005). There seem to be no functional or genetic relationship between resistance to starvation and heat shock (Minois, 2001; Wang *et al.*, 2004; Bubliy & Loeschcke, 2005). There are two potential reasons for the inconsistency of genetic correlations between resistance to different stressors among, or even within (Bubliy & Loeschcke, 2005) experiments. First, the direct response to selection may be based on different genes, with different pleiotropic effects. Second, rather than being due to pleiotropy reflecting a functional relationship, correlated responses to selection may be due to fortuitous linkage disequilibria in the selected populations. Thus, the relationship between SR and desiccation and oxidative stress resistance notwithstanding, there is no compelling evidence that SR is part of a general stress resistance mechanism.

Life history correlates of starvation resistance: the survival mode

The literature reviewed by Hoffmann & Harshman (1999) indicated a robust evolutionary association between high SR and long developmental time, large adult body mass, low fecundity and long lifespan. These traits usually show correlated responses to selection on SR and/or vice versa, a pattern also observed in a recent study (Bubliy & Loeschcke, 2005), with some interesting exceptions (see below).

The association between SR and body weight at eclosion can presumably be mostly accounted for by the increase in lipid and/or carbohydrate reserves put up by the larvae, without much increase in the structural size. This was the case for at least one set of SR-selected lines (Chippindale *et al.*, 1996); in another no significant increase in overall body weight was observed in spite of a greater proportional lipid content (Hoffmann *et al.*, 2005a). Prolonging the period of feeding (i.e. delaying pupation) is a simple way for the larvae to put up these additional reserves (Edgar, 2006), explaining the longer developmental time of SR-selected lines. This option may, however, be unavailable under high larval competition, leading to deteriorating larval nutritional environment. An increase in larval feeding rate may then be an alternative. One set of lines selected under larval crowding indeed showed a higher feeding rate, greater lipid reserves at eclosion, and greater SR (Santos *et al.*, 1997; Borash & Ho, 2001). Higher feeding rate would also be expected as a correlated response to selection for SR, but this hypothesis has, to our knowledge, not been tested.

The low fecundity and long adult lifespan (i.e. time to death under *ad libitum* food) of flies selected for high SR are unlikely to be a direct consequence of greater lipid

reserves at eclosion. Intuitively, one would expect that greater lipid stores at eclosion would lead to higher fecundity. Indeed, flies that put up higher lipid reserves as a result of artificial selection for high larval feeding rate actually have higher early fecundity and shorter lifespan (Foley & Luckinbill, 2001). Rather, together with reduced mortality and higher resistance to oxidative stress, SR seems to be part of a physiological state geared to high survival at the expense of a reduction, or even a complete arrest, of reproduction. Many heterotrophic organisms are capable of a plastic switch of their physiology from a 'reproduction mode' to such a 'survival mode', e.g. in response to poor or declining food quality (caloric restriction; Partridge *et al.*, 2005). Such a switch is also part of the inducible diapause phenotype in diverse insects (Tatar & Yin, 2001). Recent evidence indicates that in animals as diverse as flies, nematodes and mammals this switch is mediated in part by insulin signalling (Tatar *et al.*, 2003; Kapahi *et al.*, 2004; Partridge *et al.*, 2005).

Several insulin-like peptides are produced by a set of neurons in the region of fly brain called pars intracerebralis, and are involved in regulating metabolism, cell division, growth and development (Broughton *et al.*, 2005; Wu & Brown, 2006). In adult flies they reduce the level of trehalose (fly equivalent of glucose) in the haemolymph and stimulate vitellogenesis (Tatar *et al.*, 2003; Broughton *et al.*, 2005). Flies whose insulin-producing cells have been ablated at a late third instar larval stage (by locally expressing the apoptotic gene *reaper*), put up more lipid reserves, are long-lived and more resistant to starvation and oxidative stress, but show reduced fecundity, along with poorer resistance to cold and heat (Broughton *et al.*, 2005). Pharmacological application of a juvenile hormone analogue has opposite effects (Salmon *et al.*, 2001); juvenile hormone is one of the downstream effectors of the insulin signalling (Tatar *et al.*, 2003; Flatt *et al.*, 2005).

Several other candidate mechanisms that may simultaneously regulate lifespan and SR have been proposed. For example, mutants of *methuselah*, encoding a membrane receptor apparently not involved in insulin signalling, live 35% longer and are substantially more resistant to starvation and oxidative stress (Lin *et al.*, 1998). Overexpression of gene *GLaz* leads to 29% longer lifespan on *ad libitum* food and 60% greater SR (measured as time to death without food), together with higher resistance oxidative stress and desiccation; in contrast to effects mediated by the insulin pathway, dry weight, protein content and lipid content remain unchanged (Walker *et al.*, 2006). *GLaz* encodes a lipid-binding protein and so is likely to be involved in lipid metabolism, but beyond that little is known about its role.

All these results indicate an important functional relationship between SR and longevity under *ad libitum* food. However, there is also evidence of some processes affecting these two traits independently or even antagonistically. For example, target of rapamycin (TOR) signalling pathway is thought not only to act in parallel to, but also to interact with, the insulin signalling to regulate lifespan (Oldham *et al.*, 2000; Tatar *et al.*, 2003). Manipulating the expression in the fat body of several genes involved in this pathway resulted in substantial increase in lifespan, with no associated change in SR (Kapahi *et al.*, 2004). In turn, the apokinetic hormone (encoded by *Akh*) seems to regulate SR independently of lifespan. This neuropeptide modulates lipid and sugar metabolism, as well as feeding response to hemolymph trehalose level. It is produced in a subset of neurons in the neuroendocrine gland corpora cardiaca; ablation of those neurons enhances SR without an effect on longevity (Lee & Park, 2004).

These results indicate that the molecular and physiological mechanisms of SR and longevity overlap only partially. That this may have evolutionary consequences has been confirmed in some selection experiments. In one experiment, populations selected for SR showed no correlated change in longevity (Harshman *et al.*, 1999b); in another evolution of greater longevity was not associated with any change in SR (Force *et al.*, 1995). In still another study, selection for SR was initially accompanied by an increase in longevity, but in subsequent generations further increase in SR was associated with a decline in longevity (Archer *et al.*, 2003). In a somewhat complementary experiment (Vermeulen *et al.*, 2006) lines selected for long virgin lifespan showed a reduction in SR. Presumably, the response to selection on either SR or longevity initially targets mechanisms common to these two traits. However, once genetic variation affecting those mechanisms is exhausted, further response is based on genetic variation at loci showing antagonistic pleiotropy with respect to SR and longevity. Thus the evolutionary relationship between SR and longevity, and life history in general is apparently more complex than the picture suggested by the earlier studies reviewed by Hoffmann & Harshman (1999). Differences in direct and correlated responses between experiments may result from different initial gene pools. However, genetic drift, especially combined with epistasis, may even result in replicate selection lines derived from the same base population to climb alternative 'adaptive peaks' (Kawecki & Mery, 2006). Finally, the detailed way in which selection is imposed and correlated responses assayed could play a role (e.g. Ackermann *et al.*, 2001). The contribution of these factors to the variability of responses to selection for SR has not been investigated.

Evolutionary genetics of starvation resistance

A comprehensive understanding of the evolutionary biology of SR will ultimately require identification and characterization of genetic loci which contribute to heritable variation and underlie evolutionary changes

in this trait. Although mutants or gene expression manipulations that enhance SR indicate potential genetic foci of evolutionary change in this trait, it is not yet clear if they contribute to genetic variation in nature. Some insights into aspects of genetic variation in SR, such as the patterns of dominance and epistasis, can be gained from an analysis of crosses between resistant and susceptible genotypes (for general methods and interpretations see Lynch & Walsh, 1998). Kennington *et al.* (2001) used this approach to study the genetic architecture of differences between two pairs of geographically distant populations from South America and Australia. Although significant deviations from a simple additive model were found, the results were not consistent between the two continents and the sexes, the deviations being variously due to dominance, epistatic and maternal effects. Interestingly, for both sexes in South America and females in Australia positive maternal dominance effects were found: backcrossed offspring showed a higher SR if their F_1 parent was the mother rather than the father (Kennington *et al.*, 2001). With a similar approach maternal effects have been found to contribute to difference between laboratory populations selected for SR and unselected controls (Teotonio *et al.*, 2004), with less resistant mothers producing more resistant offspring of both sexes. The reason for this paradoxical result remains unknown.

Recent developments in quantitative trait locus mapping, combined with quantitative complementation tests (Mackay & Fry, 1996) pave the way to identification of specific loci responsible for differences in SR. As an early candidate, a natural polymorphism in *desaturase-2* locus was proposed to affect SR (Greenberg *et al.*, 2003), but subsequent experiments (Coyne & Elwyn, 2006; Greenberg *et al.*, 2006) failed to replicate this results. With a more comprehensive approach, Harbison *et al.* (2004) identified 13 loci (of those six with sex-specific effects) contributing to a difference in SR between two inbred laboratory strain. The list included genes involved in oogenesis (e.g. *l(2)rG270* which affects egg development); metabolism, including lipid allocation (e.g. *phosphoglucose isomerase* involved in glycolysis and gluconeogenesis) and feeding behaviours (e.g. *NaCP60E*, a cation channel implicated in olfactory avoidance behaviour). Other genes they identified had known phenotypes in cell fate specification and cell proliferation (e.g. *numb*, a protein that alters cell fate). This indicates that changes in metabolism, feeding behaviour, reproduction and resources allocation during development all contribute to improved SR.

In a complementary study, Harbison *et al.*, 2005 used the microarray technology to study changes in gene expression induced by starvation conditions. This approach is not informative about genetic variation in SR, but throws light on the molecular mechanisms of the plastic response to starvation. Under starvation conditions, genes involved in growth and maintenance processes, protein biosynthesis and hydrolase activity tend to be upregulated, suggesting that protein and organelle degradation provides substrate to starving cells. In contrast, genes involved in cross-membrane transport, immune defence and gametogenes become downregulated, suggesting that these functions are compromised in starving flies (Harbison *et al.*, 2005).

Insights provided so far by these pioneering studies are modest, but they illustrate the potential of *Drosophila* as a model system to study the genetic and molecular bases of adaptation to nutritional stress.

Ecological and evolutionary significance of starvation resistance

It is reasonable to expect that adult *Drosophila* face periods of food scarcity, and thus that SR is under natural selection. However, data that would allow quantifying the strength of this selection, or even conclusively demonstrating its existence, are missing.

Differences in the strength of selection on SR should lead to differences in this trait between populations. Evidence for such differences comes mainly from studies of larger-scale geographical variation. Hoffmann & Harshman (1999) list five studies showing latitudinal clines on the Indian subcontinent, with SR negatively correlated with latitude in five *Drosophila* species, including *melanogaster* (see also Parkash & Munjal, 2000). Analogous altitudinal clines were recently reported in two other Indian species (Parkash *et al.*, 2005). In contrast, a positive correlation between SR and latitude seems to occur in *D. melanogaster* in eastern North America (Schmidt *et al.*, 2005a) whereas no clinal variation for SR was found in South America (Robinson *et al.*, 2000) and eastern Australia (Hoffmann *et al.*, 2005b). In that last region, a correlation between SR and latitude is also absent in *D. serrata* (Hallas *et al.*, 2002), but there is a positive correlation in *D. birchii* (Griffiths *et al.*, 2005). Thus, the patterns of geographical variation in SR are less consistent than those suggested by Hoffmann & Harshman (1999).

Furthermore, it is difficult to imagine that food availability for *Drosophila* changes systematically on continental scale. Rather than reflecting differential selection on SR itself, the latitudinal and altitudinal clines in SR are likely to be due to correlated response to natural selection acting on other traits, genetically correlated with SR. For example, cold resistance varies with latitude and altitude, so variation in SR across climates could result from correlated response to selection for cold resistance (Hallas *et al.*, 2002). In the temperate zone the frequency of diapausing genotypes increases with latitude, and the tendency to enter diapause is positively genetically correlated with SR (measured on nondiapausing individuals) (Schmidt *et al.*, 2005a). As a consequence, a positive correlation between latitude and SR in eastern North America seems to be

mostly explained by a cline in the frequency of diapausing genotypes (Schmidt *et al.*, 2005a). In contrast, the negative correlation between SR and latitude shown by several species in India may reflect correlated response to selection for desiccation resistance.

One might expect that local food supply would be more important, so there would be more differentiation at a local scale. However, Hoffmann *et al.* (2001) found little variation in SR among local *D. melanogaster* populations, despite high variation within populations and moderate differences between geographically distant populations. Similarly, no differentiation for SR was found among four localities for any of eight Philippine rainforest species studied (Van Der Linde & Sevenster, 2006). These studies suggest that either selection for SR varies little at local scales, or that gene flow is strong enough to obliterate any pattern of local adaptation. The latter explanation is undermined by the fact that both studies found evidence for differentiation in other traits. Direct measurements of natural selection for SR in relation to local ecology are needed before we can understand the patterns of among-population variation at local and geographical scales.

There is no doubt that there are large differences in SR among species from the same locality and using similar resources (Hoffmann & Harshman, 1999; Bharathi *et al.*, 2003; Marron *et al.*, 2003). Such interspecific differences in SR have been proposed to play an important role in the maintenance of local *Drosophila* species diversity. Sevenster & Van Alphen (1993) argued that fast developing species are superior competitors for larval food but show lower SR as adults and thus are more affected by periods of food shortage, promoting coexistence of 'fast' and 'slow' species. Such an interspecific trade-off between fast development and SR was indeed observed among 18 fruit-feeding rainforest species from Panama (Sevenster & Van Alphen, 1993). However, no relationship between developmental time and SR has been found in a guild of 12 rainforest species from the Philippines (Van Der Linde & Sevenster, 2006). Without more data it remains unclear to what extent differences in SR contribute to the coexistence of *Drosophila* species.

Beyond *Drosophila*

Evolutionary aspects of SR have been only sporadically studied in other animal species. Among-population differences in SR were found in, e.g. house flies (Hicks *et al.*, 2004), cockroaches (Mira & Raubenheimer, 2002) and ant lions (Arnett & Gotelli, 2003), with plausible links to ecological differences. Laboratory evolution of improved SR was observed as a result of direct selection in blowflies (Cooper *et al.*, 2002), and as a correlated response to selection on body size in dung flies (Reim *et al.*, 2006) and on dispersal behaviour in spider mites (Li & Margolies, 1994). In *Tribolium* beetles a loss of SR occurred as a result of relaxed natural selection (Lomnicki & Jasienski, 2000). Kirk (1997) reported a positive correlation between SR and lifespan across species of rotifers. As an example of trans-generational adaptive plasticity, *Daphnia* mothers kept under nutritional stress have been shown to induce greater SR in their offspring (Gliwicz & Guisande, 1992). Finally, in crickets there is a trade-off between reproduction and dispersal, involving differences in lipid and amino acid metabolism (Zera & Zhao, 2006); these differences are likely to have consequences for SR, which however, were not investigated yet. These studies usefully complement the fruit fly results summarized above, but they are far from forming a comprehensive picture which begins to emerge from the *Drosophila* studies.

Conclusion

Comparison of this review with Hoffmann & Harshman (1999) reveals that the progress in our understanding of the evolutionary biology of starvation in *Drosophila* during the last decade was rather uneven. Important advances were made regarding the molecular and physiological mechanisms of SR. The recognition that SR shows adaptive plasticity and is part of a 'survival mode' regulated in part by the insulin signalling helps to make sense of the association of SR with long lifespan, low fecundity and diapause, observed at both physiological and evolutionary levels. A dozen loci contributing to natural heritable variation in SR have been identified, opening a way for studies of physiological and molecular differences between their allelic variants. Additional candidates for targets of selection on SR are loci with starvation-resistant mutants, and genes whose experimental changes in expression induce improvements in SR. The amount of data on genetic and physiological aspects of evolutionary change in SR is by far greater in *Drosophila* than in any other species.

In contrast, rather little progress has been made since Hoffmann & Harshman (1999) in understanding the ecological aspects of SR. In particular, it remains unknown how strong natural selection on SR is in nature, and to what extent among-population differences in SR are due to natural selection on SR rather than being a by-product of selection acting on some other aspects of performance.

Drosophila offers a unique opportunity for a comprehensive understanding and integration of the different facets of evolutionary response to nutritional stress. The increasing recognition that responses to nutritional stress in organisms as diverse as yeast, nematodes, flies and mammals are regulated by highly conserved physiological and cellular mechanisms, such as insulin signalling and TOR nutrient sensing pathway, means that those results are likely to apply much more generally.

Acknowledgments

We thank J. M. S. Burger and two referees for comments. This work was supported by the Swiss National Science Foundation.

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