Targeting environmental and genetic aspects affecting life history traits

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Summarising Discussion

As I am finishing up this thesis, my most important task has just begun.

Summarising Discussion

Setting the stage

In the IOP Genomics program "The genetics of longevity and disease at old age" epidemiologists, gerontologists and evolutionary biologists have integrated their knowledge and approaches to unravel the genetics underpinning longevity. The evolutionary biology projects consisted of one project on *Bicyclus anynana* and two on *Drosophila*. One of the *D. melanogaster* experiments has been conducted at the Evolutionary Genetics group from the University of Groningen and focuses on the genetics of longevity. In Leiden, I have conducted experiments concerning starvation resistance and longevity in three species of *Drosophila*, with a focus on *D. melanogaster*.

In this thesis, I have attempted to integrate knowledge from a wide variety of disciplines and levels of research. I tinkered with flies in pre-adult and adult stages, meddled with environmental and genetic factors and examined whether we could observe effects in physiology and life histories. In doing so, I compared species, selection lines, and a single gene mutant to obtain insights into the genetics underlying starvation resistance and longevity.

The focus of my thesis lies on the association between starvation resistance and longevity. Therefore, I used artificial selection, the effects of pre-adult and adult environmental manipulation on adult life history traits, genotype-by-environment interactions and micro-arrays as my tools. Throughout the thesis I attempted to unravel general patterns that may be informative about the mechanisms underlying these life history traits. Here, the accumulated knowledge is sieved, weighted, and integrated.

Starvation resistance does not necessarily mean longevity

Longevity as such is generally not a trait that is under selection in nature. This is because longevity is hidden in a so called selection shadow (Hoekstra 1993). The exceptions are mammals that provide parental and grandparental care. In these groups living longer after reproductive cessation (e.g. menopause) has an advantage in terms of provisioning offspring (e.g. Hawkes et al. 1998; Packer et al. 1998; Sherman 1998; Kirkwood 2001, 2002). This means that the chance of survival to that age is low and that those that do make it have a long-lived genotype. This may not be advantageous in the evolutionary sense, because the additional age will not result in (a lot) more offspring. Therefore, since there is no advantage, there can be no selection in favour of this trait specifically. In nature, the risk of dying early

irrespective of genotype is so large that there is no selective advantage of having a long lived genotype; selection is on optimal reproductive lifespan. Longevity is the result of a correlated response to selection on other traits that facilitate optimal reproduction. Longevity must thus hitchhike along with other traits that are under selection in nature. Since the IOP project focused on naturally occurring genetic variation in humans, I focussed on starvation resistance, which has a positive correlation with longevity. Increased resistance to adversity implies increased potential to survive adverse conditions and be prepared for better ones to invest in energy consuming processes such as reproduction and development. Starvation resistance does, however, not necessarily take longevity along proportionally (see Chapters 1, 3 and 4) and there have been reports of gradual dissociation between these traits (Archer et al. 2003; Phelan et al. 2003). Longevity is also associated with other traits, such as paraquat resistance (e.g. Vettraino et al. 2001), development time (DaCunha et al. 1995, yet see Zwaan et al. 1995a), metabolic rate (Riha and Luckinbill 1996; Braeckman et al. 2001; Lin et al. 2002, but see Hulbert et al. 2004), desiccation resistance (Graves et al. 1992; Hoffmann and Harshman 1999) and reproduction (ref Chippindale). Increased stress resistance is found in lines selected for increased longevity (Service et al. 1985; Service 1987; Leroi et al. 1994b; Harshman et al. 1999b, but not always see Force et al. 1995). More specifically, some studies showed an increase in longevity as a result of selecting on increased starvation resistance (Rose et al. 1992; Chippindale et al. 1996). In this thesis I have found that longevity and starvation resistance, though often found to be correlated, may not be as tightly linked as was previously thought.

In Chapter 1, we found that there was a general adverse effect of larval density on adult life history traits. However, we observed that in *D. melanogaster* and *D. ananassae* the effects on starvation resistance were more severe than those on longevity, whereas the reverse was observed in *D. willistoni*. Usually the life span decreased with increasing larval density, although not in all cases. This indicates that there is no strict one-on-one relationship between longevity and starvation resistance. Inducing an increase in starvation resistance by means of environmental manipulation does therefore not necessarily invoke a similar response in longevity and *vice versa*.

In our selection experiment in Chapter 3 we found that of the four selected lines with a considerable increase in starvation resistance, two showed an increase in longevity; the other two did not. Interestingly, the two lines that became long lived had also acquired an increased paraquat resistance. Apparently, by selecting on increased starvation resistance, longevity becomes selected for simultaneously in a subset of lines. Moreover, in the second principal component in Chapter 3, longevity and starvation resistance are effectively contrasted. These findings show that longevity is indeed a secondary trait that arises by selection for another trait. Again, starvation resistance and longevity were found to be not as closely related as was thought earlier.

In Chapter 4, lines selected for both traits were found to show interactions as a result of extreme environmental challenges. The long lived lines had an advantage in affluent situations, whereas the starvation resistant lines had this advantage in adverse circumstances. Relative to their controls both line types had a skew in their relative life span optimum as compared to their control lines. The differences between the lines give rise to a genotype-by-environment interaction, which indicates

that the genotypes underlying longevity and starvation resistance are to a certain extent different.

In summary, in both our genetic and environmental manipulation studies the link between starvation resistance and longevity is present, but the absence of one does not prevent the presence of the other and *vice versa*. Both traits seem to respond in a similar way to pre-adult environmental manipulation, but the associations between the traits are not tight. When examined under different adult conditions, there is a considerable genotype-by-environment interaction, which shows that the underlying genotypes are adapted to their selection environments. On the basis of the findings in Chapters 1 and 4 we state that the correlated response that is quite often found between longevity and starvation resistance is modulated by both the pre-adult and adult food conditions.

Fat content, prerequisite not cause

Fat content is often thought to be the main factor underlying genetic differences in starvation resistance (Robinson et al. 2000; Zera and Zhao 2003; Harbison et al. 2004), and the resource underpinning the phenotypic trade off between early fecundity and starvation resistance (Chippindale et al. 1993; Leroi et al. 1994a). In Chapter 1 we found that although the starvation resistance of animals is reduced with increased larval density, the relative fat content is increased simultaneously. Thus, for environmental manipulation experiments we conclude that relative fat content can not be the only resource for starvation resistance. In Chapter 3 we manipulated the genetics of starvation resistance by using artificial selection. We observed a large increase in relative fat content with a subsequent increase in starvation resistance. Metabolic rate in selection lines was comparable to controls under fed conditions and higher under starved conditions. Selection for increased starvation resistance leads to an increased build up of reserves in the pre-adult stages, so that the individuals have more resources to survive for a longer period under the absence of adult food.

We can thus state that increased relative fat content does not necessarily induce an increase in starvation resistance, but that when starvation resistance is increased, it does so through increased relative fat content. When more resources are allocated to triglyceride build up, potentially starvation resistance is higher. Yet, this is not the only factor involved; the rest of the animal's physiology also has to be set for allocation to starvation resistance. We conclude that fat is a prerequisite rather than a causal factor for increased starvation resistance.

Pre-adult conditions

The pre-adult environment is of considerable importance in determining adult longevity (see Chapters 1 and 2). Larval adversity negatively affects adult life history.

In Chapter 2, we have tested part of the Barker hypothesis. The Barker hypothesis is a medical hypothesis that states that adverse pre-adult conditions result in elevated

risk of adult metabolic syndrome, which reduces life span. We have examined what the effect was of offering little and abundant food in the pre-adult stage. The control and abundant medium-bred animals could not be distinguished in their life history traits and physiological characters. The animals reared at half medium, the adverse state, needed more time to develop, and had lower adult body weight and a reduced life span. This finding of a similar phenotype hints at the Barker hypothesis. Yet, to know this for sure, adult flies from adverse larval conditions need to be diagnosed with similar metabolic diseases as play a role in the human case. The comparability of the fruit fly model and humans on this pathological level remains to be seen, because known diabetic phenotypes have not reduced *Drosophila* lifespan, but rather enhanced it (Broughton et al 2005).

The question remains whether this effect of pre-adult stress on adult life span is a scar or adaptive plasticity. Scar is most simply explained; the individual gets damaged and consequently functions less well. Adaptive plasticity is a more complex explanation. The organism then has a “buffer” mechanism by which it is able to withstand environments other than the ideal one. During evolution these mechanisms are likely to have arisen because selective pressure never acts on a single feature. From this point of view the reduction in life span is the consequence of a programmed response in allocation. For now, we cannot answer this question, but are intrigued by the ongoing research in this field.

Reproduction, longevity and the relevance of mutant studies

Reproductive output is the ultimate measure of fitness; the traits that determine the life history of an individual are configured to maximum reproduction. The soma is the vehicle of the germ line and therefore, somatic maintenance is necessary to optimise reproductive life span. Life span is defined as the time an individual stays alive. It does not concern the condition in which the individual reaches old age. The work of Cook-Wiens and Grotewiel (2002) shows that the extended longevity of the methuselah fly does not go together with extended functional performance. This is not advantageous from a fitness point of view, especially, when this longevity comes at the cost of a reduced early fecundity, as is predicted by the antagonistic pleiotropy theory of ageing (Williams 1957). We examined this system in Chapter 6 by comparing mated and unmated individuals of the long-lived methuselah (*mth*) strain. On the whole, reproduction reduced longevity. During reproduction, the longevity advantage of the *mth* mutants is strongly reduced and their life span cannot be distinguished from that of the progenitor strain. We stimulated the reproductive output by exposing the flies to higher yeast concentrations, but made sure the flies were unable to mate. We then observed a dosage dependent decline in longevity of the long-lived mutant relative to its progenitor strain. In the most extreme case, longevity of methuselah was even lower than that of its ancestor. This shows that the validity of correlative studies is directly related to the conditions in which the correlation has been confirmed. Extrapolation of findings to nature should be done with caution in the cases where genotype-by-environment interactions have not been estimated. In addition, it shows that the stunning longevity of some mutant strains may be very condition dependent. Methuselah tends to respond more extremely to changes in the environmental reproductive cues; living longer in their absence and shorter in their presence. Their reproductive output has also shifted towards increased late-life

reproductive success and reduced early-life reproductive output. In combination with the high stochastic mortality in nature this would make fitness lower, and would lead to a lower frequency of the *mth* allele in the population after each generation and subsequently extinction. Evident from this example and literature is that mutations that seem advantageous may only be so in the laboratory. The chance that we will find a truly advantageous mutation in terms of life span and its correlated traits that is relevant to natural situations remains low, despite the great effort that is put into it. On the other hand, humans in the western society also do not live in fully natural conditions any longer, with medication, social welfare and lack of predation, making present-day society perhaps more reminiscent of the laboratory than of nature.

Correlations with paraquat resistance

Longevity is clearly not fully dependent on starvation resistance. Generally, it does show a good correlation with paraquat resistance (e.g. Arking et al. 1991; Force et al. 1995, see also Arking et al. 2000; Vettraino et al. 2001, where even different extended longevity phenotypes are found). Starvation resistance correlated in a similar way to paraquat resistance as it did to longevity in our selected lines (Chapter 3). This indicates that selection on increased starvation resistance may, but does not necessarily, induce other traits to co-evolve. We observed that paraquat resistance and longevity are increased together in lines SR1 and SR2. In the second principal component for the overall variation, we observed that paraquat resistance had a negative vector value, just as longevity, whereas all other traits show positive vector values. The paraquat resistance of the mutant line *mth* was found to be increased in the original paper by Lin et al. (1998). In Chapter 6, we found that paraquat resistance did not always differ consistently between the lines and that in most cases the progenitor line had a significantly higher paraquat resistance than the long-lived strain. Strikingly, the long lived mutant had also lost most of its longevity under the rearing condition in the Leiden laboratory.

Paraquat resistance is important because it is a measure of the resistance to oxidative damage. Protection against oxidative damage is often thought to underlie longevity. Detoxification of products that cause harm, as proposed by Gems and McElwee (2005) to be involved in longevity determination, is an important aspect of preservation and protection from oxidative damage. Starvation resistance can be the product of two types of processes: one is an increase in resources by which one can survive an adverse period, the other a reduction in expenditure, a so-called thrifty genotype (see general introduction). For both strategies, if the metabolic rate remains stable or is reduced there is no need for increased paraquat resistance, because there is no increased threat of oxidative damage. In Chapter 3, we provide evidence that metabolic rate is not increased nor decreased in the starvation resistant animals under fed conditions. Two out of four starvation resistant lines do not show an increase in paraquat resistance. The lines with increased paraquat resistance also show increased longevity, a correlation that has been shown before (Arking et al. 1991; Force et al. 1995; Arking et al. 2000; Vettraino et al. 2001). Therefore, we conclude that paraquat resistance is not causally linked to starvation resistance, but rather to longevity.

The disposable soma theory of ageing

Trade offs

The disposable soma theory of ageing has been proposed by Kirkwood (1977; Kirkwood and Holliday 1979). It states that there is a trade off between the reproductive tissues and the rest of the body. We know from the work of Chippindale et al. (1993) that such a trade off exists in *Drosophila*. The work of Patel et al. (2002) shows that germ line signalling reduces longevity and that ablation of the germ line cells increases life span in *C. elegans*. Leroi (2001) stated that such molecular signals are more likely to be the cause of the soma-germ line antagonism rather than resource allocation based trade offs. Barnes and Partridge (2003) opposed this by explaining that the findings from germ line cell ablation do not refute resource allocation based trade offs, but rather offer an additional mechanism underlying the soma-germ line contrast. Irrespective of the precise mechanism, this work underpins the antagonistic forces that separate the germ line and soma. Here, the introduction springs to mind. Life initially began as a self-reproducing unit that evolved a soma in order to convert energy more efficiently. Thus, the soma is for the germ line only a means to continue to exist over time. The fact that the germ line signals in Patel et al's work reduce longevity shows this basic interdependence.

In Chapter 3 we deduced that allocation to starvation resistance implies resources to be available for adverse times. Allocation to starvation resistance will reduce the allocation to reproduction. Therefore, reproduction and starvation resistance trade off. This was shown phenotypically by Chippindale et al. (1993). Since longevity can be correlated with starvation resistance, as was also shown in Chapter 3, longevity seems to be dependent on the allocation event (see figure 1). We have already shown that increased total fat content does not necessarily lead to an increase in starvation resistance in Chapter 1. We then concluded that there is more to starvation resistance than fat content, but it is a prerequisite for increased starvation resistance.

Mair et al. (2004) found that flies that are given less food are longer lived but do not show a reduction in reproductive rate. This may relate to the allocation of lipids. Eating a lot can be disadvantageous from a certain point onwards. A reduced access to food can therefore be relatively healthy and explain the increase in longevity. If enough resources are still available, the allocation of resources to the reproductive apparatus may not be limited by the diminished supply of food. In this way life span will be enhanced and reproductive output will remain the same. When the allocation has shifted and lipids are not allocated to reproduction but rather to the soma, the lipids will consequently be used by processes other than reproduction. One such process is starvation resistance. Here, we propose that when fat is allocated to the soma some mechanism may, but need not, simultaneously enhance potential life span. This matches the findings of Mourikis et al. (2006), who claim that lipid metabolism may play a role in life span regulation.

Because we found a large increase in fat content in the selected lines in Chapter 3, we expect that allocation in our flies is towards the soma instead of the germ line. As a result of selection under the absence of food, the animals have increased allocation to the soma at the cost of allocation to the germ line. Allocation to the germ line is not relevant in this set up, and is thus selected against.

In Chapter 5, we examined genes that were differentially expressed between a long lived starvation resistant line and its control under fed and starved conditions. Under normal, fed conditions we observed few differences between the lines, which was in sharp contrast with the large number of differentially expressed genes we found under starved conditions. We found that the starvation selected line expressed genes at a lower level that were involved in neural transmission, insulin signalling, glycolysis and reproduction. This means that the starvation selected flies have adapted to this situation by down regulating a large cascade of events that are linked to one another. Some gene categories have been discussed in more detail in Chapter 5. Here, I will focus on the low expression of reproduction related genes. Down regulation of these genes is in congruency with the hypothesis that in our selected lines reproductive output will be smaller than in the control lines.

The effect of genotype-by-environment interactions

From the above it follows that the response to starvation resistance selection has involved the trade off between soma and germ line. Leroi et al. (1994a; 1994b) showed that trade offs may be present among environments and populations and may be obscured by strong genotype-by-environment interactions. Apparently, genotype-by-environment interactions pose a caveat on the interpretation of correlative data. Hence, trade offs must always be interpreted in relation to the environment the genotypes were selected in. In Chapters 3 and 4 we found that lines selected for starvation resistance in some cases display long life span under fed conditions and in others do not. The long lived and starvation resistant lines displayed strong genotype-by-environment interactions over affluent and adverse conditions. The genotype-by-environment interactions indicated that each selected direction has its own skew in longer life span relative to the control line. Lines selected for longevity or starvation resistance should be regarded specialists that outperform in the condition they have been selected in.

Similarly, the work on the mutant methuselah revealed a strong effect of the environmental conditions on adult life histories. In Chapter 6, we showed that in conditions favouring reproduction, the longevity advantage of the mutant had faded. Also, the starvation resistance was relatively low and the longevity was not as elevated as was previously found by Lin et al. (1998). In the original study by Lin et al. (1998) little yeast was used, and methuselah lived longest. In the Leiden laboratory, we use a medium that contains a lot of yeast, which is known to enhance reproduction in fruit flies (Simmons and Bradley 1997). Under food conditions that contained even more yeast, the long-lived mutant even underperformed relative to the control. Therefore, we state that by shifting the emphasis to reproduction, longevity suffered in methuselah.

In summary, we expect that in the starvation selected lines reproductive output is reduced. This is underpinned not only by theory but also by gene expression studies. Furthermore, the trade off between starvation resistance and longevity, and reproduction is highly environment dependent, as is the relation between starvation resistance and longevity.

A new synthesis

Longevity and starvation resistance have often been found to negatively correlate with reproduction. This is in line with the disposable soma theory of ageing (Kirkwood and Holliday 1979; Kirkwood and Rose 1991). There, the allocation difference to the soma and germ line tissues, and the interaction between these determines longevity. Evidence for this hypothesis has been presented from different disciplines (Chippindale et al. 1993; Patel et al. 2002). Barnes and Partridge (2003) explained that resource allocation is at least in part responsible for life history trade offs. The initial allocation to soma or germ line causes the general observation of the link between starvation resistance and longevity. If we combine this with the skew of the life span advantage in the selected lines (Chapter 4) and the variability in the correlation found in Chapter 1, we see that longevity and starvation resistance are not intimately linked. Van Noordwijk and De Jong (1986) have shown that a positive correlation can be found among traits that are expected to trade off. Their analogy with economics provides a good insight in how these positive correlations can be found. With a certain budget one can buy a house and a car. The more one spends on the house, the less there is to spend on a car and *vice versa*. This resembles the trade off model. Yet, if the income of a family is higher, they can spend more on both housing and cars. Therefore, families having more money will probably have a large house and ditto car. This results in a positive correlation. Here, we found a similar situation. When all resources are allocated to the soma, both starvation resistance and other mechanisms can benefit. Yet, because the amount of resources is fixed there will be an allocation-fraction (cf. fraction B in Van Noordwijk and De Jong, 1986) dependent correlation between starvation resistance and longevity. On the basis of our findings and this model, I hypothesise that after allocation to the soma side there is a second allocation event that may lead to an extra increase in either starvation resistance or longevity (figure 1).

Thus far it has been found that fat and its allocation are of importance to starvation resistance (Chapters 1 and 3). Also, longevity is a by product of increased starvation resistance and is found to associate with paraquat resistance (Chapters 3 and 4). Furthermore, insulin signalling is important for the germ line-soma contrast (Chippindale et al. 1993; Patel et al. 2002; Broughton et al. 2005) and longevity is dependent on insulin signalling (Clancy et al. 2001; Tatar et al. 2001; Tatar et al. 2003; Broughton et al. 2005). Longevity and starvation resistance do not respond proportionally among species or as a result of the ablation of neurons involved in insulin signalling (Broughton et al. 2005). Thus, if present, the more subtle effects of the hypothesised secondary allocation event will depend on mechanisms other than insulin signalling. We suggest that the second allocation event between starvation resistance and longevity is dependent on lipid metabolism. Allocation to the soma side through insulin signalling makes all soma processes gain resources. The different processes on the soma side can be adjusted by selection in multiple ways. If this involves a strong increase in reserve building lipid metabolism, starvation resistance will be altered. If allocation is not targeted so specifically, other processes like somatic maintenance, will gain this energy and increase other traits, including longevity.

The fact that the high fat content in Chapter 1 did not lead to increased starvation resistance may have to do with the first allocation point. As a result of severe crowding, the surviving individuals are set to remain ahead of their competitors and

allocate their resources to reproductive output rather than to the soma. This would also explain the smaller body size at high larval density. In Chapter 3, we then observed two lines that were selected for starvation resistance on the basis of features that act before the second allocation event, taking paraquat resistance and longevity along in the selection process. The starvation resistant lines that do not show increased longevity, SR3 and SR4, will then have been selected for starvation resistance on features after the second split point. The absence of starvation resistance in the long lived lines in Chapter 4, could have arisen from selection on processes after the second split point. The single gene mutant methuselah, did not show paraquat or starvation resistance advantages in our set-up in Chapter 5, but did in the Lin et al. (1998) set-up, together with a more extreme longevity. Apparently, this mutation works on features that lie before the second split. This also explains why the extended longevity and associated features do not appear in more reproduction enhancing environments, which act on the first allocation point. A similar story may be applicable to the short lived lines from Chapter 4.

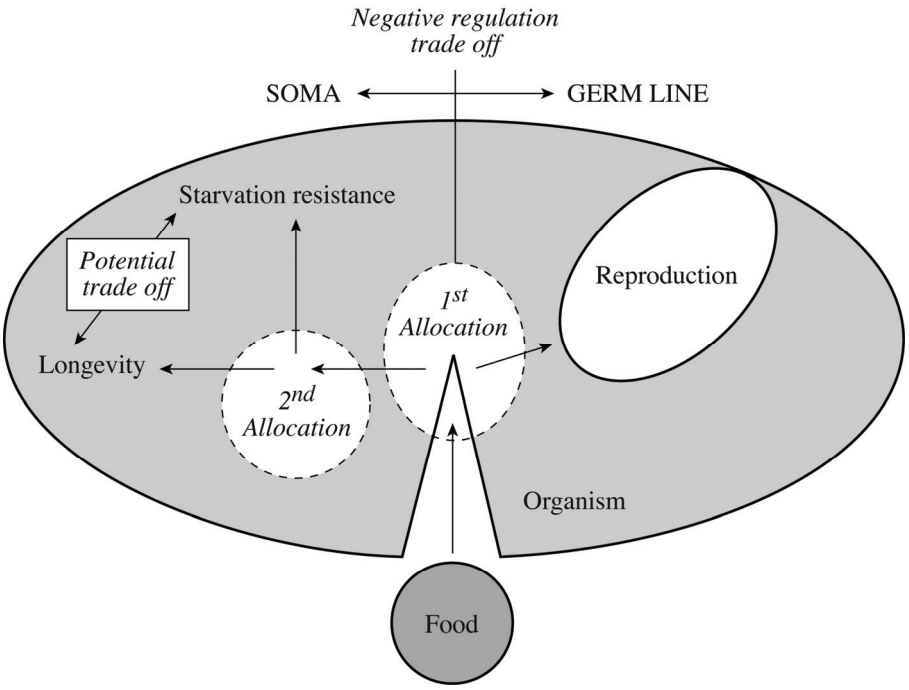

Figure 1. Hypothetical life history determination scheme. Food allocation is possibly followed by a second decision on the soma side of the organism. This process, that can be either physiological or genetic in its regulation, could lead to a trade off between starvation resistance and longevity, depending on the genotype of the organism.

In summary, I conclude that as a result of genotype and environmental cues resources are allocated to either the germ line or the soma. On the soma side at least two mechanisms act to maintain somatic stability. These traits probably trade off following an Y-model, as proposed by Van Noordwijk and De Jong (1986). One of these is starvation resistance, which acts to maintain the soma when facing adversity. The other mechanism is about maintaining the quality of the soma for a long time, which involves longevity. This last mechanism may be associated with paraquat resistance or defence from damage in general. These mechanisms trade off. The state of the trade off seems to be the result of certain genotypes, which display their phenotypic advantage only in specific environments.

Conclusion

We know that the environment is the only thing the organism, a genetic product and gene carrier, has to live up to and from which it tries to be as independent as possible. Information on the response of this genetic product to the environment provides insight in the relatedness of characters and their environmental specificity.

We have examined life history traits by environmental and genetic manipulation. These approaches sometimes yield seemingly contrasting results. Integrating the interpretation of both types of data yields novel insights. It is clear that both genetics and the environment have large impacts on life history traits. Also, conditions in the larval stages clearly affect the adult stages. Overall, the general patterns that were found earlier were also found here. Building on these, new insights into the fundamental essence of the life histories under study could be obtained. The result was that because of our findings the genetics of starvation resistance and longevity have become partly unravelled and a new and hypothetical relationship between both traits has been suggested. With this hypothesis and the data found here, I trust that the intricate genetics of longevity can be studied in more detail than before.

General statements

1. There is more than one way for a polygenic trait to adapt.

2. A prerequisite is not necessarily a causal factor. Fat is not the cause of starvation resistance. To become starvation resistant, additional processes have to be tuned for starvation resistance as well.

3. One should be very cautious in interpreting correlations among life history traits because they may represent effects of chance, environment or evolutionary history rather than common genetics.

4. The validity of correlative studies is directly related to the variation in conditions in which the correlation has been confirmed.

5. One should be very lucky to find a mutant with a truly longer life-span without a reduction in fitness.

6. Genes and genomes are ultimately dependent on environments. They have been selected to cope with them and can horribly fail if they are not adapted to a certain environment.