Chapter 4
Hypergravity in *Drosophila melanogaster*

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Introduction

Hypergravity (HG), i.e., gravity levels higher than 1 g, the Earth gravity level, can be considered as a stress because the animal subjected to a high g-load has to adapt to a higher weight: a 3 g level means that the weight of the animal is magnified thrice. Contrarily to temperature, HG is not a natural stress, because the gravity level is constant on Earth even if one can experience slight and short HG episodes in everyday life, for instance in cars or elevators during strong braking. Therefore, while species can detect the direction of the Earth's gravity vector, for instance by using the vestibular system (see Sondag 1996), they have probably not evolved specific defense mechanisms against HG. However, even if the analogy is of a limited value, it could be said that subjecting a man weighing 70 kg to a 2 g level is like subjecting him to a 70 kg extra-weight in a backpack. This analogy is of a limited value because, in HG, the increased weight is not confined to the back but is spread on each cell of the body. Thus, even if specific defense mechanisms against HG do not exist, animals can adapt to an increased weight, as it is the case in females of mammals during pregnancy, and it can be hypothetized that they would react in HG conditions as if they were carrying an extra-weight.

Beyond the study of mild stress in flies (see below), HG has been used in mammals, mainly at the US National Aeronautics and Space Administration (NASA) during the 1960s and 1970s (Miquel and Economos 1982). In one of these studies, Economos et al. (1982) reported that rats kept at 3.14 g for life had a shorter, albeit not significant, longevity than rats kept at 1 g. Using HG in mammals is however not an easy procedure, due to technical problems. The centrifuge of the NASA, designed to study aging in rats, had a 16 m diameter (for a picture, see Oyama 1982), while that used to study the vestibular system in hamsters (for a picture, see

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Sondag 1996) had a 3.5 m diameter. Only a very few laboratories can use such centrifuges, due to their size. Beyond technical problems, it is worthy of note that rodents, after a severe adaptation phase during which the body weight decreases (review in Le Bourg 1999), have still some difficulty to cope with a high g-load. Hypergravity attenuates body mass gain (e.g., Kita et al. 2006) and rats exposed to 3.6 g for 21 months or to 4.7 g for 25 months show a deformation of vertebra of the thoracic cervical region with a marked lordosis (Oyama 1982). Therefore, the use of mammals to study the effect of HG on aging and longevity may face various technical and physiological issues. These physiological issues can explain why HG has not been considered in mammals as a mild stress which could have beneficial effects on aging and longevity but, rather, as a strong stress with deleterious effects. In such conditions, turning our attention to other species than mammals can be of interest.

Various mild stresses have been used in *D. melanogaster*, such as temperature (see the chapter "Temperature induced hormesis in *Drosophila*") or irradiation (see the chapter "Irradiation and hormesis"). These stresses have also been used in mammals, sometimes with conflicting results (compare Caratero et al. 1998 and Courtade et al. 2002). These mild stresses are easy to use in flies and it is possible to vary their strength. Obviously, it would be desirable to have at hand a panel of various mild stresses that can be used in the same species, allowing to compare their effects and, possibly, to draw general rules about the effects of mild stress. While non-rodent vertebrate species have sometimes been used in HG conditions (e.g., Anken et al. 1998), most of the studies using HG have been done with *D. melanogaster* in the author's laboratory and in the Genetics Laboratory of the Catholic University of Louvain-La-Neuve, Belgium. Thanks to the tiny size of *D. melanogaster*, the diameter of the centifuges used in France or in Belgium by Lints et al. (1993) was ca. 1 m. In addition, while the centrifuges designed for mammals provide only one gravity level at a time, those designed for flies allow to place vials containing animals at various distances from the axis of the centrifuge, and thus to obtain several g levels at the same time.

Studying HG in flies has other advantages. Hypergravity increases the metabolic demand of flies at the same rearing temperature, due to the higher weight. Therefore, HG is a means to increase the metabolic demand without the adverse consequences of increased temperatures in poikilotherms (increased speed of chemical reactions, decreased longevity, increased activity level, and so on). Since a fly is a holometabolous insect, its growth is complete at emergence, and thus HG cannot have any effect on its adult size, provided development occurs at 1 g (for results on development in HG, see below). Obviously, a study of the effects of HG on aging and longevity could take advantage of the other features of the fly, such as a low longevity, well-known genetics and behavioral patterns, and so on.

This article summarizes the experiments conducted in *D. melanogaster* flies subjected to HG. These results have been published between 1989 and 2005. As a first step of these studies, flies were subjected throughout life to HG. Thereafter, flies were subjected to HG only at a young age.

The First Studies: Hypergravity Throughout Lifespan Is a Strong Stress

Hypergravity is obtained by putting the usual rearing vials of flies in a continuously rotating centrifuge (Fig. 1). If the distance to the axis is increased, the HG level is also increased. With a 102 rpm speed, it is possible to obtain 1.41, 2.16, 3.02, 3.61, 3.97 and 5.02 g. Flies of the 1 g groups are on the same table as the centrifuge and are thus subjected to the same conditions as HG flies (noise, temperature, light). Two other centrifuges have been used from time to time: one, located in France, providing 1.96, 2.33, 2.72, 3.11, 3.51 and 3.92 g levels, and another one, in Belgium, providing 2.58, 3.70, 5.14, 6.31 and 7.38 g levels. Flies are subjected from the second day of imaginal life to a given g level 24 h a day (the centrifuge is briefly stopped twice a week to transfer flies to new vials containing fresh medium). In all experiments described in this chapter, except when indicated, groups of 15 virgin males or females live in 20 mL vials containing the usual medium (corn flour, agar, sugar, dead and live yeast).

Fecundity Is Modified in Hypergravity

Fecundity has been measured for life at various gravity levels in individual females kept with a single male. Since the metabolic demand is expected to increase in HG,

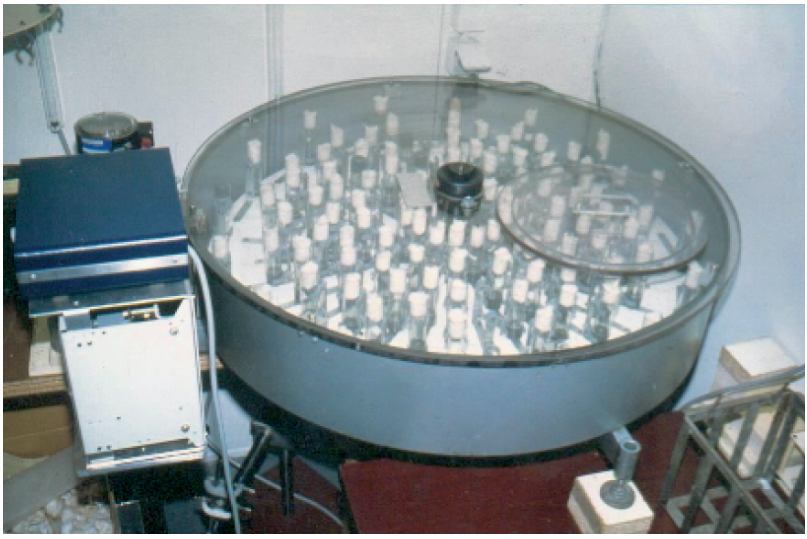

Fig. 1 Picture of the most commonly used centrifuge (1 m diameter). Flies are kept in their usual vials stored in the centrifuge. With a 102 rpm speed, the outer gravity level reaches 5 g

fecundity was expected to be modified in HG as in food restriction conditions, a rearing condition where the metabolic supply is reduced. David et al. (1971) observed that underfed females had lower lifetime, mean daily and maximal fecundities and that, with increased underfeeding, the day of maximal fecundity was delayed. Recording of individual daily fecundity in HG (1–5 g) provided similar results, except that the decrease of lifetime fecundity failed to reach significance (Lints and Le Bourg 1989). Therefore, females modulate their laying activity to cope either with a decreased metabolic supply (underfeeding) or a higher metabolic demand (HG). These first results show that living in HG is not highly detrimental to flies since they remain able to mate and lay eggs. However, the viability of their eggs could be affected by HG and it is necessary to check this point.

Viability of the Eggs Is Slightly Decreased in Hypergravity

The viability, i.e., the percentage of eggs reaching the adult stage, has been recorded to test whether HG is a stress affecting development, as it does in mammals (review in Le Bourg and Lints 1989b). Viability was recorded in eggs developing in HG (1–5 g) from parents kept at 1 g, in eggs developing at 1 g from parents kept in HG (1–5 g), and in eggs developing at the same gravity level as that used for parents (1–5 g). Hypergravity slightly decreased viability since 75% of eggs were able to reach the adult stage in the worst case (94% in the control condition: parents and eggs living at 1 g). Therefore, it can be concluded that flies can lay viable eggs in HG and that these eggs have a normal development in HG; in other words, the HG condition is not really detrimental to life. Nevertheless, even if fecundity and viability are not strongly affected, HG could decrease longevity.

Longevity Is Decreased in Hypergravity

Longevity has been recorded in several experiments. No clear deleterious effect of HG was observed in virgin flies living at a gravity level not higher than 4 g. At 5 g, longevity was shortened by around 1 week in males and 3 weeks in females (Le Bourg and Lints 1989a) but this effect could also be less important (Minois and Le Bourg 1997). Similar results were observed in an experiment testing higher g levels (1–7.38 g), both sexes having a 40 days mean longevity at 7.38 g, which is still a high value at 25 °C (Lints et al. 1993). The same experiment also tested the HG effect in mated flies. Mating strongly decreased longevity, a well-known result (Boulétreau-Merle 1988), and HG slightly decreased longevity in both sexes only above 5 g, this to a lesser extent than in virgin flies. In other words, mating by itself had a stronger impact on longevity than HG.

As for fecundity and viability, keeping flies in HG for life has some negative effect on longevity, which is however not a tragic one. It remains to know whether

HG, in addition to decrease longevity above 4 g, could also impair aging, as inferred from the study of age-related behavioral changes.

Hypergravity Seems to Accelerate Behavioral Aging

Three behaviors which are known to be affected by aging have been observed in flies of various ages living in HG (1–5 g). Flies lived in HG and were transferred at 1 g before their behavior was observed. Therefore, the behaviors were always observed at 1 g and never in HG, simply because it was impossible to observe the flies into the rotating centrifuge.

Climbing activity is the ability measured at the individual level to climb up the vertical side of a vial after having been subjected to a mechanical stimulus. The climbing score is the maximal height reached 20 s after the cessation of the mechanical stimulus. Climbing activity is impaired at older ages and this impairment was observed at younger ages in flies previously kept in HG (Le Bourg and Lints 1992a), no clear gravity effect being observed at 4 days of age.

Young flies have straight paths when they are released at the center of an arena, while older ones exhibit rather sinuous paths and do not move as far away from the center as young flies. These effects of age are increased if flies have lived in HG, no gravity effect being observed at young age (Le Bourg and Lints 1992b).

The spontaneous locomotor activity level, i.e., the number of motions recorded during a 12 h photophase, decreases with age. These effects of age are increased if flies have lived at 5 g, but no effect is observed at 3 g; no gravity effect is observed at young age (Le Bourg and Lints 1992c).

Therefore, these results show that HG seems to accelerate behavioral aging because the normal age-related changes are increased if flies have lived in HG.

However, HG does not always mimick an accelerated behavioral aging because the proboscis-extension response threshold to sucrose of males, which increases with age, is not modified if they live for 1, 4 or 7 weeks at 3 or 5 g (Le Bourg 1996). Hypergravity also decreases the speeds of habituation and learning of the inhibition of the proboscis-extension response of 1-week-old males which have lived in HG, but has no effect in middle-aged and old males that have spent 4 or 7 weeks in HG (Minois and Le Bourg 1997). These results are best explained, not by a decreased ability to learn or habituate, but rather by the consequences of an increased metabolic demand in HG inducing a mild stress to which flies must adapt. This stress was shown to have long-lasting consequences: 5 days after having been transferred at 1 g, young males still displayed a lower speed of habituation than males that always lived at 1 g (Le Bourg 1999).

In conclusion, these results on flies living permanently in HG show that HG modifies fecundity and slightly decreases viability. Longevity decreases above 4 g and behavioral aging can be accelerated in HG. It can be concluded that living in HG up to old age is rather stressful, even if not a threat to life. Since HG is a strong stress when imposed throughout life, it could be that a short stay in HG, at a young

age, would act as a mild stress with possible hormetic effects. This is the rationale which prompted all the next described studies on HG.

Hypergravity at a Young Age Is a Mild Stress with Hormetic Effects

In all following experiments flies only spent a part of their life in HG, usually the first 2 weeks of adult life, but sometimes a shorter or a longer time. After the end of the stay in HG, flies were transferred to 1 g. Except when indicated, the same temperature was used into the centrifuge and at 1 g (25 °C), flies were virgin, the gravity level was in the 1–5 g range and experiments were performed in the French laboratory. The Belgian laboratory used a gravity range from 1–7.38 g.

Two Weeks in Hypergravity Increase Longevity of Males, but Not of Females

Two weeks in HG increase longevity in males (Fig. 2, +10 to 20%), but not in females, for which a negative effect of HG can exist. These results have been observed (Le Bourg and Minois 1997; Le Bourg et al. 2000, 2002) in two laboratories (France and Belgium) with two strains differing by their mean longevity at 1 g (Fig. 2b).

Since 2 weeks in HG increase longevity of males, it was of interest to vary the duration or schedule of exposure to HG. In a first experiment, flies spent a total of 12 days in HG with 4 days in HG followed by 3 days at 1 g. No positive effect of HG was observed in either sex, showing that males must be continuously exposed to HG to live longer. In the same way, a 1-week exposure to HG failed to increase longevity. By contrast, 3 weeks in HG still increased longevity in males but not 25 days, while 14, 19 and 24 days of HG exposure (1–7.38 g) increased longevity in the Belgian lab: it thus seems that 3 weeks of HG is the longer exposure which can clearly give rise to a longevity increase. This last point was checked again in a last experiment using individual rearing after transfer to 1 g, which increased longevity, particularly in males (Table 1 in Le Bourg et al. 2000). In such conditions, 25 days of HG exposure increased longevity of males (mean longevities respectively for 1, 3 and 5 g groups: 59.27, 62.60, 64.66 days). In this last experiment, while 13 days in HG increased longevity of males living in individual vials after transfer to 1 g (respectively for 1, 3 and 5 g groups: 53.78, 61.42, 61.22 days), 3 days or 46 days in HG failed to do so.

All these results (Le Bourg and Minois 1997; Le Bourg et al. 2000, 2002) show that 2 weeks in HG increase longevity of males but not of females, a shorter or intermittent exposure being inefficient. Longer exposure can also increase longevity, but this result

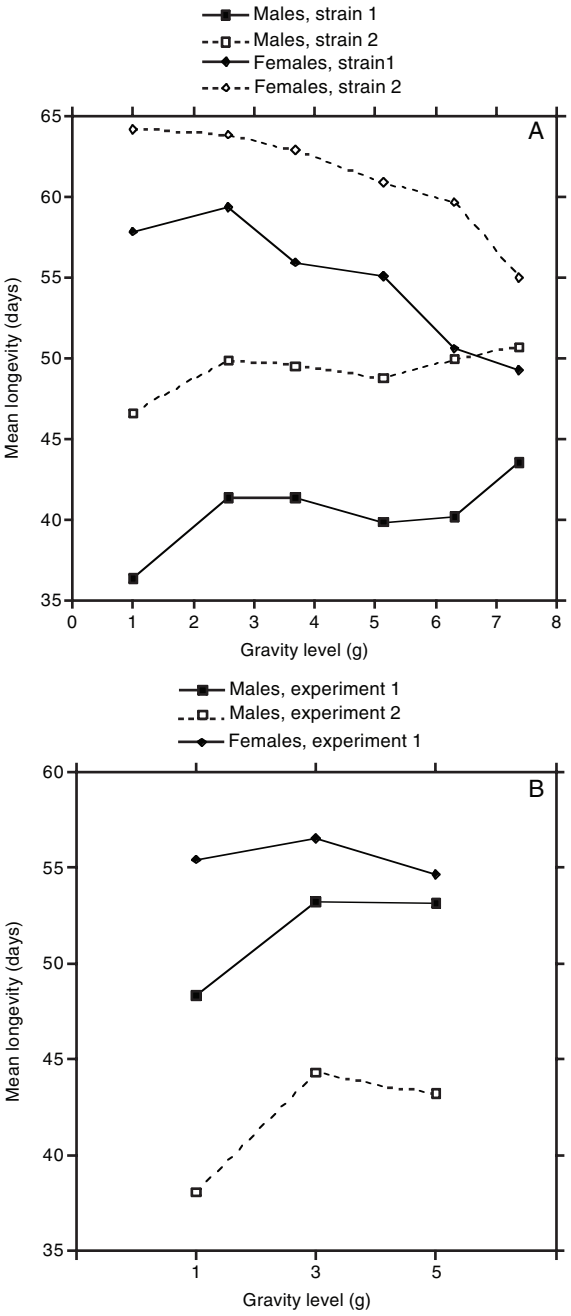

Fig. 2 Effect of a 2-week stay in HG, from the second day of adult life, on longevity of *D. melanogaster.* A. Two wild-type strains, differing by their mean longevity at 1 g, are used in a Belgian laboratory (Le Bourg et al. 2000). Each point is the mean of ca. 145 flies. B. The strain 2 (wild-type strain Meyzieu) is tested with another centrifuge in a French laboratory. The figure reports two experiments for which the longevity at 1 g was contrasted (Le Bourg et al. 2000, 2002). Each point is the mean of ca. 60 (experiment 1) or 100 flies (experiment 2)

is not stable, as it depends on the rearing condition (individual vs group rearing) and on experiments. It seems probable that 3 weeks in HG are the limit between a mild stress with hormetic effect and a strong stress with no such effect (see the chapter "What is hormesis?" for a discussion of the hormetic dose–response relationship).

As a matter of fact, the positive effect of HG on longevity can disappear if flies are subjected to a too strong stress. Mating is known to decrease longevity (Boulétreau-Merle 1988): in mated males, 2 weeks in HG have no positive effect on longevity, which indicates that subjecting flies to a living condition decreasing longevity can suppress the positive effect of HG. Similar conclusions can be reached if flies are transferred after a 2-week stay in HG at 28 °C or 30 °C: no positive HG effect on longevity of males is observed in these conditions shortening longevity (Le Bourg et al. 2004). This result is exactly the contrary of what was expected by Sacher (1977), who wrote that hormetic effects are "unlikely to occur in the healthy active individual, and are more likely to be significant in the ill or depressed animal".

These results on longevity thus show that HG may have a hormetic effect on longevity. It is of interest to know whether similar effects are observed on behavioral aging.

Two Weeks in Hypergravity May Delay Behavioral Aging, Mainly in Males

The same behaviors as those previously observed during the study of HG throughout life (see above) have been used.

Flies kept in HG for 2 weeks displayed a lower climbing score than those always kept at 1 g the day following transfer to 1 g, but higher scores some days after this transfer (Fig. 3, Le Bourg and Minois 1999; Le Bourg et al. 2002), all flies being unable to climb up the side of the vial at 5 weeks of age. This effect is mainly observed in males, but a positive effect of HG may also be shown in females. No positive effect of HG has been observed if flies were kept in HG for only the first week of adult life or for 4 weeks (Minois 1998). However, it is difficult to conclude anything from this last experiment, because climbing scores are very low in flies older than 4 weeks of age.

Old flies released at the center of a circular arena exhibit rather sinuous paths and do not move as far away as young flies. At old age, flies kept in HG for 2 weeks at young age moved more away the center of the arena than flies always kept at 1 g, but HG had no effect on the sinuosity of the path (Le Bourg and Minois 1999). There is thus only a slight tendency for a slower behavioral aging if flies have lived in HG at a young age.

By contrast, having lived in HG for 2 weeks at young age had no positive effect on spontaneous locomotor activity level at old age, i.e., the number of motions recorded during a 12 h photophase (Le Bourg and Minois 1999).

All these results indicate that, depending on the studied behavior, a 2-week stay in HG at young age can delay behavioral aging or has no effect on it. Thus, HG can increase longevity of males and delay aging: could it also protect against strong stresses?

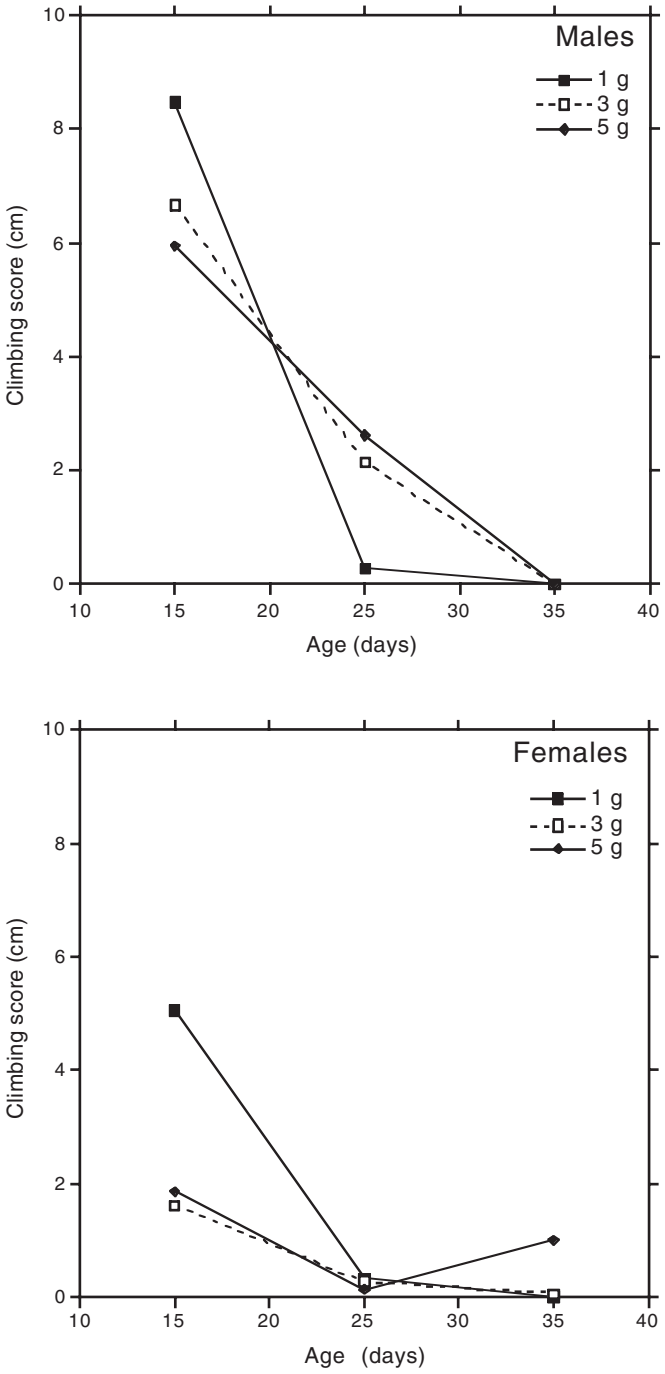

Fig. 3 Effect of a 2-week stay in HG, from the second day of adult life, on climbing scores of *D. melanogaster*, i.e., the ability, measured at the individual level, to climb up the vertical side of a vial after having been subjected to a mechanical stimulus (Le Bourg et al. 2002). The climbing score is the maximal height reached in 20 s after the cessation of the mechanical stimulus. Each point is the mean of 15 flies

One to 4 Weeks in Hypergravity Increase Survival Time to Heat but have no Effect on Other Stresses

A mild stress may increase resistance to a strong stress. For instance, a mild heat or cold stress increases survival time to heat (see the chapter "Temperature induced hormesis in *Drosophila*"). Similarly, spending 2 weeks in HG at a young age increases survival time at 37 °C in both sexes (Fig. 4). Other experiments, using either 1, 2 or 4 weeks of HG exposure starting the second day of life provided similar results, but no HG effect was observed with a 7 weeks exposure (Le Bourg and Minois 1997; Le Bourg et al. 2002; Minois and Le Bourg 1999; Minois et al. 1999).

Could this effect of HG on heat resistance be explained by a behavioral adaptation to heat? Indeed, flies previously kept in HG could be more inactive at 37 °C than flies always kept at 1 g: this reduced locomotor activity, a costly metabolic activity, could explain their higher survival time. Spontaneous locomotor activity was individually recorded at 37 °C until death in flies which spent 1 week in HG (Minois and Le Bourg 1999). These flies survived longer at 37 °C than those always kept at 1 g but the mean activity level did not vary with the gravity level (1, 3, 5 g), which shows that the high survival of flies which have lived in HG is not explained

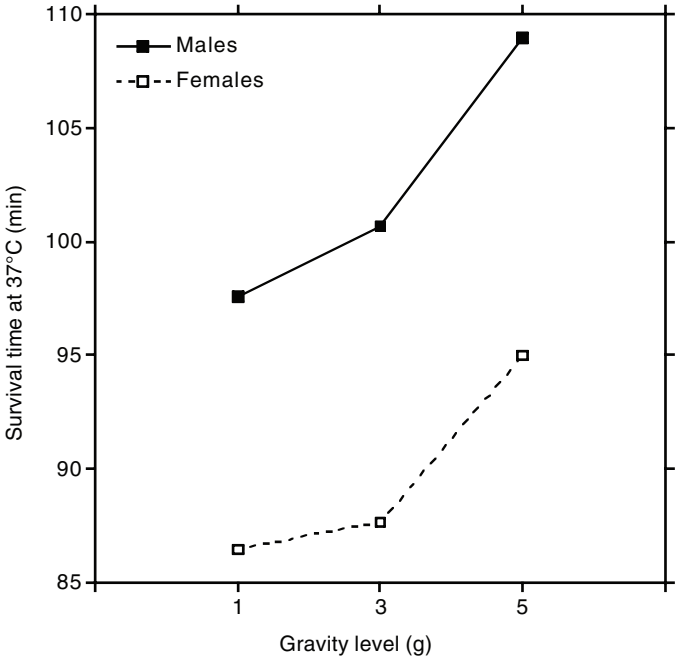

Fig. 4 Effect of a 2-week stay in HG, from the second day of adult life, on survival time at 37 °C of *D. melanogaster*. Flies are placed in tight vials in a waterbath set at 37 °C and the number of dead flies is recorded every 5 min up to the death of the last fly. Each point is the mean of ca. 45 flies

by a decreased energy expenditure. Furthermore, in each sex and gravity group, the individual activity score was positively correlated with survival time. In other words, in each sex and gravity group, flies which survived longer were also more active.

The positive effect of HG on heat resistance cannot be extended to other stresses because HG has no effect on resistance to cold (0 °C), desiccation and starvation (Minois and Le Bourg 1999).

Two Weeks in Hypergravity Help Middle-Aged Males to Cope with Simulated Heatwave

Flies kept in HG at a young age survive longer at 37 °C than flies always kept at 1 g (Fig. 4). While this result is of interest, it would be still more interesting if HG could also help to recover from a deleterious but non lethal heat stress, such as a sudden but transient temperature rise. These transient temperature rises have been of a tragic importance for elderly people during the 2003 summer heatwave.

To simulate heatwave, flies have been subjected to a 37 °C stress for 60 or 90 min, a duration decreasing longevity (–50%) after the heat shock, but not killing flies, since most of them survived to these rather long heat shocks. Males heat-shocked at 4 weeks of age which lived 2 weeks in HG at a young age survived 15% longer than 1 g ones (Fig. 5). No positive effect of HG was observed at 5 or 6 weeks of age and in 4- to 6-week-old females. The heat shock decreased climbing activity and spontaneous locomotor activity scores but HG did not counteract this effect at any age and in any sex. Therefore, HG protects against a deleterious non lethal heat shock but not against the behavioral impairments due to this shock (Le Bourg et al. 2004).

This experiment was reiterated using several shocks of a shorter duration. Flies were subjected from 4 weeks of age to 4 heat shocks (30 or 45 min at 37 °C) during a 2-week period. Males that spent 2 weeks in HG at a young age lived 15% longer than flies always kept at 1 g, no effect being observed in females (Fig. 6). Furthermore, living in HG had nearly no effect on the longevity difference between the HG and the 1 g groups when the deleterious effect of heat was moderate, i.e., when the longevity of 1 g groups was not strongly shortened by heat. By contrast, males took advantage of a stay in HG if the negative effect of heat was important (Le Bourg 2005). Therefore, these results show that a mild stress applied at young age protects against a strong stress at middle age.

In conclusion, this whole set of results shows that HG at a young age has hormetic effects on longevity, provided HG exposure is not too short or long and the rearing conditions do not decrease longevity. Hormetic effects are also observed on behavioral aging (but not on all studied behavioral traits), and on resistance to heat (but not to other stresses). Hypergravity can thus help flies, particularly males, to live a better old age and it is now of concern to know the possible mechanisms of these effects.

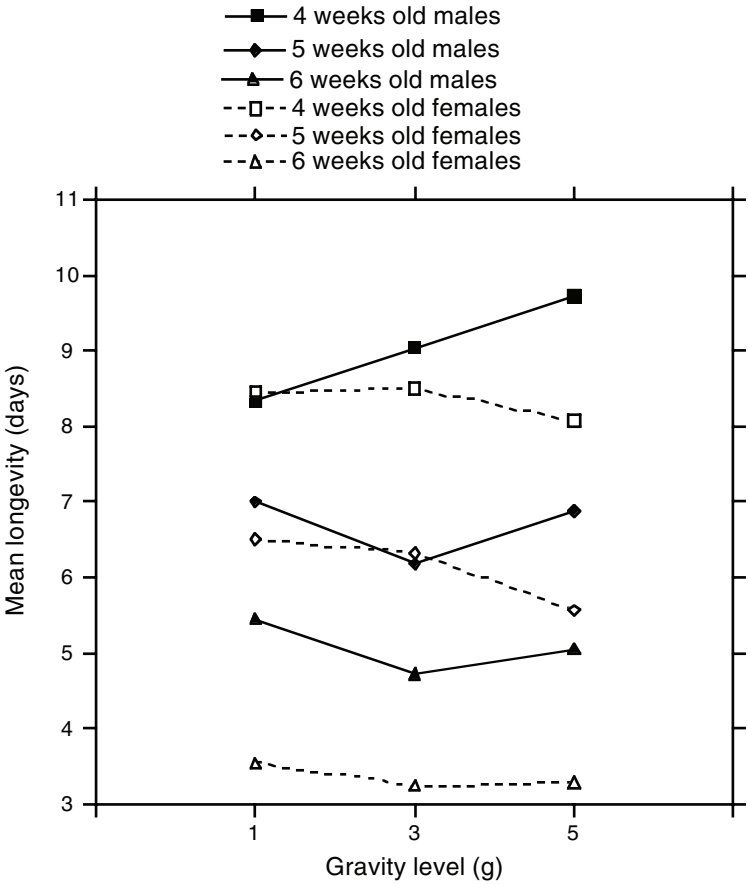

Fig. 5 Effect of a 2-week stay in HG, from the second day of adult life, on longevity of *D. melanogaster* after a 60 or 90 min 37 °C heat shock. Day 0 is the day of shock and the 60 and 90 min groups are pooled in the figure. Flies were either 4-, 5- or 6-week old the day of heat shock. Each point is the mean, respectively at 4, 5 and 6 weeks of age, of 133–170, 107–165, and 94–147 flies

A Search for the Mechanisms of the Hormetic Effects of Hypergravity

A mild stress, i.e., a low dose of an otherwise deleterious stimulus is expected to upregulate maintenance and repair pathways to induce hormesis (see the chapter "What is hormesis?"). Studies on two kinds of such repair pathways have been done in flies subjected to HG: the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), on the one hand, and the 70 kDa heat shock protein on the other hand.

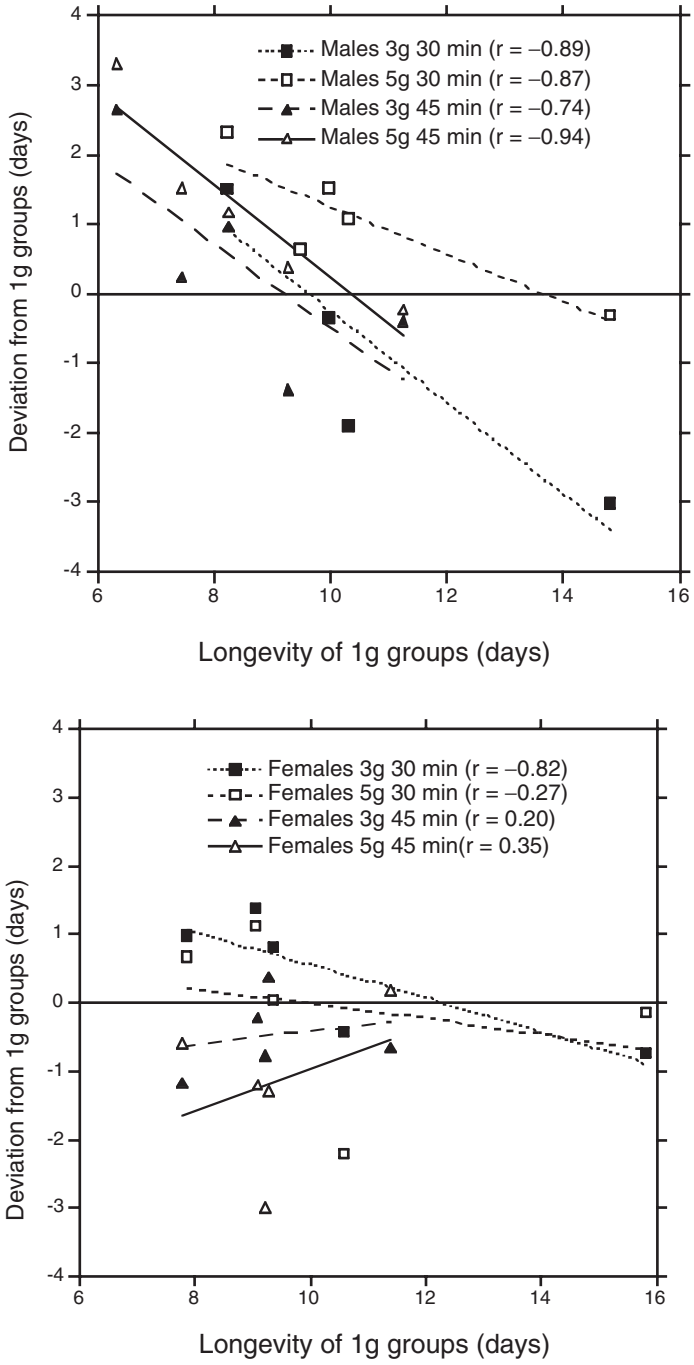

Fig. 6 Effect of a 2-week stay in HG, from the second day of adult life, on longevity of *D. melanogaster* after 4 heat shocks (30 or 45 min at 37 °C) during a 2-week period starting at 4 weeks of age. The figure shows the difference between the mean longevity of flies which lived at 3 or 5 g and that of flies always living at 1 g. For each length of heat shock, five replicates were done (total n = 2,352). Each point stands for the mean of a replicate

Antioxidant Enzymes Do Not Explain the Hormetic Effects of Hypergravity

According to the well-known free radical theory of aging (Harman 1956), aging is explained by the damages due to the free radicals produced during normal metabolism, despite the existence of antioxidant defenses. If this theory is correct, one may expect that a higher activity of antioxidant enzymes, for instance in transgenic flies overexpressing SOD or CAT, could increase longevity (review in, e.g., Sohal et al. 2002).

Since flies weigh more in HG than at 1 g, their metabolic rate could be increased in HG: the egg-laying activity decreases in flies living in HG as in food-restricted flies (see above), which suggests that the metabolic demand increases in HG. The metabolic rate of flies has not been measured in HG but a higher metabolism has been reported in HG-adapted rats than in 1 g ones (Oyama and Chan 1973; Daligcon and Oyama 1975; Wade et al. 2002). One could thus hypothetize that a higher metabolic rate of flies in HG could induce a higher activity of antioxidant enzymes in order to counteract an increased production of free radicals during the oxidative phosphorylation process. As an outcome, flies that have lived in HG could be more protected against free radical attacks than those always living at 1 g and, if this protection would persist after removal from HG, they could better resist free radicals and live longer. However, a longer life would be observed only if antioxidant defenses do have a beneficial effect on longevity, as postulated by the free radical theory (Harman 1956).

The first step to test this hypothesis is to measure the activity of antioxidant enzymes in flies that have lived in HG. SOD and CAT were measured in homogenates of individual males and females that have lived at various gravity levels (1, 3, 5 g) for the first 2 weeks of adult life. The enzymatic activity was measured at 2, 4, or 6 weeks of age, i.e., just after transfer from HG to 1 g, and 2 or 4 weeks later: the gravity level had no effect on SOD and CAT at any age and in any sex (Le Bourg and Fournier 2004).

Therefore, flies that have lived in HG are not better protected against free radical attacks than flies always living at 1 g. This result is in accordance with that of Niedzwiecki et al. (1992) who showed that a 37 °C heat shock had no clear effect on SOD and CAT activities in 2-, 24-, or 50-day-old male flies. Even if other antioxidant defenses, such as reduced glutathione, have not been measured in our studies it seems that the increased longevity of males is not linked to an increased activity of antioxidant enzymes. Since antioxidant enzymes appear not to explain the hormetic effects of HG, it is therefore needed to focus on another repair pathway, the heat shock proteins.

The 70 kDa Heat Shock Protein Could Explain the Hypergravity Effect on Heat Resistance but Not That on Longevity

Heat shock proteins (Hsp) are molecular chaperones, differing by their molecular weight, which are induced by various stresses in *D. melanogaster* and other species

(review in Morrow and Tanguay 2003). In *D. melanogaster*, the 70 kDa Hsp (Hsp70) is expressed, for instance, after a heat (e.g., Dahlgaard et al. 1998) or cold shock (Sejerkilde et al. 2003, but see also Overgaard et al. 2005). It is known that heat or cold shocks can increase longevity and resistance to some stresses, particularly in *D. melanogaster* (see the chapter "Temperature induced hormesis in *Drosophila*"). Therefore, Hsp70 could explain the hormetic effects of temperature stresses on longevity. Could a higher expression of Hsp70 also explain the hormetic effects of HG on longevity, resistance to heat and behavioral aging?

A first study (Western immunoblot procedure) measured Hsp70 expresssion in flies that lived in HG (3 or 5 g) for 1, 4 or 7 weeks before to be transferred at 1 g (Minois et al. 1999). An antibody against both the inducible and constitutive (i.e., expressed even in the absence of heat shock) forms of Hsp70 was available for this study. The comparison of the size of the blots showed that, in each sex, HG did not increase Hsp70 expresssion at any age. By contrast, flies kept in HG for 2 weeks from the second day of adult life and subjected to a heat shock (60 min at 37 °C) after their transfer at 1 g had a higher synthesis of Hsp70. Thus, HG by itself did not provoke Hsp70 synthesis, even after 7 weeks in HG, but more Hsp70 was expressed after a heat shock if flies were kept in HG before this heat shock. This study has been replicated using an antibody against the inducible form of Hsp70 only. It was confirmed (Fig. 7) that HG does not provoke Hsp70 synthesis but that more protein is synthetized after a heat shock (45 min at 37 °C) if flies lived for 2 weeks in HG at young age (Le Bourg et al. 2002).

The HG-linked increased heat resistance could thus be explained by an increased Hsp70 synthesis. However, the increased longevity or delayed behavioral aging of flies that lived in HG for 2 weeks cannot be explained by Hsp70 because this protein is not synthetized at 25 °C, the rearing temperature of flies, even after 1, 2, 4 or 7 weeks in HG. Since Hsp70 probably explains a part of the HG effects, one may wonder whether transgenic flies overexpressing *hsp70* would take advantage of more Hsp70 synthesis when subjected to HG.

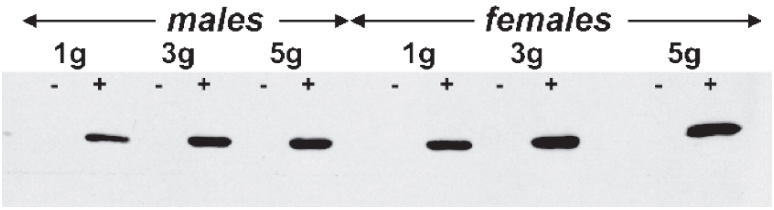

Fig. 7 Effect of a 2-week stay in HG, from the second day of adult life, on Hsp70 expression of *D. melanogaster* after a heat shock (45 min at 37 °C: + on the figure) or no heat shock (– on the figure). Fifteen flies were used for each blot

Overexpression of the 70 kDa Heat Shock Protein does not Increase the Hypergravity Effect on Heat Resistance and has no Effect on Longevity or Behavioral Aging

Thanks to transgenesis technics, flies overexpressing *hsp70* are available (Welte et al. 1993). Since HG increases Hsp70 synthesis when flies are subjected to a heat shock, it could be hypothetized that these transgenic flies would take more advantage of a stay in HG than control ones.

The longevity of flies which have lived for 1 or 2 weeks in HG (3 or 5 g) at young age was recorded in a transgenic strain with 12 extra-copies of the *hsp70* gene or in a control strain harboring the transfection vector but no extra-copies (Le Bourg et al. 2002). In both strains, no positive effect of HG was observed in males and HG decreased longevity of females. The experiment was reiterated with males only, flies being transferred to individual vials after 1 or 2 weeks in HG, which increased longevity (+50%). Here again, no positive effect of HG on longevity was observed in either strain. In all experiments, the transgenic strain lived for a shorter time than the control one. A control experiment, carried out at the same time as the experiments with the transgenic and control strains, showed that males of the wild strain used in most of experiments described in this article lived longer if they spent 2 weeks in HG. Thus, the absence of a positive HG effect on longevity in the transgenic and control strains is really strain-specific. It could be that the transgenic and control strains suffer from a heavy genetic load obscuring the positive effects of HG on longevity observed in wild-type strains (Fig. 2), because the transgenic and control strains are derived from an inbred strain. In accordance with this hypothesis, no positive effect of HG on climbing activity at middle age was observed in these strains, while such an effect was observed in the usual wild strain (Fig. 3).

By contrast, 1 or 2 weeks in HG increased survival time at 37 °C in both transgenic and control strains. This effect was due to males only (Fig. 8). Furthermore, the transgenic strain survived longer at 37 °C than the control strain at 1 week of age, but no strain effect was observed at 2 weeks of age. The positive effect of the extra-copies of the *hsp* gene on survival to heat is thus transient. Finally, HG increased survival time at 37 °C to the same extent in males of both transgenic and control strains (Fig. 8), showing that extra-copies of the *hsp70* gene increase survival time at 37 °C, but not the positive effect of HG on survival time.

This whole set of results shows that the positive effects of HG on survival time at 37 °C are not increased in flies carrying extra-copies of the *hsp* gene. The longevity of the males of the transgenic and control strains is not increased after having lived in HG and is even decreased in females, and the transgenic strain lives for a shorter time than the control one: thus, extra-copies of the *hsp* gene could be detrimental to a normal life (Krebs and Feder 1997; Klose et al. 2005). Therefore, a higher number of extra-copies of the *hsp* gene has no positive effect on longevity.

Furthermore, HG increases survival time at 37 °C and has no positive effect on longevity of these transgenic and control strains. This result observed in females of the usual wild strain (compare Figs. 2 and 4) is thus also shown in both sexes of the transgenic and control strains. The effects of HG on survival to heat and longevity

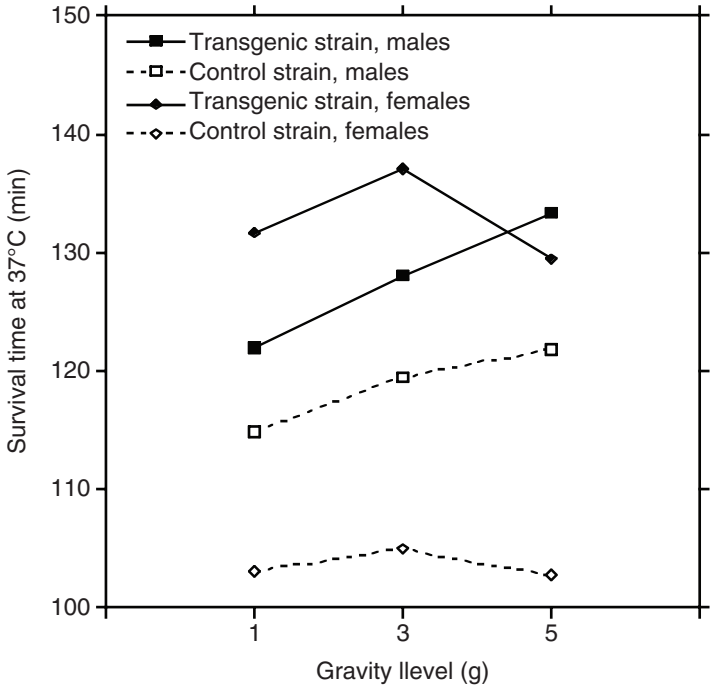

Fig. 8 Effect of a 1-week stay in HG, from the second day of adult life, on survival time at 37 °C of *D. melanogaster* transgenic and control strains. The transgenic strain has 12 extra-copies of the *hsp70* gene. Flies are placed in tight vials in a waterbath set at 37 °C and the number of dead flies is recorded every 5 min up to the death of the last fly. Each point is the mean of ca. 45 flies

are thus probably not due to a single cause: the increased resistance to heat is probably linked to the increased synthesis of Hsp70, while the cause of the increased longevity remains unknown.

In conclusion, these studies have shown that the HG effects on longevity, behavioral aging and resistance to heat are not explained by an increased activity of the antioxidant enzymes SOD and CAT. The increased synthesis of Hsp70 can probably explain, at least partly, the increased resistance to heat but not the effects on longevity and behavioral aging, since Hsp70 is not synthetized at 25 °C. Therefore, the cause of the HG effects on these traits remains unknown and more research on this question is needed.

General Conclusion: Hypergravity As a Mild Stress with Hormetic Effects on Aging, Longevity and Heat Resistance

Spending 2 weeks in HG at a young age has beneficial effects on longevity (in males), behavioral aging (mainly in males) and survival time at 37 °C (in both sexes). Furthermore, males subjected to HG at young age live longer after non-lethal

heat shocks occurring at middle age. The cause of the two first effects remains unknown while the increased resistance to heat seems to be linked to an increased Hsp70 synthesis.

Hypergravity is thus a good example of a mild stress with hormetic effects on aging and longevity but, as emphasized in the introduction of this article, it probably cannot be used to study hormesis and aging in mammals. However, this study of HG shows the possible effects of a mild stress in an organism. Other studies of mild stress in *D. melanogaster* carried out in the same laboratory and with the same strain have shown that mild heat shocks at young age could also, but very slightly, increase longevity. This study also reported a longer survival at 37 °C after mild heat shocks, but not a delayed behavioral aging (Le Bourg et al. 2001). Cold shocks at young age also increase longevity and survival time at 37 °C, decrease the deleterious effects of non-lethal heat or cold shocks on longevity, and delay an age-related behavioral change (Le Bourg 2007a). Finally, if middle-aged flies feed on a sucrose solution, a poorly nutritious medium which strongly decreases longevity, a low dose of hydrogen peroxide added to this solution reduces its negative effect on longevity (Le Bourg 2007b). This result shows that a low dose of a harmful chemical can be beneficial in some conditions.

Therefore, various mild stresses have positive effects on aging, longevity, and resistance to stress in *D. melanogaster*, but these effects are dependent on the mild stress used to provoke hormesis. For the time being, *D. melanogaster* is the species for which there is the most extended database on the effects of mild stress on aging. Since various mild stresses have rather similar positive effects on aging, it seems inescapable to conclude that hormetic effects on aging do exist and that they can be observed easily, provided the experimenter is able to identify the mild stress to use. One can thus hypothetize that hormesis has been selected during the course of evolution as a means to protect against strong stresses occurring at young age but, since in the wild most of animals do not live up to old age, it was not used to protect the old organism or to increase its longevity. Thanks to laboratory rearing methods allowing flies to live up to old age, it is possible to show that hormetic effects can also be observed at old age. Hormesis thus appears to be a rescue system designed to be used at young age that can be also used at old age, even if, in the wild, it is never used at this age due to the death at an early age of most of animals.

The antagonistic pleiotropy theory of aging (Williams 1957, review in Le Bourg 2001) tells us that alleles may have beneficial effects at young age and deleterious ones at old age, and that these undesired effects have not been selected against. There is no selection against these deleterious effects simply because at old age a very few animals are still alive and reproduce and, thus, alleles provoking such effects cannot be eliminated from the gene pool. The hormesis results seem to indicate that a rescue system used at young age can be used at old age, i.e., that favorable effects at old age have not been selected for. These favorable effects at old age have not been selected because only very few animals are still alive at this age. In a way, there is thus some irony regarding side-effects occurring at old age of the natural selection process: provided an animal is able to reach old age, side-effects of genes selected for their effects at young age may reveal to be favorable (hormesis) or not (antagonistic pleiotropy).

If the existence of hormesis can be linked to a selection process of a rescue system used at young age by various species (Minois 2000), hormetic effects at old age are probably not present only in a single species, *D. melanogaster*. Since various mild stresses have rather similar positive effects in the fly, one may wonder whether there is some hope to extend the conclusion that mild stress is beneficial to aging, longevity and resistance to strong stresses not only to other poikilotherms (e.g., in the nematode, Cypser and Johnson 2002), but also to mammals. The panel of effects observed in *D. melanogaster* is rather large, which warrants to search for stresses having similar effects in mammals. While exercise has beneficial effects on aging and longevity of mammals, including human beings (see the chapter "Physical activity: a strong stimulant for hormesis during aging"), it is necessary to discover other mild stresses than exercise because it would be of interest to have at hand several mild stresses with hormetic effects in mammals. As emphasized in the introduction of this chapter, HG cannot be such a stress for mammals, and particularly human beings, for technical and physiological reasons. However, it can be expected that, since HG, temperature (chapter "Temperature induced hormesis in *Drosophila*"), and irradiation (chapter "Irradiation and hormesis") have beneficial effects on the aging of *D. melanogaster*, several stresses could be efficient in mammals, too. Obviously, if hormesis would remain confined to lower species, it would be a very interesting phenomenon but of no application to prevent age-related diseases and maintain physical and mental abilities. The issues to resolve to use hormesis in therapy are thus important (Rattan 2004) and only experimental work will tell us whether using mild stress in aging research was a good idea or not.

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