

medium (CeMM) in comparison to the NGM one.³⁷ CR created by serial dilution of bacterial culture has been widely used in studies of worm longevity.³⁸ The advantage of this method is the possibility of testing life span in a broad range of bacterial concentrations. Finally, the complete absence of bacteria on plates also extended the life span of nematodes.^{39,40} Interestingly, in these conditions worms lived 50% longer but restriction of bacteria by 90% increased life span by 20%.³⁹

10.2.3 CR in Fruit Flies

Studies on various arthropod species show the possibility of extending the life span by CR in these animals. The most useful model is fruit fly *Drosophila melanogaster*. This model has many advantages like short generation time, life span of about 2–3 months, availability of many mutants and the possibility to manipulate genes of interest in the whole organism or very specific cells. In addition, flies can be fed on a diet with only sucrose and yeast. The combination of sucrose and yeast in the diet makes it possible to produce a diet with different caloricities or ratios of macronutrients, such as protein and carbohydrate. A few types of dietary manipulations can be distinguished for fruit flies. The first one is food dilution, which is usually achieved by simple reduction of sucrose and yeast within the diet. Dilution decreases the caloric value of the diet. The only problem is that flies eat more to compensate for the lower caloric value of the diet by increasing amount of volume eaten when given full access to food.⁴¹ Second, dietary manipulation is usually called dietary protein restriction and is experimentally realized by using different concentrations of yeast (or protein in some cases). In principle, this means that researchers use diets with different protein-to-carbohydrate ratios ($P:C$). The power of using fruit flies in studies involving dietary effects on life span was most fully used by evolvement of the geometric framework (GF) firstly introduced by Simpson and colleagues.⁷ Later GF was used on other models, including mice. GF suggests using an array of diets with varied ratios between macronutrients with different total diet caloricity. Since the caloric value of yeast and sucrose is about 4 cal gram⁻¹, an isocaloric diet can be simply prepared by adding these compounds in the same concentrations.

CR achieved by food dilution had a beneficial effect on fly life span. It was shown that restriction of the initial diet by 60% and 40% caused life extension in males and females, respectively.⁴² It was discovered that dietary restriction (DR), as well as lowering the amount of only one or a few components of the diet, affects life span by decreasing the mortality rate. This decrease can be observed in two days after changing the diet from the control to the restricted one.⁴³ Conversely, the mortality rate returned to the control values when flies were switched from a dietary restricted to an *ad libitum* diet. Taken together, these results show that DR can be implicated at any age to extend the life span. Many studies were performed to study the interaction of specific genes and diet in regulation of life span. It was shown that Sir2 mediates longevity in the same way as CR. Ubiquitous