Prolongevity effects of a botanical with oregano and cranberry extracts in Mexican fruit flies: examining interactions of diet restriction and age

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Abstract Botanicals rich with phytochemicals have numerous health benefits. Dietary restriction (DR) extends lifespan in diverse species. We previously demonstrated that an oregano–cranberry (OC) mixture can promote longevity in the Mexican Fruit fly (Mexfly, *Anastrepha ludens* Loew). However, little is known about the interaction between botanicals and DR, and the age-dependent effect of botanicals on lifespan and reproduction. Here we investigated these issues by feeding Mexflies a full or DR diet supplemented with

or without 2% OC. Lifespan and daily egg production of individual flies were recorded. The effect of short-term OC supplementation was evaluated by implementing the supplementation at three age intervals—young, middle, and old age. We found that OC increased lifespan of Mexflies on the full or DR diet when compared to their respective controls. OC increased reproduction of females on the full diet and, to a lesser extent, on the DR diet. Short-term OC supplementation was not sufficient to extend lifespan for males at all three age intervals nor for females at young and old age intervals. However, OC supplementation at the middle age interval was sufficient to extend lifespan in females, while only OC supplementation at the young age interval increased reproduction in females. Our findings suggest that OC extends lifespan and promotes reproduction partly through DR-independent pathways, and short-term supplementation have varied impact on longevity and reproduction. This also suggests a positive interaction between non-genetic interventions in promoting longevity and provides guidance for using botanicals as aging interventions in humans.

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Abbreviations

DR	Dietary restriction
OC	Oregano and cranberry
Mexfly	Mexican fruit fly

Introduction

It is challenging to identify and implement efficient and cost-effective aging interventions to promote longevity and healthspan in humans. Emerging lines of evidence indicate that consumption of botanicals, such as fruits and spices, rich in phytochemicals and antioxidants provides numerous health benefits, such as reducing cancer incidence and delaying the aging-related decline of cardiovascular and cognitive function (Joseph et al. 2007; Liu 2004; Seeram 2008). Botanicals, such as extracts from fruits to spices, have also been shown to possess prolongevity properties in model organisms. Blueberry extracts can extend lifespan in the nematode *Caenorhabditis elegans* partly through a calmodulin-dependent kinase-mediated pathway (Wilson et al. 2006). Resveratrol, a polyphenolic compound present in grapes and many plants, has been found to extend lifespan in yeast, worms, flies, and short-lived fishes (Allard et al. 2009; Pirola and Frojdo 2008; Wood et al. 2004; Valenzano et al. 2006). It should be pointed out that the longevity promoting property of resveratrol remains controversial since some other investigators have not observed lifespan extension by resveratrol in their experimental conditions (Bass et al. 2007b; Kaeberlein et al. 2005).

Several lines of evidence indicate that botanicals can interact with dietary macronutrients to modulate lifespan. For example, the prolongevity effect of resveratrol in the Mexican fruit fly (Mexfly), *Anastrepha ludens* Loew, has been shown to be limited to the Mexfly under a narrow dietary condition, which contains high sugar to protein ratio and low calorie content (Zou et al. 2009). Resveratrol has also been reported to promote survival of mice under a high-fat diet but not a standard diet (Pearson et al. 2008). A mixture of oregano and cranberry (OC) extracts promotes longevity of the Mexfly under the dietary conditions with relatively high ratios of sugar to protein (Zou et al. 2010). A freeze-dried açai extract can promote longevity of *Drosophila melanogaster* under a high-fat diet but not a standard diet condition, partly through modulating the oxidative stress response pathways (Sun et al. 2010). These findings indicate the complications of using botanicals as aging interventions, especially considering the diverse dietary customs of humans.

Lifespan and healthspan of multi-cellular organisms are influenced by genetic and environmental factors, including dietary nutrients (Kenyon 2010;

Masoro 2006; Partridge et al. 2005; Fontana et al. 2010). Dietary restriction (DR) without malnutrition has been shown to extend lifespan and promote healthspan in diverse organisms, although the effect of DR on lifespan in some cases depends on the genetic background of an organism (Liao et al. 2010; Masoro 2006; Partridge et al. 2005; Sohal et al. 2009). Health benefits of DR can be achieved even through implementation for the short-term. In *D. melanogaster*, DR is often conducted by diluting all nutrients of a full standard diet (Bass et al. 2007a). Switching flies from a full diet to a DR diet can result in a reduced age-specific mortality rate in 2–3 days, while a reverse switch results in an increased age-specific mortality (Mair et al. 2003). This temporal effect of DR has also been observed in higher organisms, such as mice, rats, and monkeys, although changes in mortality rates occur at a much slower pace. Typical average lifespan of laboratory mice and rats is approximately 2–2.5 years. Initiation of DR in mice or rats of more than 1 year old is sufficient to promote longevity and healthspan (Masoro 2006). Mean life span of the rhesus monkey is approximately 27 years in captivity. Implementation of DR in adult rhesus monkeys of 7–14 years old has been shown to reduce the age-specific mortality rate and improve health in these monkeys (Colman et al. 2009). These findings suggest that short-term DR can be effective in alleviating age-associated damage.

An ideal intervention to promote lifespan and healthspan in humans would act synergistically or additively with other prolongevity interventions and be efficient even when implemented for a relatively short period of time. Numerous pharmaceutical studies have demonstrated that dietary components can interact with drugs, and thus influence the efficacy and side effects of drugs (Fimognari et al. 2008; Ulbricht et al. 2008; Murray 2006). Despite increasing interest in the potential health benefits of consumption of botanical products, not much is known about the effects of botanical consumption and especially the interaction between botanicals and dietary macronutrients on lifespan and healthspan. Much less is known about the efficient and cost-effective ways for implementing aging interventions using botanicals, let alone the mechanisms of various intervention paradigms. We have previously demonstrated that a botanical, the OC mixture, can extend lifespan in the Mexfly fed a diet with a high ratio of

sugar to protein (Zou et al. 2010). Here we use the same Mexfly model to address the aforementioned issues by investigating the interaction between OC and DR on lifespan and reproduction. We also determine whether a short-term OC consumption is sufficient to promote longevity and affect reproduction. There are several advantages of using the Mexfly model. The Mexfly is readily available in a large number (more than a million a day) from the industrial scale Mexfly facility at a very low cost. This fly has been used extensively to study the demographics of aging, which has revealed several novel concepts in aging, most noticeably the discovery of the de-accelerated age-specific mortality rate in very old animals (Carey et al. 2008; Carey and Molleman 2010; Vaupel et al. 1998). More importantly for aging intervention studies, due to the relatively large size of the Mexfly, a method has been developed to precisely control the food intake in the Mexfly for DR or other nutritional interventions, similar to those used in rodent dietary studies (Carey et al. 2008). This allows assessing the doses of intervention agents and their interaction with dietary nutrients on lifespan and reproduction. Here we demonstrate that OC can simultaneously promote both longevity and reproduction partly through DR-independent pathways. We further demonstrate that short-term implementation of OC supplementation at the middle age interval of the Mexfly is sufficient to extend lifespan of the female Mexfly without compromising reproduction.

Materials and methods

Materials

Pupae of the Mexfly were obtained from the mass rearing facility at Metapa, Chiapas, Mexico, and placed in Petri dishes inside the 27-dm³ population cages 1 day before their maximum emergence. After 24 h, the Petri dishes with empty pupal cases and non-emerged pupae were removed. Virgin males and females were collected by a mouth aspirator and individually housed in clear Plexiglass® condominium cages (size 4×4×10 cm per cage). The OC extract was prepared by mixing 75% oregano extract with 25% cranberry extract, kindly provided by Dr. Reza Ghaedian at Decas Cranberry Products Inc. (Carver, MA, USA) (Apostolidis et al. 2006; Zou et al. 2010).

Lifespan and reproduction assays

Individually housed virgin male and female Mexflies were maintained at 24±2°C, 65±9% relative humidity and a 12:12 h light–dark cycle as described previously (Zou et al. 2007; Zou et al. 2009). Twenty microliters of a sugar–yeast extract (SY) diet supplemented with or without OC and a 20-μl droplet of water were provided daily to each fly on a glass slide through an opening in each condominium cage. The SY diet was prepared by dissolving 24 g sugar and 1 g enzymatically hydrolyzed yeast extract to every 50 ml H₂O. The ratio of sugar and yeast extract in the diet was therefore 24:1, which was called 24:1 SY or full diet in this study. To prepare the DR diet, the full diet was diluted by fourfold with H₂O. Diets supplemented with OC were prepared by adding OC into the full or DR diet to the final concentration of 2% (weight/volume). For each dietary treatment, 80 males and 80 females were randomly assigned into a 160-cage unit with the stipulation that females and males occupied alternate units to avoid mixing eggs from any two females. Each cage contained a black silicon membrane attached to the rear of the cage for females to lay eggs. Numbers of dead flies and eggs laid by individual females were recorded daily.

Duration of OC supplementation

Before OC supplementation, Mexflies were first aged on a dry diet with only sugar and yeast extract at the ratio of 24:1. For lifetime supplementation of OC, 80 male or female Mexflies were fed the 24:1 SY diet with or without 2% OC starting when they were 10 days old. For short-term supplementation of OC, Mexflies were divided into three intervention groups, young, middle, and old age interval groups. For the young age interval group, 80 male or female Mexflies of 10 days old were fed the 24:1 SY diet with or without 2% OC for 30 days and then maintained on the 24:1 SY diet without OC for the rest of their life. For the middle age interval group, Mexflies were first maintained on the dry 24:1 SY diet until they were 40 days old. Eighty surviving male or female Mexflies were then fed the 24:1 SY diet with or without 2% OC for 30 days and then maintained on the 24:1 SY diet without OC for the rest of their life. For the old age interval group, Mexflies were first maintained on the dry 24:1 SY diet until they were

70 days old. Eighty surviving male or female Mexflies were then fed the 24:1 SY diet with or without 2% OC for the rest of their lives.

Statistical analysis

Data were expressed as means $\pm$ standard errors. Log rank analyses were performed for lifespan data using the Statview Version 5.0 software (SAS, Cary, NC, USA). Gompertz model was used to assess the age-dependent mortality rate using the SAS PROC LIFETEST program by the equation, $m(t) = \alpha \exp(b \times t)$, where $m(t)$ is the mortality rate at time t , the positive parameter α is the age-independent mortality rate or the initial mortality rate and the positive parameter b is the age-dependent mortality rate. Larger values of b mean higher age-dependent mortalities. In this study, we calculated and compared the b values for Mexflies under different treatments while keeping the α values constant between two treatments in any comparison. Lifetime egg production was analyzed by two-way ANOVA for females on full or DR diet supplemented with or without 2% OC or by the zero-inflated Poisson regression for females on short-term OC supplementation and their respective controls using the SAS program. The duration of egg laying refers to the duration in days from the first egg laying day to the last egg laying day for individual female Mexflies, and analyzed by two-way ANOVA for females on full or DR diet supplemented with or without 2% OC or by t test for females on short-term OC supplementation and their respective controls. $p < 0.05$ was accepted as statistically significant.

Results

OC promotes lifespan through DR-independent pathways

We have previously demonstrated that supplementation of 2% OC could extend lifespan of the Mexfly fed a high sugar diet with the ratio of sugar and yeast extract at 24:1, called the 24:1 SY diet (Zou et al. 2010). Here we determined the effect of OC on age-dependent mortality rate by using the Gompertz model (Fig. 1a and b and Table 1). The Gompertz b values representing age-dependent mortality rates were significantly lower in both male and female Mexflies fed a full

diet supplemented with 2% OC when compared to their respective non-supplemented controls ($p < 0.01$; Table 1). Carey et al. previously demonstrated that DR without malnutrition could extend lifespan in the Mexfly fed the 24:1 SY diet (Carey et al. 2008). Here we confirmed those findings in both male and female Mexflies (Fig. 1a and b and Table 1). Analysis of Gompertz parameters indicated that the age-dependent mortality rates (Gompertz b values) were not statistically significantly different between the fully fed and DR male Mexflies ($b = 0.02466$ for full diet fed males or 0.02228 for DR males, $\alpha = 0.00475$, $p = 0.276$) or female Mexflies ($b = 0.02174$ for full diet fed or 0.01830 for DR females, $\alpha = 0.00596$, $p = 0.1237$). These findings suggest that OC supplementation and DR modulate lifespan through different pathways. To further test this hypothesis, male and female Mexflies were fed the DR diet supplemented with or without 2% OC. OC supplementation further extended lifespan of both male and female Mexflies under the DR condition ($p < 0.001$; Fig. 1a and b and Table 1). In addition, similar to the results from using the full diet, lifespan extension by OC supplementation under the DR condition was associated with a reduction of Gompertz b values and hence age-dependent mortality rates in both males and females ($p < 0.001$; Table 1). Taken together, these findings suggest that OC supplementation promotes longevity through reducing age-dependent mortality rate partly in DR-independent pathways.

OC supplementation increases reproduction

To determine the effect of OC supplementation on reproduction, eggs were counted daily for the female Mexflies fed the full or DR diet supplemented with or without 2% OC (Fig. 1c and d). Consistent with previous studies (Carey et al. 2008; Zou et al. 2009), we found that Mexflies laid eggs at various daily rates throughout their life and some females did not lay any eggs throughout life (Fig. 1d). OC supplementation increased lifetime egg laying in fertile females fed either the full or DR diet when compared to the respective non-supplemented controls ($p < 0.0001$; Fig. 1c and Table 2). However, the extent of increase in lifetime egg production was reduced from approximately 48.6% in flies fed the full diet to approximately 15.4% in those fed the DR diet.

Fig. 1 The effect of dietary calorie content and OC on lifespan and reproduction. **a** The graph shows the lifespan curves of male *Mexflies* fed the full or DR diet supplemented with or without 2% OC. **b** The graph shows the lifespan curves of female *Mexflies* fed the full or DR diet supplemented with or without 2% OC. **c** The bars indicate the mean lifetime egg production per fertile females fed the full or DR diet

supplemented with or without 2% OC. The error bars represent standard errors. **d** Life history event graphics show the daily egg laying of individual females throughout their life. The length of each light gray horizontal line indicates the lifespan of a female. Dots with different grayness represent the amount of daily egg laying. The scale bar for number of eggs is shown on the right side. *** $p < 0.001$

We further compared the egg laying duration of fertile flies. DR modestly increased the duration of egg laying in fertile females when compared to the full diet controls ($p = 0.053$; Table 2). Supplementation of 2% OC modestly extended duration of egg laying in fertile females fed the full diet ($p = 0.055$) but not the DR diet ($p > 0.5$) when compared to their respective non-supplemented controls (Table 2). Together, these findings suggest that OC supplementation increases reproduction and delays reproductive aging through DR-dependent and DR-independent pathways.

Lifespan extension by short-term OC supplementation depends on the time of the initiation of supplementation

To determine whether short-term intervention with supplementation of OC was sufficient to promote lifespan and reproduction, male and female *Mexflies* were fed the full diet supplemented with or without OC supplemented at three age intervals—young, middle, and old age intervals. The duration of OC

supplementation was 30 days for the young age group from the age of days 10 to 39, 30 days for the middle age group from the age of days 40 to 69, and the rest of their lives for the old age group from the age of day 70. Based on the log rank test, supplementation of 2% OC was not sufficient to significantly extend lifespan for males at any of the three age intervals nor for females at the young and old age intervals ($p > 0.06$; Fig. 2a–d and f and Table 1). However, supplementation of 2% OC at the middle age interval significantly increased lifespan of the female *Mexflies* when compared to the age-matched non-supplemented controls ($p < 0.05$; Fig. 2e). The demographic aging patterns of all groups of flies were analyzed by the Gompertz model. Only in the old age interval group for the male *Mexflies*, did OC supplementation significantly reduce the age-dependent mortality rate ($p < 0.001$; Table 1). Supplementation of OC at the old age interval slightly increased age-dependent mortality rate in the female *Mexflies* ($p < 0.05$; Table 1). Taken together, these findings suggest that females are more sensitive to short-term intervention by OC than males and that the

Table 1 Lifespan of Mexflies on a full or DR diet supplemented with or without OC

Diet or age interval	Gender	No. of flies	Mean lifespan $\pm$ SE (day)	Log rank p value for lifespan ^a	Gompertz α value ^c	Gompertz b value	p Value for Gompertz b parameters ^d
Diet							
Full diet	Male	80	58.3 $\pm$ 3.4			0.02827	
Full diet + 2% OC		80	83.6 $\pm$ 3.6	<0.0001	0.0035	0.02249	0.0056
Full diet	Female	80	50.6 $\pm$ 3.2			0.02159	
Full diet + 2% OC		80	81.3 $\pm$ 3.9	<0.0001	0.00606	0.01517	0.0035
DR diet	Male	80	70.0 $\pm$ 3.4	0.0097 ^b		0.03296	
DR diet + 2% OC		80	97.1 $\pm$ 3.3	<0.0001	0.00183	0.02609	0.0002
DR diet	Female	80	75.2 $\pm$ 3.5	<0.0001 ^b		0.03549	
DR diet + 2% OC		80	104.0 $\pm$ 3.8	<0.0001	0.00131	0.02709	<0.0001
Age interval							
Young (10–39 days)	Male	80	44.5 $\pm$ 2.5			0.0349	
Young (10–39 days) + 2% OC		80	51.8 $\pm$ 2.6	0.0782	0.0058	0.0319	0.3122
Young (10–39 days)	Female	80	56.9 $\pm$ 3.3			0.0256	
Young (10–39 days) + 2% OC		80	55.4 $\pm$ 2.5	0.2321	0.0053	0.0291	0.164
Middle (40–69 days)	Male	80	58.4 $\pm$ 1.9			0.0483	
Middle (40–69 days) + 2% OC		80	63.6 $\pm$ 1.9	0.1246	0.0018	0.0464	0.4127
Middle (40–69 days)	Female	80	65.3 $\pm$ 2.5			0.0335	
Middle (40–69 days) + 2% OC		80	74.0 $\pm$ 2.2	0.0343	0.0022	0.0368	0.1148
Old (70 days and over)	Male	80	81.3 $\pm$ 1.3			0.0608	
Old (70 days and over) + 2% OC		80	85.4 $\pm$ 1.7	0.0616	0.0003	0.0537	0.0001
Old (70 days and over)	Female	80	85.9 $\pm$ 2.2			0.0307	
Old (70 days and over) + 2% OC		80	89.0 $\pm$ 1.9	0.3967	0.0009	0.0408	0.0302

^a Log rank p values were calculated by comparing flies fed a sugar–yeast extract diet supplemented with 2% OC to the controls on the same diet without OC except for the p values marked by “b”

^b p values were calculated by comparing flies fed the DR diet to those on the full diet

^c The α values were calculated by combining two groups of flies fed a diet supplemented with 2% OC and their respective controls

^d p values for the Gompertz b values were calculated by comparing flies fed a sugar–yeast extract diet supplemented with 2% OC to the controls on the same diet without OC

middle age interval is a critical period for OC to promote longevity in females.

Alteration of reproduction by short-term supplementation of OC depends on the time of the initiation of supplementation

To determine the effect of short-term OC supplementation on reproduction, eggs were counted daily for the female Mexflies at all three age intervals. Supplementation of 2% OC at the middle or old age

interval did not affect the lifetime egg production per fertile female when compared to the age-matched controls, respectively ($p>0.5$; Fig. 2g and Table 2). However, supplementation of 2% OC at the young age interval significantly increased the lifetime egg production per fertile female by approximately 42% when compared to the non-supplemented age-matched controls ($p<0.001$; Fig. 2g and h, and Table 2). Analysis of the egg laying patterns revealed that the duration of egg laying was not significantly different between OC supplemented and non-

Table 2 Lifetime reproduction and egg laying duration of Mexflies

Diet or age interval	No. of fertile female flies	Mean lifetime egg + SE/Fly	<i>p</i> value for lifetime egg production ^a	Duration of egg laying + SE (day)	<i>p</i> Value of egg laying duration ^c
Diet					
Full diet	20	52.1±13.5		12.8±4.2	
Full diet + 2% OC	36	77.3±8.4	0.011	27.1±2.9	0.055
DR diet	38	92.4±12.8	<0.001 ^b	27.1±4.0	0.053 ^b
DR diet + 2% OC	51	106.6±13.7	0.020	30.2±3.7	0.524
Age interval					
Young (10–39 days)	34	85.3±14.3		15.9±3.6	
Young (10–39 days) + 2% OC	41	123.3±13.7	<0.0001	20.3±2.5	0.30
Middle (40–69 days)	34	66.1±12.0		17.4±3.2	
Middle (40–69 days) + 2% OC	36	64.7±11.6	0.8818	12.8±2.8	0.28
Old (70 days and older)	16	30.8±10.6		5.1±2.3	
Old (70 days and older) + 2% OC	20	33.3±7.7	0.519	7.3±2.3	0.52

^a The *p* values were calculated by comparing flies fed a sugar–yeast extract diet supplemented with 2% OC to the controls on the same diet without OC except for the *p* value marked by “b”

^b *p* value was calculated by comparing flies fed the DR diet to those on the full diet

^c *p* values were calculated by comparing flies fed a sugar–yeast extract diet supplemented with 2% OC to the controls on the same diet without OC except for the *p* value marked by “b”

supplemented females at any of the three age intervals. Taken together with the lifespan results, these findings indicate that OC supplementation at the young age interval is sufficient to promote reproduction but not lifespan, and OC supplementation at the middle age interval is sufficient to promote lifespan but not reproduction in the female Mexflies. These findings suggest the existence of different critical periods for OC to promote lifespan and reproduction.

Discussion

Consumption of botanical products has long been considered beneficial to human health (Greenwood and Gershwin 2010). DR is a potent dietary paradigm to extend lifespan and healthspan in many species although its efficacy in promoting longevity in humans remains to be determined (Masoro 2006; Partridge et al. 2005). In this study, we have addressed two issues related to the effectiveness of aging interventions, which are not well studied in model organisms but important for their implementation in humans. Firstly, whether a prolongevity botanical can act synergistically or additively with other prolongevity paradigms to promote lifespan and

reproduction in an organism? Secondly, what are the critical age intervals for an intervention to promote longevity and reproduction? We have found that supplementation of 2% OC can further extend lifespan in both male and female Mexflies and increase lifetime egg production in the female Mexflies under the DR condition. OC supplementation but not DR decreases age-dependent mortality rate. We have also demonstrated that supplementation of 2% OC at the middle age interval is sufficient to extend lifespan without compromising reproduction in the female Mexfly, while OC supplementation at the young age interval is sufficient to promote reproduction but not longevity. However, the magnitude of increase in lifespan and reproduction by short-term OC supplementation is much less than the lifetime supplementation. Our findings suggest that supplementation of OC can promote longevity and reproduction partly through DR-independent pathways and the extent of health benefits induced by OC depends on the age interval when the supplementation is implemented.

The soma disposal hypothesis of aging states that aging is due to diverting the resource for maintaining the integrity of somatic cells to reproduction (Kirkwood and Shanley 2010). This hypothesis is supported by numerous lines of

Fig. 2 The effect of short-term OC supplementation on lifespan and reproduction. **a** and **d** The graphs show the lifespan curves of male (**a**) or female (**d**) *Mex*flies fed the full diet supplemented with or without 2% OC from the age of 10 to 39 days old. The period of 10–39 days old refers to the young age interval. **b** and **e** The graphs show the lifespan curves of male (**b**) or female (**e**) *Mex*flies fed the full diet supplemented with or without 2% OC from the age of 40 to 69 days old. The period of 40–69 days old refers to the middle age interval. **c** and **f** The graphs show the lifespan curves of male (**c**) or female (**f**)

*Mex*flies fed the full diet supplemented with or without 2% OC starting from the age of 70 days old. The period after 70 days old refers to the old age interval. **g** The bars indicate average lifetime egg production per fertile female fed the full diet supplemented with or without 2% OC for three age intervals. The error bars represent standard errors. **h** Life history event graphics show the daily egg laying of individual females throughout their life. Only flies fed the full diet supplemented with 2% OC between 10 and 39 days and their respective controls are shown. *** $p < 0.001$

evidence and suggests that lifespan and reproduction are conversely correlated. In *Drosophila*, an increase of lifetime reproduction in some cases is associated with decreased lifespan (Chapman and Partridge 1996; Tatar 2010). Lifespan extension by DR in rats in some cases has been shown to be associated with reduced litter

size and overall fertility (Holehan and Merry 1985). However, there are numerous examples indicating the absence of a connection between lifespan and reproduction. Lifespan extension by DR in *Drosophila* in some cases is associated with increased reproduction (Tatar 2010). Long-lived mutants in *Drosophila* may

be associated with a decrease, increase, or no change in lifetime fecundity (Tatar 2010). Lifespan extension by DR in the Mexfly may be associated with increased lifetime fecundity (Carey et al. 2008), which is consistent with what we observed in this study. Mild DR is sufficient to promote longevity without affecting fertility in mice (Rocha et al. 2007). Therefore, aging interventions using botanicals can promote lifespan without compromising reproductive potential and even enhancing it.

Dietary restriction modulates lifespan and promotes healthspan through a number of genetic pathways, including those involved in sirtuin and target of rapamycin pathways (Fontana et al. 2010; Guarente 2007; Kenyon 2010). Some botanicals have been shown to modulate lifespan of an organism through DR-dependent pathways. For example, resveratrol has been shown not to further extend lifespan in *Drosophila* under a DR condition, and lifespan extension by resveratrol requires dSir2, a sirtuin involved in the DR response (Wood et al. 2004). In addition, resveratrol can activate SIRT1, a mammalian sirtuin, in mouse and human cells (Pearson et al. 2008; Allard et al. 2009; Pirola and Frojdo 2008). Therefore, resveratrol appears to modulate lifespan partly through DR-dependent pathways, although the action of resveratrol on lifespan remains controversial. We have assessed the relationship between DR and OC supplementation in this study and found that OC supplementation can further extend lifespan in the Mexfly under a longevity-promoting DR condition. OC supplementation can also increase lifetime egg production of the female Mexfly under both full and DR diet conditions. However, the extent of increase in reproduction decreases in the Mexfly under the DR diet condition when compared to those under the full diet condition. These findings suggest that OC is not a DR mimetic and can promote both longevity and reproduction through DR-independent pathways. However, we cannot rule out the likelihood that OC enhances reproductive function partially through DR-dependent pathways.

For aging interventions, it is important to identify the critical period of time for an intervention to exert health benefits. Resveratrol has been shown to be sufficient to promote survival of mice under a high-fat diet when the supplementation is initiated at the middle age (Baur et al. 2006; Pearson et al. 2008). We have found that only supplementation of OC at the

middle age interval extends lifespan, but does not change reproduction in the female Mexfly. On the other hand, OC supplementation at the young age interval significantly increases reproduction but not lifespan in the female Mexfly. The uncoupling between lifespan and reproduction that we have observed is consistent with several fly studies investigating the effects of dietary composition on lifespan and reproduction. Those studies have demonstrated that the diets optimal for lifespan and reproduction are not the same and contain sugar and protein at different ratios (Carey et al. 2008; Lee et al. 2008; Skorupa et al. 2008). Our findings suggest that in females the middle age interval is the critical period for OC and other potential interventions to promote longevity, while intervention at the young age interval is sufficient to promote reproduction.

Considering the soma disposal hypothesis of aging as the model (Kirkwood and Shanley 2010), we postulate the following actions of OC on lifespan and reproduction at least in females. At the middle age interval, cellular functions that maintain the integrity of somatic cells decline and no additional resource is funneled to somatic cell maintenance. Probably due to high contents of phytochemicals in OC, supplementation of OC at the middle age interval improves the functional capacity of somatic cells by reducing cellular damage without altering the distribution of resources between somatic cells and reproduction. This consequently results in an increase in lifespan without an alteration in reproduction. At the young age interval, endogenous cellular functions are sufficient to maintain somatic cell integrity, which allows more resources to be diverted to reproduction when OC supplementation helps maintain the integrity of somatic cells. However, for these young flies, supplementation of OC is discontinued at the middle and old age intervals in our study, when the capacity to maintain somatic cell functions may be critical for lifespan. Consequently, OC supplementation at the young age interval results in increased lifetime fecundity, but is not sufficient to extend lifespan. The ineffectiveness to promote longevity and reproduction when OC supplementation is implemented at the old age interval can be simply due to the fact that OC is not sufficient to reverse the damage that has been sustained by somatic cells and the reproductive system. Clearly, there are other possibilities to explain

our results on the short-term supplementation of OC on lifespan and reproduction. Taken together, our findings reveal not only the interaction between dietary calorie content and a longevity intervention utilizing a botanical, but also the critical age periods for an intervention to increase lifespan, reproductive capacity, and healthspan in the Mexfly. This study also provides guidance for implementing supplementation with botanical products to promote health benefits in higher organisms.

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