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Rhodiola: A Promising Anti-Aging Chinese Herb

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ABSTRACT

Using the fruit fly, Drosophila melanogaster, we investigated the effects of Rhodiola on lifespan. Rhodiola is a plant root used in traditional Chinese medicine that may increase an organism’s resistance to stress. It has been proposed that Rhodiola can extend longevity and improve health span by alleviating oxidative stress. Rhodiola supplied every other day at 30 mg/mL significantly increased the lifespan of Drosophila melanogaster. When comparing the distribution of deaths between Rhodiola-supplemented and control flies, Rhodiola-fed flies exhibited decelerated aging. Although the observed extension in lifespan was associated with statistically insignificant reductions in fecundity, correcting for a possible dietary restriction effect still did not eliminate the difference between supplemented and control flies, nor does the effect of Rhodiola depend on dietary manipulation, strongly suggesting that Rhodiola is not a mere dietary restriction mimetic. Although this study does not reveal the causal mechanism behind the effect of Rhodiola, it does suggest that the supplement is worthy of continued investigation, unlike the other Chinese herbals, Lu Duo Wei (LDW), Bu Zhong Yi Qi Tang (BZYQT), San Zhi Pian (SZP, Three Imperial Mushrooms), Hong Jing Tian (Rhodiola) that were evaluated in this study.

INTRODUCTION

In developed countries, chronological age is the most significant risk factor for noncontagious disease.1 Thus, it is understandable that many suppose that understanding the aging process might play a major role in the development of therapies to slow or alleviate age-related diseases such as cancer, cardiovascular disease, and cognitive disorders. Some further imagine that such “anti-aging” measures include dieting, weight control, exercise, and stress management. Although these are all desirable interventions, patient compliance with these measures is a considerable obstacle to overcome for health professionals.

With the advent of successful genetic interventions in aging among model organisms over the past 3 decades, pathways regulating aging and potential molecular targets for interventions have been identified.2 Although the genetic methods applied to these model systems are not appropriately applied to humans, the pathways that they reveal are considered helpful clues for pharmacological intervention. The genetic mapping of aging in animal models has thus opened the door for “anti-aging” drug discovery.

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In recent years, increases in longevity have been achieved by supplementing pharmaceuticals and botanicals with antioxidative properties to various laboratory organisms. For example, superoxide dismutase/catalase mimetics have been shown to extend the mean lifespan of wild-type worms by 44%. Furthermore, there have been recent reports that supplements such as lipoic acid, resveratrol, melatonin, and Lu-Duo-Wei (traditional Chinese medicine herb) can extend \textit{Drosophila melanogaster} lifespan, supposedly by reducing oxidative damage to macromolecules. These few cases of extended longevity resulting from drug exposure suggest both that aging can be slowed by targeted interventions and that the pharmacology of lifespan extension is a practical area of biomedical research.

Pharmacological approaches to postpone or slow aging have used several experimental systems. Nematodes, fruit flies, and mice have all provided useful data, and are candidate model systems for anti-aging pharmacology. The fruit fly, \textit{Drosophila melanogaster}, is a particularly effective model system for aging research. Fruit flies and humans share key metabolic pathways, such as superoxide metabolism, DNA repair, and insulin-like signaling. They also have similar pathophysiological outcomes of these pathways. Furthermore, \textit{Drosophila} has been used as a model system for aging for more than 90 years, with notable successes in experiments specifically focused on postponing its aging. For these reasons, we have chosen to use fruit flies as a model system for screening of candidate anti-aging compounds.

Anti-aging pharmacological intervention is likely to involve secondary effects, given the multifold pathways that affect fruit fly aging. Although various compounds that have been tested in \textit{Drosophila} have increased the lifespan of these model systems, this work has typically lacked evaluation of secondary mechanisms that could have resulted in osensible anti-aging effects artifactualy. Particular pharmaceuticals or botanicals that affect aging may do so through multiple pathways, not just the pathway that is of a priori interest. Most importantly, it is not appropriate to simply assume that a compound that affects adult survival \textit{only} has such effects. Such compounds might also have adverse secondary effects that would limit their clinical use in the treatment of aging. To date, anti-aging studies have not systematically evaluated the secondary adverse effects of potential anti-aging compounds, particularly negative effects on reproduction.

The \textit{Rhodiola} root has long been used in the traditional medical systems in Europe and Asia to increase an organism’s resistance to physical stress; currently, it is widely thought to have antioxidative properties. Studies in cell cultures have demonstrated that \textit{Rhodiola rosea} extract has a protective effect against free-radical oxidative damage in human red blood cells. This study was designed to examine the effect of \textit{Rhodiola} and other Chinese Herb mixtures on the lifespan of adult \textit{Drosophila}. In addition, we performed experiments to detect life extension confounds and artifacts.

**METHODS**

\textit{Drosophila population used}

All \textit{Drosophila melanogaster} stocks used in these experiments were ultimately derived from a sample (called “IV”) of the Amherst, Massachusetts, Ives population that was collected in 1975 and cultured at moderate to large population sizes ever since. This population has been reared at controlled densities (50–80 eggs per vial) for more than 700 generations with discrete generations cultured every 2 weeks.

\textit{Maintenance of flies}

All flies used in these assays were raised as larvae in 5 mL of standard banana-molasses food at densities of between 50 and 80 eggs per 8-dram vial. Populations were maintained at about 25°C with constant illumination. During the assays, adults were kept in standard 5-mL food vials containing banana-molasses food and 1 mg of yeast paste to promote egg laying. All Chinese herbal compounds were supplied to adults only. The Chinese Herbs were mixed into the yeast paste; the adults preferentially consume this paste. Adults were transferred to fresh vials and survivors were
counted every 2 days. All assays were conducted on flies that had undergone two generations of controlled density rearing. When flies from different treatments were compared, all preliminary rearing was carried out in parallel.

**Chinese herbal trials**

The powder of each Chinese Herb mixture was mixed into the yeast paste. The calculated dose used in our data analyses reflects the final concentration of the herb in the yeast paste that flies consumed during the experiment. Compounds were obtained from Sun Ten, Inc., in powder format. For each trial, various doses of each compound were compared to a control. We screened the Chinese Herbal mixtures at the following doses: Lu Duo Wei (LDW) at 0, 2, 10, 50, 60, 100, 200 mg/mL, Bu Zhong Yi Qi Tang (BZYQT) at 0, 60, 100, 200 mg/mL, San Zhi Pian (Three Imperial Mushrooms) at 0, 15, 30, and 60 mg/mL, and Hong Jing Tian (Rhodiola) at 0, 15, 30, 60, 100, and 200 mg/mL.

**Mortality assays**

We evaluated the impact of each compound on mortality. For each assay, three doses of each compound were compared to a control group. For each dose, 320 males and 320 females were exposed to the compound. There were 4 males and 4 females in each vial, with a total of 80 vials per dose per sex, and the flies were transferred every other day during the aging phase, which lasts 4 weeks in IV stocks. We attempted to determine a dose–response relationship during the mortality assays. This required repeating testing of the same compound over a range of doses. Compounds demonstrating a beneficial effect on mortality were subjected to fecundity assays, as described below.

The data were analyzed by examining the number of surviving flies at the end of week 4. Significant differences between the control and each drug treatment were assessed by the Pearson chi-square test.

**Survival probability**

*Rhodiola* resulted in a beneficial effect on mortality; therefore, a total survivorship assay was conducted to reaffirm the result. This assay also allowed for the determination of mean lifespan change as a result of the herb. Survivorship was determined with every-other-day transfers until every fly was dead. The most efficacious dose from the mortality assay, 30 mg/mL of *Rhodiola*, was compared to a control. For each group (control vs. drug treatment), 2400 males and 2400 females were used in sets of 8 (4 males: 4 females) per vial. The flies from one group were housed in 15 separate racks.

**Gompertz mortality.** In this formulation we will let the index *i* indicate one of the 15 racks, *j* indicate sex (1 = female, 2 = male), *k* indicates drug treatment (1 = control, 2 = drug treated), and *t* indicate age. Then the predicted mortality between ages *t* and *t* + 1 is *y*.*ijk*. The basic nonlinear model is given by,

\[
y_{ijk} = f(\Psi_{ijk}, t) + \epsilon_{ijk},
\]

where *y*.*ijk* is the vector of parameters, *t* is the age, and *ε*.*ijk* is the within population variation. The function *f* is the Gompertz model,

\[
f(\Psi_{ijk}, t) = 1 - \exp\left(\frac{A_{ijk}}{\alpha_{ijk}} \left[\exp(\alpha_{ijk} t) - \exp(\alpha_{ijk}(t + 1))\right]\right).
\]

The parameter *A* is sometimes called the age-independent parameter of the Gompertz and is a reflection of background mortality that does not change with age. On the other hand, *α* is called the age-dependent parameter and measures the rate at which mortality increases with age, e.g., senescence. We assume that the parameters of the Gompertz equation may be affected by the fixed effects, sex and drug treatment, and the random rack environment. These assumptions translate into a system of equations,

\[
A_{ijk} = \beta_1 + \gamma_1 \delta_i + \phi_1 \delta_k + b_{1i},
\]

\[
\alpha_{ijk} = \beta_2 + \gamma_2 \delta_i + \phi_2 \delta_k + b_{2i},
\]

where \(\delta_i = 0\) if \(i = 1\), or 1 otherwise. To test for significant effects of sex on *A* and *α*, we determined whether γ1 or γ2 is significantly different from 0, respectively. Likewise, a test for the
effects of drugs on A and α corresponds to a test for whether φ1 or φ2 is significantly different from 0. Model (3) could be expanded to include interactions between sex and drugs. Although we tested for such interactions; since they were never significant, we do not include them in the basic model described here.

The variance of mortality is expected to change with the mean value of mortality. The general formulation for the variance of εijkl is,

\[ \text{Var}(εijkl) ≡ σ^2g^2(ˆuijkt, t), \]

where ˆuijkt = E(yijkl|bj). In this analysis we used \( g(.) = |yijkl|^α \). The bj were distributed as,

\[ b_j \sim N\left(0, \begin{bmatrix} σ_1 & 0 \\ 0 & σ_2 \end{bmatrix} \right). \]

The parameters in Equations (3–5) were estimated from a nonlinear mixed effects model as implemented by the nlmef package of R (r-project.org; version 2.4.0; see also Pinheiro and Bates, 2000, chapter 7).18

Two-stage Gompertz mortality. Large cohorts of fruit flies exhibit departures from the Gompertz mortality dynamics in the form of a leveling off of mortality rates at advanced ages.19,20 We have developed a model, called the two-stage Gompertz, which shows Gompertz dynamics at young ages and then at an advanced age, called the break day, mortality rates plateau at a constant value.17 The discontinuity in the two-stage Gompertz model caused by the break day makes it especially difficult to obtain estimates of the parameters for this model.21 As a practical matter, the same methods used to infer the drug effects on the Gompertz model cannot be used with the two-stage Gompertz.

To overcome this difficulty, we have used bootstrap samples as a means of determining whether there were significant differences in the two-stage Gompertz parameters due to drug treatment. For the two-stage Gompertz model, the probability of surviving to age t is

\[ p(t) = \begin{cases} 
\exp(A(1 - \exp(\alpha t))/\alpha) & \text{if } t \leq bd \\
\exp(A(1 - \exp(\alpha bd))/\alpha) + A_2(bd - t) & \text{if } t > bd
\end{cases} \]

where \( bd \) is the break day, A and α have similar interpretations as in the Gompertz, and \( A_2 \) is the plateau mortality rate. The mortality rate between ages \( t \) and \( t + 1 \) is

\[ 1 - \frac{p(t + 1)}{p(t)}. \]

Least-squares estimates of the parameters \( \theta = (A, \alpha, bd, A_2) \) are obtained by minimizing the sum of squared differences between the observed and predicted mortality (Eq. 7). In the following discussion, we will also refer to the components of \( \theta \) as \( \theta_1, \theta_2, \theta_3, \theta_4 \). Because the least-squares surface can sometimes be very flat, we used four very different initial conditions to begin our numerical search for the least-squares estimates. The initial condition that leads to estimates with the smallest sum of squares is used for the final estimate of \( \theta \).

One set of observations consists of a vector of ages of death, \( T = (t_1, t_2, \ldots, t_n) \), the number of deaths at each of those ages, \( D = (d_{t_1}, d_{t_2}, \ldots, d_{t_m}) \), and a total sample size of \( N = \sum d_{ti} \). To create bootstrap samples, we need to define the probability of dying on any one of the \( m \)-days in \( T \) as \( P_i = d_{ti}/N \). One bootstrap sample then consists of \( N \) individuals whose ages of death are equal to one of the days in \( T \). Thus, the chance of choosing day \( t_i \) as the day of death in the bootstrap sample is \( P_i \).

This sampling process was used to create \( B \) bootstrap samples, \( D^\dagger, D^\dagger, \ldots, D^\dagger \). From each of these samples, a least-squares, \( \theta^\dagger \), estimate was made by the methods described above. The bootstrap parameter estimates are given by

\[ \hat{θ}^* = \left( B^{-1} \sum_i θ_i^r, B^{-1} \sum_i θ_i^s, \right. \]

\[ B^{-1} \sum_i θ_i^x, \left. B^{-1} \sum_i θ_i^y \right). \]

We generated \( B \) bootstrap samples for the control samples, \( θ^r \) and the drug-treated sample, \( θ^s \). From these we computed the difference between the drug treatment parameter values and the control treatment parameter values, \( \hat{θ}^r - θ^r \). To test for significant differences between the drug and control, we created a random permutation, without replacement, of the 2B control and drug bootstrap vectors. We then computed B differences between the first B val-
Fecundity assays

Some compounds may increase lifespan simply by substantially depressing fecundity; depressed fecundity will increase longevity by and of itself in *Drosophila*. Therefore, a fecundity assay is an important check for artifactual lifespan enhancement. We evaluated age-specific fecundities using the same dosing protocols as above, except that the flies were handled with one female and one male in each assay vial. The number of eggs laid each day by each individual female was recorded for a period of 10 days. The standard fecundity assay was used to measure fecundity rates. At less than 12 hours from pupa emergence, flies are collected as newly emergent adults. Adult flies are transferred to 8-dram charcoal-food vials containing 75 µl yeast solution at a density of one female and one male fly per vial and allowed to lay eggs for 24 hr. The experimental groups were supplemented with a mixture of yeast solution and *Rhodiola*, whereas the controls were supplemented with yeast solution only. The flies to be assayed were transferred daily at regular intervals for 10 days to fresh charcoal-food vials with *Rhodiola* supplement. For 10 days, the number of eggs laid was counted daily and recorded using 16× light stereoscopes. The number of flies assayed for fecundity rates was 160 females per experiment. Forty females per dose (low, medium, and high) were evaluated including 40 females for the control group. Thus, we examined 160 females at a time, assaying fecundity rates over the first 10 days of life, with daily transfers. We analyzed

| Replicate | Dose (mg/ml) | Mortality rate ± SD | p-value |
|-----------|-------------|---------------------|--------|
| 1         | Male        |                     |        |
| 0 (Control) | 0.61 ± 0.03 | —                   |        |
| 15 | 0.56 ± 0.03 | 0.23               |        |
| 30 | 0.38 ± 0.03 | 0.00               |        |
| 60 | 0.51 ± 0.03 | 0.01               |        |
| Female    |             |                     |        |
| 0 (Control) | 0.62 ± 0.03 | —                   |        |
| 15 | 0.48 ± 0.03 | 0.00               |        |
| 30 | 0.44 ± 0.03 | 0.00               |        |
| 60 | 0.61 ± 0.03 | 0.82               |        |
| 2         | Male        |                     |        |
| 0 (Control) | 0.61 ± 0.03 | —                   |        |
| 15 | 0.48 ± 0.03 | 0.00               |        |
| 100 | 0.43 ± 0.03 | 0.00              |        |
| 200 | 0.42 ± 0.03 | 0.00               |        |
| Female    |             |                     |        |
| 0 (Control) | 0.69 ± 0.03 | —                   |        |
| 60 | 0.50 ± 0.03 | 0.00               |        |
| 100 | 0.51 ± 0.03 | 0.00              |        |
| 200 | 0.52 ± 0.03 | 0.00               |        |
| 3         | Male        |                     |        |
| 0 (Control) | 0.63 ± 0.03 | —                   |        |
| 30 | 0.48 ± 0.03 | 0.00               |        |
| Female    |             |                     |        |
| 0 (Control) | 0.84 ± 0.02 | —                   |        |
| 30 | 0.65 ± 0.03 | 0.00               |        |
| 4         | Male        |                     |        |
| 0 (Control) | 0.28 ± 0.03 | —                   |        |
| 30 | 0.24 ± 0.03 | 0.35               |        |
| Female    |             |                     |        |
| 0 (Control) | 0.43 ± 0.03 | —                   |        |
| 30 | 0.27 ± 0.03 | 0.00               |        |
the total number of eggs laid over 10 days for each female. The impact of drugs on fecundity was assessed by a one-way analysis of variance (ANOVA). We also compared the 10-day fecundity of control females to the pooled data from all three doses using a standard t-test on the mean values.

Diet assays

Two assays were performed to evaluate the impact of interaction of diet with *Rhodiola* on the mortality rate. The same methodologies (i.e., number of flies, density in each vial, frequency of transfers, etc.) used in the mortality assay were employed for the diet assays. In one assay, control assay, flies were exposed to the following concentrations of yeast: 0.5 mg/vial, 1.0 mg/vial, and 1.5 mg/vial. The yeast solutions contained 50 mL water, 2 mL 1% acetic acid, and the respective amount of yeast. In the other, *Rhodiola*-supplemented, assay, flies were exposed to the above concentrations of yeast plus 30 mg/mL of *Rhodiola* in each vial.

Metabolic rate assays

As a further test for artefactual effects, compounds that had a beneficial effect on mortality, but did not significantly depress fecundity, were assayed for their effect on metabolic rate. This assay was used to ascertain whether there had been an artifactual decrease in mortality due to hypometabolism. In addition, metabolic rate is a useful surrogate for a number of physiological and behavioral functions that could potentially be impaired by medications, such as locomotor activity. CO₂ production in drugged flies was compared to that of a control group handled in parallel and assayed simultaneously. We used flow-through respirometry to measure the rate of CO₂ release from groups of flies following the methods of Williams et al. Room air was passed through a small chamber containing soda lime, two silica gel chambers, and a Drierite/Ascarite/Drierite column (Drierite; W.A. Hammond Drierite Co.; Ascarite (Arthur H. Thomas Co.). The air was directed by a series of computer-controlled valves (Sable Systems, Las Vegas, NV) that allowed six individual flies in separate chambers to be measured in turn. The flies were measured while in vials in the presence of food and the appropriate pharmacological agent. Measurements of CO₂ were made using a Licor LI-6260 gas analyzer. CO₂ levels were averaged and recorded every second using data acquisition software (Sable Systems). Each vial was measured for 20 minutes. During periods when they were not being measured, the flies were kept in a stream of dry, CO₂-free air by flushing with a separate air stream. The last 5 minutes of each 20-minute recording of CO₂ release was averaged to provide an estimate of relative metabolic rate. The effects of drug treatments on metabolic rates were analyzed with a one-way ANOVA using drug level treated as a fixed effect.
Mating success assay

Male virility was used as an assay for a variety of possible secondary drug effects on fly behavior, including reduced activity and other forms of central nervous system depression. In order to administer the herbs to the males, they were maintained in vials containing the herb/yeast solution for 10 days prior to the virility assay, using the same protocol as that for the mortality assays. The control (no-herb) males were maintained in parallel, with all conditions of rearing and maintenance identical except for the absence of the herb in the yeast paste given to the control males.

For each assay of virility, two male flies—one that was exposed to the Chinese Herb and one that was not exposed to the Herb—were placed in a mating vial with a virgin female fly, who had not been exposed to the Chinese Herb. Among the mating vials, half had marked drugged males; the other half had marked control males. The marking procedure used a felt-tip marker to color the tip of one wing.

Each virility assay used 120 vials, with 40 vials for each dose. Virility was scored according to the number of replicates in which the drugged male mated with the test female. The scoring of a successful mating required mounting for at least 30 seconds.

In this experiment, each male was scored according to his drug status (control or drugged), marked status (marked or not marked), and mating status (mated or not mated). The data were inserted into a contingency table that was analyzed using a log likelihood model. The basic model included only the main effects (drug status, marked status, and mating status). The fit of this model was compared to a model with the main effects and interactions between herb status and mating status as well as marked status and mating status. For each herb, if the model with interactions provided a better fit, we then determined whether this improvement was due to the herb effects, the marking effects, or both.

RESULTS

Negative results

LDW, BZYQT, and San Zhi Pian (SZP) did not result in statistically significant increases in the number of flies surviving to the end of the aging phase (Table 1). As a result of this absence of a net beneficial effect on survival during aging over the range of doses used, we did not proceed with further testing over a finer range of doses, nor did we proceed with tests of the side effects of these Chinese Herbs on fecundity, metabolic rate, or mating success.

Positive results

Mortality and confounds. Rhodiola resulted in a statistically significant decrease in mortality.
We were able to replicate these mortality results four times in four different assays (Table 2). Consequently, we proceeded with further testing of fecundity, metabolic rate, mating success, and total longevity. At the higher dose range (0, 60, 100, and 200 mg/mL) a significant mortality decrease was observed (Fig. 1) along with a dose-dependent decrease in fecundity (Fig. 2). At lower doses (0, 15, 30, and 60 mg/mL), at which a significant mortality benefit was observed (Fig. 3), there was no statistically significant difference in fecundity relative to the control (Fig. 4).

We proceeded on to a metabolic rate assay. As shown in Tables 3 and 4, *Rhodiola* did not have a significant adverse effect on metabolic rate at any dose on either sex. In fact, metabolic rate showed a statistically nonsignificant increase. Accordingly, we continued to an assay of male mating success. As shown in Table 5, there was no significant decrease in mating success from this assay.

**Longevity.** The mean longevity for the entire experiment was used to determine whether there were significant differences between the flies given drugs and the controls (Table 6). *Rhodiola* increased survival by 3.5 days in males and 3.2 days in females. All differences in longevity were statistically significant because of the very large number of flies used in these experiments.

If we use the observed variances, we can estimate the minimum required sample sizes to detect the observed differences. The largest mortality difference was 3.5 days and a sample of only 51 flies would have been sufficient to detect longevity differences of this magnitude. From the estimated confidence intervals (Table 6) it is apparent that differences in longevity as small as 0.53 days could have been detected. As discussed previously, the differences in mean longevity may be caused by a variety of changes in the age-specific mortality patterns. Making inferences about these changes is a statistically more challenging undertaking.

**Distribution of deaths.** If we plot the quantiles from the drugged flies versus the quantiles from the control flies (Fig. 5), we see that for both males and females the points are elevated above the $x = y$ line even at early ages. This suggests that drugged flies are dying more slowly even from the start of the experiment. This difference is also apparent in the survivorship curves (Fig. 6). This would be consistent with a smaller age-independent Gompertz parameter for flies treated with *Rhodiola*. In males, this departure from the $x = y$ line gets even more pronounced at later ages, suggesting a possible decline in the age-dependent parameter of the Gompertz for drugged males. We investigate these possibilities in more detail next.

**Sampling units.** A single sex, drug-level treatment consisted of 15 separate racks of flies.
adult flies, with approximately 160 flies per rack. To some extent, each rack can be considered its own microenvironment that may differ slightly from one rack to the next. There are two very different ways these data might be analyzed. The observations from all 15 racks can be pooled to form one set of observations, which can then be used to estimate age-specific mortality. Alternatively, age-specific mortality can be estimated for each rack separately and then the results from all 15 racks can be pooled.

The parameters of the Gompertz model were estimated by regression analysis and thus the number of distinct ages and the variability of the underlying mortality estimates determine the variance of the parameter estimates. Pooling all 15 racks will roughly increase the total sample size \((N)\) by a factor of 15. We expect that in the pooled sample the variance of the estimated mortalities should decrease because the variance is approximately binomial and thus proportional to the \(N^{-1}\). However, the number of different ages will not increase linearly with increasing \(N\), although it should be somewhat higher in the pooled sample than for any individual rack.

The control flies have actually been tested on two separate occasions or blocks: in the *Rhodiola* experiment and in a separate experiment not reported here testing the effects of Pioglitazone. Thus, we can analyze these results with two different models. In the first model, assume that there is random variation in the values of \(A\) and \(\alpha\) due to each rack and that this level of variation was nested within the variation from each block. The second model pools the results from all 15 racks to create two samples, one per block. The estimates of \(A\) and \(\alpha\)
are each slightly different with each procedure (Fig. 7) although the differences are not statistically significant. However, the pooling procedure results in substantially higher standard errors for both parameters (Fig. 7).

If we examine the fraction of the variance in \( A \) due to racks versus blocks, we find that 80% is due to racks. For \( \alpha \), the fraction of variance due to racks is virtually 100%. We interpret this to mean that the small sample sizes of the racks leads to uncertainty in the estimated mortality rates and that this uncertainty affects \( \alpha \) to a greater extent than \( A \). Presumably if the number of flies in racks was reduced even further, the standard error of \( \alpha \) estimated from individual racks would be higher than the variance from a pooled estimate. Although we have no general rule yet for an optimal size for racks, it appears that in our study it is still better to analyze the data by keeping results from individual racks separate. Consequently, we have chosen to analyze the results of the experiments in this study by analyzing each rack separately.

**Gompertz mortality dynamics.** Males have a significantly lower value of \( A \), the age-independent mortality parameter, than do females (\( \gamma_1 \) Table 7), but a significantly higher value of \( \alpha \) (\( \gamma_2 \) Table 7), the rate-of-aging parameter. *Rhodiola* significantly lowers \( A \) by 20% in females and 33% in males (\( \phi_1 \), Table 7, Fig. 8). The effects of *Rhodiola* on \( \alpha \) are smaller, a 5% reduction in females and a 3% reduction in males (Fig. 8). The effects of *Rhodiola* on \( \alpha \) are not statistically significant (\( \phi_2 \), Table 7).

![Graphs showing fraction of surviving males and females in cohorts exposed to food supplemented with *Rhodiola* and their controls.](image-url)
Partial data set. For a population where mortality is well described, the Gompertz model may be possible to get good estimates of the model parameters with just the first few weeks of observations rather than waiting for every fly to die. We repeated the analysis of the Rhodiola data set using only the first 28 days of data but otherwise using the same techniques as described previously. The numerical values of the parameters are different; however, the sign of the effects due to sex and drugs are the same for all the statistically significant parameters as with the full data set (Table 8). In addition, all of the significant parameters with the full data set are also significant with just 28 days of observations.

Although the parameter estimates obtained with 28 days of observation are somewhat different from those produced from the full data set, they give very similar predictions. For instance, the observed longevity of control females was 26.9 days, whereas the predicted mean from the Gompertz model utilizing all data is 26.6 days. The mean longevity predicted from the Gompertz model, utilizing only the first 28 days of data, is 24.2 days. We suggest that it may often be feasible to determine whether drugs have significant effects on longevity and mortality dynamics by using only partial datasets unless the drug effects are very small.

Two-stage Gompertz mortality dynamics. The estimation of the two-stage Gompertz parameters requires observations late in life to get reasonable estimates of the break day and plateau mortality rate. Consequently, we have not followed the previous protocol of obtaining individual estimates for each rack. Additionally, there are now four parameters to estimate rather than two and the estimation is done separately for each sex and drug treatment. We expect that our ability to infer differences between treatments when they exist will decline relative to the power available with the Gompertz model. The estimated parameters for the two-stage Gompertz show no significant differences between the control and Rhodiola-treated flies (Table 9). The control males have a higher $\alpha$, lower $A$, and earlier break day than the Rhodiola-treated males (Table 9, Fig. 9). The higher value of $\alpha$ leads to a departure in the mortality kinetics late in life (Fig. 9), but again the difference is not statistically significant.

Control females show a 50% larger value of $A$ than the Rhodiola-treated females, but this difference is not statistically significant. This difference does lead to slightly higher mortality rates at nearly all ages (Fig. 10).

One possible explanation for the increased survival of Rhodiola-treated flies is that they can sense the presence of the drug in the food and it reduces their intake of calories. The flies then experience increased survival due to caloric restriction, not due to a direct effect of the drug. In our nutrition experiment, we test this by comparing survival after 28 days at three different yeast levels, with and without Rhodiola.

### Table 7. The Parameter Estimates for the Complete Rhodiola Dataset with Model (3). Each Parameter was Tested to Determine If It Differed from Zero. The Statistical Significance of This $t$-Test Is Given in the Column Labeled p-Value

| Parameter | Value   | Standard error | d.f. | p-value  |
|-----------|---------|----------------|------|----------|
| $\beta_1$ | 0.0082  | 0.00043        | 1614 | <0.0001  |
| $\gamma_1$| -0.0034 | 0.00042        | 1614 | <0.0001  |
| $\phi_1$  | -0.0016 | 0.00036        | 1614 | <0.0001  |
| $\beta_2$ | 0.072   | 0.0022         | 1614 | <0.0001  |
| $\gamma_2$| 0.038   | 0.0026         | 1614 | <0.0001  |
| $\phi_2$  | -0.0035 | 0.0027         | 1614 | 0.18     |
We expect survival to decline as yeast levels increase. However, in the treatments with *Rhodiola*, food consumption should be reduced at all yeast levels if *Rhodiola* acts by affecting food consumption. Thus, in an analysis of survival, the effects of yeast should be radically different in *Rhodiola*-treated vials compared to vials without *Rhodiola*.

Using log linear models, we compared two models. The first model (model 1) included the main factors of yeast (low, medium, high), drug (presence, absence), sex (male, female), and

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**TABLE 8.** The parameter estimates for the partial (Day 28) *Rhodiola* dataset with model (3). Each parameter was tested to determine if it differed from zero. The statistical significance of this t-test is given in the column labeled *p*-value.

| Parameter | Value  | Standard error | d.f. | *p*-value |
|-----------|--------|----------------|------|-----------|
| $\beta_1$ | 0.0042 | 0.00031 | 805 | <0.0001 |
| $\gamma_1$ | -0.0013 | 0.00028 | 805 | <0.0001 |
| $\phi_1$ | -0.0013 | 0.00031 | 805 | <0.0001 |
| $\beta_2$ | 0.12 | 0.0041 | 805 | <0.0001 |
| $\gamma_2$ | 0.032 | 0.0049 | 805 | <0.0001 |
| $\phi_2$ | 0.0066 | 0.0051 | 805 | 0.20 |

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**FIG. 8.** The natural log of mortality versus age for control and *Rhodiola*-treated flies. The predicted values are not linear because the predictions are for 2-day intervals of mortality.
survival (to days 28 yes or no), and the two-way interactions between survival and sex, yeast, and drug. The second model (model 2) was the same as the first but also included the three-way interaction between yeast, drug, and survival. This three-way interaction is not significant (Table 10). We conclude that *Rhodiola* does not act by affecting food consumption, and thus the longevity-enhancing effects of *Rhodiola* are not produced by a caloric-restriction effect.

### DISCUSSION

The present study demonstrates that *Rhodiola rosea* increases the lifespan of adult *Drosophila* without significantly affecting any secondary physiological mechanisms that may have resulted in an artefactual extension of lifespan. The observed anti-aging property of *Rhodiola* was not associated with statistically significant reductions in fecundity, metabolic rate, or male mating success.

We extensively replicated our *Rhodiola* trials, both by wholesale replication and by subsampling from a very large cohort study. There are many ways in which such data can be analyzed, and our analyses necessarily are limited to only some of the possibilities. However, we are confident in our conclusion that *Rhodiola* does significantly increase lifespan no matter how it is calculated or inferred. Whether the other substances tested here might be found efficacious by some other type of adult survival assays is an open question.

Because caloric restriction has been consistently found to increase longevity in mammals and invertebrates, there is the bare possibility that the presence of *Rhodiola* in the food may induce lower levels of food consumption. This could, in effect, mimic an effect similar to dietary restriction. It is well established that gross depressed fecundity is associated with dietary restriction sufficient to significantly increase *Drosophila* longevity. In order to test possible caloric restriction effects in *Rhodiola*-induced

### Table 9. The Estimated Parameters Values and the Bootstrap Tests for Significant Differences Between Control and Drug Flies. The Difference is Computed as the Drug Fly Value Minus the Control Fly Value. The p-Values are Based on 1000 Bootstrap Samples

| Parameter | Control male | Drug male | Control female | Drug female |
|-----------|--------------|-----------|----------------|-------------|
| A         | 0.0092       | 0.0144    | 0.0153         | 0.0109      |
| Difference|              | 0.0051    |                |             |
| p-value   |              | 0.21      |                | 0.19        |
| α         | 0.0861       | 0.0618    | 0.0534         | 0.0570      |
| Difference|              | -0.024    | 0.0534         | 0.0036      |
| p-value   |              | 0.22      |                | 0.32        |
| A₂        | 0.358        | 0.429     | 0.129          | 0.187       |
| Difference|              | 0.0071    | 0.129          | 0.058       |
| p-value   |              | 0.37      |                | 0.36        |
| Break Day | 35.0         | 52.5      | 55.6           | 55.0        |
| Difference|              | 17        |                | -0.60       |
| p-value   |              | 0.21      |                | 0.40        |

**FIG. 9.** The mortality rates versus age for males with predictions from the two-stage Gompertz model. The filled circles are drug-treated flies and the open circles are the control flies.
Drosophila, we measured the number of eggs produced over a 10-day period. As shown in Figure 4, supplementing Drosophila cohorts with various concentrations of the drug (0, 15, 30, and 60 mg/mL) produced no significant differences in treatment groups relative to the control, nor were there any notable differences between groups. In these measurements, there was no statistically significant decrement in reproductive output due to Rhodiola feeding. Because the dietary restriction enhancement of longevity found by Chippindale et al. required a severalfold reduction in fecundity, we concluded that the extension of lifespan is not due to a dietary restriction effect mediated by reduced fecundity at doses of 15, 30, and 60 mg/mL Rhodiola. Because egg production is proportional to food level, the absence of a statistically significant decline in fecundity indicates that the Drosophila females were not lowering their levels of Rhodiola-supplemented food consumption.

However, let us numerically evaluate the possibility that the entire effect of Rhodiola is due to dietary restriction. We observed a statistically insignificant reduction in fecundity of 8.19 eggs per day in the 30 mg/mL treatment that has been our primary focus here. Taking the ratio between fecundity reduction and longevity increase found by Chippindale et al. which was 0.15 days of adult life per egg laid in a day, we can hypothetically reduce our estimate of the longevity benefit of 30 mg/mL of Rhodiola by 1.23 days. Given that the effect of this dose on average female longevity was 3.21 days with a confidence interval of ±0.68, this hypothetical, as opposed to substantiated, dietary effect is not enough to eliminate our estimate of the effect of the supplement on longevity.

As a further test of dietary artifact, we retested our flies at varying yeast levels with and without Rhodiola. Although varying yeast levels do indeed affect survival rates, they do not do so with a detectable interaction involving exposure to Rhodiola. Thus, it appears that the effect of Rhodiola on the longevity of adult flies was not mediated solely or primarily through dietary effects on reproduction.

It has been proposed that Rhodiola has versatile protective properties that counteract adverse physical, chemical, and biological stressors. Rhodiola supplementation has been recommended for the treatment of depression,

![FIG. 10. The mortality rates versus age for females with predictions from the two-stage Gompertz model. The filled circles are drug-treated flies and the open circles are the control flies.](image)

| Deviance | df | Delta (Dev) | Delta (df) | P > Delta (Dev) |
|----------|----|-------------|------------|-----------------|
| Model 1  | 15.277 | 14 | 7.449 | 0.11 | 0.65 |
| Model 2  | 7.828 | 10 | 0.000 | | |
| Saturated | 0.000 | 0 | 7.827 | | |
headaches, hypertension, amenorrhea, asthenia, schizophrenia, male sexual dysfunction, as well as colds and flus. There is also clinical evidence that *Rhodiola* has anti-fatigue, anti-stress, anti-hypoxic, anti-cancer, anti-oxidant, and immune-enhancing effects. Additionally, cardioprotective effects have been inferred from reduced cardiac damage under stress.

In conclusion, we have presented evidence that pharmacological intervention using *Rhodiola rosea* can extend lifespan without statistically significant physiological tradeoffs that could generate an artefactual longevity benefit. The lifespan extension detected here might be due to antioxidative protection against a possible accumulation of free radicals that occurs with aging, but we have not shown that.

REFERENCES

1. Lithgow GJ, Gill MS, Olsen A, Sampayo JN. Pharmacological intervention in invertebrate aging. AGE 2005;27:213–223.
2. Rubin GM, Yandell MD, Wortman JR, Gabor Miklos GL, Nelson CR, Hariharan IK, Fortini ME, Li PW, Apweiler R, Fleischmann W, Cherry JM, Henikoff S, Skupski MP, Misra S, Ashburner M, Birney E, Boguski MS, Brody T, Brokstein P, Celniker SE, Chervitz SA, Coates D, Cravchik A, Gabrielian A, Galle RF, Gelbart WM, George RA, Goldstein DB, Patrick L, Patridge L, Pickeral OK, Shue C, Vosshall LB, Zhang J, Zhao Q, Zheng XH, Lewis S. Comparative genomics of the eukaryotes. Science 2000;289:1567–1574.
3. Melov S, McGavin MJ, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Dogliotti S, Lithgow GJ. Extension of life-span with superoxide dismutase/catalase mimetics. Science 2000;289:1567–1569.
4. Bauer JH, Goupil S, Garber GB, Helfand SL. An accelerated assay for the identification of lifespan-extending interventions in Drosophila melanogaster. Natl Acad Sci 2004;101:12980–12985.
5. Bonilla E, Medina-Leendertz S, Diaz S. Extension of life span and stress resistance of Drosophila melanogaster by long-term supplementation with melatonin. Exp Gerontol 2002;37:629–638.
6. Cui X, Dai XG, Li WB, Zhang SL, Fang YZ. Effects of lu-duo-wei capsule on prolonging life span of housefly and Drosophila melanogaster. Am J Chin Med 1999;27:407–413.
7. Loeb J, Northrop JH. What determines the duration of life in Metazoa? Proc Natl Acad Sci U S A 1917;3:382–386.
8. Lin YJ, Seroud L, Benzer S. Extended life-span and stress resistance in the Drosophila mutant methuseelah. Science 1998;282:943–946.
9. Fleming JE, Spicer GC, Garrison RC, Rose MR. Two dimensional protein electrophoretic analysis of postponed aging in Drosophila. Genetica 1993;91:183–198.
10. Rose MR, Long AD. Ageing: the many-headed monster. Curr Biol 2002;12:R311–R312.
11. Fletcher SD, Macdonald SJ, Margueri R, Certa U, Stearns SC, Goldstein DB, Patridge L. Genome-wide transcript profiles in aging and calorically restricted Drosophila melanogaster. Curr Biol 2002;12:712–723.
12. Jafari M, Rose M. Rules for the use of model organisms in antiaging pharmacology. Aging Cell 2006;5:17–22.
13. de Sanctis R, De Bellis R, Scelsa C, Mancini U, Cucchiari L, Dacha M. In vitro protective effect of Rhodiola rosea extract against hypochlorous acid-induced oxidative damage in human erythrocytes. Biofactors 2004;20:147–159.
14. Brown RP, Gerbang PL, Ramazanov Z. Rhodiola rosea: a phytochemical overview. Herbal Gram 2002;56:40–52.
15. Rose MR, Dorey ML, Coyle AM, Service PM. The morphology of postponed senescence in Drosophila melanogaster. Can J Zool 1984;62:1576–1580.
16. Rose MR, Passananti HB, Mato M. Methuselah flies: a case study in the evolution of aging. Singapore: World Scientific Publishing, 2004.
17. Rose MR, Drapeau MD, Yazdi PG, Shah KH, Moise DB, Thakar RR, Rauser CL, Mueller LD. Evolution of late-life mortality in Drosophila melanogaster. Evolution 2002;56:1982–1991.
18. Pinheiro JC, Bates DM. Mixed-effects models in S and S-PLUS. New York: Springer-Verlag, 2000.
19. Carey JR, Liedo P, Orozco D, Vaupel JW. Slowing of mortality-rates at older ages in large medfly cohorts. Science 1992;258:457–461.
20. Curstinger JW, Fukui HH, Townsend DR, Vaupel JW. Demography of genotypes: failure of the limited life span paradigm in Drosophila melanogaster. Science 1992;258:461–463.
21. Steinsaltz D. Re-evaluating a test of the heterogeneity explanation for mortality plateaus. Exp Gerontol 2005;40:101–113.
22. Maynard Smith J. Sex-limited inheritance of longevity in Drosophila subobscura. J Genet 1959;56:1–9.
23. Williams AE, Rose MR, Bradley TJ. The respiratory dimension of postponed aging in Drosophila melanogaster selected for desiccation resistance is not associated with the observed evolution of decreased locomotory activity. Physiol Biochem Zool 2004;77:10–17.
24. Mueller LD, Nusbaum TJ, Rose MR. The Gompertz equation as a predictive tool in demography. Exp Gerontol 1995;30:553–569.
25. Bishop Y, Fienberg, SE, Holland PW. Discrete multivariate analysis. Cambridge: MIT Press, 1975.
26. Chippendale AK, Leroi AM, Kim SB, Rose MR. Meta-
bolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. J Evol Biol 1993;10:269–293.

27. Chippindale AK, Leroi AM, Kim SB, Rose MR. Metabolic aspects of the trade-off between fecundity and longevity in *Drosophila melanogaster*. J Evol Biol 1993;10:269–293.

28. Chapman T, Partridge L. Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. Proc Biol Sci 1996;263:755–759.

29. Chippindale AK, Leroi AM, Kim SB, Rose MR. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. J Evolution Biol 1993;6:171–193.

30. Khanum F, Bawa SB, Singh B. *Rhodiola rosea*: a versatile adaptogen. Compr Rev Food Sci Food Safety 2005;4:55–62.

31. Germano C, Ramazanov Z, Bernal Suarez M. Arctic root (*Rhodiola rosea*): the powerful new ginseng alternative. New York: Kensington Publishing Corp., 1999.

32. Darbinyan V, Kteyan A, Panossian A, Gabrielyan E, Wikman G, Wagner H. *Rhodiola rosea* in stress induced fatigue—a double blind cross-over study of a standardized extract SHR-5 with a repeated low-dose regimen on the mental performance of healthy physicists during night duty. Phytomedicine 2000;7:365–371.

33. Spasov AA, Wikman GK, Mandrikov VB, Mironova IA, Neumoin VV. A double-blind, placebo-controlled pilot study of the stimulating and adaptogenic effect of *Rhodiola rosea* SHR-5 extract on the fatigue of students caused by stress during an examination period with a repeated low-dose regimen. Phytomedicine 2000;7:85–89.

34. Lishmanov Iu B, Trifonova Zh V, Tsibin AN, Maslova LV, Dement’eva LA. Plasma beta-endorphin and stress hormones in stress and adaptation. Biull Eksp Biol Med [Russian] 1987;103:422–424.

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