© 2019. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2019) 222, jeb192534. doi:10.1242/jeb.192534

RESEARCH ARTICLE

Adult diet does not compensate for impact of a poor larval diet on stress resistance in a tephritid fruit fly

Christopher W. Weldon[1,§], Sandiso Mnguni[1,*], Fabien Démares[1,‡], Esther E. du Rand[1], Kevin Malod[1], Aruna Manrakhan[2] and Susan W. Nicolson[1]

ABSTRACT

Adult holometabolous insects may derive metabolic resources from either larval or adult feeding, but little is known of whether adult diets can compensate for deficiencies in the larval diet in terms of stress resistance. We investigated how stress resistance is affected and compensated for by diet across life stages in the marula fruit fly *Ceratitis cosyra* (Diptera: Tephritidae). Larvae were fed diets containing either 8% torula yeast, the standard diet used to rear this species, or 1% yeast (low protein content similar to known host fruit). At emergence, adults from each larval diet were tested for initial mass, water content, body composition, and desiccation and starvation resistance or they were allocated to one of two adult diet treatments: sucrose only, or sucrose and yeast hydrolysate. The same assays were then repeated after 10 days of adult feeding. Development on a low protein larval diet led to lower body mass and improved desiccation and starvation resistance in newly emerged adults, even though adults from the high protein larval diet had the highest water content. Adult feeding decreased desiccation or starvation resistance, regardless of the diet provided. Irrespective of larval diet history, newly emerged, unfed adults had significantly higher dehydration tolerance than those that were fed. Lipid reserves played a role in starvation resistance. There was no evidence for metabolic water from stored nutrients extending desiccation resistance. Our findings show the possibility of a nutrient-poor larval environment leading to correlated improvement in adult performance, at least in the short term.

KEY WORDS: Body composition, Dehydration tolerance, Desiccation resistance, Diet, Starvation resistance, Water content

INTRODUCTION

Nutrition plays an essential role in enhancing insect resistance to environmental stress (Andersen et al., 2010; Djawdan et al., 1997; Sisodia and Singh, 2012). Animals obtain nutrients for somatic maintenance, growth and reproduction, but nutrient reserves also play a large role in tolerance of desiccation and starvation (Djawdan et al., 1998). Lipids represent the main form of energy storage in insects as triglycerides in the fat body (Arrese and Soulages, 2010) but also act as a source of water upon oxidation in some species (Djawdan et al., 1998; Kleynhans and Terblanche, 2009; Nicolson, 1980; Weldon et al., 2016). Glycogen can also serve as a source of metabolic water, with the additional benefit that water bound to glycogen also becomes available when the glycogen is metabolised (Djawdan et al., 1998; Gefen et al., 2006). A range of proteins is also associated with insect tolerance of dry conditions. For example, upregulation of heat shock protein expression protects water-stressed cells from changes in pH and solute concentrations by preventing protein aggregation and preserving enzyme activity (Benoit et al., 2010; Hayward et al., 2004).

Some insects may struggle to meet their minimum nutritional requirements, which can lead to nutrient deficiency or imbalance (Raubenheimer and Simpson, 1999). This detrimental effect hinders the fitness and everyday activities of insects (Andersen et al., 2010). This is largely due to the fact that insect body tissues require a specific amount and composition of nutrients to function adequately (Boggs, 2009), but dietary sources of nutrients, particularly plant-based diets, may have low nutrient content or unbalanced nutrient composition (Simpson and Raubenheimer, 2000). In response to nutritional stress, insects may extend their growth periods by changing their energy allocation to somatic maintenance (Boggs, 2009). This prolongs the time needed to reach maturity, reduces total body size and changes allometric relationships between body regions (Boggs and Niitepõld, 2016; Gefen et al., 2006). Alternatively, an insect may exhibit compensatory feeding and dietary selection (e.g. Abisgold and Simpson, 1987; Fanson et al., 2009; Maklakov et al., 2008; Malod et al., 2017) to achieve a nutrient intake that approaches an optimum defined by its evolved life history strategy. However, it has also been found that restriction of food intake (calories) can lead to 'hormetic' effects, whereby individuals experiencing a mild stress exhibit improved stress resistance and life extension (Le Bourg, 2009).

Larval nutrition determines how adult insects cope with various kinds of stress because the larval stage contributes to metabolic reserves in adults. When reared on a protein-rich larval medium, adult *Drosophila melanogaster* exhibited increased desiccation resistance and heat tolerance compared with those reared on a carbohydrate-enriched medium (Andersen et al., 2010). Larval development of *D. melanogaster* in a field diet of mostly decomposing apples led to lower critical thermal maximum, dry body mass and fat content, but higher starvation resistance, in comparison with development on a standard laboratory diet (Kristensen et al., 2016). Mexican fruit flies (*Anastrepha ludens*) reared on a carbohydrate-based larval diet exhibited longer larval and pupal development than those reared on a highly protein-biased diet (Pascacio-Villaf et al., 2016). However, pupae were larger, and adult desiccation and starvation resistance was improved when flies developed on the carbohydrate-biased larval diet. In some cases, feedback has been detected between environmental temperature and

[1]Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa. [2]Citrus Research International, PO Box 28, Nelspruit 1200, South Africa.
*Present address: DST-NRF Centre of Excellence in Palaeosciences, University of the Witwatersrand, Private Bag 3, Wits 2050, South Africa. ‡Present address: Emerging Pathogens Institute, University of Florida, Gainesville, FL 32608, USA.

§Author for correspondence (cwweldon@zoology.up.ac.za)

C.W.W., 0000-0002-9897-2689; S.M., 0000-0002-3077-2162; F.D., 0000-0002-2638-4247; E.E.d., 0000-0002-1893-5934; K.M., 0000-0002-6811-2717; A.M., 0000-0003-2954-4450; S.W.N., 0000-0001-6024-9383

Received 26 September 2018; Accepted 25 February 2019

nutritional outcomes in developing insects (Coggan et al., 2011). Adult diet can also play a role in stress tolerance. For example, in *D. melanogaster*, dietary restriction on a low protein to carbohydrate ratio, which favors longevity over reproduction, also led to higher starvation resistance and associated elevated lipid deposition (Lee and Jang, 2014). Dietary restriction during the adult life stage in the oriental fruit fly (*Bactrocera dorsalis*) led to increased resistance to desiccation, starvation, and heat and cold shock, as well as improved longevity, but came at a cost of reduced fecundity (Chen et al., 2017). However, nutrient restriction did not have any consistent effects on thermal tolerance traits in the Mediterranean fruit fly (*Ceratitis capitata*) (Mitchell et al., 2017).

As mentioned above, compensatory feeding within an insect developmental stage to counteract the consequences of a poor diet is well documented, especially for adult stages (Nestel et al., 2016 and references therein). However, the capacity of holometabolous insects to compensate and correct for nutritional deficiencies in an earlier life stage has received much less attention (Nestel et al., 2016). In the mustard leaf beetle (*Phaedon cochleariae*), a larval diet of watercress led to slower development and reduced adult fecundity in comparison with beetles that developed on a highly nutritious cabbage diet (Müller and Müller, 2016). However, when beetles that had developed on watercress were switched to an adult diet of cabbage, their fecundity did not differ from those that had developed on cabbage, providing clear evidence for adult dietary compensation for reproductive output (Müller and Müller, 2016). When reared on a low quality larval diet, adult females of the map butterfly (*Araschnia levana*), were smaller but showed a significant preference for a nectar mimic with amino acids in comparison with females developed on a high quality larval diet (Mevi-Schütz and Erhardt, 2003). In contrast, adult feeding on a source of amino acids in another nymphalid butterfly, *Bicyclus anynana*, resulted in only limited reproductive compensation, in terms of increased egg size, for a brief period of larval starvation (Bauerfeind and Fischer, 2009). In two tephritid fruit fly species, *A. ludens* and the guava fruit fly *Anastrepha striata*, copulations achieved by males with access to an adult diet high in protein did not differ among males of different size, although size is affected by larval developmental environment (Aluja et al., 2008). Similar results were again obtained for *A. ludens* as well as the West Indian fruit fly (*Anastrepha obliqua*) (Aluja et al., 2009). Males with constant access to protein and sucrose, regardless of size, mated more often, had shorter copulations and induced longer refractory periods in females than males fed a low quality diet as adults (Aluja et al., 2009). These examples support the suggestion that larval nutritional conditions leading to deficiencies in adult stress tolerance traits can be compensated for by the adult diet.

The aim of this study was to determine the effect of larval and adult diets on desiccation resistance and starvation resistance of the marula fruit fly *Ceratitis cosyra* (Walker 1849) (Diptera: Tephritidae). By doing so, we could test for the potential for compensation for deficiencies in nutrition across life stages. Body nutrient reserves (total carbohydrates, glycogen, lipids and proteins) were determined to identify potential links between diet, nutrient storage, and desiccation and starvation resistance. We predicted that stress resistance would be improved by larval development in a nutrient-rich diet, leading to greater body reserves, but consumption of a nutrient-rich diet as an adult would compensate for poor stress resistance associated with a suboptimal larval diet. *C. cosyra* was chosen as the focus of this study due to availability of data on its adult nutritional intake targets and life history (Malod et al., 2017), its close phylogenetic relationship to other fruit fly species that have been the subject of water balance studies (Weldon et al., 2016, 2018, 2013) and because it exhibits relatively high desiccation resistance (Weldon et al., 2016). In addition, *C. cosyra* is an important quarantine pest across sub-Saharan Africa where it affects production of mangoes (Vayssières et al., 2009; White and Elson-Harris, 1992). An increased understanding of how the stress tolerance traits of *C. cosyra* vary with diet will contribute to predicting its distribution in a changing climate using physiological models that account for flows of energy and conservation of mass (Rosenblatt and Schmitz, 2016).

MATERIALS AND METHODS

Flies and colony maintenance

Flies were obtained from a *C. cosyra* culture from the Department of Zoology and Entomology, University of Pretoria, South Africa. The culture had been maintained for approximately 12 generations with 300 adults in each generation. Adults were kept in 5 liter clear plastic containers with one side replaced with an insect screen for ventilation. The lid of the plastic containers was replaced with a sleeve of white voile curtain fabric to permit access to the cage. Each cage was furnished with sugar and yeast hydrolysate (HG000BX6.500, Merck, Wadesville, South Africa) in separate Petri dishes. Each cage was also supplied with water-soaked cotton wool as a water source. When culture flies were more than 10 days old, oviposition substrates were provided into which females laid eggs. The oviposition substrates comprised 100 ml plastic cups containing water-soaked tissue paper and 3 ml guava concentrate (Halls; Tiger Consumer Brands Limited, Bryanston, South Africa). These were covered with a double layer of Parafilm (Bemis Flexible Packaging, Neenah, WI, USA) that was pierced multiple times with an insect pin. Females were given 2–3 h to lay eggs. Eggs were then washed off the Parafilm with distilled water into a glass dish where they were permitted to settle, then taken up with a 3 ml disposable plastic Pasteur pipette and again allowed to settle at the tip of the pipette. One drop of densely packed eggs, containing approximately 300 eggs, was transferred to each larval diet (see below). The cultures and all experiments described below were kept at a constant temperature of 24±1°C in a climate room with buffered relative humidity (40–60%) and a 12 h light:12 h dark cycle with a simulated dawn and dusk for the first and last hour of the light phase. Lights in the climate room turned on at 06:00 h (South Africa Standard Time; GMT+2) and turned off at 18:00 h.

Choice of larval diets

Before determining the effect of larval diet on adult stress resistance traits, it was first necessary to identify larval diets that contained high and low protein, but still permitted sufficient development and survival to obtain adults for testing. The standard larval diet used to rear *C. cosyra* comprises a blend of carrot powder, torula yeast and sodium benzoate as a preservative (Citrus Research International, Nelspruit, South Africa). This standard diet, plus a range of diets with different proportions of carrot powder (a source of carbohydrates and some amino acids) and torula yeast (a source of protein and other nutrients) were screened for development time, pupal production and adult eclosion. The diets included 8% (standard), 4%, 1% and 0% torula yeast by wet mass. As torula yeast content decreased, the quantity of carrot powder was increased. Protein content of known fruit hosts for *C. cosyra* is 0.5% for marula fruit (*Sclerocarya birrea*) (Wehmeyer, 1966) and 0.2% for mango (*Mangifera indica*) (Kansci et al., 2008). To 100 g of the dry mix of each diet, 250 ml of boiling water was added and combined to form a smooth paste. A quantity of 100 g of the wet mix of each diet was

Journal of Experimental Biology (2019) 222, jeb192534. doi:10.1242/jeb.192534

transferred to 100 ml plastic cups. There were 5 replicates for each diet.

After diets were prepared, *C. cosyra* eggs were obtained from the laboratory culture. Approximately 2700 eggs (three Petri dishes, each having three drops of eggs) were allowed to hatch on moist filter paper in covered Petri dishes. Using a dissecting microscope, 100 freshly hatched larvae were transferred with a moist, very fine paintbrush to each replicate of the four diets (i.e. a total of 2000 freshly hatched larvae). The inoculated diets were then placed on a 2 cm layer of dry sand in individual ventilated containers (with a volume of 2 liters). After 18 days, when all surviving larvae had completed development, migrated from the diets and entered the pupal stage, the sand from each container was sifted to count the number of pupae produced from each replicate of each diet. The pupae were returned to the ventilated containers and covered with 2 cm of dry sand. The containers were observed on a daily basis to record the date of first adult emergence and main emergence. The adults were left to starve until death; the number and sex of adults was then determined by counting them under a dissecting microscope.

Experimental protocol

Based on the results of the trials described above, the standard larval diet (8% torula yeast) and the larval diet containing 1% torula yeast were used for subsequent diet manipulations. These larval diets were referred to as 'high protein' and 'low protein', respectively. Approximately 2400 eggs were obtained from the laboratory culture, as described above. Rates of adult emergence from the pilot study were used to estimate the number of eggs needed to obtain the adults for planned desiccation and starvation resistance assays. A droplet containing approximately 300 eggs was inoculated into each of three 100 ml containers of high protein larval diet and of five 100 ml containers of low protein larval diet (to allow for the lower adult emergence of *C. cosyra* from the latter). These were placed on a 2 cm layer of dry sand in separate 2 liter plastic containers with ventilated lids. Larvae emerged out of the larval diets at the end of the third instar and entered the pupal stage. The sand was sifted after 18 days to obtain pupae. Pupae were then placed in an empty cage to emerge as adults. Upon emergence, the adults from each larval diet were allocated to assays for initial mass, water content, and desiccation and starvation resistance (described below) or transferred to one of two cages where they were allocated to one of two adult diet treatments: sucrose only, or sucrose and yeast hydrolysate (YH). Sucrose and YH were offered in separate Petri dishes so that flies could select their intake of each. YH is a rich source of amino acids, lipids and micronutrients (Taylor et al., 2013). Each cage was also supplied with water-soaked cotton wool as a water source. Flies provided with food as adults were assayed for initial mass, water content, and desiccation and starvation resistance 10 days after adult emergence. On emergence, females and males on each larval and adult diet were kept in the same cage. No oviposition substrates were provided for egg-laying by females.

Initial mass and water content

At 0 days and 10 days after adult emergence, 20 flies (10 females and 10 males) from each larval and adult diet treatment were placed in centrifuge tubes of known weight and weighed on a microbalance (to 0.001 mg; CPA2P, Sartorius AG, Germany). Initial mass of each fly was obtained by subtracting tube weight from tube+fly weight. These adult insects were frozen and stored at −20°C for several days. Flies were then freeze-dried using an automated freeze-drier (2KBTXL-75 Benchtop SLC Freeze Dryer, Virtis, Los Angeles, California, USA) for approximately 48 h before being weighed again on a microbalance to determine dry mass. Water content was determined by subtracting dry mass from body mass. Freeze-dried flies were stored at 4°C for later analyses of body composition.

Desiccation and starvation resistance

At 0 days and 10 days, 30 female and 30 male flies from each larval and adult diet treatment were placed into individual, numbered, 2 ml microcentrifuge tubes that had been pierced with 12 holes (diameter ∼1 mm) and then pre-weighed. Flies in tubes were then weighed (to 0.001 mg) and initial body mass of each fly was calculated by subtracting tube mass. Half of the tubes from each larval and adult diet treatment were then placed onto racks in airtight containers over anhydrous silica gel to maintain relative humidity below 5%. The other half were placed onto racks in airtight containers over distilled water to maintain relative humidity close to 100%. All airtight boxes were maintained at 25°C for the duration of the experiment. Desiccation and starvation resistance assays commenced at 10:30 h and all tubes were checked for mortality every 3 h by viewing them through the clear plastic top of the airtight containers. Dead flies were removed from the tubes and weighed to record body mass at death (to 0.001 mg). These were then placed in new, intact, labeled tubes for storage in a freezer at −20°C. Later, all flies were freeze-dried and weighed as described above to determine dry mass at death and body water at death (expressed as percentage of initial water content, this is referred to as dehydration tolerance; Gibbs et al., 1997). Higher values of water at death are interpreted as lower dehydration tolerance.

Measurement of whole-insect nutrient reserves

The methods used for the body composition analyses were adapted from Foray et al. (2012) to determine carbohydrate, glycogen, lipid and soluble protein content from the same individual. Five to seven freeze-dried flies were initially selected at random for each combination of larval diet, adult diet, sex and stress (desiccation or starvation). These were then randomly processed to avoid all individuals from the same treatments being assayed synchronously. Additional tests for carbohydrates only were also run in three to five additional randomly selected flies to verify low quantities that were detected in some groups. Precise sample sizes are available in the publicly available data file (http://hdl.handle.net/2263/66649). Each insect was individually homogenised in 180 µl lysis buffer using a microtube homogeniser (BeadBug™ 3 Position Bead Homogeniser, Benchmark Scientific, Sayreville, NJ, USA) for 30 s at the maximum setting. The lysis buffer consisted of 100 mmol l^{-1} potassium dihydrogen phosphate, 1 mmol l^{-1} DTT and 1 mmol l^{-1} EDTA (pH 7.4). After homogenisation, the samples were centrifuged at 14,000 ***g*** for 10 min.

For soluble protein determinations, 1.5 µl supernatant was transferred to a 96-well plate in triplicate. Total protein content was determined by the Bradford assay (Bradford, 1976) using Bradford Reagent (Sigma-Aldrich, St Louis, MO, USA) and serial dilutions of bovine serum albumin standard (Sigma-Aldrich) to establish a standard curve according to the manufacturer's instructions. Optical density was measured at 595 nm using a microplate reader (Eon™ High Performance Microplate Spectrophotometer, BioTek, Winooski, VT, USA).

To determine the carbohydrate and lipid content, 4.5 µl lysis buffer and 20 µl of 20% (w/v) sodium sulphate was added to the remaining homogenate (to reach a final volume of 200 µl). After adding 1500 µl of chloroform:methanol (1:2 v/v) to each sample, samples were vortexed vigorously and centrifuged at 14,000 ***g*** for 10 min. Three hundred microliters of the supernatant was aliquoted

for the lipid analysis and the pellet was kept for the determination of the glycogen content. The remainder of the supernatant was evaporated at room temperature overnight in a fumehood and made up to a final volume of 250 µl using chloroform:methanol (1:2 v/v). The anthrone method was employed to determine the water-soluble carbohydrate content of these samples. A glucose (Merck, Billerica, MA, USA) dilution range (0.1, 0.3, 0.5, 1.0, 2.5 and 5 mg ml^{-1}) was used as calibration standard. Two hundred microliters of standard or sample were pipetted into a 15 ml tube and 4.8 ml anthrone reagent (1.42 g l^{-1} anthrone dissolved in 70% sulphuric acid) added (Anthrone, ACS reagent 97% was obtained from Sigma-Aldrich). The reaction mixture was vortexed and incubated at 90°C in a water bath for 15 min before the optical density was measured at 625 nm using the microplate reader. Measurements were performed in duplicate using 250 µl reaction mixture.

The anthrone method was also used to determine the total glycogen content of each sample. The pellet was washed with 400 µl of 80% (v/v) methanol and centrifuged at 16,000 ***g*** for 5 min. The supernatant was discarded before adding 400 µl distilled water to the pellet. Subsequently, the samples were incubated at 70°C in a water bath for 5 min. Two hundred microliters of standard or sample were pipetted into a 2 ml Eppendorf tube and 1 ml anthrone reagent (1.42 g l^{-1} dissolved in 70% sulphuric acid) added. The reaction mixture was vortexed and incubated at 90°C in a water bath for 15 min. The reaction mixtures were cooled on ice and filtered using 0.22 µm, PVDF syringe filters (Millex®-GV, Merck, Billerica, MA, USA) before the optical density was measured at 625 nm using the microplate reader. Measurements were performed in triplicate using 200 µl reaction mixture. A glucose dilution range (0.05, 0.1, 0.25, 0.5, 0.75 and 1 mg ml^{-1}) was used as calibration standard.

The total amount of lipids in each insect was determined with the vanillin colorimetric assay. Glycerol trioleate (Sigma-Aldrich) dissolved in chloroform:methanol (1:2 v/v) was used as calibration standard (dilution range 0.1, 0.2, 1.0 and 2.0 µg $µl^{-1}$). One hundred microliters of standard or aliquoted supernatant was transferred to a 96-well microplate in triplicate. Following the complete evaporation of the samples at 90°C, the samples were incubated with 10 µl of 98% sulphuric acid at 90°C for 2 min. After cooling the samples on ice, 190 µl vanillin reagent (1.2 g l^{-1} vanillin dissolved in 68% orthophospohoric acid) were added to each sample (Vanillin, ReagentPlus® 99% was obtained from Sigma-Aldrich). The microplate was placed in the microplate reader and the shake function was used to mix the reaction mixtures before incubation for 15 min at room temperature and subsequent measurement of the optical density at 525 nm.

Data analysis

Analyses of variance were applied to data obtained from the preliminary study to select suitable larval diets. Development time (from egg inoculation to main adult emergence), number of pupae and adult emergence (expressed as a percentage of the number of pupae) were dependent variables, and larval diet torula yeast content (8%, 4%, 1% and 0% of wet mass) was the predictor variable. When we excluded the 0% torula yeast treatment from all analyses (after yielding 0 pupae and thus 0 adults), data met the assumptions of parametric tests. *Post hoc* multiple comparisons were performed using Tukey's honest significant difference tests to identify which diets differed from each other. A contingency analysis was used to determine if the sex ratio differed with larval diet.

Initial mass and water content of adults were related to larval diet, adult diet and sex using generalized linear models (GLZ) with normal distribution and identity link. Owing to the hierarchical nature of the experimental design, a nested model was used, with sex within adult diet [none (age: 0 days), sugar only and sugar+YH (age: 10 days)], and adult diet within larval diet (low yeast, high yeast). For the water content model, initial mass was included as a covariate to account for the effects of body size. Model residuals indicated that the data did not violate assumptions of constancy of variance and normality of errors. Model coefficients were inspected to identify simple effects. A significant effect of body mass was found on initial water content, so a series of ordinary least squares regressions were used to establish the linear relationship between initial mass and initial body water content for each combination of larval diet, adult diet and sex. The resulting regression equations (Table S1) were used to estimate initial body water content based on measurements of initial mass in later analyses.

Cox proportional hazards regression was used to determine the effects of larval diet, adult diet, stress, sex and initial mass of adult *C. cosyra* on time to death in desiccation and starvation resistance assays. Again, due to the hierarchical nature of the study design, a nested model was used, with sex within assay, assay within adult diet, adult diet within larval diet and initial mass as a covariate. Adjustment for ties was estimated using Breslow likelihood. Based on inspection of scaled Schoenfeld residuals over time, the data did not violate the assumption of proportionality of hazards. Model coefficients were inspected to identify simple effects. Model results were used to plot survivor functions.

The nested effects of larval diet, adult diet, stress, and sex on dehydration tolerance were determined using a GLZ with gamma distribution and log link function. Based on comparison of Akaike's information criterion (AIC), this model provided a better fit for the data than a normal distribution with identity or log link, and did not violate assumptions of constancy of variance and normality of errors. The dependent variable was body water content at death, which was related to estimated body water (from the regression equations in Table S1) by inclusion of the latter as a covariate in the model. Survival time during desiccation and starvation resistance assays were also included as a covariate in the model.

Dry mass, and whole insect carbohydrate, glycogen, lipid and protein content (expressed as µg mg^{-1} dry mass) were analysed using five separate GLZs with gamma distribution and log link function. In each model, the predictors were sex nested within stress, stress nested within adult diet, and adult diet nested within larval diet. In the model for dry mass, the interaction of initial mass with sex was also included in the model. Although it was often the case that the content of each nutrient was determined for a single fly, in some cases it was necessary to obtain carbohydrate content estimates from additional flies to verify very low levels detected in some treatments. For this reason, not all nutrients were measured in all flies, and it was consequently not possible to run a multivariate analysis of covariance model. In addition, the obtained data did not fit a normal distribution; based on AIC values, a gamma distribution with log link provided the best fit for the data.

All data analyses were performed using Statistica 64-bit v13 (Dell Inc., Tulsa, OK, USA).

RESULTS

Choice of larval diets

No pupae were obtained from the 0% torula yeast larval diet (Table 1). There was a significant difference between the number of pupae obtained from the remaining three larval diets ($F_{3,16}$=265.42, P< 0.0001). The number of pupae from the 4% and 8% torula yeast larval diets was significantly higher than from the 1% torula yeast diet. Larval diet also had a significant effect on adult emergence

Journal of Experimental Biology (2019) 222, jeb192534. doi:10.1242/jeb.192534

Table 1. Effect of larval diets varying in torula yeast content on mean (± 1 s.e.) larval survival, juvenile development time and adult emergence of *Ceratitis cosyra*

	Torula yeast content in larval diet			
Performance trait	0%	1%	4%	8%
Larval survival (%)	0	47.6±4.3^a	86.2±1.5^b	80.4±1.7^b
Juvenile development time (days)	0	32.6±0.2^a	28±0.0^b	27.6±0.2^b
Adult emergence (%)	0	76.9±7.7^a	90.4±1.2^b	91.0±3.1^b

Each replicate (n=5) began with 100 newly emerged larvae. Adult emergence is expressed as the percentage of pupae obtained from each diet that emerged as adults. Means for each performance trait with different superscript letters are significantly different (Tukey's HSD tests; P>0.05).

expressed as a percentage of adults emerged over pupae obtained from each diet ($F_{3,16}$=70. 39, P<0.0001). Adult emergence from the diets containing 4% and 8% torula yeast was significantly higher than from the 1% torula yeast larval diet. There was a significant effect of larval diet on development time ($F_{3,16}$=7374.60, P<0.0001), with the adult stage reached in a shorter period of time by individuals reared on 4% and 8% torula yeast diet in comparison with those reared on the 1% torula yeast diet. Larval diet had no significant effect on the sex ratio of *C. cosyra* (χ^2=3.13, d.f.=2, P=0.209), with the sex ratio being 1:1 in all diets where adults emerged. Based on these results, and because it is the standard diet used to rear *C. cosyra* in the laboratory, the 8% torula yeast larval diet was selected as the high protein larval diet, and the 1% torula yeast larval diet was selected as the low protein larval diet for stress resistance experiments.

Initial condition

Adult *C. cosyra* reared from a high yeast larval diet were significantly heavier (predicted mean±1 s.e.=9.24±0.12 mg) than those reared on a low yeast larval diet (7.92±0.12 mg) (GLZ: Wald χ^2=34.69, d.f.=1, P<0.001). Adult diet nested within larval diet also affected adult initial mass (GLZ: Wald χ^2=12.68, d.f.=4, P=0.013). That is, among flies fed a high yeast larval diet, those fed sugar+YH for 10 days after adult emergence were significantly heavier than those with no opportunity to feed as adults (0 days) or fed only sugar for 10 days (Fig. 1A). In contrast, among flies fed a low yeast larval diet, there was no significant difference in initial mass between the adult diet treatments. Within adult diet, sex had a significant effect on initial mass (GLZ: Wald χ^2=85.31, d.f.=3, P<0.001), with females consistently heavier than males within each adult diet treatment (0 days: female=9.08±0.21 mg, male=7.59±0.21 mg; 10 days sugar: female=9.14±0.21 mg, male=7.50±0.21 mg; 10 days sugar+YH: female=9.86±0.21 mg, male=8.29±0.21 mg).

Water content of adult *C. cosyra* significantly increased with every unit increase in initial mass (GLZ: Wald χ^2=1298.69, d.f.=1, P<0.001; parameter estimate: 0.682±0.019). Larval diet had a significant effect on water content (GLZ: Wald χ^2=5.433, d.f.=1, P=0.020). However, when accounting for initial mass (mean=8.57 mg) there was no overall difference in water content of adults reared on a high yeast (5.78±0.03 mg) or low yeast larval diet (5.78±0.03 mg). Among adults reared on a high yeast or low yeast larval diet, the nested effect of adult diet significantly predicted water content (GLZ: Wald χ^2=26.20, d.f.=4, P<0.001). This was apparent when adults were reared from a high yeast larval diet because water content of newly emerged adults was significantly higher than those fed for 10 days on sugar only or sugar+YH (Fig. 1B). In contrast, when reared on a low yeast larval diet, water content did not differ between adult diet treatments. The

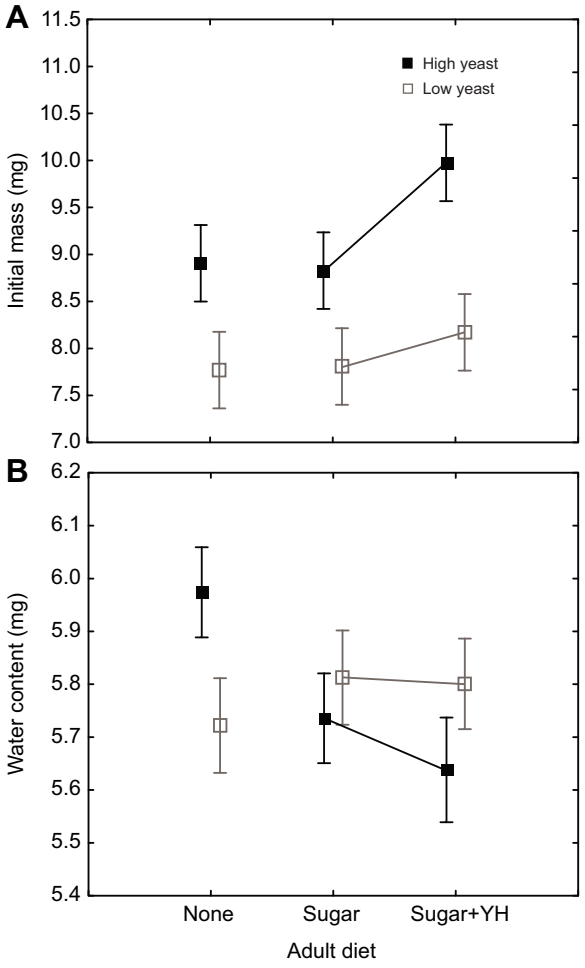

Fig. 1. Effect of adult diet nested within larval diet on body mass and water content of *Ceratitis cosyra*. Body mass (A) and water content (B) in flies from larvae fed a high yeast or low yeast diet. Flies receiving no adult diet were tested on the day of adult emergence from the puparium (none), whereas the two other adult diet treatments comprised flies given unrestricted access to sugar only (sugar) or sugar and yeast hydrolysate (sugar+YH), plus water, for 10 days following emergence. Mean water content was predicted from a generalised linear model with normal distribution and identity link, and initial mass at its mean (8.56 mg). For each combination of larval diet, adult diet and sex, n=10. Error bars indicate ±1 s.e.

effect of sex nested within adult diet had no significant effect on water content (GLZ: Wald χ^2=3.40, d.f.=3, P=0.335).

Desiccation and starvation resistance

Larval diet had a significant effect on mortality risk (Cox PHR: Wald χ^2=37.24, d.f.=1, P<0.001), as did adult diet nested within larval diet (Cox PHR: Wald χ^2=21.26, d.f.=2, P<0.001), and stress nested within adult diet (Cox PHR: Wald χ^2=109.19, d.f.=5, P<0.001). Adult *C. cosyra* mortality risk during desiccation and starvation resistance assays was significantly greater when flies had been reared on a high yeast larval diet (Fig. 2). Mortality risk during desiccation assays was 1.5–2.1 times greater (Fig. 2A,B) than during starvation resistance assays (Fig. 2C,D). In both assays, there was a highly significant decrease in mortality risk of newly emerged flies that had been reared on a low yeast larval diet when compared with those reared on a low yeast larval diet and fed as adults (Fig. 2B,D). Every unit increase in initial mass significantly decreased the likelihood of mortality (Cox PHR: Wald χ^2=19.00, d.f.=1, P<0.001;

Journal of Experimental Biology (2019) 222, jeb192534. doi:10.1242/jeb.192534

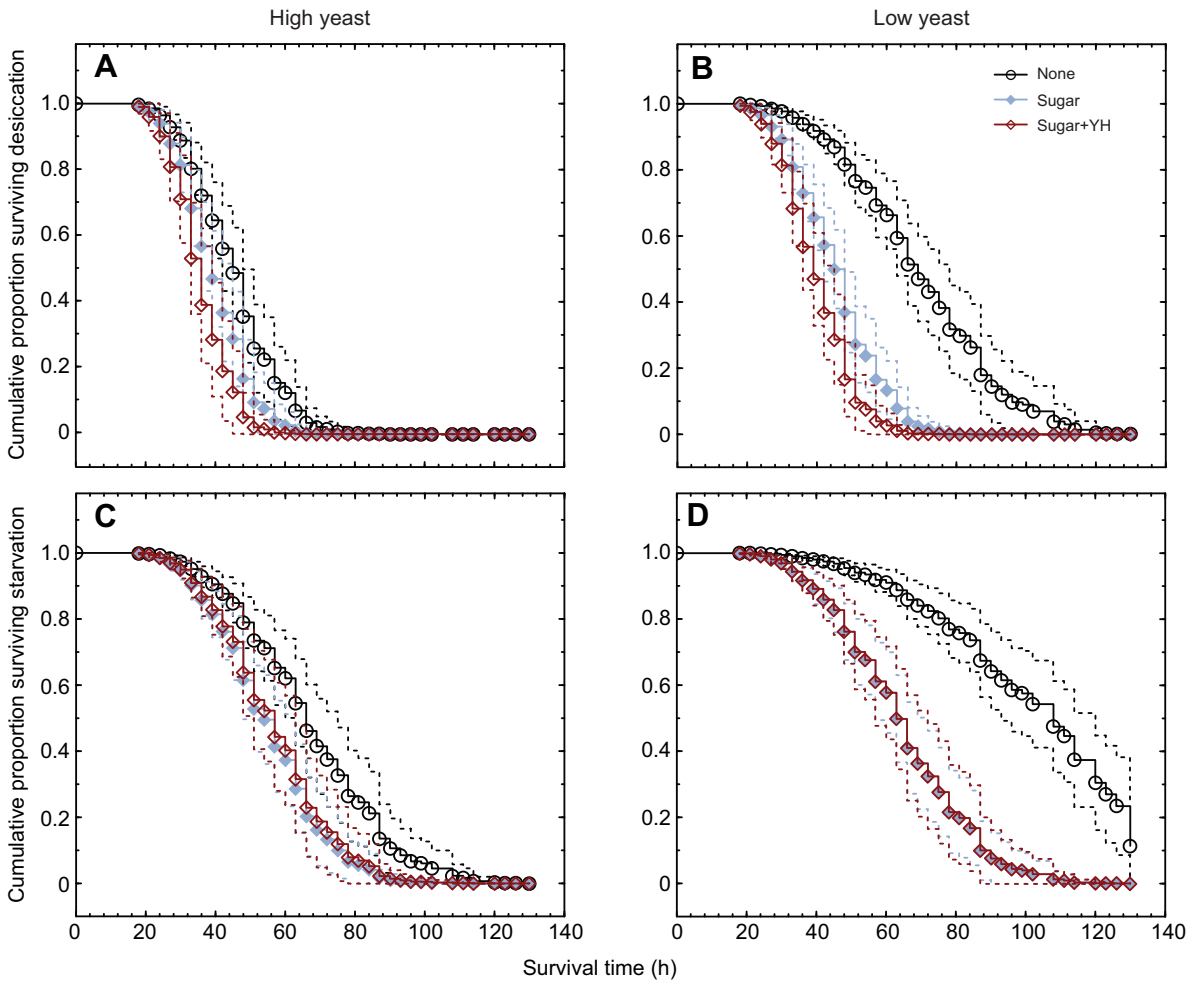

Fig. 2. Modeled survival functions for adult *C. cosyra* during desiccation and starvation resistance assays in relation to larval diet and adult diet. Survival during desiccation when reared on a high yeast (A) and low yeast (B) larval diet and survival during starvation when reared on a high yeast (C) and low yeast (D) larval diet. Adult diets as described in Fig. 1. Survival functions were estimated from a Cox proportional hazards model with initial mass held at its mean (8.56 mg). Dotted lines represent the upper and lower 95% confidence intervals for each survival function. Model plotted based on n=15 for each combination of larval diet, adult diet, sex and stress.

parameter estimate: −0.232±0.053; hazard ratio=0.793). Mortality risk of adults fed sugar or sugar+YH did not differ, irrespective of larval diet history. The effect of sex nested within stress did not significantly affect mortality risk (Cox PHR: Wald χ^2=1.31, d.f.=2, P=0.519).

Dehydration tolerance

Water content at death, a measure of dehydration tolerance (with higher water at death interpreted as lower dehydration tolerance), increased with predicted initial water content (GLZ: Wald χ^2=183.17, d.f.=1, P<0.001; parameter estimate: 0.134±0.010). Conversely, water content at death decreased slightly but significantly as fly longevity increased during stress resistance assays (GLZ: Wald χ^2=25.68, d.f.=1, P<0.001; parameter estimate: −0.002±0.000). Larval diet did not have a significant effect on water content at death (GLZ: Wald χ^2=0.83, d.f.=1, P=0.363). However, adult diet nested within larval diet did have a significant effect on water content at death (GLZ: Wald χ^2=29.56, d.f.=4, P<0.001), with newly emerged, unfed *C. cosyra* having significantly lower water content at death than those fed as adults (Fig. 3A). Within each adult diet, desiccated flies had lower water content at death than those that were starved (Fig. 3B; GLZ: Wald χ^2=125.78, d.f.=3, P<0.001). Females had significantly higher body water content at death than males following both the desiccation (female: 3.29±0.01 mg; male: 3.00±0.01 mg) and starvation resistance assays (female: 4.08±0.01 mg; male: 3.78±0.02 mg) (GLZ: Wald χ^2=183.17, d.f.=1, P<0.001).

Whole-insect nutrient reserves

Adult dry mass was significantly reduced when flies were reared on a low yeast larval diet (high yeast: 2.21±0.01 mg; low yeast: 2.17±0.01 mg; GLZ: Wald χ^2=21.00, d.f.=1, P<0.001), but this was largely due to adults fed for 10 days with sugar or sugar+YH being significantly lighter than newly emerged, unfed adults (Fig. 4A; GLZ: Wald χ^2=38.364, d.f.=4, P<0.001). When fed an adult diet of only sugar, dry mass at death did not differ among *C. cosyra* after desiccation or starvation, but when fed an adult diet of sugar+YH, dry mass of desiccated flies was significantly higher than those that had been starved (Fig. 4B; GLZ: Wald χ^2=61.29, d.f.=6, P<0.001). There was also an interaction of sex and initial mass within stress (GLZ: Wald χ^2=1530.74, d.f.=6, P<0.001). Female dry mass was greater than that of males (Fig. S1), but the relationship between initial mass and dry mass in unstressed flies had a steeper gradient (parameter estimate: female=0.106±0.006, male=0.106±0.007)

Journal of Experimental Biology (2019) 222, jeb192534. doi:10.1242/jeb.192534

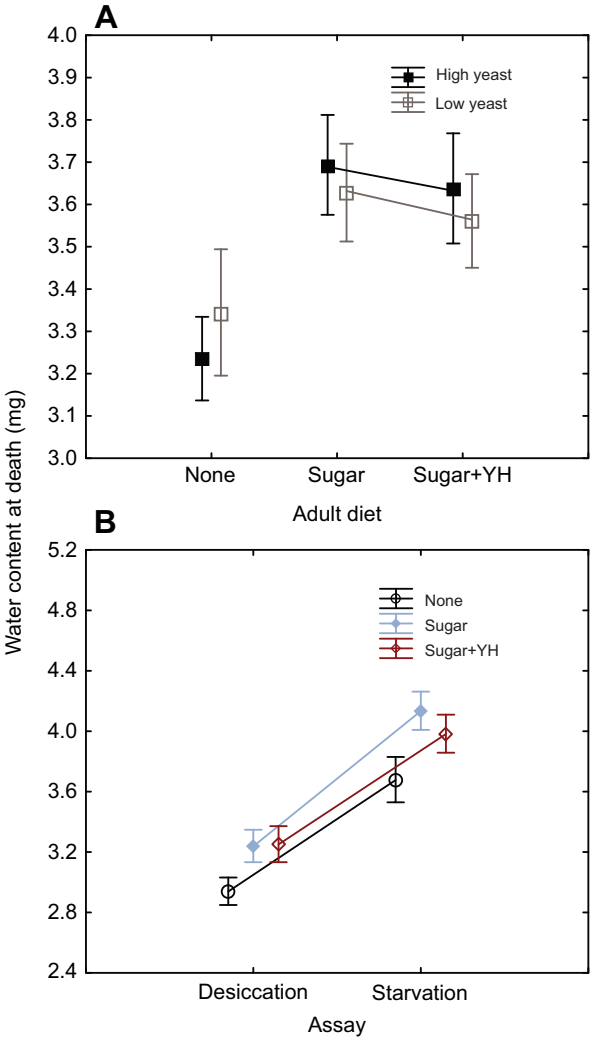

Fig. 3. Effect of adult diet or stress on water remaining at death in adult *C. cosyra*. (A) Water content in flies from larvae fed a high yeast or low yeast diet, which were then fed different adult diets. (B) Water content in flies fed different adult diets and exposed to either starvation or desiccation stress assays. Flies receiving no stress represent the condition of flies before being desiccated or starved to death. Adult diets as described in Fig. 1. Mean water content at death was predicted from a generalised linear model with Gaussian distribution and log link, and longevity (57.50 h) and estimated initial water content (5.76 mg) at their mean. For each combination of larval diet, adult diet, sex and stress, n=15. Error bars indicate ±1 s.e.

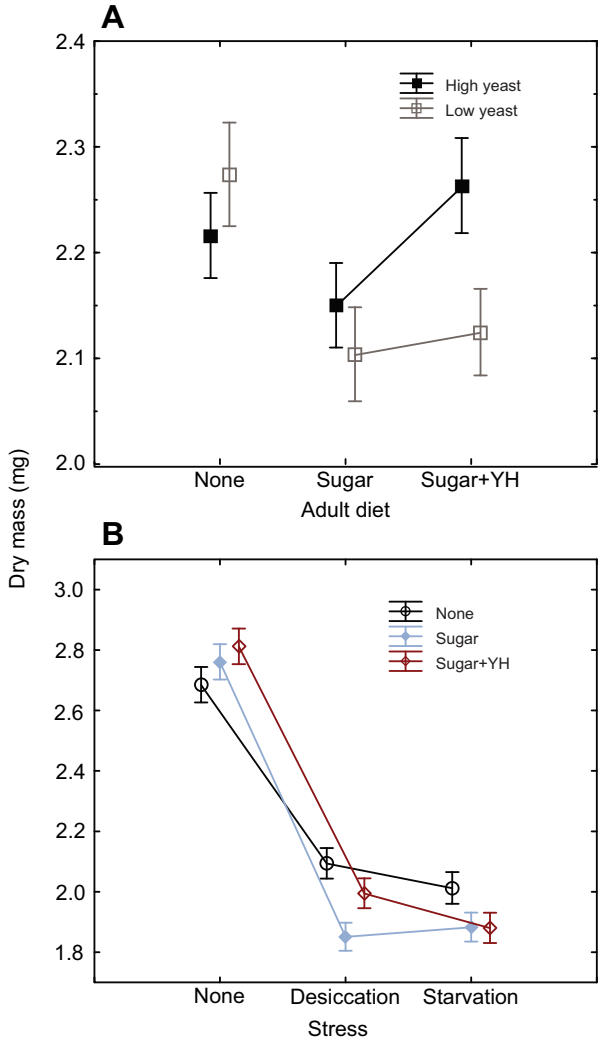

Fig. 4. Effect of adult diet or stress on dry mass of adult *C. cosyra*. (A) Dry mass of flies from larvae fed a high yeast or low yeast diet, which were then fed different adult diets. (B) Dry mass of flies fed different adult diets and then exposed to either starvation or desiccation stress assays. Adult diets as described in Fig. 1. Flies receiving no stress represent the condition of flies before being desiccated or starved to death. Mean dry mass is predicted from a generalised linear model with Gaussian distribution and log link, and initial wet body mass at its mean (8.56 mg). For each combination of larval diet, adult diet, sex and stress, n=15. Error bars indicate ±1 s.e.

than those that had been desiccated (parameter estimate: female=0.092±0.006, male=0.082±0.007) or starved to death (parameter estimate: female=0.104±0.006, male=0.096±0.007).

Water-soluble carbohydrate content of *C. cosyra* was not significantly affected by larval diet (GLZ: Wald χ^2=0.32, d.f.=1, P=0.573), or adult diet nested within larval diet (GLZ: Wald χ^2=6.64, d.f.=4, P=0.156). Within adult diet treatment, stress had a significant effect on carbohydrate content per unit dry mass (GLZ: Wald χ^2=176.27, d.f.=6, P<0.001). Initial carbohydrate content of newly emerged, unfed adult *C. cosyra* did not differ from carbohydrate content after desiccation or starvation to death (Fig. 5A). In contrast, initial carbohydrate content of flies fed sugar or sugar+YH for 10 days after emergence was approximately double the levels remaining after desiccation and starvation. Regardless of the adult diet, there was no difference in the carbohydrate remaining per unit dry mass after desiccation or starvation. The effect of sex nested within stress had no effect on water-soluble carbohydrate content (GLZ: Wald χ^2=3.93, d.f.=3, P=0.269).

Total glycogen content of *C. cosyra* per unit dry mass was not significantly affected by larval diet (GLZ: Wald χ^2=0.01, d.f.=1, P=0.926) or adult diet nested within larval diet (GLZ: Wald χ^2=8.10, d.f.=4, P=0.088). Total glycogen content was significantly affected by stress nested within adult diet (GLZ: Wald χ^2=36.37, d.f.=5, P<0.001). Newly emerged, unfed *C. cosyra* had very low glycogen content, even before being desiccated or starved to death (Fig. 5B). No glycogen was detected in newly emerged flies that were starved to death. A similar result was found for those desiccated to death, with the exception of a single individual (total glycogen: 1.58 µg; 0.85 µg mg^{-1} dry mass). Unstressed flies fed sugar for 10 days after emergence had a glycogen content not significantly different from that of flies starved to death, but

Journal of Experimental Biology (2019) 222, jeb192534. doi:10.1242/jeb.192534

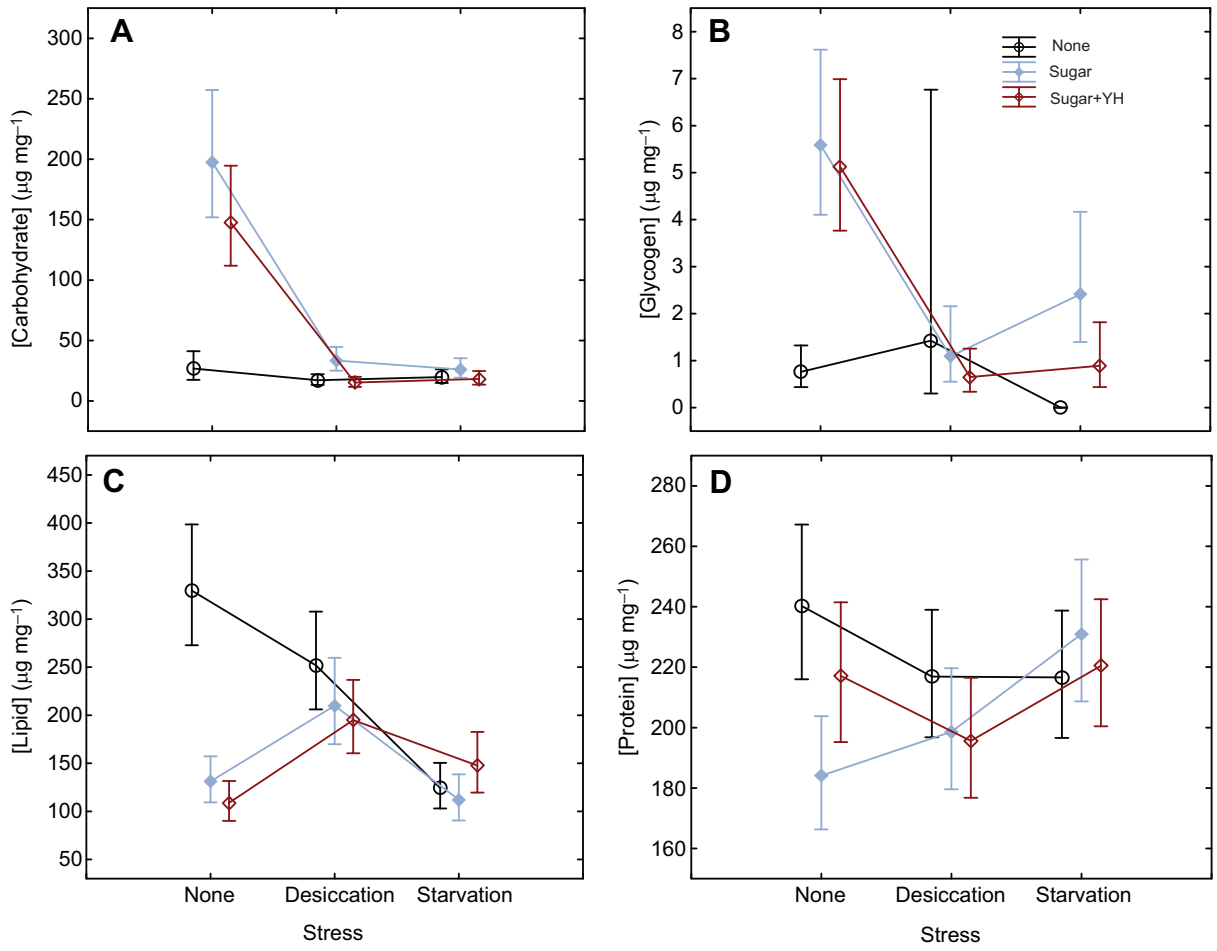

Fig. 5. Effect of adult diet and stress on carbohydrate, glycogen content, lipid and protein content of adult *C. cosyra*. Concentration of each water-soluble carbohydrate (A), total glycogen (B), total lipid (C) and soluble protein (D) is expressed per unit dry mass. Adult diets as described in Fig. 1. Flies receiving no stress represent the condition of flies before being desiccated or starved to death. For each combination of larval diet, adult diet, and stress, n=6–18. Means are predicted from generalized linear models with gamma distribution and log link function. Error bars indicate ±1 s.e.

glycogen content of desiccated flies fed the same adult diet were significantly lower than those that were starved. When fed sugar+YH for 10 days after adult emergence, unstressed *C. cosyra* had significantly higher initial glycogen content than those that were desiccated or starved to death. Sex nested within stress significantly affected glycogen content (GLZ: Wald χ^2=9.41, d.f.=3, P=0.024). Females had significantly higher glycogen content than males after desiccation (female=2.14±0.38 µg mg^{-1}, male=0.46±0.41 µg mg^{-1}), but there was no difference in glycogen content between the sexes before stress (female=2.47±0.15 µg mg^{-1}, male=3.16±0.17 µg mg^{-1}) or after being starved to death (female=0.90±0.37 µg mg^{-1}, male=1.26±0.37 µg mg^{-1}).

There was no significant effect of larval diet (GLZ: Wald χ^2=0.28, d.f.=1, P=0.597), or adult diet nested within larval diet (GLZ: Wald χ^2=36.37, d.f.=4, P=0.383) on total lipid content of *C. cosyra*. However, the effect of stress within adult diet did significantly affect total lipid content per unit dry mass (GLZ: Wald χ^2=61.809, d.f.=6, P<0.001). Among newly emerged, unfed *C. cosyra*, initial lipid content and lipid content of flies that had been desiccated to death was significantly higher than in flies starved to death (Fig. 5C). Among flies fed an adult diet of sugar only for 10 days after emergence, lipid content of those desiccated to death was significantly higher than lipid content in unstressed flies or those starved to death. The apparent elevation of total lipid content following the desiccation assay was due to lower depletion of lipids relative to total dry mass loss in these flies (Fig. 4B) in comparison with starved flies. When fed an adult diet of sugar+YH, initial lipid content was significantly higher than in flies that had been desiccated or starved to death. Total lipid content was not significantly affected by sex nested within stress (GLZ: Wald χ^2=1.01, d.f.=3, P=0.799).

Soluble protein content of adult *C. cosyra* was significantly affected by larval diet (GLZ: Wald χ^2=4.689, d.f.=1, P=0.030), with protein content per unit dry mass in adults reared on a low yeast larval diet being lower (204.77±0.02 µg mg^{-1}) than when they were reared on a high yeast larval diet (221.03±0.02 µg mg^{-1}). The effect of adult diet nested within larval diet did not have a significant effect on soluble protein content (GLZ: Wald χ^2=2.01, d.f.=4, P=0.734). Stress nested within adult diet had a significant effect on soluble protein content (GLZ: Wald χ^2=15.30, d.f.=6, P=0.018). Among newly emerged *C. cosyra* that were not fed as adults, there was no difference in protein content before or after desiccation or starvation to death (Fig. 5D). Similarly, stress treatment did not affect protein content among flies fed for 10 days on sugar+YH after emergence. Among adults fed only sugar for 10 days, initial protein content per unit dry mass was significantly lower than in flies starved to death, with desiccated flies intermediate between those treatments. Sex nested within stress had a significant effect on soluble protein

content (GLZ: Wald χ^2=20.47, d.f.=3, P<0.001). Among unstressed flies, there was no difference in protein content per unit dry mass of females (206.75±0.04 µg mg^{-1}) and males (218.52±0.04 µg mg^{-1}). However, after desiccation, female protein content per unit dry mass (187.07±0.04 µg mg^{-1}) was less than in males (221.37±0.04 µg mg^{-1}), and the same pattern between the sexes was found after starvation (female: 201.84±0.04 µg mg^{-1}; male: 245.51±0.04 µg mg^{-1}).

DISCUSSION

There was no evidence for compensation by the adult diet for poor stress resistance resulting from the larval diet. Regardless of adult diet over a period of 10 days following emergence, *C. cosyra* was at greater risk of desiccation and starvation than newly emerged adults. Other studies on dietary compensation across arthropod life stages have focused on its effect on fitness traits such as body size, fecundity, egg size and longevity (Aluja et al., 2008, 2009; Bauerfeind and Fischer, 2009; Kleinteich et al., 2015; Michaud and Jyoti, 2007). With the exception of the bridge spider (*Larinioides sclopetarius*) (Kleinteich et al., 2015), juvenile deprivation of food or feeding on a less nutritious diet led to reduced juvenile survival, slower development and lower adult body mass. This was also noted in our study among *C. cosyra* fed on the low yeast larval diet. In the case of the butterfly *B. anynana*, subsequent adult feeding on nutritious diets led to improved female fitness (Bauerfeind and Fischer, 2009; Geister et al., 2008), whereas the improved number of egg clutches and fecundity of the lady beetle *Coleomegilla maculate*, depended on a switch between the source of protein consumed between the larval and adult life stage (Michaud and Jyoti, 2007). Male *A. ludens* fed a high quality adult diet mated sooner, secured more copulations, had shorter copulations and inhibited female re-mating for longer than males receiving a poor adult diet, regardless of reduced size resulting from a poor larval diet (Aluja et al., 2009).

While effects on fitness traits have been examined, there have been no explicit tests for the potential compensatory effects of adult diet on insect environmental tolerance traits. When insects have been reared on a constant larval diet but experienced differences in their adult diet, the effect on environmental tolerance traits is equivocal. Following a short starvation treatment as adults, *D. melanogaster* were more desiccation and starvation resistant (Bubliy et al., 2012). However, starvation-acclimated *D. melanogaster* also suffered reduced survival. In *C. capitata*, a starvation pre-treatment had no significant effect on chill-coma recovery time or heat knockdown time (Mitchell et al., 2017). In contrast, in the same species and in the Natal fly (*Ceratitis rosa*), a short starvation treatment significantly improved the critical thermal maximum and minimum (Nyamukondiwa and Terblanche, 2009).

Development on a low yeast larval diet rather than a high yeast larval diet led to improved desiccation and starvation resistance in newly emerged adult *C. cosyra*. This was despite development on a high yeast larval diet leading to higher body water content on adult emergence. Similar results have been found in *D. melanogaster*, where adults reared from a ‘field diet’ of decomposing apples rather than a standard diet used in the laboratory were more resistant to starvation, while also being smaller and leaner (Kristensen et al., 2016). In addition, rearing on a standard laboratory diet led to higher adult tolerance of high temperatures in comparison with *D. melanogaster* reared on the field diet (Kristensen et al., 2016). When reared on a protein-enriched larval diet, adult *D. melanogaster* exhibit higher heat and desiccation tolerance, but impaired recovery from chill-coma, in comparison with those fed a carbohydrate-enriched diet (Andersen et al., 2010). The same patterns were found in *Drosophila ananassae* (Sisodia and Singh, 2012). These results are surprising because initial body mass is positively associated with water content and survival of water stress in tephritid flies in this and other studies (Tejeda et al., 2014; Weldon et al., 2016, 2013). A higher pool of standing water should provide a protection from dehydration. However, in addition to differences in desiccation resistance in *C. cosyra*, water content at death was very similar in flies reared on high and low yeast larval diets. This suggests that water loss rates were higher in flies reared on the high yeast diet. These results point to the possibility of a stressful larval environment leading to correlated improvement in adult performance, at least in the short term. This ‘acclimation’ or ‘hardening’ could be associated with increased lipid reserves (discussed in more detail below) or reduced metabolic rate (Bubliy et al., 2012). Investigation of the mechanisms leading to this cross-life stage hardening or acclimation in stress tolerance will be a fascinating area for future research.

Decreased desiccation resistance in older *C. cosyra* corresponded to a decrease in dehydration tolerance at an age of 10 days, as indicated by higher water content at death following both desiccation and starvation resistance assays. This result aligns with previous studies showing that desiccation resistance declines with age in another tephritid, the Queensland fruit fly (*Bactrocera tryoni*) (Weldon and Taylor, 2010). The causes underlying this observation still require further study but several plausible explanations can be posited. It may be that older tephritids exhibit lower desiccation resistance due to higher rates of water loss as a result of elevated metabolic rate to sustain foraging, sexual development, mating behaviour and reproduction. It has been reported in *C. capitata* that transcription of genes associated with growth, development and energy metabolism is elevated in young females and males (Gomulski et al., 2012). Similarly, in *B. dorsalis*, starch, sucrose and galactose metabolism pathways, and D-glucose levels (obtained by conversion from more complex carbohydrates) were associated with improved male competitive abilities during mating (Cheng et al., 2014). Reduced desiccation resistance in older tephritids may be caused by changes in the water-proofing properties of the cuticle (Gibbs, 1998) during the adult life stage. This could occur due to abrasion and damage to the cuticle as flies age (Johnson, 2000; Johnson and Gibbs, 2004). Alternatively, the composition of cuticular lipids may change as flies mature. Flexibility in the composition of cuticular hydrocarbons within the adult life stage has been found in *D. melanogaster*, where rapid desiccation hardening led to a reduced proportion of unsaturated and methylated hydrocarbons relative to controls (Stinziano et al., 2015). In blood-feeding insects, blood feeding leads to plasticisation of the cuticle through the breakdown of weak intermolecular bonds between proteins, and cuticular lipids increase in abundance in preparation for dry off-host conditions (Benoit and Denlinger, 2010).

There was little evidence for a role of metabolic water from lipid or glycogen stores in extending desiccation resistance of *C. cosyra*. This result is similar to that reported for *C. cosyra* by Weldon et al. (2016). However, lipid reserves do appear to play a role in starvation resistance, with high levels of lipids in newly emerged *C. cosyra* being associated with high starvation resistance early in life. Lipids are the main store of energy in insects, with lipids being converted to carbohydrates, particularly trehalose in the hemolymph and glycogen in the cells, to support energetic requirements during periods of low food intake (Arrese and Soulages, 2010). Body lipid reserves were also positively correlated with starvation resistance in four distinct populations of *Drosophila simulans* (Ballard et al., 2008). In *D. melanogaster* selected for starvation resistance, high

levels of starvation resistance relative to those from unselected lines were evident immediately after adult emergence, which suggested that metabolites permitting this phenotype were obtained during the larval stage (Chippindale et al., 1996). Subsequently, it has been shown that the contents of fat cells acquired during larval feeding permit survival after eclosion and before feeding in adult *D. melanogaster* (Aguila et al., 2013, 2007). Protein and carbohydrate content had no noticeable association with desiccation or starvation resistance. Inclusion of yeast in the larval diet and hydrolysed yeast in the adult diet of tephritids is usually linked with their need for protein (or more specifically, amino acids) during larval (Kaspi et al., 2002; Nestel et al., 2016), pupal (Nestel et al., 2004) and adult reproductive development (Taylor et al., 2013; Yuval et al., 2007). This was supported in *C. cosyra* with higher soluble protein content resulting from both the high yeast larval diet and inclusion of hydrolysed yeast in the adult diet. Development of *C. cosyra* was slower in the low yeast larval diet, so the limiting effect of fewer amino acids may have been overcome to some extent by compensatory feeding to acquire sufficient resources for successful pupal development, as in *C. capitata* (Nestel et al., 2016).

The findings of this study indicate that caution must be taken when extrapolating results from laboratory-reared insects to the field. In our study, the low yeast larval diet had a protein content similar to a preferred host fruit for *C. cosyra*, but the high yeast larval diet is the standard diet used to rear *C. cosyra* and other tephritid species in the laboratory. However, the standard diet is too rich and results in adults with lower stress resistance. Unfed flies lived longer when fed on a protein-restricted diet as larvae. Similarly, adults of a neriid fly, *Telostylinus angusticollis*, had shorter lifespans when fed protein-restricted diets as larvae (Runagall-McNaull et al., 2015). As noted earlier, similar results have also been found in *D. melanogaster* (Kristensen et al., 2016). Consequently, it is important to consider the influence of the larval diet when measuring stress resistance traits of newly emerged adults. Our results may also have implications for other species reared on high protein larval diets for sterile insect technique (SIT) programmes. The success of SIT programmes to achieve population suppression or extirpation relies on high survival rates of released insects in the potentially stressful field environment (Lance and McInnis, 2005), so the benefits of a larval diet that trades high rates of larval development for lower stress resistance need to be quantified. This will also affect the cost of fly production because yeast is the most expensive tephritid larval diet component (Nestel and Nemny-Lavy, 2008).

Conclusions

Larval diet has a short-term effect on desiccation and starvation resistance of *C. cosyra*. Adult diet does not compensate for deficiencies in desiccation or starvation resistance resulting from a larval diet suboptimal for these tolerance traits. On the contrary, over the period of time that *C. cosyra* were fed adult diets, their desiccation and starvation resistance declined irrespective of the diet offered to them. Water content plays a role in the ability of *C. cosyra* to withstand water loss, but this may be moderated by water loss rates and there is a need to investigate the pathways for water loss in this and other tephritid species. This study provided no evidence for metabolic water from stored nutrients extending survival under water stress, but showed that elevated lipid reserves likely contribute to starvation resistance.

Acknowledgements

We thank Matshidiso Hlalele for technical and research support during the experiment. The Department of Biochemistry at the University of Pretoria provided access to a freeze-drier.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: C.W.W., S.M., S.W.N.; Methodology: C.W.W., S.M., F.D., E.E.d.R., A.M., S.W.N.; Validation: C.W.W., E.E.d.R.; Formal analysis: C.W.W., F.D.; Investigation: S.M., F.D., E.E.d.R., K.M.; Resources: C.W.W., A.M., S.W.N.; Data curation: C.W.W.; Writing - original draft: C.W.W., E.E.d.R.; Writing - review & editing: C.W.W., S.M., K.M., A.M., S.W.N.; Visualization: C.W.W.; Supervision: C.W.W., S.W.N.; Project administration: C.W.W.; Funding acquisition: C.W.W., A.M., S.W.N.

Funding

This research was supported by grants from Citrus Research International (project 1075), HortGro Science, and the South African Tablegrape Industry to C.W.W. Matching funds were provided by the South African government through its Technology and Human Resources for Industry Programme (THRIP; project 1207132909). S.M. received a Scarce Skills Honours Block Grant scholarship from the South African National Research Foundation (NRF) (PGS01) and a Department of Zoology and Entomology Honours Support Bursary. F.D. and E.E.d.R. were supported by postdoctoral fellowships from the University of Pretoria. K.M. received a grant holder-linked PhD bursary associated with an NRF Competitive Support for Rated Researchers grant to C.W.W. and S.W.N. (93686).

Data availability

Data are deposited and publicly available on UPSpace, the digital repository of the University of Pretoria: http://hdl.handle.net/2263/66649.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.192534.supplemental

References

Abisgold, J. D. and Simpson, S. J. (1987). The physiology of compensation by locusts for changes in dietary protein. *J. Exp. Biol.* **129**, 329-346.
Aguila, J. R., Suszko, J., Gibbs, A. G. and Hoshizaki, D. K. (2007). The role of larval fat cells in adult *Drosophila melanogaster*. *J. Exp. Biol.* **210**, 956-963.
Aguila, J. R., Hoshikazi, D. K. and Gibbs, A. G. (2013). Contribution of larval nutrition to adult reproduction in *Drosophila melanogaster*. *J. Exp. Biol.* **216**, 399-406.
Aluja, M., Pérez-Staples, D., Sivinski, J., Sánchez, A. and Piñero, J. (2008). Effects of male condition on fitness in two tropical tephritid flies with contrasting life histories. *Anim. Behav.* **76**, 1997-2009.
Aluja, M., Rull, J., Sivinski, J., Trujillo, G. and Pérez-Staples, D. (2009). Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: Tephritidae) with contrasting life histories. *J. Insect Physiol.* **55**, 1091-1098.
Andersen, L. H., Kristensen, T. N., Loeschcke, V., Toft, S. and Mayntz, D. (2010). Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *J. Insect Physiol.* **56**, 336-340.
Arrese, E. L. and Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annu. Rev. Entomol.* **55**, 207-225.
Ballard, J. W. O., Melvin, R. G. and Simpson, S. J. (2008). Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. *J. Insect Physiol.* **54**, 1371-1376.
Bauerfeind, S. S. and Fischer, K. (2009). Effects of larval starvation and adult diet-derived amino acids on reproduction in a fruit-feeding butterfly. *Entomol. Exp. Appl.* **130**, 229-237.
Benoit, J. B. and Denlinger, D. L. (2010). Meeting the challenges of on-host and off-host water balance in blood-feeding arthropods. *J. Insect Physiol.* **56**, 1366-1376.
Benoit, J. B., Lopez-Martinez, G., Phillips, Z. P., Patrick, K. R. and Denlinger, D. L. (2010). Heat shock proteins contribute to mosquito dehydration tolerance. *J. Insect Physiol.* **56**, 151-156.
Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Funct. Ecol.* **23**, 27-37.
Boggs, C. L. and Niitepõld, K. (2016). Effects of larval dietary restriction on adult morphology, with implications for flight and life history. *Entomol. Exp. Appl.* **159**, 189-196.
Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Chem.* **72**, 248-254.
Bubliy, O. A., Kristensen, T. N., Kellermann, V. and Loeschcke, V. (2012). Plastic responses to four environmental stresses and cross-resistance in a laboratory population of *Drosophila melanogaster*. *Funct. Ecol.* **26**, 245-253.
Chen, E.-H., Hou, Q.-L., Wei, D.-D., Jiang, H.-B. and Wang, J.-J. (2017). Phenotypic plasticity, trade-offs and gene expression changes accompanying

dietary restriction and switches in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Sci. Rep.* **7**, 1988.
Cheng, D., Chen, L., Yi, C., Liang, G. and Xu, Y. (2014). Association between changes in reproductive activity and D-glucose metabolism in the tephritid fruit fly, *Bactrocera dorsalis* (Hendel). *Sci. Rep.* **4**, 7489.
Chippindale, A. K., Chu, T. J. F. and Rose, M. R. (1996). Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* **50**, 753-766.
Coggan, N., Clissold, F. J. and Simpson, S. J. (2011). Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. *Proc. R. Soc. B* **278**, 2745-2752.
Djawdan, M., Rose, M. R. and Bradley, T. J. (1997). Does selection for stress resistance lower metabolic rate? *Ecology* **78**, 828-837.
Djawdan, M., Chippindale, A. K., Rose, M. R. and Bradley, T. J. (1998). Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol. Zool.* **71**, 584-594.
Fanson, B. G., Weldon, C. W., Pérez-Staples, D., Simpson, S. J. and Taylor, P. W. (2009). Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell* **8**, 514-523.
Foray, V., Pelisson, P.-F., Bel-Venner, M.-C., Desouhant, E., Venner, S., Menu, F., Giron, D. and Rey, B. (2012). A handbook for uncovering the complete energetic budget in insects: the van Handel's method (1985) revisited. *Physiol. Entomol.* **37**, 295-302.
Gefen, E., Marlon, A. J. and Gibbs, A. G. (2006). Selection for desiccation resistance in adult *Drosophila melanogaster* affects larval development and metabolite accumulation. *J. Exp. Biol.* **209**, 3293-3300.
Geister, T. L., Lorenz, M. W., Hoffmann, K. H. and Fischer, K. (2008). Adult nutrition and butterfly fitness: effects of diet quality on reproductive output, egg composition, and egg hatching success. *Front. Zool.* **5**, 10.
Gibbs, A. G. (1998). Water-proofing properties of cuticular lipids. *Am. Zool.* **38**, 471-482.
Gibbs, A. G., Chippindale, A. K. and Rose, M. R. (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **200**, 1821-1832.
Gomulski, L. M., Dimopoulos, G., Xi, Z., Scolari, F., Gabrieli, P., Siciliano, P., Clarke, A. R., Malacrida, A. R. and Gasperi, G. (2012). Transcriptome profiling of sexual maturation and mating in the Mediterranean fruit fly, *Ceratitis capitata*. *PLoS ONE* **7**, e30857.
Hayward, S. A. L., Rinehart, J. P. and Denlinger, D. L. (2004). Desiccation and rehydration elicit distinct heat shock protein transcript responses in flesh fly pupae. *J. Exp. Biol.* **207**, 963-971.
Johnson, R. A. (2000). Water loss in desert ants: caste variation and the effect of cuticle abrasion. *Physiol. Entomol.* **25**, 48-53.
Johnson, R. A. and Gibbs, A. G. (2004). Effect of mating stage on water balance, cuticular hydrocarbons and metabolism in the desert harvester ant, *Pogonomyrmex barbatus*. *J. Insect Physiol.* **50**, 943-953.
Kansci, G., Koubala, B. B. and Mbome, I. L. (2008). Biochemical and physicochemical properties of four mango varieties and some quality characteristics of their jams. *J. Food Process. Preserv.* **32**, 644-655.
Kaspi, R., Mossinson, S., Drezner, T., Kamensky, B. and Yuval, B. (2002). Effects of larval diet on development rates and reproductive maturation of male and female Mediterranean fruit flies. *Physiol. Entomol.* **27**, e2606.
Kleinteich, A., Wilder, S. M. and Schneider, J. M. (2015). Contributions of juvenile and adult diet to the lifetime reproductive success and lifespan of a spider. *Oikos* **124**, 130-138.
Kleynhans, E. and Terblanche, J. S. (2009). The evolution of water balance in *Glossina* (Diptera: Glossinidae): correlations with climate. *Biol. Lett.* **5**, 93-96.
Kristensen, T. N., Henningsen, A. K., Aastrup, C., Bech-Hansen, M., Bjerre, L. B., Carlsen, B., Hagstrup, M., Jensen, S. G., Karlsen, P., Kristensen, L. et al. (2016). Fitness components of *Drosophila melanogaster* developed on a standard laboratory diet or a typical natural food source. *Insect Sci.* **23**, 771-779.
Lance, D. R. and McInnis, D. O. (2005). Biological basis of the sterile insect technique. In *Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management* (ed. V. A. Dyck, J. Hendrichs and A. S. Robinson), pp. 69-94. Dordrecht, Netherlands: Springer.
Le Bourg, E. (2009). Hormesis, aging and longevity. *Biochim. Biophys. Acta* **1790**, 1030-1039.
Lee, K. P. and Jang, T. (2014). Exploring the nutritional basis of starvation resistance in *Drosophila melanogaster*. *Funct. Ecol.* **28**, 1144-1155.
Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessman, J., Clissold, F., Raubenheimer, D., Bonduriansky, R. and Brooks, R. C. (2008). Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr. Biol.* **18**, 1062-1066.
Malod, K., Archer, C. R., Hunt, J., Nicolson, S. W. and Weldon, C. W. (2017). Effects of macronutrient intake on the lifespan and fecundity of the marula fruit fly, *Ceratitis cosyra* (Tephritidae): Extreme lifespan in a host specialist. *Ecol. Evol.* **7**, 9808-9817.
Mevi-Schütz, J. and Erhardt, A. (2003). Larval nutrition affects female nectar amino acid preference in the map butterfly (*Araschnia levana*). *Ecology* **84**, 2788-2794.
Michaud, J. P. and Jyoti, J. L. (2007). Dietary complementation across life stages in the polyphagous lady beetle *Coleomegilla maculata*. *Entomol. Exp. Appl.* **126**, 40-45.
Mitchell, K. A., Boardman, L., Clusella-Trullas, S. and Terblanche, J. S. (2017). Effects of nutrient and water restriction on thermal tolerance: a test of mechanisms and hypotheses. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **212**, 15-23.
Müller, T. and Müller, C. (2016). Adult beetles compensate for poor larval food conditions. *J. Insect Physiol.* **88**, 24-32.
Nestel, D. and Nemny-Lavy, E. (2008). Nutrient balance in medfly, *Ceratitis capitata*, larval diets affects the ability of the developing insect to incorporate lipid and protein reserves. *Entomol. Exp. Appl.* **126**, 53-60.
Nestel, D., Nemny-Lavy, E. and Chang, C. L. (2004). Lipid and protein loads in pupating larvae and emerging adults as affected by the composition of Mediterranean fruit fly (*Ceratitis capitata*) meridic larval diets. *Arch. Insect Biochem. Physiol.* **56**, 97-109.
Nestel, D., Papadopoulos, N. T., Pascacio-Villafán, C., Righini, N., Altuzar-Molina, A. R. and Aluja, M. (2016). Resource allocation and compensation during development in holometabolous insects. *J. Insect Physiol.* **95**, 78-88.
Nicolson, S. W. (1980). Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib Desert. *J. Insect Physiol.* **26**, 315-320.
Nyamukondiwa, C. and Terblanche, J. S. (2009). Thermal tolerance in adult Mediterranean and Natal fruit flies (*Ceratitis capitata* and *Ceratitis rosa*): effects of age, gender and feeding status. *J. Therm. Biol.* **34**, 406-414.
Pascacio-Villafán, C., Williams, T., Birke, A. and Aluja, M. (2016). Nutritional and non-nutritional food components modulate phenotypic variation but not physiological trade-offs in an insect. *Sci. Rep.* **6**, 29413.
Raubenheimer, D. and Simpson, S. J. (1999). Integrating nutrition: a geometrical approach. *Entomol. Exp. Appl.* **91**, 67-82.
Rosenblatt, A. E. and Schmitz, O. J. (2016). Climate change, nutrition, and bottom-up and top-down food web processes. *Trends Ecol. Evol.* **31**, 965-975.
Runagall-McNaull, A., Bonduriansky, R. and Crean, A. J. (2015). Dietary protein and lifespan across the metamorphic boundary: protein-restricted larvae develop into short-lived adults. *Sci. Rep.* **5**, 11783.
Simpson, S. J. and Raubenheimer, D. (2000). The hungry locust. *Adv. Study Anim. Behav.* **29**, 1-44.
Sisodia, S. and Singh, B. N. (2012). Experimental evidence for nutrition regulated stress resistance in *Drosophila ananassae*. *PLoS ONE* **7**, e46131.
Stinziano, J. R., Sove, R. J., Rundle, H. D. and Sinclair, B. J. (2015). Rapid desiccation hardening changes the cuticular hydrocarbon profile of *Drosophila melanogaster*. *Compar. Biochem. Physiol. A Mol. Integr. Physiol.* **180**, 38-42.
Taylor, P. W., Pérez-Staples, D., Weldon, C. W., Collins, S. R., Fanson, B. G., Yap, S. and Smallridgde, C. (2013). Post-teneral nutrition as an influence on reproductive development, sexual performance and longevity of Queensland fruit flies. *J. Appl. Entomol.* **137**, 113-125.
Tejeda, M. T., Arredondo, J., Pérez-Staples, D., Ramos-Morales, P., Liedo, P. and Díaz-Fleischer, F. (2014). Effects of size, sex and teneral resources on the resistance to hydric stress in the tephritid fruit fly *Anastrepha ludens*. *J. Insect Physiol.* **70**, 73-80.
Vayssières, J.-F., Korie, S. and Ayegnon, D. (2009). Correlation of fruit fly (Diptera Tephritidae) infestation of major mango cultivars in Borgou (Benin) with abiotic and biotic factors and assessment of damage. *Crop Prot.* **28**, 477-488.
Wehmeyer, A. S. (1966). The nutrient content of some edible wild fruits found in the Transvaal. *S. Afr. Med. J.* **40**, 1102-1104.
Weldon, C. W. and Taylor, P. W. (2010). Desiccation resistance of adult Queensland fruit flies *Bactrocera tryoni* decreases with age. *Physiol. Entomol.* **35**, 385-390.
Weldon, C. W., Yap, S. and Taylor, P. W. (2013). Desiccation resistance of wild and mass-reared *Bactrocera tryoni* (Diptera: Tephritidae). *Bull. Entomol. Res.* **103**, 690-699.
Weldon, C. W., Boardman, L., Marlin, D. and Terblanche, J. S. (2016). Physiological mechanisms of dehydration tolerance contribute to the invasion potential of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) relative to its less widely distributed congeners. *Front. Zool.* **13**, 15.
Weldon, C. W., Nyamukondiwa, C., Karsten, M., Chown, S. L. and Terblanche, J. S. (2018). Geographic variation and plasticity in climate stress resistance among southern African populations of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Sci. Rep.* **8**, 9849.
White, I. M. and Elson-Harris, M. M. (1992). *Fruit Flies of Economic Significance: their Identification and Bionomics*. Wallingford, UK: CAB International and Australian Centre for International Agricultural Research.
Yuval, B., Maor, M., Levy, K., Kaspi, R., Taylor, P. W. and Shelly, T. E. (2007). Breakfast of champions of kiss of death? Survival and sexual performance of protein-fed, sterile Mediterranean fruit flies (Diptera: Tephritidae). *Fla. Entomol.* **90**, 115-122.

Table S1. Linear regression for the relationship between body mass (mg) and body water content (mg) for cohorts of *Ceratitis cosyra* that were fed different larval (high yeast, low yeast) or adult diets (none, sugar, sugar + YH) and tested for desiccation resistance and starvation resistance. The equation for each relationship was used to estimate initial body water content from initial body mass for flies subjected to desiccation and starvation resistance, and then used to calculate dehydration tolerance.

Cohort	Equation	R^2	$F_{(1,8)}$	P-value
High yeast				
None				
Female	y = 0.979x – 0.560	0.958	183.574	<0.001
Male	y = 0.969x – 0.137	0.939	122.461	<0.001
Sugar				
Female	y = 0.935x – 0.884	0.874	55.275	<0.001
Male	y = 0.943x + 0.587	0.889	64.019	<0.001
Sugar + YH				
Female	y = 0.961x + 1.670	0.924	97.515	<0.001
Male	y = 0.763x + 1.173	0.583	11.176	0.010
Low yeast				
None				
Female	y = 0.978x – 0.711	0.956	172.481	<0.001
Male	y = 0.971x + 0.081	0.943	131.611	<0.001
Sugar				
Female	y = 0.979x – 0.600	0.959	186.434	<0.001
Male	y = 0.980x + 0.025	0.961	198.735	<0.001
Sugar + YH				
Female	y = 0.965 – 0.237	0.930	106.758	<0.001
Male	y = 0.936 + 1.049	0.877	57.038	<0.001

Journal of Experimental Biology • Supplementary information

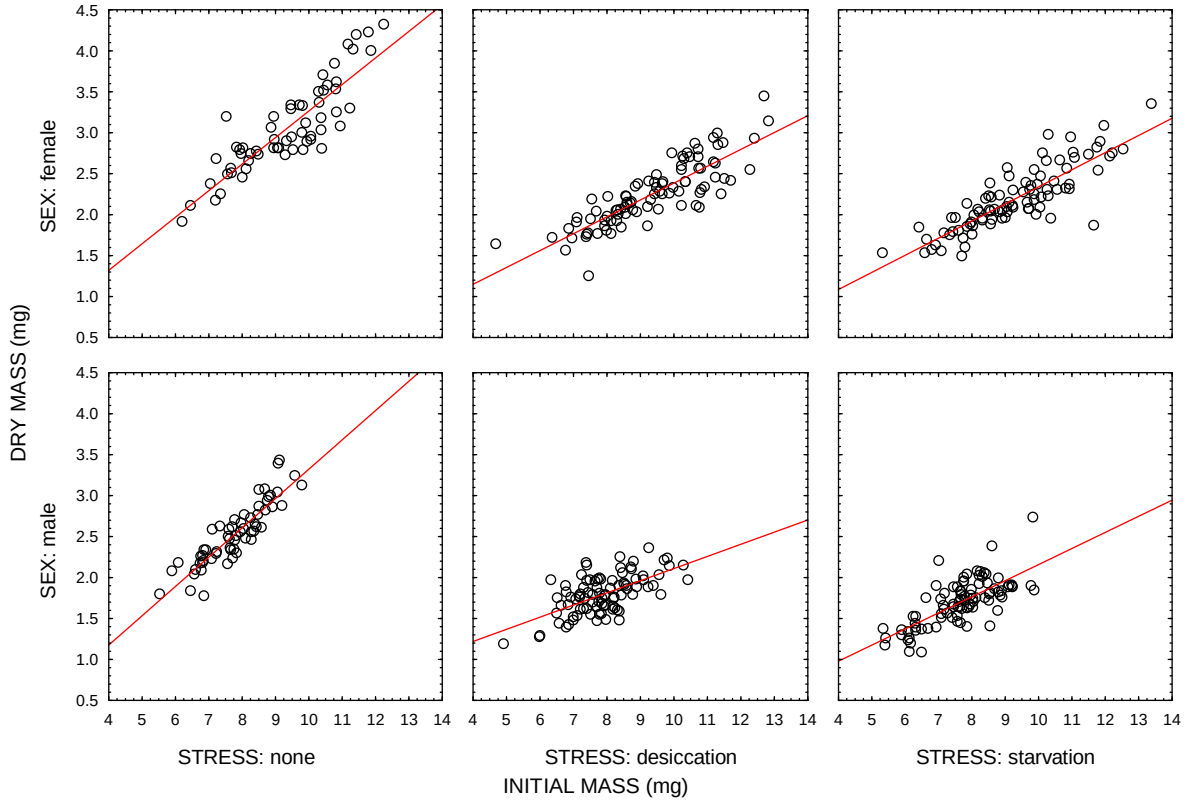

Fig. S1. Relationship between initial and dry mass for female and male *C. cosyra*. Flies receiving no stress (none) represent the condition of flies before being desiccated or starved to death. Dry mass was also determined after each stress (desiccation, starvation) led to death.

Journal of Experimental Biology • Supplementary information