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Fruit flies may face a nutrient-dependent life-history trade-off between secondary sexual trait quality, survival and developmental rate

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ABSTRACT

Optimal life-history strategies are those that best allocate finite environmental resources to competing traits. We used the geometric framework for nutrition to evaluate life-history strategies followed by *Drosophila melanogaster* by measuring the condition-dependent performance of life-history traits, including the morphology of male secondary sexual characters, sex combs. We found that depending on their rearing environment flies faced different forms of trait trade-offs and accordingly followed different life-history strategies. High-energy, high-carbohydrate, low-protein diets supported development of the largest and most symmetrical sex combs, however, consistent with handicap models of sexual selection these foods were associated with reduced fly survival and developmental rate. Expressing the highest quality sex combs may have required secondary sexual trait quality to be traded-off with developmental rate, and our results indicated that flies unable to slow development died. As larval nutritional environments are predominantly determined by female oviposition substrate choice, we tested where mated female flies laid the most eggs. Mothers chose high-energy, high-protein foods associated with rapid larval development. Mothers avoided high-carbohydrate foods associated with maximal sex comb expression, showing they may avoid producing fewer 'sexy' sons in favour of producing offspring that develop rapidly.

1. Introduction

Optimal life-history strategies are those that best allocate finite resources to competing traits, and manage potential trade-offs to maximise fitness (Calow, 1982; Stearns and Koella, 1986; Arendt, 1997; Kokko, 1998; Roff and Fairbairn, 2007; Zeller and Koella, 2016). Two strategies animals can follow to maximise their fitness are, 1) averaging available resources equally across all fitness components in all environments, or 2) by trading off the quality of one trait against another in an environment dependent manner (see Fig. 1). The second may be achieved by managing the relative expression of life-history traits by diverting resources away from traits that yield lower fitness in a given environment, to those that yield higher fitness. The life history strategy followed by animals that bear secondary sexual characters is of particular interest to evolutionary biologists because maximal expression of secondary sexual traits can come at the expense of other fitness components. For insects that bear secondary sexual traits, experimental evidence for species following either the trait averaging or the trait trade-off strategy has been found (Sentinella et al., 2013; House et al., 2016).

Secondary sexual traits encompass a diverse range of features subjected to intra- and/or inter-sexual selection (Johnstone, 1995; Berglund et al., 1996). By definition these traits are a characteristic of one sex, typically males, and have evolved due to sexually selective processes additional to those of the primary sexual characters. Such traits may influence individual access to mates for reproduction (Heisler, 1984; Prokop and Drobniak, 2016), by functioning as weapons, social signals, or quality indicators (Eberhard, 1985; Berglund et al., 1996; Polak et al., 2004; Emlen, 2008).

When secondary sexual trait expression is correlated with an individual's quality or 'condition', they can provide evidence of a prospective mate's quality (Zahavi, 1977; Andersson, 1982, 1994; Johnstone, 1995; Cotton et al., 2004; Polak, 2003; Hill, 2015; Kodric-Brown, 1989; Järvi, 1990; Jennions et al., 2001; Jones et al., 2015; Polak et al., 2016). A degree of honesty necessarily underpins the evolution and maintenance of this relationship, with high-quality traits able to be borne only by high-quality individuals (Johnstone, 1995; Berglund et al., 1996; Giery and Layman, 2015; Hill, 2015). Trait 'condition dependence' (Andersson, 1994; Emlen, 2008; Hill, 2015) can occur because the traits impose some form of cost on the bearer,

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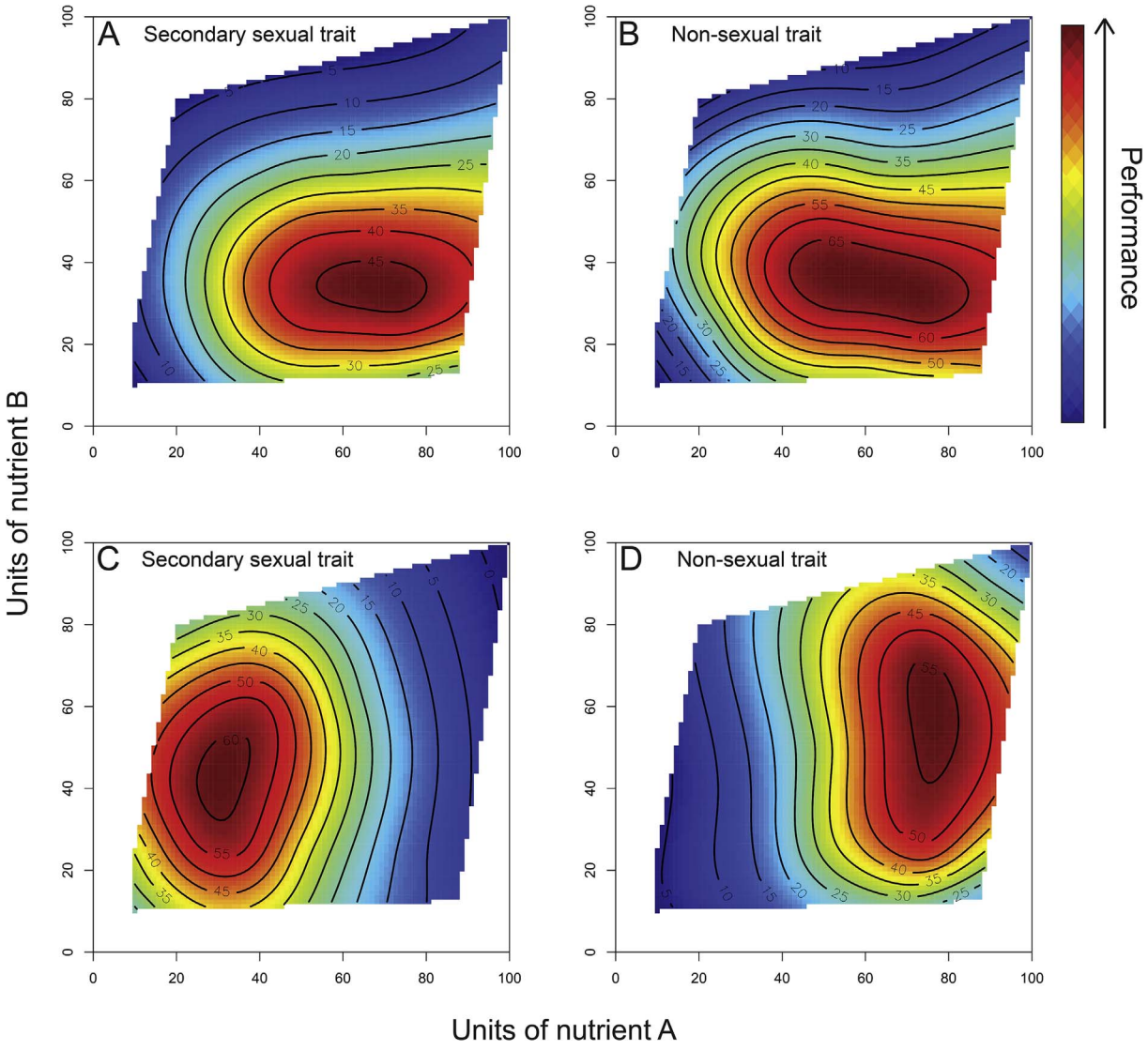

Fig. 1. Theoretical nutrient-dependent response surfaces for secondary sexual and non-sexual life-history traits. The uppermost panels depict the trait averaging life-history strategy where the performance of life-history trait A (the secondary sexual trait), and B (a non-sexual trait) are both maximised in the same region of nutrient space. For animals following the trait averaging strategy, there is no trade-off between traits A and B and the performance of both traits is similar across all nutrient-dependent conditions. There is no apparent cost incurred by other traits when the secondary sexual trait is maximally expressed. The lower panels show the trait trade-off strategy. Here secondary sexual trait quality is traded-off with non-sexual trait quality. For animals following the trait trade-off strategy, the secondary sexual trait C is optimised in a different region of nutrient space to the non-sexual trait D. Following the trade-off strategy means maximal expression of the secondary sexual trait C is associated with decreased performance of trait D and *vice versa*. For this strategy to be expressed, the overall fitness benefits of optimally expressing the secondary sexual trait C must outweigh the fitness costs associated with lowered performance of trait D.

therefore acting as 'handicaps' (Zahavi, 1975, 1977; Johnstone, 1995; Andersson and Simmons, 2006; Emlen, 2008; Roulin, 2016). In more stressful environments only inherently higher-quality individuals are able to maintain 'optimal' secondary sexual trait expression in the face of the environmental demands (Grafen, 1990; Folstad and Karter, 1992; Roulin, 2016; Kodric-Brown, 1989; Rowe and Houle, 1996; Tomkins et al., 2004; Giery and Layman, 2015).

Male *Drosophila melanogaster* have unusual chitinous, multi-bristled structures on their fore tarsi called sex combs that are used in a range of sexual behaviours. They are used to grasp female genitalia during copulation (Hurtado-Gonzales et al., 2014) and during the 'lunging' phase of intra-sexual fights (Chen et al., 2002; Hoyer et al., 2008) where they appear to be used for grabbing and ruffling the wings of other males. Sex combs may also be displayed to females during the 'tapping' phase of *D. melanogaster* courtship (Suzuki et al., 1997; Sokolowski, 2001). Sex combs exhibit remarkable diversity across *Drosophila* species (Kopp and True, 2002), evidence they are subject to diversifying sexual selection (Eberhard, 1985; Andersson, 1994; Panhuis et al., 2001) and significant sexual selection for increasing comb size has been detected in Australian populations of *D. bipectinata* (Polak et al., 2004; Polak and Simmons, 2009). There is evidence *Drosophila* sex combs are condition dependent, showing a greater size reduction and increased fluctuating asymmetry compared to non-secondary sexual traits under developmental nutritional stress (Ahuja et al., 2011), and increased developmental instability under developmental thermal stress (Polak and Tomkins, 2012, 2013).

In *D. melanogaster*, depending on the social context, the quality of sex combs influences male mating success. Males whose sex combs have been experimentally reduced in size at the distal end have a less than 5% probability of successfully mating when competing with intact males. However, when other males are absent, small-combed males express normal, high-levels of mating success (Hurtado-Gonzales et al., 2014). This indicates males with complete combs can dominate males with smaller combs, preventing access to females, and/or that females eschew males with smaller combs when larger combed males are present. Females could do this through directly rejecting males with low

quality combs based on their relative size or through decamping a male once mating has commenced (Suzuki et al., 1997). In closely related *D. silverstris*, females show preference for males with a higher number of tibial bristles (Spiess and Carson, 1981). It is therefore possible male *D. melanogaster* with low quality sex combs face reduced fighting capacity, are less attractive to mates, and/or are more readily rejected by females after mating has commenced.

We used the geometric framework (GF) for nutrition to evaluate the life-history strategies followed by *D. melanogaster* by measuring the condition-dependent performance of life-history traits, including that of the sex comb. Our goal was to identify whether *D. melanogaster* follow a trait averaging life-history strategy, or a trait trade-off strategy (Fig. 1). The GF allows for all environmental influence on condition to be reduced to nutritional variation only (e.g. as in South et al. (2011), Sentinella et al. (2013), and House et al. (2016)), and for the individual and interactive effects of multiple nutrients and other food components on multiple animal responses to be evaluated and analysed concurrently (Simpson and Raubenheimer, 2012). Here we define any measurable variation in a trait due to the nutritional environment experienced by the individual as 'condition dependence'. Traits we examined were larvae to adult developmental rate, survivorship to adult, male body size, and sex comb quality. We also incorporated a measure of inherent quality in males, developmental instability (DI). Individuals may vary in quality as determined by non-environmental factors, like their genetics. Measuring DI provides a means to analyse this variation. Finally, as a major determinant of a *Drosophila* larva's nutrition is the substrate it is laid upon as an egg (Thompson, 1988; Refsnider and Janzen, 2010), we also tested for correspondence between conditions associated with high-trait performance and the oviposition substrate choice of mother flies.

2. Materials and methods

2.1. Fly stocks

We used the long-term culture of Canton-S used by Lee et al. (2008), sourced in 2006 from the Bloomington Stock Centre. Prior to experimentation, flies were maintained with overlapping generations on a 1 protein (P):1.23 carbohydrate (C), 161.4 kcal·100 g^{-1} laboratory diet comprised of 250 g raw semolina (P = 9.8%, C = 66.2%), 175 g hydrolysed bakers yeast (P = 45%, C = 24%, Lowan Whole Foods, Glendenning, NSW, Australia), 425 g treacle (P = 0.3%, C = 99.7%, CSR, Yarraville, VIC, Australia), 24 g agar (P = 0.06%, C = 75%, A9799, Sigma-Aldrich, Castle Hill, NSW, Australia) that was mixed well and then boiled for 1 min in 2.1 L of distilled water. Following cooling, 0.5 g of methylparaben antifungal 'Nipagin' was added (H5501, Sigma-Aldrich, Castle Hill, NSW, Australia). The culture was maintained, and all experiments were conducted at 25 °C, under a 12L:12D light cycle, with lights on at 0700 h.

2.2. No choice feeding experiment

Larvae were raised from hatchling to adult on 24 diets that ranged over eight P to C ratios (P:C: 1:1, 1:2, 1:4, 1:9, 1:16, 2:1, 4:1 and 7:1), and three energy densities (75, 200, and 400 g.kg^{-1} P + C), generating a comprehensive P-C nutrient space (*sensu* Simpson and Raubenheimer, 2012) to map fly responses on (Fig. S1). Diets were made with hydrolysed bakers yeast (as above), casein (P = 73%, C = 0.03%, C7078, Sigma-Aldrich, Castle Hill, NSW, Australia), sucrose (S9378, Sigma-Aldrich, Castle Hill, NSW, Australia), and agar (as above). Simultaneous contribution of P and C by each ingredient to each diet was accounted for. Distilled water diluted the P:C mixes to appropriate energy levels and 10 mL of each were poured into separate 60 mL, 3 cm diameter plastic vials. Diet surfaces were scratched prior to introducing larvae to facilitate their feeding.

Sets of the 24 diets were maintained in 25 × 25 × 7 cm plastic containers and fitted with perforated lids. The experiment was conducted in two rounds. The first consisted of 5 replicate sets of the 24 diets, and the second, 6 replicate sets.

For each round, larvae were sourced from three replicate parent-vials containing ~100 female and male flies 5 days post-eclosion. Parent flies mated, and females oviposited in fresh vials capped with a Petri dish containing a 1.8 g agar, 50 mL distilled water and 1 g treacle oviposition medium topped with 0.5 g fresh yeast paste. The Petri dishes were replaced after 24 h. Experimental larvae were then age staged to 3 ≤ h from hatching by removing all adults and all visible hatchlings from the Petri dish after another 12 h. Following another 3 h, twelve larvae selected from across all three parent vials were introduced into each experimental diet vial. Diet vials were then capped with foam stops.

In round one, all males that emerged were collected within 24 h under light CO_2 anesthesia and transferred to 70% ethanol. Thorax length (used to quantify body size), sex comb size and sex comb fluctuating asymmetry were measured. An ocular micrometer was used to measure thorax length. This was taken as the anterior thorax edge to the scutellum distal end. Sex comb size was quantified as each male's total number of sex comb teeth. Body size corrected (BSC) sex comb size, was determined as the residuals from a linear model of sex comb size regressed over body size. The model results are: $F_{(1,\ 43)} = 95.04$, $P < .001$, adjusted $R^2 = 0.17$. Sex comb symmetry was calculated by subtracting the number of sex comb teeth on one side of the body from the number on the other. If the number of teeth differed from one side to the other, the tooth number on the side with fewer teeth was subtracted from the side with more. Individual flies were considered the replication unit.

We used sex comb fluctuating asymmetry to quantify male developmental instability (DI). Trait DI is a marker of whether physiological stress was incurred by an individual during development (Bradshaw, 1965; Møller, 1990; Palmer and Strobeck, 1986; Simmons, 1995; Polak, 2003; Polak and Tomkins, 2012) either through trade-offs arising from investment in a non-sexual trait, like growth rate (Calow, 1982; Reznick, 1983; Arendt, 1997; Robinson and Wardop, 2002), and/or because of the eco-physiological costs imposed by certain foods (Odum and Pinkerton, 1955; Raubenheimer and Simpson, 2009). Measurement error (Merilä and Björklund, 1995; Palmer and Strokeck, 2003) in sex-comb tooth number is negligible (Polak et al., 2004). Comb asymmetry data in *D. melanogaster* (Markow et al., 1996; Sharma et al., 2011) and other species (Polak et al., 2004; Polak and Taylor, 2007) do not exhibit directional asymmetry, so comb asymmetry in *Drosophila* can be assumed to be fluctuating asymmetry and therefore to reflect developmental instability (Waddington, 1957; Palmer and Strobeck, 1986). Our sex comb fluctuating asymmetry measure thus allowed us to evaluate sex comb quality and male quality.

From round two we measured survivorship to eclosion and developmental rate of male and female flies. Survivorship was the percentage of flies that successfully enclosed on each larval rearing diet from each replicate container, therefore $N = 6$ for each diet. Developmental rate was calculated as the inverse of the number of days for a fly to reach eclosion.

2.3. Individual response surface visualization and statistical analysis

To visualise the response variables, the Fields package (ver. 6.8, R ver. 3.0.2) was used to plot a thin plate spline response surfaces over P-C space. For P-C coordinate points in nutrient space which were not represented by specific experimental diets (see Fig. S1 for these points), the response variable value was interpolated during surface fitting by Fields. Generalised linear models (GLM) with Gaussian probability distributions where used to determine linear, quadratic and cross product effects of P and C on trait surfaces. A Quasi-Poisson distribution was used for the sex comb symmetry values as they were not normally distributed. Body size corrected sex comb size values had a constant of

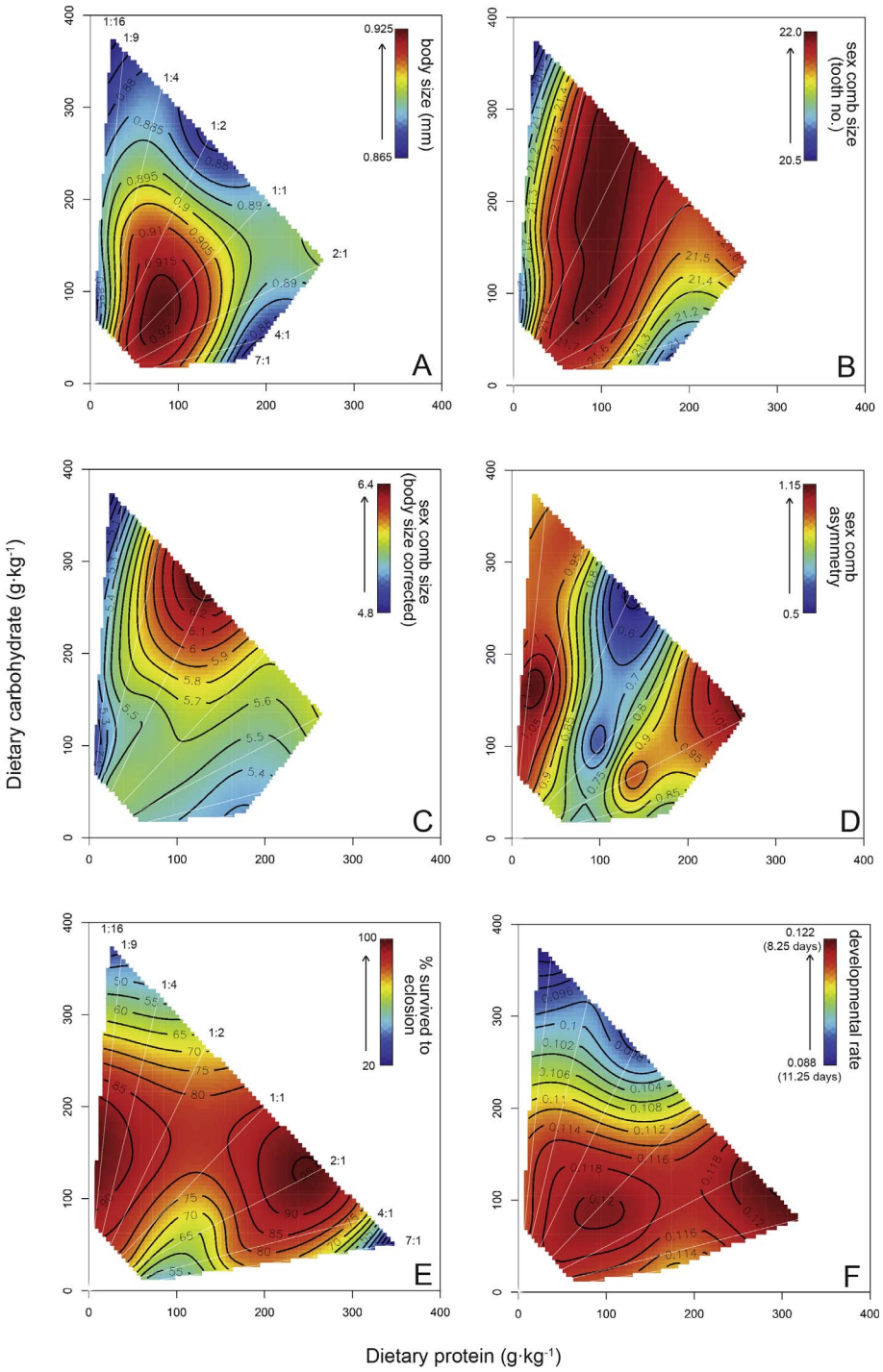

Fig. 2. Thin plate spline response surfaces for *Drosophila melanogaster* raised from hatchling to eclosion on 24 experimental diets that varied in their dietary protein to carbohydrate ratio and energy density. Response surfaces were measured for the following traits, (a) male *D. melanogaster* body size, (b) male sex comb size (measured as total tooth number), (c) male body size corrected sex comb size, (d) male sex comb symmetry, (e) *D. melanogaster* survivorship, and (f) developmental rate.

10 added so they appeared positive values on the response surface, however, raw values were analysed. (See the online Supplementary material for the *N* of each diet group represented on each response surface).

To interpret surface shape, regions of deep red indicated areas of higher response values, while deep blue indicated lower values. Description of response surface shape by GLM was interpreted as follows: positive linear macronutrient coefficients indicated linear increase in the response variable with dietary increase in the nutrient, and a negative coefficient indicated the opposite. Positive quadratic coefficients indicated 'concave' trait responses to a nutrient increase, while negative quadratic nutrient coefficient indicated a 'convex' response. Positive P × C cross product coefficients indicated that surfaces increased in elevation as covariance between P and C increased, and a negative coefficient indicated the opposite.

2.4. Statistical comparison between response surfaces

To evaluate fly life-history strategies, we used partial *F*-tests and sequential GLM to test for significant shape differences between response surfaces due to linear, quadratic and cross product effects of diet macronutrients [see Bunning et al. (2015)]. We compared: survivorship vs. developmental rate, survivorship vs. body size, developmental rate vs. body size, and sex comb symmetry vs. sex comb size (body size corrected). We then compared a combined sex comb symmetry and size surface first against the survivorship surface, and then the

developmental rate surface.

Prior to analyses, we scaled our data using the R scale function. For the sex comb symmetry surface, a fly with 'optimal' combs expressed a score of zero (i.e. symmetrical combs). To allow meaningful statistical comparisons to be made between the symmetry response surface and the other variables, the sex comb symmetry dataset was inverted. As the dataset was negatively skewed, a constant of 1 was added to all values, and the dataset was log-transformed to achieve normality prior to scaling (all above analyses were conducted in R ver. 3.0.2). See Supplementary material for *N* of each response variable under each dietary condition.

2.5. Maternal oviposition substrate choice

To determine whether mated female *D. melanogaster* lay eggs on nutritional substrates that potentially bias offspring toward expressing particular life-history strategies, mothers were given the choice between five diets of the following compositions: 1P:1C at 200 g P + C.kg^{-1}, 1P:2C at 400 g P + C.kg^{-1}, 2P:1C at 200 and 400 g P + C.kg^{-1}, and 4P:1C at 400 g P + C.kg^{-1}. These diet compositions were chosen as response surface fitting indicted these P-C space locations where at least one trait was maximised. The diets were formulated as above and 3 mL of each were poured into 1 cm-deep, 2 cm-diameter replicate dishes. One dish of each of the five diets was presented simultaneously to 10 mated female flies. Dishes and females were housed in a 30 × 25 × 8 cm plastic container, fitted a non-air-tight lid. The setup was replicated 10 times. Mothers were sourced using the above described technique for obtaining age-staged larvae and were obtained from five separate parental vials. When ≤3 h old, 100 hatchlings from each parent vial were transferred to five separate vials containing standard culture food. Adults (which were male and female) were maintained on the standard food until 1.5 days post-eclosion. Following brief CO_2 anesthesia 10 mated females (two from each parent vial) were then introduced into each of the 10 choice arenas and allowed to oviposit for 12 h. The number of eggs on each food-dish was then counted. The percentage of the total number of eggs laid during the whole experiment on each individual dish was calculated. Kruskal-Wallis testing with *post hoc* Bonferroni corrected multiple comparisons in SPSS (ver. 21) was used to identify which food mothers laid the most eggs upon.

3. Results

3.1. Individual trait responses to larval rearing diet macronutrient content

3.1.1. Body size

The response surface indicated the male *D. melanogaster* body size maximum was predicted to be located between the 1P:2C and 2P:1C diet rails where 75–110 g.kg^{-1} of P and 30–110 g.kg^{-1} of C were available in the larval diet. Larval diets offering less than 25, or more than 125 g.kg^{-1} of P and more than 200 g.kg^{-1} of C were predicted to be associated with males achieving smaller adult body sizes (Fig. 2a). This position of the body size optimum was due to male *D. melanogaster* body size being significantly influenced by a positive linear (t_{451} = 2.01, P < .05, Table 1) and a negative quadratic effect of larval dietary P on male fly body size (t_{451} = −3.02, P < .01), and C (t_{451} = −2.535, P < .05, Table 1).

3.1.2. Sex comb size – total tooth number

While sex comb size did not vary strongly across larval diets, the sex comb size surface featured a distinct ridge which commenced at lower energy 1P:1C (~75 g.kg^{-1} of P + C) diets and then extended along the upper, high-energy half of the 1P:2C diet rail. High-C or -P diets either side of this ridge were predicted to be associated with smaller combs (Fig. 2b). This visual trend was supported statistically. The positive cross product of larval dietary P and C was significantly related to an increase in sex comb size (t_{444} = 2.54, P < .05, Table 1), while the position of the ridge along the P axis was described by a significant negative quadratic term of larval dietary P (t_{444} = −2.654, P < .01, Table 1).

3.1.3. Body size corrected (BSC) sex comb size

The response surface fit indicated that the area of nutrient space around the high-energy 'tip' of the 1P:2C larval diet rail was most likely to be associated with the largest *D. melanogaster* BSC sex combs. Larval diets offering more than 175 or less than 75 g.kg^{-1} P were predicted to be associated with smaller BSC combs, with very low percentage P larval foods associated with the smallest BSC combs (Fig. 2c). Statistically, this surface pattern was driven by the simultaneous contribution of both P and C to the larval diet, as indicated by the significant, positive P × C term (t_{444} = 2.526, P < .05, Table 1).

3.1.4. Sex comb symmetry (DI)

Similar to the surface pattern for BSC sex comb size, the response surface fit predicted that high-energy 1P:2C larval rearing diets were associated with the most symmetrical sex combs. Nutrient space was dissected by a strong trough, associated with diets that supported more symmetrical combs which deepened into a region of maximal symmetry at the tip of the 1P:2C rail (Fig. 2d). The trough co-localised in nutrient space with the sex comb size surface ridge, and was deepest where the BSC sex comb size surface was highest (compare Fig. 2d–b and c). The unique shape of the sex comb symmetry surface was driven by a significant, positive quadratic effect of larval dietary protein (t_{446} = 2.019, P < .05, Table 1).

3.1.5. Survivorship

The response surface fit for survivorship indicated that highest *D. melanogaster* survivorship was likely to be associated with 80 to 200 g.kg^{-1} C diets with either a very low or very high-P content (Fig. 2e). This 'bi-modal' response is visible on the surface as two peaks where diets were predicted to support more than 90% survivorship. One peak was predicted to be at the tip of the 2P:1C diet rail and the other across the 1P:4C, 1P:9C and 1P:16C rails where diets comprised less than 20 g.kg^{-1} P. The response surface indicated there was a 'saddle' connecting these peaks that ran parallel to the P axis where diets offered between 110 and 200 g.kg^{-1} C where 80% of flies would be likely to survive (Fig. 2e). Generalised linear modeling showed this response surface shape was driven by a significant negative quadratic relationship between larval dietary carbohydrate and survivorship, indicating that the C content of larval rearing diets influenced survivorship while dietary P content did not (t_{100} = −2.832, P < .01, Table 1).

3.1.6. Developmental rate

The fit of the developmental rate surface also featured two trait peaks. The fit predicted flies would reach eclosion fastest (in 8 days) on either ~1P:1C diets offering between 75 and 125 g.kg^{-1} P and C, or 4P:1C and 2P:1C diets that offered more than 250 g.kg^{-1} P and less than 125 g.kg^{-1} C. Again a 'saddle' representing flies that reached eclosion in less than 9 days was predicted to run roughly parallel to the P axis. The response surface shape was statistically described by a significant positive P quadratic term (t_{1243} = 3.313, P < .001, Table 1). Modeling also showed the positive linear (t_{1243} = 5.975, P < .001, Table 1) and negative quadratic C terms (t_{1243} = −12.37, P < .001, Table 1) significantly influenced developmental time. Diets that contained up to 100 g.kg^{-1} C were predicted to increase fly developmental rate, while those that containing ≥200 g.kg^{-1} C were predicted to progressively slow development.

3.2. Sequential models statistically comparing trait response surfaces

3.2.1. Survivorship vs developmental rate

The survivorship and developmental rate surfaces differed due to

Table 1
Individual generalised linear models describing the linear, quadratic and cross product effects of larval dietary protein and carbohydrate on the shape of *D. melanogaster* trait response surfaces.

Trait	Linear effects		Quadratic effects		Cross product effects
	P	C	P^2	C^2	P * C
Body size					
Gradient ± SE	2.6e−04 ± 1.3e−04	1.5e−04 ± 1.2e−04	−1.5e−06 ± 5.0e−07	−7.7e−07 ± 3.0e−07	6.1e−07 ± 5.6e−07
t_{451}	2.010	1.233	−3.018	−2.535	1.091
P	< .05	=.218	< .01	< .05	=.276
Sex comb size					
Gradient ± SE	5.6e−03 ± 4.2e−03	1.5e−03 ± 3.9e−03	−4.3e−05 ± 1.6e−05	−1.4e−05 ± 9.9e−06	4.6e−05 ± 1.8e−05
t_{444}	1.315	0.390	−2.654	−1.410	2.544
P	=.19	=.697	< .01	=.159	< .05
Corrected sex comb size					
Gradient ± SE	2.1e−03 ± 3.8e−03	−2.4e−03 ± 3.8e−03	−2.5e−05 ± 1.5e−05	6.3e−08 ± 9.5e−06	4.3e−05 ± 1.7e−05
t_{444}	0.565	−0.629	−1.673	0.007	2.526
P	=.929	=.572	=.529	=.09	< .05
Sex comb symmetry					
Gradient ± SE	−4.1e−03 ± 2.5e−03	2.1e−03 ± 2.2e−03	1.8e−05 ± 9.2e−06	−4.1e−06 ± 5.7e−06	−6.4e−06 ± 1.1e−05
t_{446}	−1.654	0.958	2.019	−0.718	−0.602
P	=.659	=.099	< .05	=.473	=.547
Survivorship					
Gradient ± SE	3.9e−05 ± 7.3e−04	1.2e−03 ± 6.6e−04	6.6e−07 ± 2.7e−06	−4.36e−06 ± 1.5e−06	−1.4e−06 ± 2.8e−06
t_{100}	0.053	1.826	0.245	−2.832	−0.496
P	=.958	=.071	=.807	< .01	=.621
Developmental rate					
Gradient ± SE	−2.1e−05 ± 1.5e−05	8.4e−05 ± 1.4e−05	1.3e−07 ± 4.0e−08	−4.2e−07 ± 3.4e−08	−9.4e−08 ± 6.2e−08
t_{1243}	−1.410	5.975	3.313	−12.369	−1.532
P	=.159	< .001	< .001	< .001	=.126

Table 2
Partial *F*-test results derived from sequential generalised linear models that tested for statistical differences between trait response surfaces due to the linear, quadratic and cross product effects of larval dietary macronutrients.

Trait surfaces compared	Effects	Partial F test	
		F, SS	P
Survivorship vs. developmental rate	Linear	20.84, 1091.5	< .001
	Quadratic	7.20, 920.9	< .001
	Cross product	1.99, 918.76	=.158
Survivorship vs. body size	Linear	1.59, 580.11	=.204
	Quadratic	7.23, 531	< .001
	Cross product	0.023, 529.2	=.88
Developmental rate vs. body size	Linear	27.73, 1404.63	< .001
	Quadratic	19.13, 1259.82	< .001
	Cross product	3.24, 1257.102	=.072
Sex comb symmetry vs. sex-comb size (= A)	Linear	0.567, 886.6	=.567
	Quadratic	0.94, 879.48	=.3911
	Cross product	0.96, 871.72	=.9602
A vs. survivorship	Linear	5.69, 1021.65	< .01
	Quadratic	15.48, 978.5	< .001
	Cross product	0.098, 970.78	=.7545
A vs. developmental rate	Linear	70.88, 1845.31	< .001
	Quadratic	42.5, 1707.31	< .001
	Cross product	10.224, 1698.7	< .01

linear ($F = 20.84$, $P < .001$, Table 2) and quadratic ($F = 7.2$, $P < .001$, Table 2) macronutrient effects. The negative linear effects of P differed between the surfaces ($t_{1378} = -3.924$, $P < .001$, Table 3), as did the negative quadratic effects of dietary P and C (P^2, $t_{1378} = -3.06$, $P < .01$; C^2, $t_{1378} = -2.337$, $P < .05$, Table 3), and the linear effect of dietary C ($t_{1378} = 4.536$, $P < .001$).

3.2.2. Survivorship vs. body size

Response surface peaks in male body size and *D. melanogaster* survivorship significantly differed in shape due to macronutrient quadratic effects ($F = 7.23$, $P < .001$, Table 2). Associated GLM showed shape differences were driven by the dietary C negative quadratic term ($t_{586} = -3.476$, $P < .001$, Table 3).

3.2.3. Developmental rate vs body size

These surfaces differed due to linear ($F = 27.7$, $P < .001$, Table 2) and quadratic macronutrient effects ($F = 19.1$, $P < .001$, Table 2). The linear effects of P ($t_{1690} = 2.168$, $P < .05$, Table 3) and negative linear effects of C ($t_{1690} = -7.196$, $P < .001$, Table 3) significantly differed, as did the positive quadratic P term ($t_{1686} = 4.398$, $P < .001$, Table 3) and negative quadratic C term ($t_{1686} = -2.89$, $P < .01$, Table 3).

3.2.4. Body size corrected sex comb size vs sex comb symmetry (DI)

There were no significant differences in the linear ($F = 0.5$, $P = .567$, Table 2), quadratic ($F = 0.94$, $P = .391$, Table 2) or cross product ($F = 0.96$, $P = .960$, Table 2) dietary effects on these traits, indicating there was no trade-off between these traits.

3.2.5. Sex comb quality vs survivorship and developmental rate

We combined the statistically equivalent BSC sex comb size and the sex comb symmetry surfaces into a single 'sex comb quality' dataset and compared this to each of the *D. melanogaster* survivorship and development rate surfaces. This was to investigate trade-offs between the 'optimal' expression of sex combs and other traits.

3.2.6. Sex comb quality vs survivorship

The sex comb quality surface statistically differed to the survivorship surface due to macronutrient linear ($F = 5.7$, $P < .01$, Table 2) and quadratic effects ($F = 15.5$, $P < .001$, Table 2). The positive linear effect of C ($t_{1030} = 2.364$, $P < .05$, Table 3) differed significantly

Table 3
Results from sequential generalised linear models (GLM) used to identify statistical differences between response surface shapes due to the linear and non-linear effects of larval dietary protein and carbohydrate. These GLM results are associated with the Partial *F*-test results shown in Table 2.

Surfaces compared	Linear effects		Quadratic effects		Cross product effects
	P	C	P^2	C^2	P * C
Survivorship vs. *developmental rate*					
Gradient ± SE	−0.0032	0.0033	−2.405e−05	−1.593e−05	2.104e−05
t, df	−3.924, 1378	4.536, 1378	−3.063, 1378	−2.337, 1378	1.414, 1376
P	< .001	< .001	< .01	< .05	= .158
Survivorship vs. *body size*					
Gradient ± SE	−0.0018	−0.0006	1.327e−05	−3.142e−05	2.886e−06
t, df	−1.698, 590	−0.690, 590	1.1, 586	−3.476, 586	0.151, 584
P	= .0901	= .4902	= .2737	< .001	= .8797
Developmental rate vs. *body size*					
Gradient ± SE	0.0014	−0.004	3.732e−05	−1.549e−05	−1.815e−05
t, df	2.168, 1690	−7.196, 1690	4.398, 1686	−2.891, 1686	−1.800, 1684
P	< .05	< .001	< .001	< .01	= .072
Sex comb symmetry vs. *sex-comb size* (=*A*)					
Gradient ± SE	−0.0006	−0.0005	−4.843e−06	8.962e−06	−1.436e−05
t, df	−0.702, 886	−0.742, 886	−0.376, 882	1.135, 882	2.520, 880
P	= .483	= .459	= .707	= .256	= .3274
A vs. *survivorship*					
Gradient ± SE	0.0025	0.0020	−1.229e−06	4.716e−05	5.712e−06
t, df	2.644, 1030	2.364, 1030	−0.115, 1026	5.532, 1026	0.313, 1024
P	< .01	< .05	= .9087	< .001	= .7545
A vs. *developmental rate*					
Gradient ± SE	−0.0006	0.0054	−2.528e−05	3.123e−05	2.675e−05
t, df	−1.251, 2130	11.881, 2130	−3.810, 2126	7.046, 2126	3.198, 2124
P	= .2111	< .001	< .001	< .001	< .01

between the surfaces and the positive quadratic effect of dietary C ($t_{1026} = 5.532$, $P < .001$, Table 3) differed. The positive linear effect of P also significantly differed ($t_{1030} = 2.644$, $P < .01$, Table 3).

3.2.7. Sex comb quality vs developmental rate

The sex comb quality surface differed to the developmental rate surface due to linear ($F = 70.9$, $P < .001$), quadratic ($F = 42.5$, $P < .001$, Table 2), and cross product ($F = 10.2$, $P < .01$, Table 2) effects. The positive linear effect of C differed between the surfaces ($t_{2130} = 11.881$, $P < .001$, Table 3) as did the negative quadratic P ($t_{2126} = -3.81$, $P < .001$, Table 3) and positive quadratic C terms ($t_{2126} = 7.046$, $P < .001$, Table 3). The correlational effect of C and P also significantly differed ($t_{2124} = 3.198$, $P < .01$, Table 3).

3.3. Maternal oviposition choice

We compared maternal oviposition substrate choice among five rearing diets associated with variation in *D. melanogaster* performance. Kruskal-Wallis analysis showed that the nutritional content of the laying substrate significantly influenced oviposition choice ($\chi^2 = 32.812$, d.f. = 4, $p < .001$, Fig. 3, Table 4) with *post-hoc* Bonferroni multiple comparisons showing mother flies laid over 60% of all experimental eggs on the highest protein, highest energy food; 4P:1C at 400 g P + C.kg^{-1} (Fig. 3, Table 5).

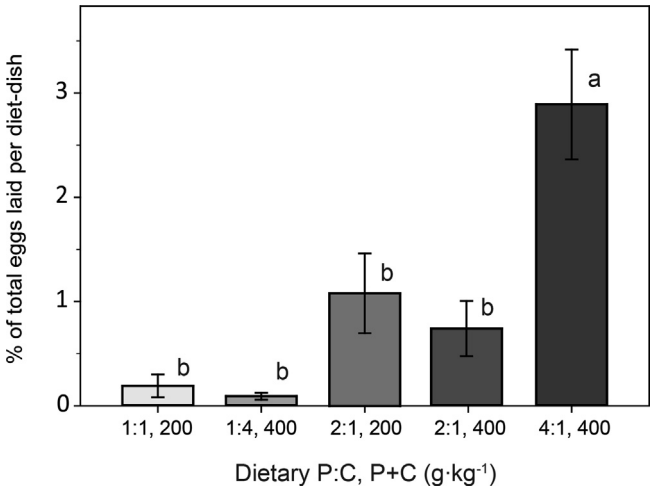

Fig. 3. Oviposition substrate selection by mated, adult female *Drosophila melanogaster*. Bars display the mean percentage (± SEM) of total eggs laid during the experiment on each food dish containing one of five experimental foods varying in P:C ratio.

4. Discussion

Our results indicate sex comb bearing *D. melanogaster* may follow the trait trade-off life-history strategy. They did not follow the trait averaging strategy (Fig. 1) where flies attempt to optimise all traits equally in every nutrient-dependent condition experienced (as followed by *Gnatocerus cornutus* broad-horned beetles (House et al., 2016)). Instead, depending on their rearing environment, flies faced different forms of trade-offs (as in *Telostylinus angusticollis* nerid flies (Sentinella et al., 2013)). All trait response surfaces we statistically compared differed in their shape except for those describing the sex comb. Therefore the nutritional-environment experienced during development (i.e. condition) dictated which traits where traded-off and the characteristics of the trade-off. Our measurement of male inherent quality (DI) also indicated that there is variation among flies in how well they can manage condition-dependent trade-offs. Here we focus the discussion on the trade-off between sex comb quality, developmental rate and survivorship and how this may influence male life history. It should be noted that our developmental rate and survivorship data include values from males and females. The patterns we have identified require verification by comparing male body size and sex comb quality data against survivorship and developmental rate data from male larvae only.

The BSC sex comb size and sex comb symmetry surfaces were

Table 4
Result from the Kruskal-Wallis test that compared the percentage of total eggs laid by mother *Drosophila melanogaster* on each dish from each experimental diet group.

Ranks		
Diet	*N*	Mean rank (% total eggs laid on each dish of each diet)
1P:1C, 200 g P + C	20	34.13
1P:2C, 400 g P + C	20	38.8
2P:1C, 200 g P + C	20	51.45
2P:1C, 400 g P + C	20	49.33
4P:1C, 400 g P + C	20	78.8
Total	100	

Test statistics	
	% total eggs laid on each dish of each diet
χ^2	32.812
df	4
Significance	$p < .001$

statistically equivalent to one another in shape (Table 2). Response surface fitting indicated high-energy diets associated with the ~1P:2C diet rail supported development of the largest and most symmetrical sex combs (Fig. 2c and d). However, consistent with handicap models (see Grafen, 1990) these high-energy, high-carbohydrate (~1P:2C) diets were associated with reduced hatch to eclosion survival, and reduced developmental rate specifically due to their high-carbohydrate content (*Survivorship, Development rate* Table 1). While these results require verification with survivorship and developmental rate data from males only, the lack of co-localisation of these performance traits indicates development of high-quality sex combs may be costly for males raised on high-carbohydrate foods.

Our measure of sex comb symmetry simultaneously quantified male DI, and provided an indicator of male inherent quality (Van Dongen, 2006). Following this theory, males with symmetrical combs have suffered less developmental instability due to their higher inherent (presumably genetic) quality. High-carbohydrate, high-energy diets (predicted by surface fitting to be ~1P:2C diets) were associated with reduced fly survivorship and developmental rate yet highest sex comb symmetry. Therefore these diets appear to disproportionately support survival of high quality flies who have successfully traded-off developmental rate with viability (i.e. they have slowed their development in order to survive). The low survivorship of individuals with high sex comb asymmetry (high-DI) raised in the high-energy ~1P:2C environment indicates that lower quality males are less able to manage the trade-off in the ~1P:2C environment than high-quality males. The trade-off between developmental rate and DI is well documented in animals (Sibly and Calow, 1984; Robinson and Wardop, 2002: Morris et al., 2012). Similar to the findings of Morris et al. (2012), our experiment indicates that there is substantial intra-specific variation in how well males manage this trade-off under environment stress. To verify this pattern we need to separately measure male and female *D. melanogaster* development and survival under diets of ~1P:2C compositions as we did not measure the sexes separately. It would be important to conduct these experiments using diets formulated from different ingredients to those used here, and/or by using recently developed holidic *D. melanogaster* foods (Piper et al., 2015). We gradually increased the proportion of casein across our diet recipes to

Table 5
Post-hoc Bonferroni multiple-comparisons for the % total eggs laid on each dish of each diet.

(I) Diet	(J) Diet	Mean difference (I-J)	Std. error	Significance (α adjusted)	95% C.I.	
					Lower	Upper
1:1, 200	1:2, 400	0.098	0.45006	> 0.999	−1.1955	1.3915
	2:1, 200	−0.8955	0.45006	0.495	−2.189	0.398
	2:1, 400	−0.551	0.45006	> 0.999	−1.8445	0.7425
	4:1, 400	−2.7	0.45006	< 0.001	−3.9935	−1.4065
1:2, 400	1:1, 200	0.098	0.45006	> 0.999	−1.3915	1.1955
	2:1, 200	−0.9935	0.45006	0.297	−2.287	0.3
	2:1, 400	−0.649	0.45006	> 0.999	−1.9425	0.6445
	4:1, 400	−2.798	0.45006	< 0.001	−4.0915	−1.5045
2:1, 200	1:1, 200	0.8955	0.45006	0.495	−0.398	2.189
	1:2, 400	0.9935	0.45006	0.297	−0.3	2.287
	2:1, 400	0.3445	0.45006	> 0.999	−0.949	1.638
	4:1, 400	−1.8045	0.45006	< 0.001	−3.098	−0.511
2:1, 400	1:1, 200	0.551	0.45006	> 0.999	−0.7425	1.8445
	1:2, 400	0.649	0.45006	> 0.999	−0.6445	0.9425
	2:1, 200	−0.3445	0.45006	> 0.999	−1.638	0.949
	4:1, 400	−0.2149	0.45006	< 0.001	−3.4425	−0.8555
4:1, 400	1:1, 200	2.7	0.45006	< 0.001	1.4065	3.9935
	1:2, 400	2.798	0.45006	< 0.001	1.5045	4.0915
	2:1, 200	1.8045	0.45006	0.001	0.511	3.098
	2:1, 400	2.1	0.45006	< 0.001	0.8555	3.4425

achieve higher P:C ratios. If casein protein is more difficult to digest than that from yeast or other high-protein foods, our current findings could be confounded.

Stress experienced by males developing on high-energy, high-carbohydrate foods (of approximate ~1P:2C composition) could be due to costs incurred from ingesting excessive carbohydrate when feeding to meet protein-dependent growth targets and/or an inability to reduce developmental rate (Arendt, 1997; Thompson et al., 2003), processes that can cause 'obesity' in insects (Simpson et al., 2002; Lee et al., 2004; Warbrick-Smith et al., 2006; Skorupa et al., 2008). As in other animals, insect obesity is associated with compromised innate immunity (Schilder and Marden, 2006). As secondary sexual trait quality is understood to reflect immune state in animals (Folstad and Karter, 1992; Roberts et al., 2004) it is possible only high-quality males can withstand the immunosuppression of heightened adiposity and large and symmetrical sex combs act as 'status badges' associated with higher relative fitness (Hansen and Rohwer, 1986). Interestingly, through food-choice behaviour, larval *D. melanogaster* may cause themselves to face the developmental rate and survival vs. sex comb quality trade-off in nature. Larvae self-select a ~1P:1.5C diet (Rodrigues et al., 2015; Lihoreau et al., 2016; de Carvalho and Mirth, 2017) suggesting the diet contributes to fitness (Simpson and Raubenheimer, 2012) such that lower-quality individuals will risk their viability. In invertebrate species, including *Drosophila*, ~1P:2C foods are associated with the high-performance of adult male reproductive traits (Rapkin et al., 2015; Bunning et al., 2015; House et al., 2016), and larval learning capacity (Lihoreau et al., 2016) which is relevant to male *D. melanogaster* as elements of male courtship are learnt (Dickson, 2008; Keleman et al., 2012). Experiments have found adult male *D. melanogaster* also self-regulate P:C intake between 1P:1.5C (Morimoto and Wigby, 2016) to 1P:2.5C (Reddiex et al., 2013), with the later P:C intake maximising male competitive fitness (Reddiex et al., 2013), and lifetime offspring production rate (Jensen et al., 2015). Working with adults rather than larvae, Morimoto and Wigby (2016) found adult males fed compositions ~1P:1.5C were highly attractive to females. This food composition was also associated with males siring more offspring than males fed either higher or lower carbohydrate foods (Morimoto and Wigby, 2016). The relationships among *D. melanogaster* larval self-selection of ~1P:2C foods and the universality of ~1P:2C foods in supporting male reproductive traits in insect species warrants further investigation.

To verify these patterns, male mating success experiments are needed. Similar to experiments by Hurtado-Gonzales et al. (2014) competitive and non-competitive mating environments should be used. To test that males with relatively larger more symmetrical sex combs are 'higher quality' the copulation success and reproductive fitness of surviving males from a range of food environments, including high-energy ~1P:2C larval diets should be measured. We predict surviving males with larger, symmetrical sex combs will out-perform those with smaller, asymmetrical sex combs. We also predict the relative increase in performance from males with smaller, asymmetrical sex combs to that of larger, symmetrical sex combed males will be greatest when these males are from ~1P:2C environments. The bases of any increased performance of males with symmetrical combs also needs confirmation. It is possible that as in *Rheumatobates* water striders, sex combs act as sexually antagonistic traits (Abderrahman et al., 2012) and sexual selection alone does not control mating success. Given that mating is costly for female *D. melanogaster* (Chapman et al., 1995) it is possible symmetrical combs could increase a male's ability to resist female decamping.

When presented five oviposition-environments, mothers laid the majority of their eggs on a high-energy, 4P:1C food. Unlike the exclusively high-quality survivors from the ~1P:2C environment (who appeared to slow their development to 'preserve' their sex comb quality), our modeling indicated that survivors of the maternally selected 4P:1C food were not disproportionately high quality individuals. Our results show that as dietary protein concentration is increased and carbohydrate decreased, flies are likely to emerge with smaller bodies and smaller, less symmetrical sex combs (i.e. with higher DI experience). By laying eggs on the 4P:1C food and not the ~1P:2C food, mothers appear to choose to generate very rapidly maturing offspring rather than promoting survival of only her 'highest quality' sons who are capable of growing the largest, most symmetrical sex combs.

Offspring that develop rapidly would benefit mothers through reducing offspring exposure to predation (Sokolowski and Turlings, 1987; Kraaijeveld and van der Wel, 1994; Arendt, 1997; Vonesh and Warkentin, 2006), or through maximising larval survival in situations subject to developmental time constraints (Johansson et al., 2001; De Block and Stoks, 2005) like the discrete pieces of rotting fruit within which larvae grow (Sokolowski, 1985; Rodrigues et al., 2015; Lihoreau et al., 2016). It should also be noted that female *D. melanogaster* are not known to discriminate between oviposition sites for female or male offspring, therefore selection of high-protein environments could reflect mothers averaging fitness costs across their male and female offspring (Uller, 2008). Rodrigues et al. (2015) and Lihoreau et al. (2016) found *D. melanogaster* mothers chose oviposition sites of much higher carbohydrate composition than we did; over 1P:8C. While we did not include a similar high-carbohydrate oviposition option in our experiment, our response surface analyses indicated that fixed-composition ~1P:8C larval foods would have supported larvae with a similar life-history traits to the high-energy 4P:1C food, albeit with higher viability. It is possible 4P:1C substrates are only utilised when high-energy high-carbohydrate foods are unavailable.

Here we measured condition-dependent performance of fly life-history traits, including those of secondary sexual characters. We have shown that flies do not average food resources evenly across fitness components, nor is there a single nutrient-dependent condition that supports the co-optimisation of all measured traits. Instead, condition which supports the maximisation of one trait, leads to reduction in the performance of another due to trade-offs (Stearns, 1992). Our inclusion of a measure of male and secondary sexual trait quality (sex comb DI) allowed us to demonstrate that even closely related flies (such as Canton S *D. melanogaster*) vary in their capacity to manage trade-offs and highlights the need for explicit description and quantification of individual genetic quality in future studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2017.11.010.

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