

The nutritional geometry of parental effects: maternal and paternal macronutrient consumption and offspring phenotype in a neriid fly

Russell Bonduriansky*, Aidan Runagall-McNaull and Angela J. Crean

Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney, NSW 2052, Australia

Summary

1. Although the ecological and evolutionary importance of environmentally induced parental effects is now widely recognized, such effects are still typically studied by contrasting just two environments in a single parental sex. Yet, parental effects should generally be viewed as reaction norms, and a more complete understanding of their ecological role therefore requires examining continuously varying and interacting environmental variables in both parental sexes.

2. We used nutritional geometry to investigate linear, nonlinear and interactive effects of protein and carbohydrate in maternal and paternal larval diets on offspring juvenile development and viability and adult body size and shape in the fly *Telostylinus angusticollis* (Diptera: Neriidae).

3. We found that egg hatching success was enhanced by protein in the maternal larval diet but reduced by protein in the paternal larval diet, while other juvenile traits were unaffected by parental diets.

4. Maternal effects on offspring adult body size and head elongation (a secondary sexual trait in males) were mediated by linear and quadratic effects of protein, and were consistent in sons and daughters. In contrast, paternal effects on offspring body size and head elongation were mediated by carbohydrate effects or carbohydrate–protein interactions, and varied by offspring sex.

5. Our findings show that macronutrients in the parental larval diet can have complex, nonlinear and interactive effects on offspring traits, and that the effects of maternal and paternal diets can be strikingly different. Effects of parental diet on offspring represent important fitness consequences of variation in nutrient intake, with potential implications for the evolution of foraging and reproductive strategies.

Key-words: carbohydrate, condition, development, developmental plasticity, diet composition, egg hatching success, maternal effect, optimal foraging, paternal effect, protein

Introduction

It is now clear that both maternal and paternal environments can affect a variety of offspring traits in both simple and complex organisms. Empirical studies show that maternal and paternal effects can contribute substantially to phenotypic variation in fitness-related traits, and play important ecological roles by allowing parents to transfer their phenotypic condition to their offspring, or to optimize the phenotype of their offspring for the anticipated

conditions in variable environments (Mousseau & Fox 1998; Qvarnström & Price 2001; Galloway & Etterson 2007; Marshall & Uller 2007; Crean & Marshall 2009; Bonduriansky, Crean & Day 2012; Crean, Dwyer & Marshall 2013; Ezard, Prizak & Hoyle 2014). Theoretical analyses suggest that, like other forms of nongenetic inheritance, parental environment effects (also called ‘transgenerational effects’) can have important consequences for both the dynamics and outcome of adaptive evolution (Lande & Kirkpatrick 1990; Wolf, Moore & Brodie 1997; Ginzburg 1998; Bonduriansky & Day 2013). There is thus a pressing need to understand how such

*Correspondence author. E-mail: r.bonduriansky@unsw.edu.au

effects are induced by environmental factors, how they are conveyed across generations, and how they influence the phenotypes of descendants.

Although numerous examples of maternal and paternal effects have been described (Roach & Wulff 1987; Mousseau & Dingle 1991; Maestriperieri & Mateo 2009; Crean & Bonduriansky 2014), the existing literature has a number of important limitations. In particular, effects of parental environment have almost always been studied by contrasting just two or three levels of an environmental variable. This approach can be appropriate for systems comprising only two or three discrete and biologically relevant environmental states (e.g. alternative hosts: see Fox, Waddell & Mousseau 1995; Newcombe *et al.* 2013, 2015; Newcombe, Moore & Moore 2015), but is problematic in the more typical case where environments vary along a continuous gradient. Like direct effects of environment on phenotype (developmental plasticity), environmentally induced parental effects should generally be viewed as norms of reaction – i.e. functions describing the dependence of offspring phenotype on maternal or paternal environment – representing a transgenerational form of developmental plasticity. Reaction norms are typically nonlinear, and characterizing such functions therefore requires examination of multiple levels of the inducing environment (Rocha & Klaczko 2012).

Very little is known about how offspring traits vary along gradients of continuously varying factors in the parental environment (such as temperature, diet composition or social experience), or how different factors in the parental environment might interact in their effects on offspring. Studies based on contrasts of two or three parental environments suggest the possibility of nonlinear parental effects (e.g. parental rearing temperature in *Drosophila melanogaster*: Gilchrist & Huey 2001; maternal rearing temperature in *Daphnia magna*: Garbutt *et al.* 2014) or context-dependent parental effects (e.g. paternal social environment and larval diet in the fly *Telostylinus angusticollis*: Adler & Bonduriansky 2013; parental age and shaking stress in the butterfly *Pieris brassicae*: Ducatez *et al.* 2012). However, it remains unclear whether effects of parental environment are typically threshold-dependent (i.e. a parental effect occurs only if an environmental variable exceeds a particular value), or continuous functions of parental environment. It also remains unknown whether the effects of a given factor in the parental environment are generally context-dependent, or largely independent of other factors. Such questions can be addressed most effectively by studies that treat parental effects as reaction norms.

Furthermore, because few studies have directly compared maternal and paternal effects, it is generally unknown whether maternal and paternal exposures to a similar environmental factor will tend to have similar consequences for offspring phenotype. Such effects may be expected to differ as a result of the distinct channels of influence available to mothers and fathers, and understanding such differences is important because theory

suggests that maternal and paternal effects can have very different evolutionary consequences (Crean & Bonduriansky 2014). Indeed, several studies have shown that maternal and paternal effects can differ (e.g. in the beetle *Callosobruchus maculatus*: Fox, Waddell & Mousseau 1995; Fox & Savalli 1998; Hallsson, Chenoweth & Bonduriansky 2012; in *T. angusticollis*: Bonduriansky & Head 2007; in *D. melanogaster*: Valtonen *et al.* 2012; in *D. serrata*: Magiafoglou & Hoffmann 2003; in *P. brassicae*: Ducatez *et al.* 2012; in humans: Pembrey *et al.* 2006; in the weed *Campanula americana*: Etterson & Galloway 2002). However, to our knowledge, no previous study has investigated the effects on offspring of continuous variation in multiple factors in both maternal and paternal environments.

In this study, we apply the nutritional geometry framework to investigate the effects and interactions of continuously varying factors in the parental nutritional environment and directly compare maternal and paternal effects on offspring phenotype. Nutritional geometry is an experimental technique whereby two components of the diet are varied simultaneously, resulting in multiple diets composed of different ratios and total amounts of the two nutrients (Simpson & Raubenheimer 1995). Typically, diets are arrayed along several ‘nutritional rails’ corresponding to particular nutrient ratios, each represented by multiple diets varying in total nutrient concentration. This makes it possible to examine the linear, nonlinear and interactive effects of the two nutrients on the phenotype, and represent these effects as a response surface. Nutritional geometry has become a standard technique in experimental nutrition research (Simpson & Raubenheimer 2012) but, to our knowledge, has never before been applied to the study of parental effects.

In the Australian neriid fly *Telostylinus angusticollis*, larval dietary nutrients enhance growth and promote expression of male secondary sexual traits (head and legs) (Bonduriansky 2007; Cassidy *et al.* 2014). Using nutritional geometry, we have previously shown that larval growth is enhanced by consumption of both protein and carbohydrate, while protein consumption also enhances male secondary sexual traits but reduces larval viability (Sentinella, Crean & Bonduriansky 2013), and has a nonlinear effect on adult longevity (Runagall-McNaull, Bonduriansky & Crean 2015). Besides these direct effects, we have also detected effects of parental larval diet on offspring. When reared on a nutrient-rich larval diet, females laid larger eggs that hatched into faster-developing offspring, whereas males produced offspring that attained a larger body size (Bonduriansky & Head 2007). The effects of male larval diet on offspring can be modulated by social environment (Adler & Bonduriansky 2013), and can extend to offspring sired by other males that mate subsequently with the same female (Crean, Kopps & Bonduriansky 2014). These effects are particularly interesting because *T. angusticollis* males transfer a tiny ejaculate that lacks any obvious nuptial gift (Bonduriansky & Head 2007).

However, all previous parental effects studies on this species have contrasted just two parental larval diets representing different concentrations of the same macronutrient ratio.

Here, we simultaneously manipulated protein and carbohydrate content in the larval diet of *T. angusticollis*, generating 20 larval diets varying in both macronutrient ratio and total concentration (Fig. 1a). The direct effects of larval diet from this experiment are reported in Runagall-McNaull, Bonduriansky & Crean (2015). In this study, we examine effects of protein and carbohydrate in maternal and paternal larval diets on offspring juvenile performance (egg hatching success, larva-to-adult viability and development time) and adult body size and shape. This experiment enabled us to address several questions: What is the shape of the functions relating variation in protein and carbohydrate concentrations in maternal and paternal larval diets to variation in offspring traits? Do protein and carbohydrate concentrations interact in their effects on offspring? Do the effects of maternal and paternal larval diets on offspring differ? Do male and female offspring respond differently to parental larval diets?

Materials and methods

SOURCE AND REARING OF FLIES

Telostylinus angusticollis aggregates and breeds on rotting bark of *Acacia longifolia* and other trees in eastern Australia. Males fight for access to females, which feed and oviposit on damaged spots on the bark, and larvae develop in rotting tissue between the bark and the tree trunk. Flies for this experiment were derived from a laboratory stock originating from Fred Hollows Reserve (Sydney, Australia) that had been recently supplemented with wild-caught individuals from the same source population. Our laboratory stock is maintained as a large, outbred population with overlapping generations. As a result of variation in density and oviposi-

tion substrate quality, stock larvae experience a range of nutritional conditions, and the resulting phenotypic variation is comparable to that observed in the natural population. Flies from this stock were reared on a 'rich' larval diet (consisting of 30 mL molasses, 30 mL barley malt and 32 g soy protein per litre of dry cocopeat and 600 mL water; see Bonduriansky 2007), which is the standard rearing medium used to maintain our stocks, at controlled larval density for one generation prior to the experiment described here in order to minimize maternal and paternal effect variation. Newly emerged males and females (F_0) were placed into separate population cages and provided with food (brown sugar and yeast), water, and oviposition containers to collect eggs for the experiment described below.

EXPERIMENTAL DIETS

We created twenty larval diets, consisting of different quantities of brown sugar and soy protein (Nature's Way brand, Pharm-a-care Pty. Ltd., Warriewood, NSW, Australia) thoroughly mixed with 1 L of dry cocopeat (Galuku Pty. Ltd., Sydney, NSW, Australia) and 600 mL of water. The brown sugar consists of 98% fructose (by weight), in addition to sucrose and other sugars, and trace quantities of minerals (especially sodium) and protein (0.2%). The soy protein consists of 18 amino acids (Alanine, Arginine, Aspartic Acid, Cysteine, Glutamic Acid, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine). Cocopeat provides a substrate similar to the bark pulp in which *T. angusticollis* larvae naturally develop, but has no nutritional value for neriid larvae. The larval diets comprised six protein:carbohydrate ratios ('nutritional rails'), each represented by multiple concentrations (Fig. 1a). These diets were based on an earlier study (Sentinella, Crean & Bonduriansky 2013), but high-protein diets that resulted in very low adult emergence were replaced with other diets (see Runagall-McNaull, Bonduriansky & Crean 2015).

EXPERIMENTAL DESIGN

Each F_1 larval diet was replicated across five larval containers, each containing ~100 g of larval medium (Fig. 1b). We transferred 20 freshly laid eggs into each larval (F_1) container, taking care to

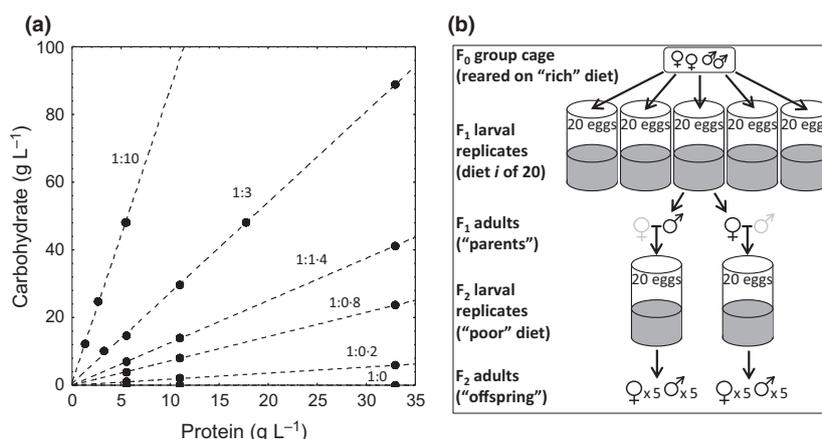


Fig. 1. Experimental design: (a) Larval diets (closed points) used in the experiment, arrayed along six nutritional rails (dashed lines) representing varying protein:carbohydrate ratios (numbers along dashed lines). (b) Initial generation flies (F_0) were all reared on a 'rich' larval diet (see text), and eggs laid by these flies were transferred to 20 experimental larval diets, with 20 eggs transferred to each of five F_1 larval replicate containers for each diet. From each replicate container, we then obtained eggs from two F_1 adults ('parents'), one male and one female (shown in black), both of which were paired with opposite-sex individuals reared on a 'rich' larval diet (shown in grey). From each parent (F_1), 20 eggs were transferred to one F_2 larval replicate container filled with 'poor' larval diet (see text). From each F_2 replicate, five adult offspring of each sex were phenotyped.

divide clutches of eggs laid by individual females among multiple larval containers and to alternate between treatments in order to minimize variation associated with parental genotype and age. Containers were placed into an environment chamber set at 27 °C and 50% humidity, and moistened with water every 3 days, for 21 days (i.e. until most larvae had pupated). The containers were then removed from the environment chamber, and each container was placed into a 2 L jar containing moist cocopeat and petri dishes of brown sugar and yeast as food for adults, and covered with a stocking. Adult flies were allowed to emerge into these 2 L containers. We obtained offspring (F_2) from one male and one female (where possible) from each F_1 replicate larval container. However, to maximize the probability of getting eggs for the F_2 generation, three individuals of each sex from each F_1 replicate were given the opportunity to breed: The first three flies of each sex to emerge in each F_1 container were transferred individually to 250 mL containers with moist cocopeat, brown sugar and yeast as food. At age 7 days, each of these F_1 individuals was paired with an opposite-sex individual (all reared on a 'rich' larval diet and *c.* 7 days of age), and provided with a petri dish of oviposition medium ('rich' larval diet that was allowed to grow mouldy and then mixed thoroughly to encourage oviposition). From each F_1 replicate container, 20 eggs from one randomly selected individual of each sex were transferred into a container of 100 g of 'poor' larval medium (a 1/3 dilution of the 'rich' medium; see Bonduriansky 2007). Poor larval medium was used to rear all offspring (F_2) because previous research suggested that parental larval diet effects are more pronounced when offspring are reared in a resource-limited environment (Bonduriansky & Head 2007). These containers were maintained in an environment chamber and then transferred to 2 L jars as described above. Ten days after the first emergence in each container, all adult offspring (F_2) were frozen at -20 °C.

MEASUREMENT OF VIABILITY AND DEVELOPMENT TIME

Egg hatching success was quantified as the number of eggs that hatched out of 20 randomly selected eggs laid by each F_1 focal female or each F_1 focal male's female partner. Larva-to-adult viability was quantified as the number of adult offspring emerging from each F_2 replicate larval container out of the number of hatched eggs. For hatching success and larva-to-adult viability, separate data were not collected for each offspring sex because we could not determine the sex of unhatched eggs or dead juveniles. Development time was quantified as the number of days from oviposition to emergence of the first adult in each F_2 replicate larval container. We did not record separate development times for male and female offspring because these are highly correlated among replicates (A. J. Crean and R. Bonduriansky, unpublished data).

MORPHOMETRIC DATA

From each F_2 brood, five individuals of each sex (where possible) were sampled at random for collection of morphometric data. All legs and both wings were removed, and flies were placed on their side into a petri dish lined with graph paper and imaged from above using a Leica DFC420 camera attached to a Leica MS5 stereoscope. IMAGEJ software (Rasband 1997–2012) was then used to measure thorax length (distance from the anterior margin of the scutum to the posterior tip of the scutellum) and head length (length of the head capsule from the posterior margin to the anterior tip, excluding the antenna) of each individual from these images. Adult body size was quantified as thorax length because this trait loads strongly on the first principal component in both sexes in this species (Bonduriansky 2006, 2007). Individuals that showed signs of damage or

decomposition were not measured. A total of 1055 F_2 individuals (516 females, 539 males) were measured.

STATISTICAL ANALYSIS

We examined linear (P, C), quadratic (P^2 , C^2) and interactive ($P \times C$) effects of protein and carbohydrate concentrations ($g L^{-1}$) in the maternal and paternal (F_1) larval diets on several juvenile and adult traits in their offspring (F_2). Protein and carbohydrate concentrations were centred at zero to reduce the collinearity of linear and nonlinear predictors (see Schielzeth 2010), and divided by 100 for ease of display of effect estimates. Responses were examined in three juvenile traits (egg hatching success, larva-to-adult viability and development time) and two adult traits (body size and head elongation).

For egg hatching success, larva-to-adult viability and development time, we first fitted a full model including nutrient effects (P, C, P^2 , C^2 , $P \times C$), parental sex and the full set of interactions among these factors as fixed effects, and F_1 replicate larval container identity as a random effect. Development time was included in the model of larva-to-adult viability, while hatching success was included in the model for development time, but neither of these effects approached statistical significance, and excluding them has no qualitative effect on results. We then tested the contribution of interactions with parental sex to model fit by excluding this set of interactions and comparing the reduced model to the full model using a likelihood ratio test (see Chenoweth & Blows 2005), which compares the variance explained by the reduced model to that explained by the full model based on a Chi-squared statistic. If the contribution of a set of interactions to model fit was found to be far from statistical significance based on a conservative cut-off ($P > 0.15$), these interactions were removed from the model. Otherwise, we interpreted the effects of particular significant interactions.

Offspring thorax length and head length were centred by z-transformation (mean = 0, standard deviation = 1) within the four parental sex \times offspring sex combinations prior to analysis. In order to insure that comparisons of maternal and paternal effects on morphology were based on comparable data sets, we excluded diets for which <2 matrilineal or patrilineal (F_1) replicates yielded adult offspring [P/C concentrations ($g L^{-1}$): 5.5/1, 11/0, 16.5/49.4, 33/41.3, 33/89] because of low emergence in either the F_1 or F_2 generation. The resulting data set for adult morphology consisted of 955 offspring (468 females, 487 males).

For each morphological trait, we first fitted a full model including nutrient effects (P, C, P^2 , C^2 , $P \times C$), parental sex, offspring sex and the full set of interactions among these factors as fixed effects, and F_1 and F_2 replicate larval container identities as random effects. Development time was initially included in these models, but neither its main effect nor its interactions approached statistical significance (results not shown), so all models were refitted without this factor. We then tested the contribution of interactions with parental sex and interactions with offspring sex to model fit using likelihood ratio tests, as described above. These models allowed us to test for overall differences between nutrient-dependent maternal and paternal effects (interactions of nutrients with parental sex), overall differences between nutrient effects on male and female offspring (interactions of nutrients with offspring sex), and differences between maternal and paternal nutrient effects on male and female offspring (nutrient \times parental sex \times offspring sex interactions). We further investigated maternal and paternal effects for morphological traits by fitting separate models to the matrilineal and patrilineal data sets, allowing us to investigate maternal and paternal effects separately. For these models, we tested the contribution of interactions with offspring sex by LRT, as described above.

We analysed effects on head elongation in two ways. First, we fitted models of offspring head length, following the same

procedure as described above for thorax length. However, interpretation of such models is not straight-forward because variation in absolute head length reflects a combination of variation in body size and body shape. We therefore also investigated treatment effects on relative head elongation by fitting models like those described above, but with the addition of thorax length as a fixed covariate. Thorax and head lengths were centred (z-transformed) within each of the four parental sex \times offspring sex combinations because equality of covariate means is a key assumption of analysis of covariance, but sex is a classification factor for which equality of covariate means cannot be assumed (Schneider, Avivi-Reich & Mozuraitis 2015). These models allowed us to investigate deviations in head elongation from the expectation based on head length – thorax length scaling. We first tested the assumption of homogeneity of slopes by fitting a model that included parental sex, offspring sex, thorax length and all interactions among these predictors. This model yielded little evidence of heterogeneity of slopes, so we fitted models that included thorax length but not its interactions with other predictors. As described above, we began by fitting a model to the full data set and testing interactions involving parental sex and offspring sex. We then fitted separate models to matriline and patriline.

All analyses were carried out in R version 3.0.1 (R Core Team 2013). For egg hatching success, larva-to-adult viability and development time, we fitted Poisson models using lme4/glmer. For morphological traits (thorax length and head length), we fitted Gaussian models using lme4/lmer (Bates *et al.* 2015). Effects were tested by *F*-tests based on the Satterthwaite approximation using the lmerTest package. Response surfaces were visualized as thin-plate splines using the fields package and Tps function. A lambda value of 0.2 was chosen by inspection to optimize visualization of response surface shape.

Results

JUVENILE TRAITS

For egg hatching success, there was strong support for interactions between dietary nutrient composition and par-

ental sex (LRT: $\chi^2 = 15.95$, d.f. = 5, $P = 0.0070$), suggesting differential effects of maternal and paternal nutrient consumption. In particular, we observed a significant protein \times parental sex interaction (Estimate = 2.52, $P = 0.0056$): maternal protein consumption increased egg hatching success, whereas paternal protein consumption reduced egg hatching success (Table 1; Fig. 2).

For larva-to-adult viability, interactions with parental sex did not contribute significantly to model fit (LRT: $\chi^2 = 1.35$, d.f. = 5, $P = 0.93$), suggesting that maternal and paternal nutrient consumption did not have differential effects on juvenile viability. No significant effects of nutrients were observed in the simplified model (Table 1).

Likewise, for development time, interactions with parental sex did not contribute significantly to model fit (LRT: $\chi^2 = 0.47$, d.f. = 5, $P = 0.99$), suggesting that maternal and paternal nutrient consumption did not have differential effects on development time, and no significant effects of nutrients were observed in the simplified model (Table 1).

ADULT BODY SIZE AND SHAPE

For thorax length, we found support for interactions with parental sex (LRT: $\chi^2 = 26.04$, d.f. = 11, $P = 0.0064$), as well as interactions with offspring sex (LRT: $\chi^2 = 26.10$, d.f. = 11, $P = 0.0063$), suggesting that maternal and paternal nutrient consumption had different effects on offspring, and that male and female offspring responded differently to parental nutrients. The full model included significant C \times offspring sex and C² \times offspring sex interactions (Table 2).

In separate models fitted to matriline, we found weak overall support for interactions with offspring sex (LRT:

Table 1. Effects of protein and carbohydrate concentration in the maternal and paternal larval diets, and their quadratic terms and cross product, on egg hatching success and offspring larva-to-adult viability and development time. Parental sex denotes matriline vs. patriline. Effect estimates (and their standard errors) are shown from mixed models with F₁ larval replicate fitted as a random effect. Fixed effects were tested based on Type III sums of squares and the Satterthwaite approximation. Simplified models are shown (see text for details), with significant effects highlighted in bold

Effect	Egg hatching success [†]	Larva-to-adult viability [‡]	Development time [§]
Protein (P)	-1.160 (0.945)	0.242 (0.444)	0.034 (0.283)
Carbohydrate (C)	0.405 (0.403)	-0.144 (0.194)	-0.058 (0.130)
P ²	7.882 (7.561)	-1.666 (3.561)	-0.820 (2.249)
C ²	-1.797 (1.249)	-0.073 (0.610)	0.097 (0.434)
P \times C	-0.423 (2.642)	0.755 (1.314)	0.033 (0.908)
Development time	-	-0.005 (0.012)	-
Egg hatching success	-	0.091 (0.006)***	-0.003 (0.003)
Parental sex (ParSex)	0.049 (0.111)	-	0.004 (0.033)
P \times ParSex	2.524 (0.910)**	-	-
C \times ParSex	-0.135 (0.418)	-	-
P ² \times ParSex	-6.565 (7.272)	-	-
C ² \times ParSex	0.024 (1.475)	-	-
P \times C \times ParSex	4.930 (2.959)	-	-

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[†] $N = 130$ families.

[‡] $N = 124$ families.

[§] $N = 124$ families.

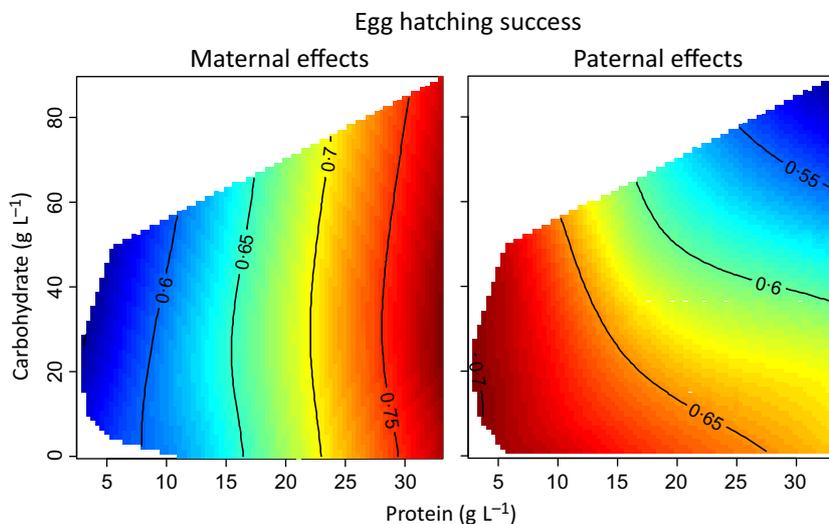


Fig. 2. Response surfaces for effects of protein and carbohydrate content (g L^{-1}) in maternal and paternal larval diets on egg hatching success, represented by colour (blue = low, red = high). Solid black lines and numbers within each plot represent isoclines for the proportion of eggs hatched out of 20.

$\chi^2 = 8.74$, d.f. = 5, $P = 0.120$). The maternal effects model included a significant positive effect of P and significant negative effect of P^2 , as well as a marginally nonsignificant $P \times C$ interaction (Table 2), indicating that maternal protein consumption increased offspring body size, subject to diminishing returns, and suggesting a $P \times C$ interaction effect in daughters only (Fig. 3). In separate models fitted to patriline, we likewise found weak overall support for interactions with offspring sex (LRT: $\chi^2 = 8.13$, d.f. = 5, $P = 0.149$). The paternal effects model included a significant positive $C \times$ offspring sex interaction and a significant negative $C^2 \times$ offspring sex interaction (Table 2), suggesting that the negative effect of paternal carbohydrate consumption on offspring body size was stronger for female offspring (Fig. 3).

For absolute head length, we found statistical support for interactions with parental sex (LRT: $\chi^2 = 30.96$, d.f. = 11, $P = 0.0011$), as well as interactions with offspring sex (LRT: $\chi^2 = 29.46$, d.f. = 11, $P = 0.0019$). The full model included a significant $C \times$ offspring sex interaction and a near-significant $C^2 \times$ offspring sex interaction, as well as a near-significant $C \times$ parental sex interaction and a significant $P \times C \times$ parental sex \times offspring sex interaction (Table S1, Supporting information). For matriline, we found weak support for interactions with offspring sex (LRT: $\chi^2 = 8.49$, d.f. = 5, $P = 0.131$), and the maternal effects model included a significant $P \times C \times$ offspring sex interaction (Table S1, Supporting information). For patriline, we found significant support for interactions with offspring sex (LRT: $\chi^2 = 13.14$, d.f. = 5, $P = 0.0221$). The paternal effects model included a marginally nonsignificant negative main effect of carbohydrate, a significant $C \times$ offspring sex interaction, and a near-significant $C^2 \times$ offspring sex interaction (Table S1, Supporting information).

For relative (i.e. body size-corrected) head length, we found overall support for interactions with parental sex (LRT: $\chi^2 = 22.00$, d.f. = 11, $P = 0.0244$), and weaker overall support for interactions with offspring sex (LRT:

$\chi^2 = 17.29$, d.f. = 11, $P = 0.0996$). The full model included a significant effect of $P \times C$, and significant or near-significant $P \times C \times$ offspring sex, $P^2 \times$ parental sex, $P \times C \times$ parental sex, $P^2 \times$ parental sex \times offspring sex and $P \times C \times$ parental sex \times offspring sex interactions (Table 2).

For matriline, we found no overall support for interactions with offspring sex (LRT: $\chi^2 = 4.03$, d.f. = 5, $P = 0.55$). The simplified maternal effects model included a significant negative effect of P and positive effect of P^2 (Table 2), indicating that relative head length of both male and female offspring decreased nonlinearly with maternal protein consumption (Fig. 4). For patriline, interactions with offspring sex were strongly supported (LRT: $\chi^2 = 18.09$, d.f. = 5, $P = 0.0028$). The paternal effects model included a significant effect of $P \times C$, a near-significant positive $P^2 \times$ offspring sex interaction, and a significant $P \times C \times$ offspring sex interaction (Table 2), indicating that relative head length of offspring decreased with paternal consumption of diets rich in both protein and carbohydrates, but this reduction was less pronounced in male offspring (Fig. 4).

Discussion

We employed the nutritional geometry framework to investigate and compare maternal and paternal effects, using the neriid fly *T. angusticollis*. Our study yielded several insights. First, we found that nutrient consumption by females and males can have strikingly different effects on offspring viability, development and growth. In particular, protein consumption by females and males had nearly opposite effects on egg hatching success. Moreover, maternal effects on adult traits were largely mediated by protein in the larval diet, whereas paternal effects were primarily mediated by carbohydrates. While several studies have reported differential effects of macronutrients (especially protein) on male and female performance (see below), we show that such effects can extend across generations. Sec-

Table 2. Effects of protein and carbohydrate concentration in the maternal and paternal larval diets, and their quadratic terms and cross product, on offspring thorax length and relative head length within the full data set, and separately within parental sexes (matrilines and patriline). Effect estimates (and their standard errors) are shown from mixed models with F₁ and F₂ larval replicates (full data set) or F₂ larval replicates (separate analyses of maternal and paternal effects) fitted as random effects. Thorax length was included in models of relative head length. Fixed effects were tested using Type III sums of squares based on the Satterthwaite approximation. Simplified models are shown (see text for details), with significant effects highlighted in bold

Effect	Thorax length			Relative head length		
	Full data set [†]	Maternal effects [‡]	Paternal effects [§]	Full data set [†]	Maternal effects [‡]	Paternal effects [§]
Thorax length	—	—	—	0.885 (0.018) ***	0.878 (0.028) ***	0.910 (0.022) ***
Protein (P)	-0.040 (2.783)	6.932 (2.909) *	-0.030 (2.728)	-0.387 (0.715)	-2.002 (0.804) *	-0.436 (0.677)
Carbohydrate (C)	-2.366 (1.539)	0.923 (1.629)	-2.304 (1.508)	-0.503 (0.392)	0.279 (0.443)	-0.455 (0.373)
P ²	3.073 (18.484)	-43.294 (19.780) *	2.606 (18.113)	-3.801 (4.749)	13.841 (5.330) *	-3.403 (4.493)
C ²	8.657 (7.874)	-9.210 (8.505)	8.597 (7.716)	0.577 (2.001)	1.056 (2.368)	0.431 (1.903)
P × C	11.857 (14.484)	9.825 (15.156)	12.634 (14.205)	-8.693 (3.653) *	1.415 (3.880)	-8.989 (3.478) *
Offspring sex (OffspSex)	0.247 (0.084) **	-0.173 (0.073) *	0.246 (0.094) **	-0.073 (0.079)	0.086 (0.095)	-0.075 (0.063)
Parental sex (ParSex)	0.956 (0.354) **	—	—	-0.165 (0.102)	—	—
OffspSex × ParSex	-0.416 (0.120) ***	—	—	0.144 (0.112)	—	—
P × OffspSex	1.342 (0.853)	-1.096 (0.787)	1.345 (0.953)	-0.325 (0.803)	0.412 (1.006)	-0.360 (0.645)
C × OffspSex	1.108 (0.471) *	0.580 (0.440)	1.118 (0.527) *	0.161 (0.444)	-0.238 (0.561)	0.148 (0.357)
P ² × OffspSex	-8.799 (5.659)	4.375 (5.188)	-8.796 (6.323)	8.061 (5.331)	-7.328 (6.672)	8.241 (4.280)
C ² × OffspSex	-6.253 (2.387) **	-1.463 (2.350)	-6.276 (2.669) *	0.484 (2.255)	-1.404 (3.009)	0.520 (1.812)
P × C × OffspSex	-5.038 (4.370)	-7.191 (3.843)	-5.090 (4.887)	9.511 (4.130) *	-6.497 (4.999)	9.479 (3.312) **
P × ParSex	6.916 (3.610)	—	—	-1.567 (1.054)	—	—
C × ParSex	3.288 (2.015)	—	—	0.865 (0.583)	—	—
P ² × ParSex	-46.172 (24.245)	—	—	17.181 (7.002) *	—	—
C ² × ParSex	-17.934 (10.432)	—	—	0.334 (3.049)	—	—
P × C × ParSex	-2.206 (18.897)	—	—	9.804 (5.261)	—	—
P × OffspSex × ParSex	-2.405 (1.259)	—	—	0.711 (1.174)	—	—
C × OffspSex × ParSex	-0.514 (0.700)	—	—	-0.471 (0.652)	—	—
P ² × OffspSex × ParSex	12.947 (8.331)	—	—	-15.014 (7.780)	—	—
C ² × OffspSex × ParSex	4.758 (3.656)	—	—	-1.612 (3.410)	—	—
P × C × OffspSex × ParSex	-2.136 (6.294)	—	—	-15.700 (5.914) **	—	—

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[†] $N = 955$ individuals from 112 parents.

[‡] $N = 452$ individuals from 54 parents.

[§] $N = 503$ individuals from 58 parents.

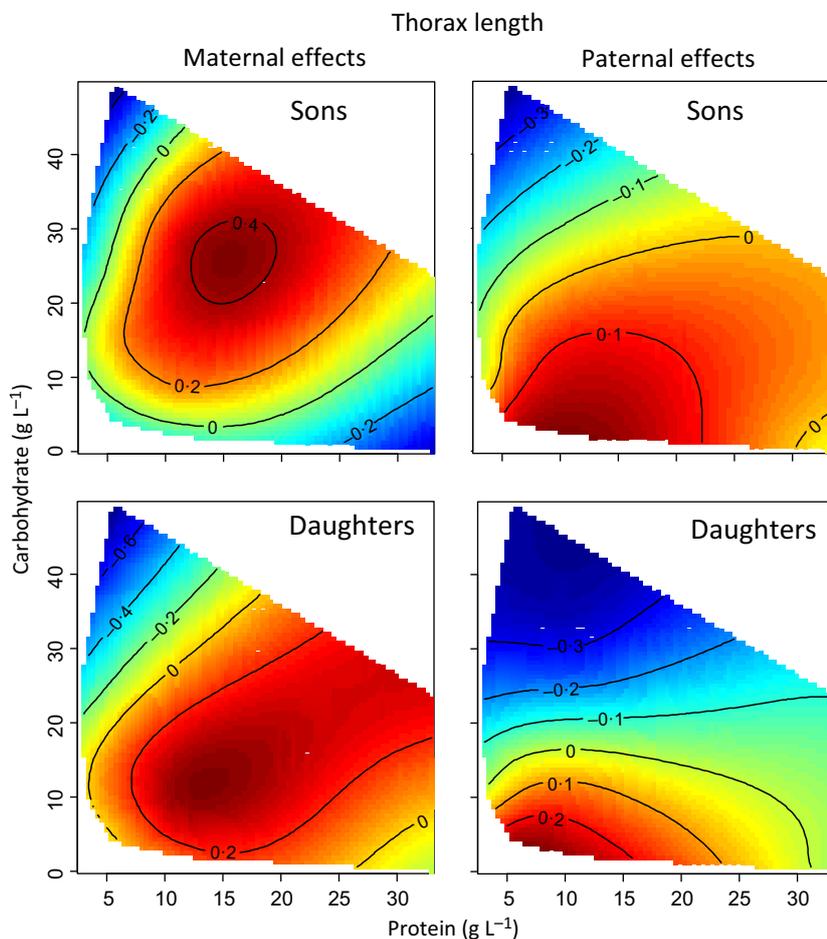


Fig. 3. Response surfaces for effects of protein and carbohydrate content (g L^{-1}) in maternal and paternal larval diets on body size (thorax length) of sons and daughters. Within each panel, body size is represented by colour (blue = low, red = high), with solid black lines showing isoclines (in standard deviations from the mean).

ond, effects of parental dietary nutrients were nonlinear for most offspring traits and interactive for some traits, highlighting the importance of treating parental effects as reaction norms in systems where environmental parameters vary along continuous gradients. Third, male and female offspring responded differently to some aspects of parental diet, suggesting that parental effects can be subject to trade-offs between performance of sons and daughters. Our findings highlight the ecological complexity of parental effects, and point to potential evolutionary implications.

COMPARING MATERNAL AND PATERNAL EFFECTS

Maternal and paternal effects may be expected to differ because the contrasting reproductive strategies of the sexes confer differential opportunities for mothers and fathers to influence their offspring (Crean & Bonduriansky 2014). In internally fertilizing, oviparous animals, both parents can influence their offspring via environmentally induced epigenetic modifications to the egg- or sperm-borne haploid genome (Gapp *et al.* 2014; Hoile *et al.* 2014; Mashoodh & Champagne 2014; Soubry *et al.* 2014; Bohacek & Mansuy 2015), but mothers can also influence offspring development via variation in the yolk and cytoplasmic content (hormones, carotenoids, amino acids, defensive com-

pounds, etc.) of eggs (Newcombe *et al.* 2013; Peluc *et al.* 2014; Warner & Lovern 2014; Newcombe *et al.* 2015; Newcombe, Moore & Moore 2015), while fathers can affect offspring development via proteins and RNA in the sperm and seminal fluid (Wong *et al.* 2007; Vojtech *et al.* 2014). Paternal effects in such systems may often be mediated by maternal responses (Bromfield *et al.* 2014; Crean & Bonduriansky 2014), including partitioning of male-derived resources between maternal soma and eggs or embryos (Simmons 2011), and differential allocation of maternal resources to offspring based on paternal phenotype (Sheldon 2000). Although maternal effects have been recognized for decades (Roach & Wulff 1987; Mousseau & Dingle 1991), and recent studies have provided abundant evidence of paternal effects in many species (Crean & Bonduriansky 2014; Mashoodh & Champagne 2014; Soubry *et al.* 2014), few studies have undertaken direct comparisons of maternal and paternal effects.

Previous research on *T. angusticollis*, based on a contrast between two larval diets differing in overall nutrient concentration, yielded evidence of paternal but not maternal larval diet effects on offspring body size (Bonduriansky & Head 2007). This study provides evidence that both maternal and paternal larval diets affect offspring growth. However, we found that macronutrients can play strikingly different roles in maternal and paternal effects.

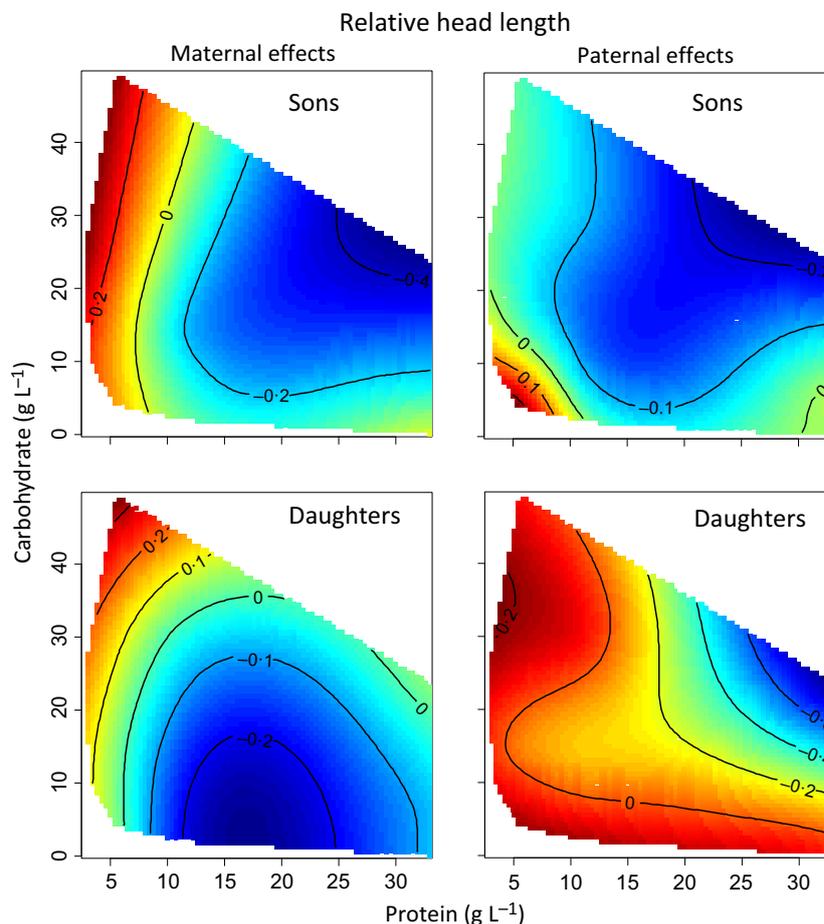


Fig. 4. Response surfaces for effects of protein and carbohydrate content (g L^{-1}) in maternal and paternal larval diets on relative head length of sons and daughters. For illustrative purposes, relative head length is depicted as residuals of head capsule length on thorax length, calculated separately for male and female offspring. Within each panel, relative head length is represented by colour (blue = low, red = high), with solid black lines showing isoclines (in standard deviations from the mean).

Several previous studies have shown that protein consumption has contrasting direct effects on some fitness-related traits in the sexes. Work on the cricket *Teleogryllus commodus* (Maklakov *et al.* 2008) and the fly *D. melanogaster* (Zajitschek *et al.* 2013; Jensen *et al.* 2015) suggests that, while protein consumption by adults tends to reduce longevity in both sexes, protein consumption by adult females enhances fecundity, perhaps by furnishing resources for the production of yolk, while protein consumption by adult males has little effect on male reproductive success. Similarly, in *T. angusticollis*, protein restriction in the adult diet extended longevity by 67% in both sexes, and rendered females completely infertile, while having little effect on male reproductive capacity (Adler *et al.* 2013). In this study, we found that protein availability in the female larval diet had positive effects on egg hatching success as well as offspring growth. Because females fed a nutrient-rich larval diet produce larger eggs (Bonduriansky & Head 2007), positive effects of female protein consumption might be mediated by increased yolk provisioning, which could enhance embryo viability (Azevedo, French & Partridge 1997; Warner & Lovern 2014). In contrast, protein in the male larval diet appeared to have largely negative consequences for offspring, reducing egg hatching success but having no independent effect on offspring growth. Increased protein consumption by male

T. angusticollis larvae results in disproportionate resource allocation to secondary sexual traits (see Sentinella, Crean & Bonduriansky 2013), and it is possible that a resource allocation trade-off limits investment in testes and accessory glands in such larvae, thereby reducing their capacity to enhance the viability and growth of their offspring via paternal effects. The molecular mechanism mediating paternal larval diet effects on offspring in *T. angusticollis* is not known, but the effect appears to be mediated by the seminal fluid (Crean, Kopps & Bonduriansky 2014), potentially implicating accessory gland proteins or non-coding RNA (Wong *et al.* 2007; Bromfield 2014; Stoeciun, Grun & Rajewsky 2014; Vojtech *et al.* 2014; Eaton *et al.* 2015).

PARENTAL EFFECTS AS REACTION NORMS

Nonlinear and interactive effects of macronutrients can be ecologically important because they can result in an optimal diet, represented by a peak or plateau in the response surface for fitness. Such peaks have been reported in several nutritional geometry studies examining direct effects of nutrient intake (e.g. Lee *et al.* 2008; Maklakov *et al.* 2008; Cotter *et al.* 2011; Fanson & Taylor 2012; Sentinella, Crean & Bonduriansky 2013). We found evidence of nonlinear parental effects mediated by both protein (particularly

in the maternal larval diet) and carbohydrate (particularly in the paternal larval diet), as well as interactive effects of the two macronutrients, resulting in apparent peaks for offspring growth at intermediate values of maternal protein consumption and paternal carbohydrate consumption. Indeed, the maternal effect surfaces for both male and female offspring body size appeared to peak at intermediate values of protein and carbohydrates, although we did not detect significant protein—carbohydrate interactions.

We also found that offspring relative head length was a nonlinear function of maternal protein consumption, and was subject to interactive effects of protein and carbohydrate in the paternal larval diet. In this experiment, all offspring were reared on a nutrient-poor larval diet, which results in diminished expression of male secondary sexual traits such as head elongation, and reduced sexual dimorphism (Bonduriansky 2007). We found that relative head elongation was maximized on maternal diets low in protein, and paternal diets low in both protein and carbohydrate. Investment in the female homologues of male secondary sexual traits is always suppressed in *T. angusticollis*, such that increasing body size is associated with reduction in relative head length, reflected in a negative static allometry for this trait in females (Bonduriansky 2006, 2007). In males reared on a nutrient-poor larval diet, relative head length increases slightly with increasing body size (from this experiment, static allometry slope for males = 1.13). However, it is possible that, when male larvae experience a nutrient-poor environment, any contribution of resources or growth factors via parental effects is allocated mainly to body growth, resulting in opposite effects of parental nutrient consumption on offspring body size and relative head length.

Performance peaks at particular nutrient concentrations or combinations suggest that natural selection may favour fine-grained oviposition site choice by females, and foraging strategies by larvae (Cotter *et al.* 2011). Our findings suggest that, in addition to optimizing direct effects on viability, growth and development, such strategies may also be selected to optimize nongenetic parental effects on offspring. Given that intake ‘targets’ for different traits may differ, this can present a very complex optimization problem, and understanding the evolution of oviposition and foraging strategies will require knowledge of the effects of diet composition within and across generations and the relative importance of these effects for fitness in both sexes. Optimal diet composition may also depend on the ecological and social conditions that determine, for example, the relative importance of allocation to body size vs. the development of male secondary sexual traits. Such effects could play a role in the evolution of sexual strategies. For example, females may benefit by selecting males that developed in an optimal larval patch because such males confer increased offspring fitness (Bonduriansky & Day 2013).

COMPARING PARENTAL EFFECTS ON SONS AND DAUGHTERS

Interestingly, maternal effects were consistent for male and female offspring body size and relative head elongation, while paternal effects appeared to influence male and female offspring unequally for these traits. Paternal consumption of carbohydrates reduced the body size of offspring of both sexes, but had a stronger negative effect on daughters than on sons. Likewise, the relative head length of male and female offspring was affected unequally by the interaction of protein and carbohydrate in the paternal larval diet. Body growth and the development of secondary sexual traits are both controlled in insects by insulin-like growth factor signalling, which is stimulated by nutrient intake (Shingleton *et al.* 2007; Shingleton, Mirth & Bates 2008; Emlen *et al.* 2012). Because body size and shape are sexually dimorphic traits in *T. angusticollis*, both overall growth rate and the growth of particular adult tissue primordia (imaginal discs) may be differentially sensitive to insulin-like growth factor in male and female larvae. Paternal effects that stimulate insulin-like growth factor signalling may therefore also have differential effects on male and female larvae, although it is not clear why maternal effects do not affect male and female larvae differently as well.

USING NUTRITIONAL GEOMETRY TO STUDY PARENTAL EFFECTS

In addition to effects of parental larval diet on offspring, nutritional geometry could also be used to investigate the effects of parental adult diet (Brown *et al.* 2014; Peluc *et al.* 2014), interactions between parental and offspring diet, and effects on grand-offspring and beyond. The geometric approach could also be used to investigate more fine-grained variation in larval or adult diets, such as the ratios of particular amino acids (see Grandison, Piper & Partridge 2009; Zajitschek *et al.* 2013). In addition, the logic of nutritional geometry can be extended to investigate effects of three nutrients (Hawley, Simpson & Wilder 2016), and adapted to study the effects of other parameters, such as parental social environment (Adler & Bonduriansky 2013).

The geometric approach also imposes certain practical limitations on study design. In particular, it may be impractical to use the geometric approach to test for interactions between maternal and paternal effects (e.g. see Nystrand & Dowling 2014): if several levels of an environmental factor are examined in each sex, testing for maternal—paternal interactions would require an extremely large factorial experiment involving all possible combinations of crosses. Likewise, geometric designs are challenging to overlay on a quantitative-genetic framework, but this problem can be overcome through the use of clones or inbred lines (see Reddix *et al.* 2013).

Conclusions

Our findings highlight the potential importance of examining the effects of continuous variation in parental environment, whereby parental effects are characterized as norms of reaction for offspring phenotype. In systems where biologically relevant environmental parameters vary along continuous gradients, studies relying on contrasts between two environmental states can produce misleading results because they can only detect linear effects. Moreover, such studies can fail to detect parental effects if the contrasted environments happen to occupy a flat region of the maternal and/or paternal effect surface. Likewise, evidence that a particular environment can induce very different maternal and paternal effects points to the need for direct comparisons of maternal and paternal effects. The geometric approach offers considerable scope for further investigation of parental effects.

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Data accessibility

Data deposited in the Dryad Digital Repository: <http://doi:10.5061/dryad.205jp> (Runagall-McNaull, Crean & Bonduriansky 2016).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. Effects of macronutrients in maternal and paternal larval diets on offspring head length.