doi:10.1111/j.1420-9101.2007.01419.x

# Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae)

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*Keywords:*

body size;
condition;
diet;
environmental effects;
maternal effects;
Neriidae;
paternal effects;
sexual selection;
*Telostylinus angusticollis*.

## Abstract

It is widely recognized that maternal phenotype can have important effects on offspring, but paternal phenotype is generally assumed to have no influence in animals lacking paternal care. Nonetheless, selection may favour the transfer of environmentally acquired condition to offspring from both parents. Using a split-brood, cross-generational laboratory design, we manipulated a key environmental determinant of condition – larval diet quality – of parents and their offspring in the fly *Telostylinus angusticollis*, in which there is no evidence of paternal provisioning. Parental diet did not affect offspring survival, but high-condition mothers produced larger eggs, and their offspring developed more rapidly when on a poor larval diet. Maternal condition had no effect on adult body size of offspring. By contrast, large, high-condition fathers produced larger offspring, and follow-up assays showed that this paternal effect can be sufficient to increase mating success of male offspring and fecundity of female offspring. Our findings suggest that both mothers and fathers transfer their condition to offspring, but with effects on different offspring traits. Moreover, our results suggest that paternal effects can be important even in species lacking conventional forms of paternal care. In such species, the transfer of paternal condition to offspring could contribute to indirect selection on female mate preferences.

## Introduction

In insects and many other animals, diet quality is a key environmental determinant of individual body size, which often reflects condition (Blanckenhorn, 2000). Environmental variation in diet may select for environment-dependent maternal or paternal effects (as opposed to potentially environment-independent parent-of-origin effects, such as genomic imprinting, e.g. see Fitch *et al.*, 1998; Lloyd, 2000), for two nonexclusive reasons. First, individuals that acquire high condition from a resource-rich environment may benefit by transferring their condition to their offspring, thus enhancing offspring fitness (Mousseau & Fox, 1998; Pál & Miklós, 1999; Qvarnström & Price, 2001). This predicts that offspring of high-condition parents will do better in any environment, but especially in a poor-quality environment where enhanced condition is most beneficial. Second, if the environment (e.g. diet) that the parents experience predicts the environment that their offspring will encounter, parents may be selected to optimize offspring phenotype for that environment (Mousseau & Dingle, 1991; Rossiter, 1996; Mousseau & Fox, 1998; Fox & Czesak, 2000; Gilchrist & Huey, 2001; Rotem *et al.*, 2003; Holbrook & Schal, 2004). This predicts that offspring will do best in an environment similar to that experienced by their parents.

Environment-dependent maternal effects, reflecting variation in maternal provisioning, have been reported in many insects and other animals (Rossiter, 1996; Mousseau & Fox, 1998). Similarly, environment-dependent paternal effects have been reported in species where males provision their offspring through gifts of nutrients or other important substances transferred to females (Zeh & Smith, 1985; Dussourd *et al.*, 1988; Gwynne, 1988;

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Smedley & Eisner, 1996; Vahed, 1998), or through direct contributions to offspring feeding (Griffith *et al.*, 1999; Hunt & Simmons, 2000; Rauter & Moore, 2002). However, paternal effects are generally assumed to be absent, or much less important than maternal effects, in animals that lack paternal provisioning (Falconer & Mackay, 1996).

Nonetheless, several recent studies suggest that environment-dependent paternal effects can occur in the absence of paternal provisioning in the conventional sense. In *Drosophila*, the ambient temperature experienced by males affects life-history traits in their offspring (Huey *et al.*, 1995; Watson & Hoffmann, 1995; Magiafoglou & Hoffmann, 2003) and, in locusts, paternal social environment influences offspring colour and behaviour (Islam *et al.*, 1994). Such paternal effects could be mediated by small doses of accessory gland products (García-González & Simmons, 2007; Ivy, 2007). Moreover, there is mounting evidence that environment can induce epigenetic modifications (e.g. changes in methylation patterns or chromatin structure) in the germ line, resulting in epigenetic 'reprogramming' of sperm (Jablonka & Lamb, 1995, 2005; Fitch *et al.*, 1998; Pembrey, 2002; Chang *et al.*, 2006; Pembrey *et al.*, 2006). In mice, paternal and maternal diets can affect offspring phenotype (Anderson *et al.*, 2006; Cooney, 2006; Cropley *et al.*, 2006). In rats, an environmentally induced low-fertility male phenotype was transmitted through the male line for four generations (Anway *et al.*, 2005; Anway & Skinner, 2006). However, very few studies have tested for environment-dependent paternal effects in species lacking conventional forms of paternal provisioning, and the ecological and evolutionary importance of such effects remains uncertain in such species.

The possibility of such paternal effects has interesting implications for theory. In species lacking paternal provisioning, environmental variation is assumed to diminish heritability (i.e. offspring–paternal resemblance) for male quality, and thus reduce the indirect benefits of choice for females (Hunt *et al.*, 2004). Nonetheless, environmental variation could play an important role in evolution if it could be transmitted to offspring through paternal effects. If environmental variation augments offspring–paternal resemblance through the transfer of paternal condition to offspring, it may contribute to indirect selection on female preferences (see *Discussion*).

In the fly *Telostylinus angusticollis* (Neriidae), larval diet quality affects a suite of condition-dependent traits, including larval survival, development rate, and adult body size and shape, and these effects are particularly strong in males (Bonduriansky, 2007). Similar phenotypic variation is observed in the wild (Fig. 1), and probably reflects natural variation in larval diet quality. Thus, environmental variation in body size (and, presumably, condition) appears to be an ecologically important parameter in natural populations. Females may be capable of transmitting environmental variation to offspring through maternal effects (e.g. via variation in egg provisioning). However, it is not clear whether environment-dependent paternal effects are possible in this species, because there is no evidence of any conventional form of paternal provisioning: mean copulation duration is only 43 s; there is no external or internal spermatophore, no visible insemination reaction and mean ejaculate size is $< 0.01\%$ of male body volume (R. Bonduriansky, unpublished data). By contrast, nuptial gifts of nutrients or other diet-derived substances are typically manifested in large ejaculates that constitute at least 1% (and often $> 5\%$) of male body mass (Dussourd *et al.*, 1988; Wedell, 1993; Smedley & Eisner, 1996; Vahed, 1998; Bonduriansky, 2001), and produce an insemination reaction (Markow & Ankney, 1988; Pitnick *et al.*, 1997). The tiny ejaculate and lack of insemination reaction in *T. angusticollis* thus suggest that ejaculates are unlikely to function as nuptial gifts in this species.

**Fig. 1** *Telostylinus angusticollis* males collected from the wild population, illustrating the naturally occurring range of body sizes.

We investigated the effects of environmental variation in maternal and paternal condition on four offspring traits in *T. angusticollis*: egg size, larval survival rate, development time and adult body size. To do this, we manipulated larval diet quality over two generations, and tested for effects of maternal, paternal and offspring diets and their interactions on offspring. We found that both

mothers and fathers transfer their condition to offspring, but that maternal and paternal effects influence different aspects of offspring phenotype. Moreover, we observed a paternal diet effect on offspring body size and, through follow-up assays, found that this effect is probably sufficient to enhance offspring fitness. The paternal diet effect may be mediated by a cryptic form of paternal investment.

## Materials and methods

### Source and rearing of flies

A laboratory population of *T. angusticollis* (Enderlein) was established from ~100 flies collected from aggregations on trunks of *Acacia longifolia* trees at the Fred Hollows Reserve in Sydney, Australia, and maintained through random, nonsibling crosses for three generations prior to this study. Males and females were paired in 250-mL cages containing a substrate of moist 'cocopeat' (Galuku Pty. Ltd, Sydney, Australia) and a Petri dish filled with 'rich' larval medium for oviposition (see below). Eggs were transferred to vials containing fresh rich larval medium provided *ad libitum* (> 5 g per egg) inside cages with mesh lids, where adult offspring emerged after ~20 days. Adults were maintained at 27 °C and ~60% humidity under a combination of incandescent and broad-spectrum fluorescent lighting (16 h light per 24 h). Larval vials were watered occasionally. Experimental flies were maintained in the same way, except as indicated below.

### Larval diet

The larval feeding substrate used by the wild source-population consists of moist, rotting bark and the frass of wood-boring beetles impregnated with mould and tree sap (R. Bonduriansky, unpublished data). The artificial larval diet medium used to maintain the laboratory stock, and in the experiments described below, was designed to approximate the texture and composition of the natural larval substrate, and consisted of water, sugars and protein added to a base of cocopeat (pulverized skin of coconut husks). Larvae appear to feed on the mould that grows in this medium. Rich larval medium consisted of 30 mL of 'black strap' sugar cane molasses (Conga Foods Pty. Ltd, Preston, Vic., Australia), 30 mL of liquid barley malt (Colonial Farms brand, Select Foods Pty. Ltd., Smithfield, NSW, Australia) and 32 g of soy protein powder (Nature's Way brand, Pharm-a-care Pty. Ltd., Warriewood, NSW, Australia) per litre of dry cocopeat hydrated with 800 mL of water purified by reverse osmosis. Poor larval medium consisted of 10 mL of molasses, 10 mL of malt and 10 g of soy protein per litre of dry cocopeat and 800 mL of water. Mixtures were thoroughly homogenized with a hand-held blender, and frozen at −20 °C until the day of use.

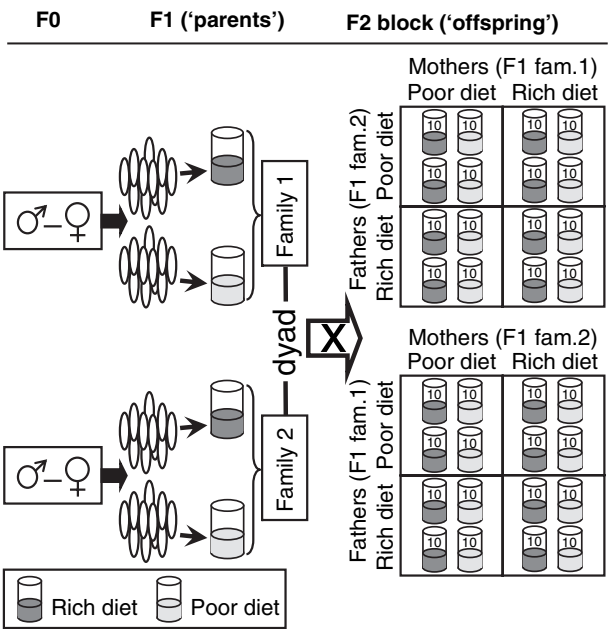


**Fig. 2** Experimental design used to test for effects of parental and offspring larval diets on offspring. From each dyad of 'parental' (F1) families, males and females reared on rich and poor larval diets were crossed in all combinations of diet treatment, and offspring (F2) from each cross were reared on rich and poor larval diets.

### Experiment to test for parental diet effects

Fourteen male–female pairs (F0), sharing no more than one grandparent within or between pairs, were allowed to oviposit in dishes containing rich medium (Fig. 2). Prior to hatching (i.e. < 48 h after oviposition), 20 randomly selected eggs from each pair were transferred to rich larval medium and 20 eggs to poor larval medium (both media provided *ad libitum*) using a probe. This yielded 14 full-sib families of F1 flies (parents), each comprising individuals reared on rich and poor larval diets. F1 families were grouped randomly into 10 dyads and, within each dyad, crosses were established in all diet treatment × family combinations (excluding sibling crosses). From each F1 cross, 10 eggs were transferred (where possible) to each of two vials of rich medium and two vials of poor medium (both media provided *ad libitum*). Females often laid < 40 eggs per oviposition bout, in which case two clutches were used, each contributing eggs to rich and poor diet vials to control for clutch effects. Three F1 dyads yielded very few eggs or adult offspring, and data from these dyads were included in the egg size and number analyses only (see below). The remaining seven dyads comprised 56 F1 (parental) crosses, of which 51 crosses yielded F2 adults (offspring), although some offspring diet × sex combinations are missing for some crosses because of larval mortality or skewed sex ratio (see below).

All parents and offspring (24–48 h after adult emergence) were killed by freezing, glued to entomological pins by the right mesopleuron, and the body size (thorax length) of each fly was measured using a Leica MS5 stereoscope (Leica Microsystems, Heerbrugg, Switzerland) fitted with an ocular micrometer by a technician unfamiliar with the aims of the study. Offspring egg-to-adult development time (days from oviposition to first adult emergence) and survival rate were also recorded. Data from different vials within F1 crosses were averaged to obtain four estimates per cross for body size (one for each offspring diet × sex combination), and two estimates for offspring viability and development time (one for each offspring diet; we did not keep track of offspring sex for these variables). Data were then log-transformed and standardized within F2 block to remove block effects on means and variances. All variables were approximately normally distributed. We analysed offspring survival rate, development time and body size data by repeated measures ANOVA, with maternal and paternal diets as independent factors, and offspring diet and sex (body size analysis) as factors within cross. Only those F1 crosses that yielded offspring for each treatment combination were included in analyses: for survival rate and development time, 43 crosses that yielded offspring for both offspring diet treatments ($N$ crosses for maternal × paternal diet combinations: $N_{\text{Rich×Rich}} = 9$, $N_{\text{Rich×Poor}} = 10$, $N_{\text{Poor×Rich}} = 12$, $N_{\text{Poor×Poor}} = 12$); for body size, 40 crosses that yielded adult offspring for all offspring diet × sex combinations ($N_{\text{Rich×Rich}} = 9$, $N_{\text{Rich×Poor}} = 10$, $N_{\text{Poor×Rich}} = 9$, $N_{\text{Poor×Poor}} = 12$). These F1 crosses yielded 1007 adult offspring (479 females and 528 males). Data were analysed using Statistica 7 (© StatSoft, Inc., Tulsa, OK, USA).

### Body size and fitness

We investigated the effects of body size on male mating success by pairing sexually mature (10- to 15-day-old) males, matched by visual inspection for similar body size, in 1-L cages containing a female and a 250-mL container of oviposition substrate. Males were drawn from the same stock as the flies used in the parental diet experiment, and were reared on diets of varying quality to generate a range of adult body sizes. Cages were observed repeatedly over several days, until a clear 'winner' was established (i.e. when one male and the female were both present on the oviposition substrate and engaged in mating/oviposition, whereas the other male was located on the top or side of the cage). Both males were then removed, killed and measured (see above). To determine the minimum difference in body size between rivals that yielded a significant size-advantage for winners, we compared winner and loser thorax lengths for a series of subsamples generated by excluding male–male pairs in which the thorax length difference between the rival males exceeded an upper limit that was reduced successively by increments of 1 ocular micrometer unit (0.04 mm).

To test for parental diet effects on egg size and quantify the relation between female body size and egg number, we dissected 60 F1 females, counted mature eggs in their ovaries, and measured lengths of five randomly selected eggs from each female, following collection of their eggs for experimental treatments. Dissections were performed by severing the abdomen, placing it into a droplet of saline solution on a microscope slide on the stereoscope stage, and removing the ovaries using microprobes. Mean egg lengths from each female were log-transformed and standardized within F2 block, and were analysed by factorial ANOVA, with female and male diets as independent variables.

## Results

### Effects of larval diet within and across generations

*Egg size*

Females reared on a rich larval diet produced larger eggs, but there was no effect of male diet on mean egg size (Table 1; Fig. 3). Qualitatively identical results (not shown) are obtained if the sample is restricted to females that produced adult offspring.

*Survival rate*

Egg-to-adult survival rate was lower for parental-generation (F1) flies reared on a poor larval diet (64%) than for those reared on a rich diet (88%) (paired $t$-test: $N = 14$ F1 families, $t_{13} = 3.67$, $P = 0.0028$). Offspring (F2) survival rate was similarly affected by offspring diet quality (Table 1). Offspring of rich-diet mothers exhibited a marginally nonsignificant trend towards enhanced survival ($P = 0.09$), but there was no evidence of a paternal diet effect (Table 1).

*Development time*

Egg-to-adult development time was shorter for offspring reared on a rich larval diet (Table 1). Offspring of rich-diet mothers exhibited a marginally nonsignificant trend towards faster development overall ($P < 0.06$), and developed significantly faster on a poor-quality larval diet (maternal diet × offspring diet interaction, Table 1; Fig. 4). There was no evidence of a paternal diet effect.

*Adult body size*

Within the parental (F1) generation, diet treatment strongly affected body sizes of males (mean thorax length ± SD: rich diet: 2.73 ± 0.26 mm; poor diet: 1.78 ± 0.13 mm; Mann–Whitney $U$-test: $N_{\text{rich}} = 27$, $N_{\text{poor}} = 24$, $Z = 6.11$, $P < 0.0001$) and females (rich diet: 2.47 ± 0.14; poor diet: 1.87 ± 0.17; Mann–Whitney $U$-test: $N_{\text{rich}} = 26$, $N_{\text{poor}} = 25$, $Z = 6.08$, $P < 0.0001$). Similarly, offspring (F2) body size was affected by offspring diet, offspring sex and an offspring diet × sex interaction reflecting a diet effect on sexual size dimorphism (Tables 1 and 2). Offspring body size was also

**Table 1** Effects of maternal diet, paternal diet, offspring diet and offspring sex* on egg size, egg-to-adult survival rate, development time and adult body size of offspring†.

| | Egg size‡ | | Survival rate§ | | Development time§ | | Adult body size¶ | |
|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| Maternal diet | 9.907 | **0.0026** | 2.982 | 0.0921 | 3.853 | 0.0568 | 0.067 | 0.7969 |
| Paternal diet | 0.024 | 0.8771 | 1.009 | 0.3214 | 2.216 | 0.1446 | 4.154 | **0.0489** |
| MD × PD | 0.069 | 0.7933 | 0.002 | 0.9625 | 0.001 | 0.9786 | 0.020 | 0.8881 |
| Offspring diet | | | 74.671 | **< 0.0001** | 166.612 | **< 0.0001** | 337.595 | **< 0.0001** |
| OD × MD | | | 0.010 | 0.9193 | 7.367 | **0.0098** | 0.078 | 0.7814 |
| OD × PD | | | 0.430 | 0.51579 | 2.032 | 0.1620 | 2.512 | 0.1217 |
| Offspring sex | | | | | | | 10.482 | **0.0026** |
| OS × MD | | | | | | | 0.205 | 0.6537 |
| OS × PD | | | | | | | 0.040 | 0.8430 |
| OS × OD | | | | | | | 53.2341 | **< 0.0001** |

*Three- and four-way interactions are not shown (all $P > 0.35$).
†Significant effects are highlighted in bold.
‡Error d.f. = 56.
§Error d.f. = 39.
¶Error d.f. = 36.

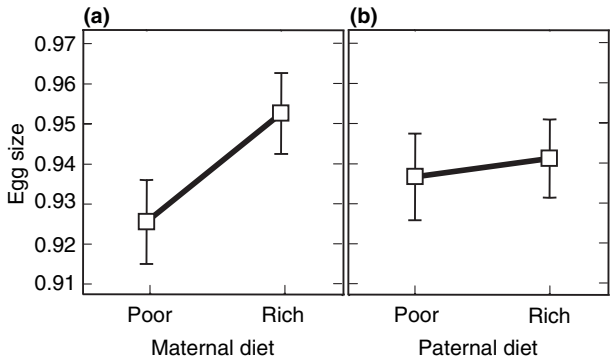


**Fig. 3** Effects of female (a) and male (b) larval diets on egg length in mm (bars show 95% confidence limits). Note that the analysis (Table 1) is based on transformed data (see *Materials and methods*).

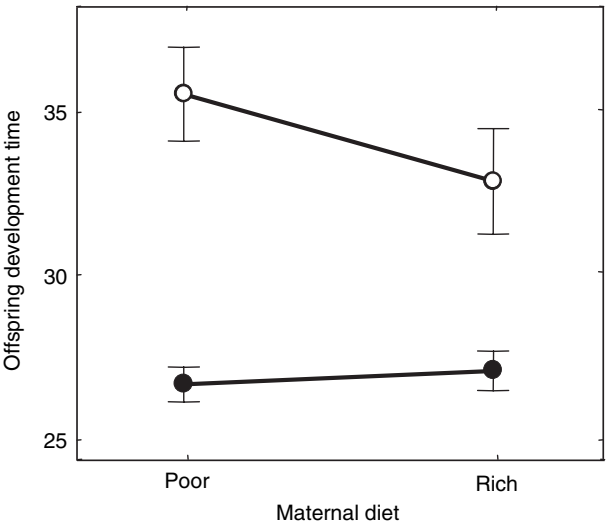


**Fig. 4** Maternal diet effect on egg-to-adult development time (days) of offspring reared on rich (closed circles) and poor (open circles) larval diets (bars show 95% confidence limits). Note that the analysis (Table 1) is based on transformed data (see *Materials and methods*).

affected by paternal diet (Tables 1 and 2): rich-diet (i.e. large) fathers produced significantly larger offspring. Removing the nonsignificant maternal diet effect ($P > 0.7$) strengthens the paternal diet effect ($F_{1,38} = 5.00$, $P = 0.0315$).

Because our design controls for genetic and maternal sources of variance (see *Discussion*), the magnitude of the paternal effect on offspring phenotype (i.e. the paternal effect coefficient) can be estimated from the least-squares slope of a regression of offspring body size on mean body sizes of rich- and poor-diet fathers (see Lande & Price, 1989). This paternal effect thus generates an offspring–paternal resemblance, analogous to a heritability for body size. However, whereas heritability reflects the transfer of genetic variation to offspring, the paternal effect reflects the transfer of environment-dependent variation in paternal condition to offspring. To facilitate comparison of this paternal effect with heritability estimates, which can be computed as twice the slope of a regression of offspring phenotype on paternal phenotype (Falconer & Mackay, 1996), we doubled the paternal effect coefficient, yielding a quantity that we will call *e*. Because diet treatment and sex affected phenotypic variances, we standardized the data within offspring diet × sex combinations prior to this analysis. For offspring reared on a rich larval diet, we obtained $e = 0.48$ (males) and $e = 0.49$ (females) and, for offspring reared on a poor larval diet, $e = 0.52$ (males) and $e = 0.62$ (females). Pooling across offspring diets and sexes yielded $e = 0.53$ (SE 0.312) (Fig. 5).

**Table 2** Mean and standard deviation (SD)* of offspring thorax length (TL) for each combination of paternal diet (PD), offspring diet (OD) and offspring sex (OS).

| Paternal diet | Offspring diet | Offspring sex | Offspring TL (mm) | SD |
|---|---|---|---|---|
| Rich | Rich | M | 3.01 | 0.133 |
| Rich | Rich | F | 2.64 | 0.056 |
| Rich | Poor | M | 1.83 | 0.301 |
| Rich | Poor | F | 1.87 | 0.249 |
| Poor | Rich | M | 2.97 | 0.177 |
| Poor | Rich | F | 2.62 | 0.095 |
| Poor | Poor | M | 1.71 | 0.211 |
| Poor | Poor | F | 1.73 | 0.185 |

*Based on $N = 18$ and 22 crosses involving rich- and poor-diet fathers, respectively, with one estimate of mean TL for each paternal diet × offspring diet × offspring sex combination.

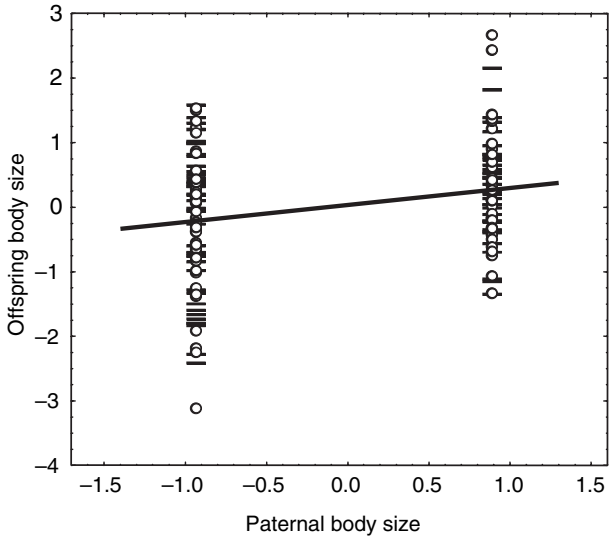


**Fig. 5** Paternal diet effect on offspring body size: regression of mean thorax length of offspring (bars: rich offspring diet, open circles: poor offspring diet) on mean thorax lengths of fathers reared on rich and poor larval diets (both axes in standard deviation units). Thorax length data were standardized within each offspring diet × sex combination to control for unequal phenotypic variances.

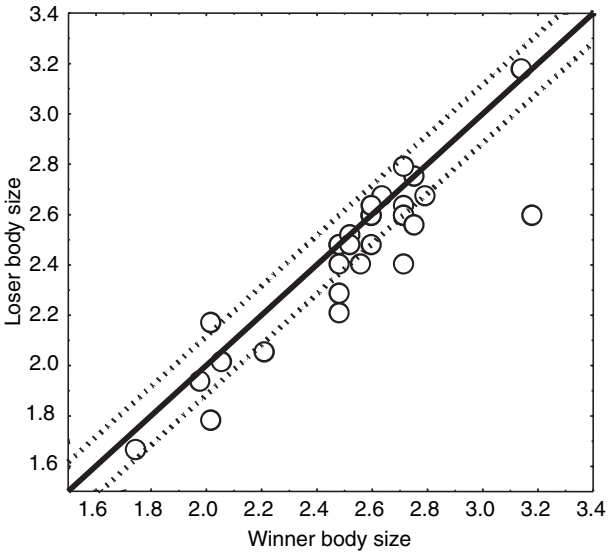


**Fig. 6** Body size and performance in sexual competition of rival males matched for similar body size: the plot shows 'winner' vs. 'loser' body size (thorax length in mm). The solid line represents equal body size, and the dotted lines show the minimum difference in body size between the paired males that resulted in a significant body size advantage for winners.

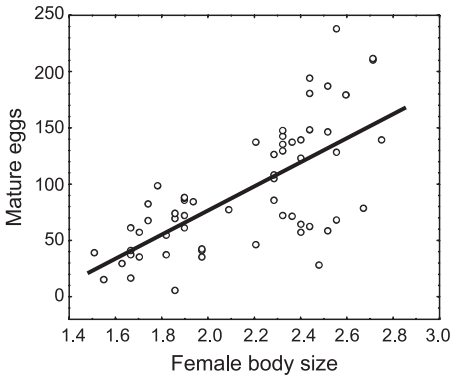


**Fig. 7** The number of mature eggs carried by females as a function of body size (thorax length in mm), with a fitted least-squares linear regression (see text).

## Body size and fitness

*Male mating success*

Successful males (winners) were larger than their rivals (Wilcoxon test: $N = 30$ male pairs, $Z = 3.26$, $P = 0.0011$; Fig. 6). The minimum difference in body size between rivals that yielded a significant size-advantage for winners was 0.116 mm (Wilcoxon test: $N = 21$ male pairs, $Z = 2.33$, $P = 0.0199$). This is less than the mean effect of paternal diet on body size of sons reared on a poor-quality diet (0.120 mm), but more than the effect on body size of sons reared on a rich larval diet (0.04 mm) (Table 2). Thus, sons of rich-diet fathers may have an advantage in sexual competition if they experience a poor larval diet.

*Female fecundity*

The number of mature eggs carried was a linear function of female thorax length (least-squares regression: $Y = -139 + 108X$; $N = 60$ females, $r^2 = 0.47$, $F_{1,58} = 51.3$, $P < 0.0001$; Fig. 7). The mean effect of paternal diet on daughters' thorax length was 0.140 mm for poor-diet daughters and 0.02 mm for rich-diet daughters (Table 2). Thus, the paternal diet effect could produce a fecundity differential per reproductive cycle of 15 eggs (27%, on

average) in daughters that experience a poor larval diet and two eggs (1.5%, on average) in daughters that experience a rich larval diet.

## Discussion

We found that variation in a key environmental determinant of condition – larval diet quality – is transferred across generations through maternal and paternal effects in *T. angusticollis*. We manipulated the environmental component of condition, while controlling for genotype, by randomly dividing eggs from each clutch between rich and poor larval diets. In the absence of selection, this yields sets of full siblings that are in high and low condition but genetically identical, on average. This manipulation was performed over two generations to test for effects of maternal, paternal and offspring conditions, and their interactions, on offspring. We found that females reared on a rich larval diet produced larger eggs, and their offspring developed more rapidly when reared on a poor larval diet. Males reared on a rich larval diet sired larger adult offspring, and this paternal effect appeared to be sufficient to increase offspring fitness, at least when the offspring are malnourished. Our findings suggest that maternal and paternal condition affect different aspects of offspring phenotype. Moreover, our findings support the emerging view that environment-dependent paternal effects can occur, and affect offspring phenotype and fitness, in species lacking conventional forms of paternal investment.

Although our experiment was designed to control for genotype, unequal larval survival rates on rich and poor larval diets may have resulted in differential selection that could confound our results by introducing genetic differences between flies from different diet treatments. However, differential selection appears to have had little effect on our results. Differential selection on genetic quality predicts that poor-diet (i.e. strongly selected) parents will produce high-quality offspring, but our results suggest the opposite: poor-diet parents produced smaller eggs, and their offspring developed more slowly on a poor larval diet, possibly had a higher larval mortality rate, and were smaller as adults. A related possibility is that paternal diet selected on a genetic polymorphism, whereby one genotype enhanced survivorship on a poor larval diet, and produced smaller body size through pleiotropy. This predicts that parents reared on a poor larval diet will produce offspring that perform better (e.g. have a higher survival rate, faster development or larger adult body size) on a poor larval diet. Contrary to this prediction, offspring of rich-diet parents appeared to perform better on both diets, but especially on a poor diet.

### Evolution and proximate basis of maternal and paternal effects

Wild insects often exhibit enormous within-population variation in condition, and this variation probably results, to a large extent, from variation in diet during the growth period. Food quality and quantity are likely to vary temporally (e.g. seasonally, or over much shorter time-scales for ephemeral resources such as rotting vegetation), as well as between patches (Kause *et al.*, 1999). Wild *T. angusticollis* larvae, which breed in rotting vegetation, must encounter substantial variation in diet quality, reflecting the intensity of larval competition, moisture, vegetation type, decomposition stage and other factors. However, mothers' and fathers' abilities to assess and predict such variation are probably limited because larvae feed deep inside the substrate, conditions may change considerably over the period of larval development, and individual flies are constrained by the ambient conditions and resource availability near their emergence site. Thus, there may be little opportunity in this system for parents to optimize offspring phenotype based on expected environmental conditions. Nonetheless, individuals that experience rich larval environments may benefit by transferring their phenotypic condition to their offspring (Qvarnström & Price, 2001). This may be especially true for males, which vary much more in body size than females (Bonduriansky, 2006, 2007). Indeed, our results suggest that parental effects in *T. angusticollis* function primarily in the transfer of parental condition to offspring, rather than in optimization of offspring phenotype for the conditions experienced by the parents, because offspring of high-condition parents performed better in both environments, and especially so in the resource-poor environment. Offspring of rich-diet mothers developed faster when those offspring were reared on poor diet. Offspring of rich-diet fathers were larger in both environments, and this effect may have been larger (albeit nonsignificantly so) for offspring reared on a poor larval diet (see *Results*).

Although both sexes may be selected to transfer their phenotypic condition to their offspring, maternal and paternal effects may be mediated by different proximate mechanisms, and affect different phenotypic traits and fitness components in offspring, as a result of sex differences in reproductive strategy and physiology. In particular, mothers have the ability to modulate egg size and resource content, and this is particularly likely to affect traits expressed in early ontogeny, such as embryonic and larval viability, and development rate (Mousseau & Fox, 1998; Fox & Czesak, 2000). Such maternal effects have been observed in many species (Mousseau & Dingle, 1991; Rossiter, 1996). We detected maternal (but not paternal) effects on egg size and development rate. We also observed a trend towards enhanced survival of offspring of rich-diet mothers.

In many insect species, males transfer nuptial gifts to females (Cumming, 1994; Vahed, 1998), or contribute directly to offspring provisioning (Hunt & Simmons, 2000; Rauter & Moore, 2002). Nupital gifts often appear to enhance female survival or fecundity, and are generally regarded as male mating investment (Markow, 1988;

Vahed, 1998; Savalli & Fox, 1999). For example, in the flies *Drosophila mojavensis* and *Prochyliza xanthostoma*, males transfer large ejaculates that increase female oviposition rate (Markow & Ankney, 1984; Markow, 1988; Markow *et al.*, 1990; Bonduriansky *et al.*, 2005). Environment-dependent paternal effects may also occur in many such species (e.g. Dussourd *et al.*, 1988; Gwynne, 1988; Smedley & Eisner, 1996; Hunt & Simmons, 2000) because variation in paternal phenotype can influence the quality or quantity of paternal provisioning, or modulate paternal behaviour towards offspring, and thus affect offspring phenotype and fitness. Even in such species, however, relatively few studies have tested for paternal effects (Fox & Czesak, 2000).

Even less is known about paternal effects in species where males make no obvious material contribution to offspring. However, recent evidence suggests that males may be able to influence their offspring through alternative mechanisms. Variation in male accessory gland products has been implicated in paternal effects on offspring viability in early ontogeny (García-González & Simmons, 2007; Ivy, 2007). Moreover, epigenetic 'reprogramming' of sperm DNA, which appears to occur in *Drosophila* and other animals (Jablonka & Lamb, 1995; Fitch *et al.*, 1998; Jablonka & Lamb, 2005), has been implicated in paternal transmission of environmental effects in mice (Anway *et al.*, 2005, 2006) and humans (Pembrey, 2002; Pembrey *et al.*, 2006). Because it functions in fathers only, this mechanism may represent an environment-dependent form of genomic imprinting (Fitch *et al.*, 1998; Anway *et al.*, 2005, 2006; Anway & Skinner, 2006; Chang *et al.*, 2006). *Telostylinus angusticollis* males transfer tiny ejaculates that are unlikely to convey nuptial gifts in the conventional sense. However, they could affect offspring growth rate via diet-dependent variation in accessory gland products or in the sperm epigenome. In either case, the inability of *T. angusticollis* males reared on poor larval diet to increase the body size of their offspring in the same way as males reared on rich larval diet suggests that the mechanism is costly and condition dependent. The apparent benefits incurred by offspring of high-condition males suggest that this paternal effect may be viewed as a form of paternal investment.

An alternative explanation for the observed effects of parental condition on offspring is an elevation of the germ-line mutation rate in stressful environments (Agrawal, 2002). This would account for reduced quality of offspring from parents reared on poor larval diet. However, this mechanism seems inconsistent with the finding that maternal and paternal diets had qualitatively different effects on offspring phenotype.

Another alternative explanation for the paternal diet effect is differential allocation by females, whereby females apportion more nutrients to eggs fertilized by males in high condition (Burley, 1986; Sheldon, 2000; Uller *et al.*, 2005). However, this is unlikely for two reasons. First, male diet had no effect on egg size (Table 1), so the quantity of resources allocated to eggs was unaffected by male phenotype (although we cannot rule out differential allocation through variation in resource quality). Second, *T. angusticollis* females do not appear to have an opportunity for differential allocation in the wild, and are thus unlikely to have evolved an ability to allocate differentially. This is because, as in most insects (Wigglesworth, 1972), eggs are fully chorionated prior to fertilization (R. Bonduriansky, unpublished data). Moreover, the association between mates in the wild is very brief, female re-mating rate is high (up to five matings per 30 min in the laboratory), and copulation is often followed immediately by oviposition (R. Bonduriansky, unpublished data). Hence, females probably cannot allocate differentially to eggs laid soon after mating, whereas ovules still in the process of formation at the time of mating are likely to be fertilized by a different male.

## Parental effects and the evolution of mating preferences

*Telostylinus angusticollis* females may benefit by mating with large males because such males transfer their condition to their offspring. In other words, because larger fathers produce larger offspring, and increased body size is likely to enhance fitness, this effect may strengthen indirect selection on female preferences. Similarly, effects of maternal condition on egg size, development rate and viability could select for male preferences for indicators of female condition (Bonduriansky, 2001).

The paternal diet effect observed in this study, and previous reports of environment-dependent paternal effects in species lacking conventional forms of paternal provisioning (Islam *et al.*, 1994; Huey *et al.*, 1995; Watson & Hoffmann, 1995; Magiafoglou & Hoffmann, 2003; Anway *et al.*, 2005; Anderson *et al.*, 2006; García-González & Simmons, 2007), provide a potential solution for the 'lek paradox' – the persistence of female preferences despite apparently low heritability of male quality, resulting from the depletion of additive genetic variation by directional selection (Kirkpatrick & Ryan, 1991; Van Homrigh *et al.*, 2007). Environment-dependent parental effects offer two potential solutions to this paradox. First, theory suggests that condition-dependent displays could act as honest advertisements of good genes for maternal or paternal investment via indirect genetic effects on trait expression (Wolf *et al.*, 1997, 1999). Females will then benefit by mating with attractive males because such males transmit good genes for maternal or paternal care to their offspring. Second, even stochastic variation in ambient conditions that is not influenced by genetic variation in parents (i.e. 'purely environmental' variation) could contribute to indirect selection on female preferences through the transfer of paternal condition to offspring. This mechanism is potentially important

because both direct and indirect genetic effects may diminish as directional selection depletes genetic variation (Miller & Moore, 2007), whereas purely environmental variation affects phenotypically plastic traits regardless of genetic variance. If such effects can occur in species lacking conventional forms of paternal provisioning, then purely environmental variation could provide an additional source of selection on female preferences in many systems. For similar reasons, environment-dependent paternal effects could inflate estimates of additive genetic variance in quantitative genetic analyses.

## Acknowledgments

We thank Tam-Chi Tran for excellent technical assistance, and Rob Brooks for providing laboratory facilities for this research. Funding was provided by the Australian Research Council through a postdoctoral fellowship and Discovery grant to RB.

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*Received 3 May 2007; revised 28 June 2007; accepted 10 July 2007*