**RESEARCH ARTICLE** 

# Paternal Effects on Offspring Fitness Reflect Father's Social Environment

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Abstract In many species, males influence phenotypic traits in their offspring through non-genetic paternal effects. Such effects can represent a form of paternal investment, and males may benefit by adjusting the effects depending on environmental parameters, such as operational sex ratio, so as to maximize offspring fitness. In the neriid fly Telostylinus angusticollis, fathers reared on a nutrient-rich larval diet produce larger offspring, independent of the rearing environment of the offspring. Here we asked whether this paternal effect was influenced by the social environment to which fathers were exposed. We found significant interactions of the effects of paternal larval diet quality and social environment (same-sex vs. mixed-sex groups) on offspring fitness-related traits. Fathers reared on a nutrient-rich diet produced larger male offspring when housed in mixed-sex groups. However, fathers reared on a nutrient-rich diet produced more viable offspring (or more viable sperm) when housed in same-sex groups prior to mating. These results suggest that fitnessenhancing paternal effects can trade off, consistent with parental investment theory on the offspring size-number trade-off, which suggests that these traits represent alternative investment options and parents are selected to optimize the balance based on a range of environmental variables. This is the first study to show that males can facultatively modulate paternal effects based on the social environment.

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**Keywords** Paternal effects · Social environment · Adaptive phenotypic plasticity · Development · Larval diet · Offspring size

# Introduction

Mounting evidence suggests that the environment experienced by a male can have important consequences for the phenotype and fitness of his offspring (Qvarnström and Price 2001; Bonduriansky and Head 2007; Ng et al. 2010; Bonduriansky and Day 2012). Such paternal effects have been demonstrated in the Australian neriid fly Telostylinus angusticollis, in which males fed a nutrient-rich larval diet are not only themselves larger but also produce larger offspring than males fed a nutrient-poor diet, and large body size confers important fitness advantages for both sexes, including competitive morphological traits in males and increased fecundity in females (Bonduriansky and Head 2007). However, the fitness value of a trait depends on multiple environmental variables. Given that a male's environment may predict the environment that his offspring are likely to encounter (Uller 2008), high-condition males may be selected to tailor paternal effects to the environment so as to confer the greatest advantage to their offspring.

The trade-off for parents between investment in offspring size versus number is well established (Smith and Fretwell 1974; Lloyd 1987), and theory predicts that the parental environment will inform optimal investment decisions (Mousseau and Fox 1998). The social environment is an important cue that can drive plasticity in many fitness-linked traits (Kokko and Rankin 2006; Immler et al. 2010; Kasumovic and Brooks 2011; Lemaître et al. 2011), and may influence the direction of parental effects,

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particularly when the same environment is likely to persist in the next generation. For males, competitive traits such as large body size may be particularly important when the operational sex ratio is moderately male-biased, creating intense competition for access to females. Fathers encountering such an environment may do well to invest in the production of large male offspring, likely to succeed in competition. However, if the sex ratio is very heavily malebiased (i.e. females are rare), investing in offspring numbers may pay off more than investing in offspring size, since finding a mate by chance may be more important than competitive ability. This sort of situation is predicted by Allen et al. (2008), who offer an alternative to the classic theory that as environments become more competitive, parents should invest in larger offspring, in a linear relationship. Rather, Allen et al. (2008) suggest that the relationship may be hyperbolic, with smaller, more numerous offspring favoured at either end of a competitive gradient.

Here, we examine the interaction of a male's developmental diet and his adult social environment (sex ratio) on two offspring fitness-related traits, egg-to-adult viability and adult body size, in T. angusticollis. This species exhibits a high degree of sexual dimorphism that is dependent on the developmental diet-males reared on nutrient-rich larval diets are on average 7 % longer than females reared on the same diet, and 35 % longer than males reared on nutrientpoor larval diets; there is no significant sex difference in body size for flies reared on poor larval diets (Bonduriansky 2007). Males reared on rich larval diets also develop secondary sexual characters, which are absent when reared on poor larval diets (Bonduriansky 2007). In the wild, these flies exhibit tremendous variation in body size and shape which can be easily observed on any given day within a single habitat (M. Adler and R. Bonduriansky, personal observations), suggesting that nutritional quality and/or degree of larval crowding is highly variable in natural populations. Thus, the larval diet manipulation used here is likely to mimic natural variation in developmental resource availability, suggesting that life-history responses to this manipulation reflect adaptive plasticity.

In addition, the social environment of *T. angusticollis* varies both temporally and spatially in the wild, as females tend to aggregate at oviposition sites on rotting tree bark, and this territory and the females that occupy it are defended fiercely by one or a few dominant males. Subordinate males tend to be found in locations where females are sparse or absent, such that the two sex ratios males encounter in the experiment reported here (all-male or mixed-sex) are fairly reflective of a natural situation. Male-male fights over dominance are common and often spectacular, commencing with mutual assessment and sometimes escalating to intense and prolonged combat if the males are of similar body sizes (M. Adler and

R. Bonduriansky, personal observations). Perhaps in part because these fights entail obvious costs to males, changes in the social environment of *T. angusticollis* have sex-specific effects on lifespan and ageing (Adler and Bonduriansky 2011).

#### **Materials and Methods**

Eggs were collected from cages of approximately 30 males and 30 females, obtained upon eclosion from stock cages and maintained in the lab as described in Adler and Bonduriansky (2011). Eggs were transferred alternately into 250-mL containers of fresh "Rich" or "Poor" larval medium provided ad libitum, with 50 eggs per container, enough to minimize any effects of density in either larval diet treatment. Rich and poor larval media differed threefold in concentration of protein and carbohydrates (see Bonduriansky 2007 for details).

Within each larval treatment, adult flies (which attain sexual maturity a few days after eclosion) were assigned randomly to adult social environment treatments immediately after emergence. All flies (n = 720) were transferred in groups of 10 into 1-L cages. Within each larval diet (rich and poor), adult social environment treatments consisted of flies in groups of 10 males, groups of 10 females, and equal-sex-ratio groups of 5 males and 5 females. Each adult social treatment was replicated 12 times, for a total of 36 cages within each of the 2 larval diet treatments (n = 72cages in total). The effects of treatments on longevity and other fitness components, including in all-female cages, are reported in a companion manuscript (M. Adler, E. Cassidy, C. Fricke, R. Bonduriansky, in preparation). Adult diet was also manipulated, in a full-factorial design, by providing flies with either protein and sugar or sugar only. Adult diet had no significant effect on its own or as a term in any interactions, so it was removed from the models.

Experimental cages were covered with mesh stockings to allow for ventilation, and polyester fabric, moistened every other day as a source of water for the flies, was used to cover the floor of each cage. Flies were kept on a 12 h–12 h light– dark cycle using a combination of broad-spectrum fluorescent and incandescent lighting, at an approximately constant temperature of 25 °C at 50 % humidity.

# Male Reproductive Performance Assay

One male (aged 23 days from eclosion) from each experimental cage was paired with a 21-day-old virgin female (reared on rich larval medium and provided with sugar, yeast and rich oviposition medium ad libitum and housed in one of five 2-L cages each containing about 20 females since eclosion) inside a 250-mL cage containing a dish of sugar and a 12-mL petri dish of pre-moulded poor larval medium sprinkled with brown sugar for oviposition. The male was removed after 24 h, and the oviposition container was checked daily for eggs. Twenty eggs (where possible) were collected from each female and transferred to a jar of poor larval medium and kept in an environment chamber at an alternating 12 h–12 h cycle of 25 and 23 °C at 70 % humidity. Upon eclosion, adult flies (offspring) were frozen and later sexed and photographed (with wings removed) under a Leica MS5 stereoscope fitted with a Leica DFC420 camera. Image J software (National Institutes of Health) was used to measure thorax length as an index of body size (all imaging and measurements performed by MIA).

#### Statistical Analysis

Analyses of variation in offspring body size and viability were performed for individual males as observational units, since each male was from a separate cage. Prior to analysis, offspring viability (the proportion of eggs that survived to adulthood) was arcsine-transformed, whereas offspring body size was standardized (i.e., transformed to Z-scores). Both variables conformed to the assumptions of parametric testing after transformation. We first performed an ANOVA on the mean body size of offspring from each male, pooling offspring sexes by computing weighted means (with the available sex substituted in five cases in which only one offspring sex was obtained). We then performed separate ANOVAs for offspring of each sex. In all analyses, paternal larval diet (Rich or Poor), paternal social environment (same-sex or mixed-sex groups) and their interaction were fitted as fixed factors.

# Results

See Table 1 for ANOVA results. Fathers reared on a rich larval diet produced larger offspring than fathers reared on a poor larval diet, but only when the fathers were housed in

mixed-sex groups prior to the mating assay. However, when male and female offspring were analysed separately, this interaction was significant only for male offspring, which were larger by about 1.3 standard deviations when their father was reared on a rich larval diet and maintained in a mixed-sex group than when their father was reared on a poor larval diet and maintained in a mixed-sex group (see Fig. 1a). In female offspring, this interaction was non-significant, although the direction of the trend was the same (female offspring of fathers reared on a rich larval diet and housed in mixed sex groups were about 0.7 standard deviations larger than female offspring of fathers reared on poor larval diet, but almost no difference in body sizes was observed for female offspring of fathers maintained in same-sex groups).

Fathers reared on a rich larval diet produced offspring with higher egg-to-adult viability (proportion of adults emerging out of 20 eggs transferred), but only when those fathers were housed in same-sex groups prior to the mating assay (Fig. 1b).

# Discussion

We show that a father's developmental (larval) diet interacts with his social environment in affecting offspring viability and body size. Males developing on a rich larval diet gain a reproductive advantage over males on a poor larval diet: Males from rich larval diets are larger, and have relatively larger secondary sexual traits (Bonduriansky 2007). However, these "high-condition" males enhance different aspects of the fitness of their offspring, depending on the social environment. For fathers housed in mixed-sex groups prior to mating, where the operational sex ratio would be moderately male-biased as a result of females being more reproductively limited than males, fathers reared on a rich larval diet have larger male offspring than fathers reared on a poor larval diet. For fathers housed in same-sex groups prior to mating, where the operational sex ratio is highly male-biased, fathers reared on a rich larval

Table 1 Results of ANOVAs for effects of paternal larval diet and sex ratio on offspring egg-to-adult viability and body size

Predictor	Egg-to-adult viability <sup>a</sup>		Offspring body size (both sexes) <sup>b</sup>		Male offspring body size <sup>c</sup>		Female offspring body size <sup>d</sup>	
	F	Р	F	Р	F	Р	F	Р
Larval diet	0.785	0.389	0.901	0.349	2.599	0.117	0.692	0.411
Sex ratio	0.006	0.938	0.599	0.444	0.022	0.882	0.141	0.710
Larval diet $\times$ Sex ratio	4.401	0.042	6.399	0.016	5.366	0.027	1.886	0.178

Values in bold are statistically significant

<sup>a</sup> Error DF = 39

<sup>b</sup> Error DF = 36

<sup>c</sup> Error DF = 31

<sup>d</sup> Error DF = 36



**Fig. 1** Interactions of father's larval diet and adult social environment on offspring fitness traits: (a) Rich larval diet fathers (*black lines*) have larger male offspring than poor larval diet fathers (*grey lines*) when housed in mixed-sex groups (standardized body size +- SE); (b) Rich larval diet fathers (*black lines*) have a greater proportion of eggs eclosing as adult flies than poor larval diet fathers (*grey lines*) when housed in same-sex (all-male) groups

diet have more offspring surviving to eclosion (or are able to fertilize more eggs) compared with fathers reared on a poor larval diet.

In the case of egg-to-adult viability, we note that female *T. angusticollis* can lay unfertilized eggs, and so it is also possible that females mated to males reared on a rich larval diet and housed in same-sex cages simply laid more fertilized eggs. This could happen, for example, if mating in the mixed-sex treatment depletes male sperm or ACP reserves or initiates an adaptive strategy to transfer smaller ejaculates per mating given an increased chance of future mating opportunities. However, we suggest that in this case it is unlikely that the difference in eggto-adult viability between males from the two sex-ratio treatments is driven solely by a difference in the number of sperm transferred. If males from the same-sex treatments had increased sperm stores or were investing more per mating, then we would also expect their offspring to be large when the males were reared on a rich larval diet (as observed for males housed in mixed-sex groups). Since this was not the case, we suggest that the most likely explanation for our results is that the rich-larval-diet males were investing in different ejaculate traits on the basis of their social environment. Males reared on a rich larval diet and maintained in mixed-sex groups apparently invested in a paternal effect that conferred increased offspring body size, but at the cost of reduced offspring viability or reduced fertilization capacity. Conversely, males reared on a rich larval diet but maintained in samesex groups invested in ejaculates that were more effective at fertilizing eggs, or conferred higher survival probability in the offspring, but at the cost of reduced offspring body size

An effect of paternal larval diet on offspring body size was demonstrated by Bonduriansky and Head (2007). That study found no parental effects on viability, but all parents were maintained as male–female pairs since shortly after eclosion.

Paternal effects in T. angusticollis do not appear to involve transfer of nutrients to offspring (Bonduriansky and Head 2007), and appear to be conferred through seminal products (such as accessory gland proteins, or ACPs) rather than sperm (A. Crean and R. Bonduriansky, in preparation). ACPs can be affected by the male's developmental diet (e.g. Baker et al. 2003), and can influence multiple aspects of female reproduction, including egg development and incorporation of the yolk into oocytes (Gillott 2003). Because ACPs can be incorporated into a female's eggs and ovaries, they have the potential to affect offspring phenotype and fitness (Sirot et al. 2006), and are thought to mediate a paternal effect on embryo viability in the Australian field cricket Teleogryllus oceanicus (Garcia-Gonzalez and Simmons 2005, 2007). Although ACPs are a likely mechanism for the paternal effects reported here, it remains unclear exactly how ACPs may mediate such effects. However, ACPs have sometimes been seen as "nuptial gifts" in species which transfer costly nutrients with the semen (Sirot et al. 2006), potentially allowing males to boost the survival chances or nutrient-limited development of their offspring. The fact that the effect we report on offspring body size was significant only in male offspring suggests that the sexes may differ in sensitivity to ACPs during development. Sexspecific sensitivity to developmental environment has been demonstrated, for example, in effects of larval nutrition on viability in Drosophila melanogaster (Andersen et al. 2010).

Our results suggest that fitness-enhancing paternal effects can trade off, such that fathers may invest in either increased offspring number (via enhanced fertilization success or enhanced survival prospects of developing offspring) or in increased offspring body size, but not both. This is consistent with parental investment theory on the offspring size-number trade-off (Smith and Fretwell 1974), which suggests that these traits represent alternative investment options and parents are selected to optimize the balance based on a range of environmental variables. Moreover, it has been demonstrated that female bryozoans, Bugula neritina, produce larger offspring in moderately competitive environments, but many, smaller offspring in environments with few conspecific individuals (Allen et al. 2008). While nongenetic parental effects have traditionally been thought to be restricted to females, recent theory and evidence support our findings that such effects may originate from fathers as well (Bonduriansky and Day 2009). For *T. angusticollis*, a male that does not encounter females early in adulthood may opt to allocate extra resources to offspring number, which may increase the chance that some of his offspring will find a mate when females are rare. Males that do encounter females early, however, may be better served by putting their extra resources into making their offspring more competitive as adults by investing in larger offspring body size. As body size affects reproductive success in T. angusticollis (Bonduriansky and Head 2007), this strategy is likely to pay off for fathers that can afford it. T. angusticollis males are often found in the wild in groups without females, and may have evolved plasticity in resource-use based on the availability of females (Adler and Bonduriansky 2011). Our findings suggest that the social environment experienced by a male informs his optimal use of resources acquired during the larval stage, which he may translate to benefits to his offspring via non-genetic paternal effects.

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