biology letters
Evolutionary biology

Biol. Lett. (2011) 7, 269–272
doi:10.1098/rsbl.2010.0872
Published online 27 October 2010

Dietary protein content affects evolution for body size, body fat and viability in *Drosophila melanogaster*

Torsten N. Kristensen[1,2], Johannes Overgaard[1], Volker Loeschcke[1] and David Mayntz[1,2,*]

[1]*Department of Biological Sciences, Aarhus University, Ny Munkegade, Building 1540, 8000 Aarhus C, Denmark*
[2]*Department of Genetics and Biotechnology, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark*
**Author for correspondence (david.mayntz@biology.au.dk).*

The ability to use different food sources is likely to be under strong selection if organisms are faced with natural variation in macro-nutrient (protein, carbohydrate and lipid) availabilities. Here, we use experimental evolution to study how variable dietary protein content affects adult body composition and developmental success in *Drosophila melanogaster*. We reared flies on either a standard diet or a protein-enriched diet for 17 generations before testing them on both diet types. Flies from lines selected on protein-rich diet produced phenotypes with higher total body mass and relative lipid content when compared with those selected on a standard diet, irrespective of which of the two diets they were tested on. However, selection on protein-rich diet incurred a cost as flies reared on this diet had markedly lower developmental success in terms of egg-to-adult viability on both medium types, suggesting a possible trade-off between the traits investigated.

Keywords: body composition; experimental evolution; nutrition; plasticity; viability

1. INTRODUCTION

The amount and quality of nutrients consumed by organisms have a strong impact on life-history traits, such as disease vulnerability, fertility, reproduction, longevity and stress resistance [1–3]. Studies concerned with the impact of nutrition often assess the physiological and morphological responses of individuals exposed to different quality and amount of nutrients. These include studies of the impact of nutrition on body composition [4,5], stress tolerance [6,7], and reproduction and longevity [8–11]. Less is known about the long-term/evolutionary consequences of transitions to different diets, although the potential interactions between 'historical' and 'present' diets will ultimately determine the fitness of the resulting phenotype. A classical example of this interaction is seen in humans where native Americans, Inuit and Polynesian populations, which have been adapted to specific food sources for centuries, are more prone to lifestyle-related diseases such as diabetes and cardiovascular diseases when exposed to 'modern' diets with highly processed energy-dense food [12–14]. Artificial selection experiments have also shown that the selection response is dependent on the nutritional environment, for instance in mice selected for larger body size [15]. Within insects, studies have used experimental evolution to study the impact of diet composition and it has been shown that rearing under alternative diet regimes for multiple generations results in genetically based changes in life-history traits [16,17].

Drosophila melanogaster is known to feed and breed on ripe or rotting fruit [18] where protein/carbohydrate ratios vary temporarily and spatially [4,19]. Together with the fact that *D. melanogaster* has a short generation time, this makes this species ideal for experimental studies aiming at investigating how diet composition shape the evolution of life-history traits. Here, we test how laboratory selection on diets with varying protein content affect body composition and viability. We investigate the interaction between plastic and evolutionary responses to diets with different protein and lipid content, using replicated experimental evolution approaches.

2. MATERIAL AND METHODS

(a) *Fly populations and larvae diets*

Five independent replicate lines of *D. melanogaster* were used in the experiment. They originated from a genetically diverse mass population reared in the laboratory since 2002. Prior to the experiment flies were kept on a basic 'Leeds medium', composed of sucrose (40 g l^{-1} water), yeast (60 g l^{-1} water), agar (16 g l^{-1} water), oatmeal (30 g l^{-1} water), nipagen (12 ml l^{-1} water) and acetic acid (1.2 ml l^{-1} water). In all generations prior to the experiment reported here, lines were held at high census size (greater than 1000). In June 2008, flies from all lines were transferred to two novel types of diets; either instant Carolina medium (Carolina Biological Supply, Burlington, NC, USA, formula 4–24 plain) or a protein-enriched diet. Carolina fly medium is a widely used larvae diet for long-term maintenance of *D. melanogaster*. The protein-enriched diet was made by mixing 60 per cent casein (Sigma C-5890, Sigma-Aldrich) and 40 per cent Carolina medium on the basis of dry weight. Water was added to the medium on a 1 : 1 volume ratio (5 ml water to 1 g medium).

Five independent replicate lines were set-up on either the standard diet (Carolina medium) or the protein-enriched diet and maintained for 17 generations. In each generation five bottles with approximately 200 flies per bottle were set-up per line and diet type. Throughout the experiment flies were reared at 20°C and 12 h light/12 h dark cycles. After 17 generations of rearing on the two diet types, flies from both diet regimes were transferred back to Leeds medium, where they were kept for three generations before testing. This procedure was followed to enable testing of flies in a common garden set-up.

(b) *Traits investigated*

After three generations on Leeds medium (density controlled by allowing 100 flies to lay eggs for 6 h in each of 10 bottles per line), we collected eggs and distributed these onto two experimental rearing diets, consisting of either standard diet or protein-enriched diet. For each of the 20 experimental groups (two selection diets each with five independent lines tested on two test diets), we collected eggs for three replicate vials with 60 eggs in each 40 ml vials. All vials contained 7 ml of diet medium, and egg-to-adult viability was determined from the number of emerging flies.

Approximately 10 female flies (in a few cases we did not get 10 females) were sampled from each combination of selection diet, test diet and line for determination of body size and lipid content. Flies were dried at 60°C for 48 h and dry mass was measured to the nearest 10 μg. Body fat was removed by placing individual flies in 1 ml pure petroleum ether for 24 h after which the supernatant was removed. This procedure was repeated, after which the flies were dried again before determination of lean body mass. The total body fat was estimated as initial dry mass minus lean body mass.

Received 21 September 2010
Accepted 6 October 2010

Table 1. Results from the nested ANOVA testing effects of line, selection diet, test diet and interaction between selection and test diets on dry body mass, lean body mass, per cent body lipid and egg-to-adult viability (per cent emerged flies).

source of variation	d.f.	sum of squares	*F*-ratio	*p*-value
dry mass of flies				
line (selection diet, test diet)	14	0.07	3.56	<0.0001
selection diet	1	0.01	7.47	0.007
test diet	1	0.24	180.90	<0.0001
selection diet × test diet	1	0.00	0.84	0.36
error	158	0.19		
lean body mass of flies				
line (selection diet, test diet)	14	0.04	2.69	0.002
selection diet	1	0.005	4.23	0.04
test diet	1	0.25	234.7	<0.0001
selection diet × test diet	1	0.00	0.78	0.38
error	158			
per cent body fat of flies				
line (selection diet, test diet)	14	0.11	3.21	0.0002
selection diet	1	0.01	4.69	0.03
test diet	1	0.08	31.52	<0.0001
selection diet × test diet	1	0.00	0.00	0.99
error	158	0.33		
per cent emerged flies				
line (selection diet, test diet)	16	9.25	13.15	<0.0001
selection diet	1	3.06	69.73	<0.0001
test diet	1	0.04	0.86	0.36
selection diet × test diet	1	0.11	2.42	0.12
error	100	4.39		

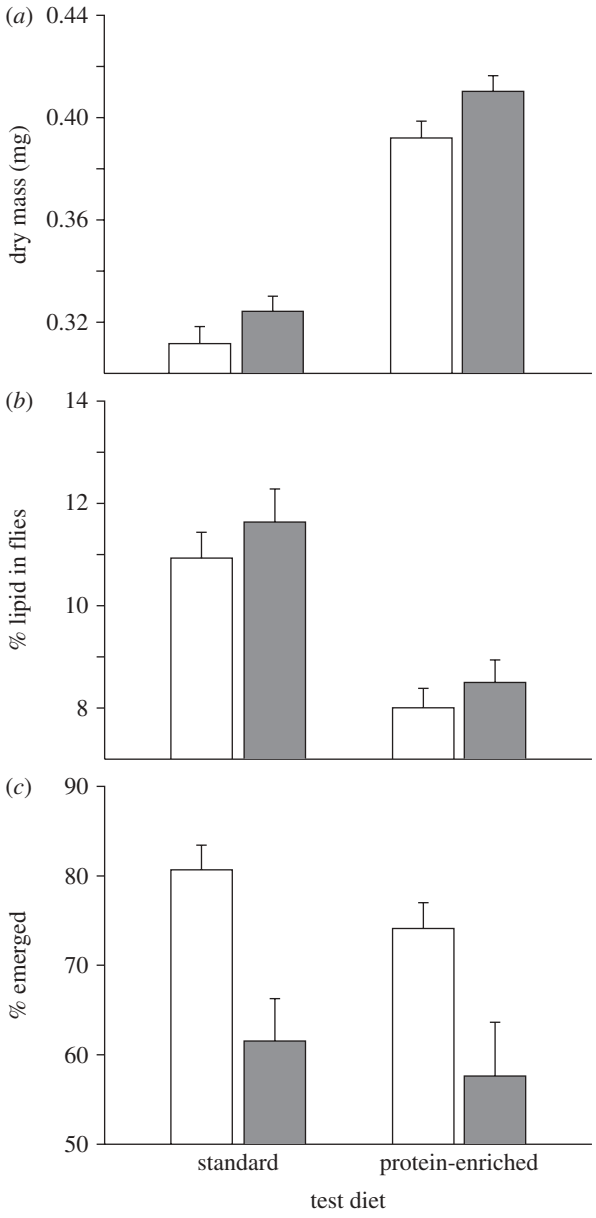

Figure 1. Dry body mass, per cent body lipid and egg-to-adult viability of flies developing on different test diets (standard diet and protein-enriched diet). Dark columns are lines reared for 17 generations at the protein-enriched diet and white columns are lines reared for 17 generations at the standard diet prior to testing. Bars represent mean + s.e.

(c) *Statistical analyses*

Nested analysis of variances (ANOVAs) were used to test the effects of selection diet (Carolina or protein-enriched), test diet (Carolina or protein-enriched) and their interaction on egg-to-adult viability, body dry mass, lean body mass and relative fat composition. Line was nested within selection diet and test diet. Data on viability and relative body fat were arcsin square root transformed before further analyses.

3. RESULTS

For all traits investigated, line effects were highly significant (table 1) suggesting that lines responded variably to rearing diet and test diet. Dry body mass and body fat composition were both significantly affected by selection diet with dry mass and lipid content being higher in flies maintained on the protein-enriched diet (table 1 and figure 1). Flies tested on protein-enriched diet had a higher dry mass and relative lower lipid content compared with flies tested on standard diet (figure 1). This was seen regardless of whether flies had been reared on protein-enriched diet or standard diet for 17 generations prior to testing. Lean body mass was significantly affected by line, selection diet and test diet (table 1). Egg-to-adult viability was significantly affected by selection diet with lower viability observed in lines selected on the protein-enriched diet (figure 1 and table 1). We never observed significant interactions between selection diet and test diet (figure 1 and table 1). Thus, the evolved responses under different dietary protein compositions were consistent across test diets.

4. DISCUSSION

Our results demonstrate both plastic and evolutionary consequences on life-history traits when *D. melanogaster* was exposed to nutritional environments with different protein composition. Rearing flies on diets with different protein content resulted in a clear genetic differentiation after 17 generations. Although flies

adapted to the two diet types had different dry mass, lipid content and egg-to-adult viability, our results show that the plastic response to the diet was generally greater than the genetic response for dry mass and per cent lipid (table 1 and figure 1). The average difference caused by evolutionary changes between flies adapted to the two diet types were 6 and 5 per cent for dry mass and body lipid composition, respectively, whereas the acute plastic response to a change in diet type were 25 and 36 per cent for those traits (figure 1). For egg-to-adult viability flies maintained on the protein-enriched diet for 17 generations maintained on average three quarters of the viability of flies at the standard diet whereas the effect of test diet was non-significant for this trait.

Our study raises the question as to why higher environmental protein availability facilitated directional selection for larger body mass. It has been suggested that larger flies have higher reproductive success [20,21], or improved competitive ability [22]. As amino acids are often the limiting building materials during periods of high mass increment, a protein-poor environment may have impaired selection for flies of large size, while in the protein-enriched environment this constraint is absent and therefore directional selection for higher body mass is possible.

We also found that flies maintained on the protein-enriched diet for 17 generations evolved to become relatively fatter than flies maintained on the standard diet. One mechanism that may have allowed such change could be selection for different utilization efficiency of carbohydrates [16]. Flies maintained on the protein-enriched diet have experienced relatively low access to easy accessible energy sources (carbohydrates). Under such conditions, flies with improved ability to store lipids might have a selective advantage. In contrast, when surplus carbohydrates but limited protein are available a decreased utilization efficiency of carbohydrates may have allowed higher consumption of food to satisfy protein needs without providing the same costs of carrying large-fat deposits.

The fact that the flies evolving under protein-rich/carbohydrate poor conditions had reduced egg-to-adult viability suggests a trade-off between the investigated traits. The trade-off between egg-to-adult survival and body mass could suggest that a limiting shared resource is divided between the two traits. However, the trade-off was found on both diet types. Thus, it is more likely that the trade-off is caused by antagonistic pleiotropy, whereby alleles coding for larger body size which is advantageous under protein-enriched conditions, at the same time have a negative effect on physiological processes that affect survival.

The novel aspect of this study is that natural laboratory selection on a protein-enriched diet favours genetically larger and fatter flies with lower survival probabilities during juvenile life-stages. If this result can be extrapolated to livestock and humans, it introduces interesting challenges and potentials in relation to breeding strategies and diet recommendations. In livestock production, variation in diet composition between farms may affect 'natural selection' of important production traits related to fertility and body composition. Furthermore, results from this experiment indicate that trade-offs between fitness traits may exist when dietary protein content is varied. This may potentially have consequences for populations (including human populations) that in recent times have changed their diet fundamentally. Our data suggest that such a change may simply provide an immediate challenge to the generations exposed to the change. Evolutionary adaptation to the new diet may potentially produce an additional risk through unfavourable trade-offs.

We thank Doth Andersen and Marie Rosenstand Hansen for laboratory assistance and the Danish Research Councils for economic support to T.N.K., V.L., J.O. and D.M.

1 Hoffmann, A. A. & Parsons, P. A. 1991 *Evolutionary genetics and environmental stress*. Oxford, UK: Oxford University Press.
2 Rion, S. & Kawecki, T. J. 2007 Evolutionary biology of starvation resistance: what we have learned from *Drosophila*. *J. Evol. Biol.* **20**, 1655–1664. (doi:10.1111/j.1420-9101.2007.01405.x)
3 Lee, K. P., Simpson, S. J. & Wilson, K. 2008 Dietary protein-quality influences melanization and immune function in an insect. *Funct. Ecol.* **22**, 1052–1061. (doi:10.1111/j.1365-2435.2008.01459.x)
4 Markow, T. A., Raphael, B., Breitmeyer, C. M., Dobberfuhl, D., Elser, J. & Pfeiler, E. J. 1999 Elemental stoichiometry of *Drosophila* and their hosts. *Funct. Ecol.* **13**, 78–84. (doi:10.1046/j.1365-2435.1999.00285.x)
5 Kaun, K. R., Chakoborty-Chatteriee, M. & Sokolowski, M. B. 2008 Natural variation in plasticity of glucose homeostasis and food intake. *J. Exp. Biol.* **211**, 3160–3166. (doi:10.1242/jeb.010124)
6 Smith, E. M., Hoi, J. T., Eissenberg, J. C., Shoemaker, J. D., Neckameyer, W. S., Ilvarsonn, A. M., Harshman, L. G., Schlegel, V. L. & Zempleni, J. 2007 Feeding *Drosophila* a biotin-deficient diet for multiple generations increases stress resistance and lifespan and alters gene expression and histone biotinylation patterns. *J. Nutr.* **137**, 2006–2012.
7 Andersen, L. H., Kristensen, T. N., Loeschcke, V., Toft, S. & Mayntz, D. 2010 Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult *Drosophila melanogaster*. *J. Insect Physiol.* **56**, 336–340. (doi:10.1016/j.jinsphys.2009.11.006)
8 Bross, T. G., Rogina, B. & Helfand, S. L. 2005 Behavioral, physical, and demographic changes in *Drosophila* populations through dietary restriction. *Aging cell* **4**, 309–317. (doi:10.1111/j.1474-9726.2005.00181.x)
9 Broughton, S. J. *et al.* 2005 Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc. Natl Acad. Sci. USA* **102**, 3105–3110. (doi:10.1073/pnas.0405775102)
10 Pijpe, J., Brakefield, P. M. & Zwaan, B. J. 2007 Phenotypic plasticity of starvation resistance in the butterfly *Bicyclus anynana*. *Evol. Ecol.* **21**, 589–600. (doi:10.1007/s10682-006-9137-5)
11 Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W. O., Taylor, P. W., Soran, N. & Raubenheimer, D. 2008 Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc. Natl Acad. Sci. USA* **105**, 2498–2503. (doi:10.1073/pnas.0710787105)

12 Bennett, P. H., Burch, T. A. & Miller, M. 1971 Diabetes mellitus in American (Pima) Indians. *Lancet* **298**, 125–128. (doi:10.1016/S0140-6736(71)92303-8)
13 Galanis, D. J., McGarvey, S. T., Quested, C., Sio, B. & Afele-Fa'amuli, S. A. 1999 Dietary intake of modernizing Samoans: implications for risk of cardiovascular disease. *J. Am. Diet. Assoc.* **99**, 184–190. (doi:10.1016/S0002-8223(99)00044-9)
14 Kittler, P. G. & Sucher, K. P. 2007 *Food and culture*, 5th edn. Belmont, CA: Wadsworth Publishing.
15 Nielsen, B. V. H. & Andersen, S. 1987 Selection for growth on normal and reduced protein diets in mice: direct and correlated responses for growth. *Genet. Res. Camb.* **50**, 7–15. (doi:10.1017/S0016672300023272)
16 Warbrick-Smith, J., Behmer, S. T., Lee, K. P., Raubenheimer, D. & Simpson, S. J. 2006 Evolving resistance to obesity in an insect. *Proc. Natl Acad. Sci. USA* **103**, 14 045–14 049. (doi:10.1073/pnas.0605225103)
17 Kolss, M., Vijendravarma, R. K., Schwaller, G. & Kawecki, T. J. 2009 Life-history consequences of adaptation to larval nutritional stress in *Drosophila*. *Evolution* **63**, 2389–2401. (doi:10.1111/j.1558-5646.2009.00718.x)
18 Elser, J. J., Dobberfuhl, D. R., MacKay, N. A. & Schampel, J. H. 1996 Organism size, life history, and N : P stoichiometry—toward a unified view of cellular and ecosystem processes. *Bioscience* **46**, 674–684. (doi:10.2307/1312897)
19 Arvanitoyannis, I. S. & Mavromatis, A. 2009 Banana cultivars, cultivation practices, and physicochemical properties. *Crit. Rev. Food Sci. Nutr.* **49**, 113. (doi:10.1080/10408390701764344)
20 Santos, M., Ruiz, A., Barbadilla, A., Quezada-Diaz, J. E., Hasson, E. & Fontdevila, A. 1988 The evolutionary history of *Drosophila buzzatii*. XIV. Larger flies mate more often in nature. *Heredity* **61**, 255–262. (doi:10.1038/holy.1988.113)
21 Partridge, L. & Fowler, K. 1993 Responses and correlated responses to artificial selection on thorax length in *Drosophila melanogaster*. *Evolution* **47**, 213–226. (doi:10.2307/2410130)
22 Warren, M., McGeoch, M. A., Nicolson, S. W. & Chown, S. L. 2006 Body size patterns in *Drosophila* inhabiting a mesocosm: interactive effects of spatial variation in temperature and abundance. *Oecologia* **149**, 245–255. (doi:10.1007/s00442-006-0434-z)