Nutritional geometry of mitochondrial genetic effects on male fertility

M.F. Camus[1], J. Moore[1], M. Reuter[1]

[1] *Research Department of Genetics, Evolution and Environment, University College London, Gower Street, London, WC1E 6BT, United Kingdom*

Words: 2495

Keywords: reproduction, mitochondria, nutrition, nutritional geometry

Abstract

Organismal fitness is partly determined by how well the nutritional intake matches sex-specific metabolic requirements. Metabolism itself is underpinned by complex genomic interactions involving products from both nuclear and mitochondrial genomes. Products from these two genomes must coordinate how nutrients are extracted, utilised and recycled; processes vital for fuelling reproduction. Given the complicated nature of metabolism, it is not well understood how the functioning of these two genomes is modulated by nutrients. Here we use nutritional geometry techniques on *Drosophila* lines that only differ in their mtDNA, with the aim to understand if there is nutrient-dependent mitochondrial genetic variance for male reproduction. We first find genetic variance for diet consumption, indicating that flies are consuming different amounts of food to meet new physiological requirements. We then find an interaction between mtDNA and diet for fitness, suggesting that the mtDNA plays a role in modulating diet-dependent fitness. Our results enhance our basic understanding of nutritional health and our chimeric genomes.

Introduction

A large determinant of organismal fitness is the acquisition of nutrients that fuel reproductive efforts (1). In nature, species have to tailor their behaviour and physiology to maximise the metabolic and energetic functions underlying fitness, while working within the constraints of the resources available (2). Beyond this external constraint, there are internal (genetic) constraints that modulate how nutrients are extracted, utilised and recycled; all of which have downstream fitness consequences. There are two main steps that influence nutrient metabolism. The first is the behavioural regulation that determines how much food is consumed (3). This is based on environmental cues relating external diet quality/quantity and feedback about the animal's internal state that is provided by nutrient sensing pathways (4-6). The second, and potentially more important aspect is how nutrient composition shapes metabolic flux, with downstream effects on cellular processing, ultimately affecting fitness (7). Both steps rely on a large number of genetically encoded elements and accordingly, there can be genetic variation in behavioural and metabolic processes. For instance, previous work has documented genetic variation in sex-specific nutritional requirements (8).

Importantly however, genetic variation in the above study was restricted to the nuclear genome. Nuclear genes (nuDNA) are not the only genetic determinant of metabolic function. Genes encoded within the mitochondria (mtDNA) also play a major role in metabolism, signalling and its regulation (9). We would therefore expect fitness to depend on the interaction between both genomes, and this supposition has been validated across several studies (10-12). Despite the importance of mitochondrial function for metabolic regulation and efficiency, the effects of mitochondrial genetic variation on diet-dependent fitness remain under explored. Previous work in *Drosophila* has shown mtDNA-specific effects on mitochondrial physiology, with these effects being contingent on the diet that flies had been reared on (13). More recently, studies have shown that changes in the mtDNA genome can also have diet-dependent effects on longevity (14, 15). What remains to be established is how changes in diet-dependent fitness are modified by the mtDNA genome. We can predict from these studies that changing the composition of dietary macronutrients (by changing environments and/or nutritional availability) can have drastic consequences on mitochondrial function by altering the production of mitochondrial metabolites and signalling molecules (16). This process will ultimately have serious impacts on metabolic flux and, in turn, feed back into the evolutionary processes shaping mito-nuclear genotypes (17).

Here we aim to understand the effects of mtDNA variation and diet on male reproductive fitness in *D. melanogaster*. We use seven fly lines with an isogenic nuclear genome but each carrying a different naturally occurring mtDNA haplotype. We then apply nutritional geometry techniques to identify diets that maximise male fitness for a given line (see supplementary methods for a brief summary of nutritional geometry principles). We recover previously described male-specific nutritional optima on carbohydrate-rich food when averaging across all lines, however we find significant mitochondrial genetic variance underpinning optimal male nutrition. These results allude to complex genetic and nutritional interactions influencing life history trait evolution.

Materials and Methods

Drosophila stocks and maintenance

All flies were reared at 25°C and 50% humidity, on a 12: 12 h light: dark cycle, 10mL glass vials, on a cornmeal-molasses-agar medium (see TableS1 for recipe), with *ad libitum* live yeast added to each vial to promote female fecundity. For each line, flies were propagated by adult 4-day old parents, with eggs laid kept at maximum 100 eggs.

For the experiment, we used seven Drosophila strains, all which had the same isogenic nuclear background (w^{1118} – Bloomington Stock Center #5905) coupled to six different mtDNA haplotypes from around the world (18). These were: w^{1118} (coevolved -WE), Barcelona (BAR); Dahomey (now called Benin) (DAH); Madang, Papua New Guinea (MAD); Mysore, India (MYS); Oregon, USA (ORE); Zimbabwe (ZIM).

Synthetic diet and nutritional geometry

We used a modified liquid version of the synthetic diet described in Piper et al. (7), that is prepared entirely from synthetic components to enable precise control over nutritional value (see Table S1-S3). Four different diets were synthesised, which varied in the ratio of protein (P, individual amino acids) and carbohydrate (C, sucrose), while all other nutritional components were provided in fixed concentrations. Nutrient ratios used were [P:C] – 1:1, 1:2, 1:4, and 1:16, with the final concentration of each diet being 32.5g/L.

Groups of three virgin males from each line were collected and placed in vials that contained 0.8% agar and kept at 80% RH for 12h to acclimatise to the vial. Following this period, all flies were supplied with one of the four artificial liquid diets using a 5 µL capillary tube. Feeding vials were changed daily during the four-day feeding trial, and daily diet consumption was recorded. Diet consumption was summed across all days, to give one datapoint. Each tray contained five evaporation control vials which contained no flies.

Non-competitive fertility

Following four days of feeding on experimental diets, non-competitive fertility was measured for all male flies. Females of the w^{1118} coevolved genotype were placed individually in vials containing standard yeast-molasses-cornmeal medium and left for 1h to acclimatise. Following this period, a focal male was transferred to the vial directly from the feeding vial and left to mate with the tester female for 24h. This timing was chosen to maximise the chance for mating to occur (96%) between the fly pair. While there is a chance that a double mating could have occurred, previous pilot experiments show the tester females to have a long refractory period. Focal males were then removed and discarded, and females were left to oviposit over two vials (48-h in the first vial and 48-h in the second). Total number of eclosing adult offspring 14 days following mating was counted and summed over both vials per female. Coevolved tester females were used in this experiment, as previous work has found mitochondrial genetic variance for female fitness components (10). We therefore chose to keep the tester female genotype consistent across all treatments to avoid the female genotype influencing the male fitness response.

Statistical analyses

We used a sequential model building approach (19) to determine if there was mitochondrial genetic variance for i) total consumption of diets and ii) diet-mediated fitness (for a full description of models and the nutritional geometry framework, see Supplementary methods). Models were fitted with maximum likelihood and compared in a pairwise manner using parametric bootstrap analysis using the *PBmodcomp* function implemented in the package pbkrtest (20). We ran an analysis of variance (ANOVA) with type III sums of squares on the full model in order to assess the significance of fixed terms in the model. We visualized nutritional landscapes based on untransformed data using non-parametric thin-plate splines implemented in the *fields* (21) package.

We used a permutation approach to assess to which degree fitness variation between mitochondrial lines is due to differences in diet consumption responses rather than metabolic differences independent of consumption. This approach has been previously described (8), and is detailed in the Supplementary methods. The rationale is that if lines differ in fitness because they alter their consumption in line with the diet available and their physiological requirements, then breaking the association between consumption and line by permutation should result in lower mean predicted fitness than in the observed dataset.

Results

We find significant variation between the consumption of different diets, with protein-rich diets being consumed in larger quantities across all genotypes than carbohydrate-based diets ($p < 0.05$, Figure 1). We also found significant mitochondrial genetic variation in consumption across diets ($p = 0.048$, 1.38% of variance in consumption, Table 1A) and in diet-specific consumption ($p < 0.001$, 4.24% of variance, Table 1A).

Analysing the relationship between diet and fitness, we recovered previous results whereby across all genotypes, male fitness is maximised by a moderate carbohydrate bias in the diet's macronutrient composition (P:C – 1:4, Figure 2). However, we also found significant variation around this average ($p = 0.0018$, 3.67% of variance in fitness; Table 1B, Figure 2). Furthermore, there was evidence for genotypes showing differential fitness responses to diet variation ($p = 0.0495$, 6.11% of variance; Table 1B, Figure 2). For example, the fitness of male flies harbouring the DAH haplotype is maximised on a more carbohydrate-rich diet (P:C – 1:16, Figure 2B), whereas ORE haplotype requires higher levels of protein to maximise fitness (P:C – 1:1, Figure 2B). We further analyse this data using reaction norms (see Supplementary) and find support for our nutritional geometry analysis.

Using our permutation approach, we found that uncoupling behaviour (intake) and physiology tended to result in a reduction in fitness, but not statistically significantly so ($p = 0.082$). Thus, differences between genotypes in the behavioural responses to food composition might make some contribution to diet-dependent fitness (resulting in a tendency for reduced fitness when behaviour and physiology are dissociated), but genetic variation in fitness responses is dominated by the physiological and metabolic properties of the mitochondrial lines (resulting in a non-significant result).

Discussion

Nutrient acquisition and metabolism are important determinants of fitness components and phenotypic trait expression (22). Genetic variation in fitness responses to nutrition are the result of two underlying processes. First, organisms with different genotypes can vary in how they change their behaviour and consume different amounts of food. Second, genotypes can differ in the functioning and efficiency of metabolism; a process critical for allocating resources to reproduction. Here we investigated this proposition in relation to the effects of mitochondrial genetic variation and nutrition on male fitness. By using lines of *D.melanogaster* that couple diverse mtDNA haplotypes to the same isogenic nuclear background, we were able to isolate the effects of mitochondrial genetics on nutrient-dependent fitness. We applied nutritional geometry techniques across our mitochondrial panel and found evidence that different mtDNA lines require divergent nutrient compositions to maximise male fitness.

In line with previously described behavioural responses to holidic media (8), we found that flies consumed more of the protein than the carbohydrate diet. These results suggest that the diet-dependent modulation of consumption in our study was aimed at ensuring an adequate carbohydrate intake. In addition to these general responses, we found a small amount of genetic variation in total consumption (across diets) between the mtDNA haplotypes (Table 1A, term 'mito'), as well as more significant genetic variation for diet-specific feeding responses (term 'diet-dependent mito'). We also found significant levels of genetic variation for fitness across diets (Table 1B, term 'mito'), as well as variation in diet-dependent fitness responses between mitochondrial haplotypes (term 'diet-dependent mito'). We also note that although our model explains only about a quarter of the variance in the measured responses—as expected for noisy traits like behaviour and male mating success—mitochondrial effects make a significant contribution to this figure (consumption: 5.62% of total variance, or 22.6% of the variance explained; fitness: 9.78% of total variance, 37% of variance explained).

Our results provide evidence that mitochondrial DNA influences male feeding behaviour and reproductive success, most likely due to their central role in metabolism and metabolic regulation. Consistent with previous work (8), the permutation analyses we performed suggests that genetic variation in fitness is more likely due to the physiological and metabolic

properties of our mitochondrial genotypes than a consequence of altered feeding behaviour (non-significant permutation test). Nevertheless, the border-line P-value (P=0.08) does not allow us to categorically completely rule out a contribution of mitochondria via the modulation of feeding.

The presence of diet-dependent effects of haplotypes on feeding and fitness reinforces the view that mitochondria are more than merely subordinate energy producers. They integrate metabolic flux and stress, signalling the physiological status of the cell to the nucleus (23, 24). Accordingly, changing the dietary composition (changing nutritional environments) will have a significant impact on Krebs cycle intermediates and ultimately impact the metabolic flux balance of the cell (13, 25). Trying to pinpoint pathway(s) being impacted by the complex interaction between diet and mito-nuclear genetics will require further experimentation. We can predict that Complex I is a very likely candidate to respond to both nutrition and mitochondrial effects as it's the start point of OXPHOS and requires components encoded in both genomes. Moreover, many previous studies have linked this complex to many environmental responses (26-28).

Our finding of fitness variation among mitochondrial lines also supports the supposition that mtDNA variants may be a direct target of selection imposed by variation in dietary macronutrients (17). Our study therefore contributes to a body of evidence suggesting that mtDNA is not just an "evolutionary bystander" (29). Indeed, empirical work by Aw et al. (27) has provided insights into mitonuclear mechanisms that are affected by nutrition. Their study used similar *Drosophila* strains to our study; isogenic w^{1118} nuclear background coupled to haplotypes from Dahomey (DAH) and Australia (neither haplotype coevolved with the nuclear background). These authors performed cage experiments with populations composed from the two strains across several nutritional environments and found that the DAH haplotype increased in frequency on a carbon-rich diet, but decreased on diets with higher protein content. Interestingly, in our study we also found the DAH haplotype to perform best in a high carbohydrate environment. As frequency change in Aw et al.'s (27) experiment are due to performance differences in females (who transmit mitochondria), the consistent carbohydrate-bias in the performance of DAH across their and our study suggests that the effects of the haplotype are similar in the two sexes.

While we use naturally occurring mtDNA haplotypes in our study, a caveat is that we only use a single nuclear background. As a consequence, we cannot differentiate between phenotypic effects that are due to mitochondrial haplotype alone, and those that arise from epistatic interaction between the haplotypes and the fixed nuclear background. Mossman and colleagues (30) have previously examined the role of interactions between mitochondrial genotype, nuclear genotype and diet (GxGxE) on development time in *Drosophila*. They used twelve nuclear backgrounds from the DGRP panel (31) coupled to a cross-species panel of six different mtDNA haplotypes (3×*D. melanogaster*, 2×*D. simulans*, 1×*D. mauritiana*). They found significant G×G×E effects on development time; flies that developed on higher protein diets had shorter development times than those on higher carbohydrate foods, but the magnitude of this response depended on both the flies' mitochondrial and nuclear genotype. Nonetheless, authors did find mitochondrial genetic variance for development time. It remains to be seen whether mtDNA variation will alter the dietary response in natural populations which have high levels of both nuDNA and mtDNA genetic variance. Future work should aim to investigate the complex interaction between genomes and nutrition that drives life history evolution in natural environments.

Acknowledgements: We thank Mark Hill and Filip Ruzicka for insightful discussions. Rebecca Finlay for help with running experiments.

References

1. Stearns SC. The Evolution of Life Histories: OUP Oxford; 1992.

2. Raubenheimer D, Simpson SJ. Nutritional Ecology and Human Health. Annu Rev Nutr. 2016;36:603-26.

3. Garlapow ME, Huang W, Yarboro MT, Peterson KR, Mackay TFC. Quantitative genetics of food Intake in *Drosophila melanogaster*. PLoS ONE. 2015;10(9):e0138129.

4. Sun JH, Liu C, Bai XB, Li XT, Li JY, Zhang ZP, et al. Drosophila FIT is a protein-specific satiety hormone essential for feeding control. Nat Commun. 2017;8.

5. Corrales-Carvajal VM, Faisal AA, Ribeiro C. Internal states drive nutrient homeostasis by modulating exploration-exploitation trade-off. eLife. 2016;5:e19920.

6. Itskov PM, Ribeiro C. The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in *Drosophila*. Frontiers in Neurobiology. 2013;7(12).

7. Piper MD, Blanc E, Leitao-Goncalves R, Yang M, He X, Linford NJ, et al. A holidic medium for Drosophila melanogaster. Nat Methods. 2014;11(1):100-5.

8. Camus MF, Fowler K, Piper MWD, Reuter M. Sex and genotype effects on nutrient-dependent fitness landscapes in Drosophila melanogaster. P Roy Soc B-Biol Sci. 2017;284(1869).

9. Wallace DC. Mitochondrial diseases in man and mouse. Science. 1999;283(5407):1482-8.

10. Camus MF, Dowling DK. Mitochondrial genetic effects on reproductive success: signatures of positive intrasexual, but negative intersexual pleiotropy. Proc Biol Sci. 2018;285(1879).

11. Immonen E, Collet M, Goenaga J, Arnqvist G. Direct and indirect genetic effects of sex-specific mitonuclear epistasis on reproductive ageing. Heredity. 2016;116(3):338-47.

12. Jelic M, Arnqvist G, Kurbalija Novicic Z, Kenig B, Tanaskovic M, Andelkovic M, et al. Sex-specific effects of sympatric mitonuclear variation on fitness in *Drosophila subobscura*. BMC Evolutionary Biology. 2015;15(1):135.

13. Pichaud N, Messmer M, Correa CC, Ballard JWO. Diet influences the intake target and mitochondrial functions of Drosophila melanogaster males. Mitochondrion. 2013;13(6):817-22.

14. Nagarajan-Radha V, Rapkin J, Hunt J, Dowling DK. Interactions between mitochondrial haplotype and dietary macronutrient ratios confer sex-specific effects on longevity in Drosophila melanogaster. The Journals of Gerontology: Series A. 2019.

15. Camus MF, O'Leary M, Reuter M, Lane N. Impact of mitonuclear interactions on life-history responses to diet. Philosophical Transactions of the Royal Society B: Biological Sciences. 2020;375(1790):20190416.

16. Aw WC, Youngson NA, Ballard JW. Can we alter dietary macronutrient compositions and alleviate mitochondrial disease? Journal of Rare Diseases Research and Treatment. 2016.

17. Ballard JWilliam O, Youngson Neil A. Review: can diet influence the selective advantage of mitochondrial DNA haplotypes? 2015;35(6):e00277.

18. Clancy DJ. Variation in mitochondrial genotype has substantial lifespan effects which may be modulated by nuclear background. Aging cell. 2008;7(6):795-804.

19. Reddiex AJ, Gosden TP, Bonduriansky R, Chenoweth SF. Sex-specific fitness consequences of nutrient intake and the evolvability of diet preferences. Am Nat. 2013;182(1):91-102.

20. Halekoh U, Højsgaard S. A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models: The R package pbkrtest. Journal of Statistical Software. 2014;59(9):32.

21. Nychka DF, R.l; Paige, J.; Sain, S. fields: Tools for spatial data. 2015.

22. Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO, Taylor PW, et al. Lifespan and reproduction in *Drosophila*: New insights from nutritional geometry. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(7):2498-503.

23. Chandel NS. Evolution of Mitochondria as Signaling Organelles. Cell Metab. 2015;22(2):204-6.

24. Chandel NS. Mitochondria as signaling organelles. Bmc Biology. 2014;12.

25. Solon-Biet Samantha M, McMahon Aisling C, Ballard JWilliam O, Ruohonen K, Wu Lindsay E, Cogger Victoria C, et al. The Ratio of Macronutrients, Not Caloric Intake, Dictates Cardiometabolic Health, Aging, and Longevity in Ad Libitum-Fed Mice. Cell Metab. 2016;19(3):418-30.

26. Camus MF, Wolff JN, Sgrò CM, Dowling DK. Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian *Drosophila melanogaster*. Molecular Biology and Evolution. 2017;34(10):2600-12.

27. Aw WC, Towarnicki SG, Melvin RG, Youngson NA, Garvin MR, Hu YF, et al. Genotype to phenotype: Diet-by-mitochondrial DNA haplotype interactions drive metabolic flexibility and organismal fitness. Plos Genetics. 2018;14(11).

28. Morales HE, Pavlova A, Amos N, Major R, Kilian A, Greening C, et al. Concordant divergence of mitogenomes and a mitonuclear gene cluster in bird lineages inhabiting different climates. Nat Ecol Evol. 2018;2(8):1258-67.

29. Ballard JWO, Pichaud N. Mitochondrial DNA: more than an evolutionary bystander. Funct Ecol. 2014;28(1):218-31.

30. Mossman JA, Biancani LM, Zhu CT, Rand DM. Mitonuclear Epistasis for Development Time and Its Modification by Diet in Drosophila. Genetics. 2016;203(1):463-84.

31. Mackay TF, Richards S, Stone EA, Barbadilla A, Ayroles JF, Zhu D, et al. The Drosophila melanogaster Genetic Reference Panel. Nature. 2012;482(7384):173-8.

Figures and Tables

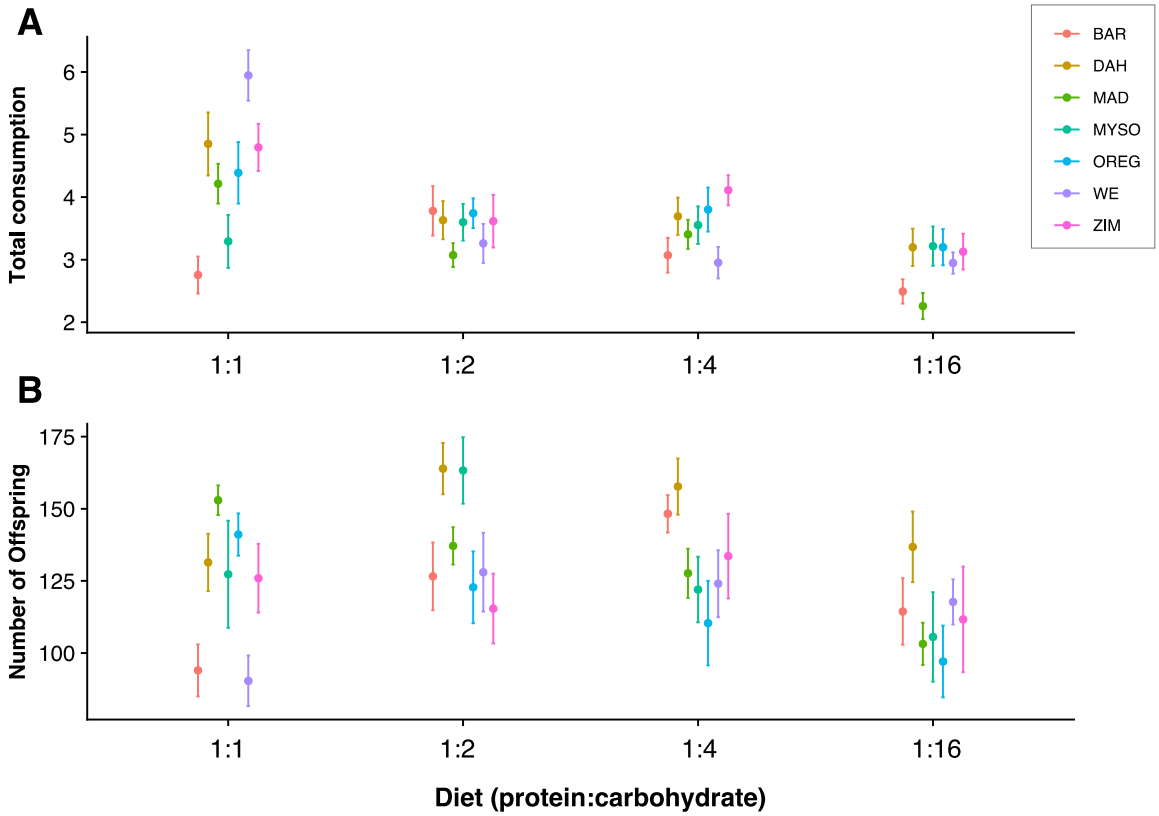

Figure 1: (A) Total liquid consumption (µl) for the four different diets across all mitochondrial genotypes used in the experiment. **(B)** Total number of offspring sired for all mitochondrial genotypes used in this study across all diet treatments.

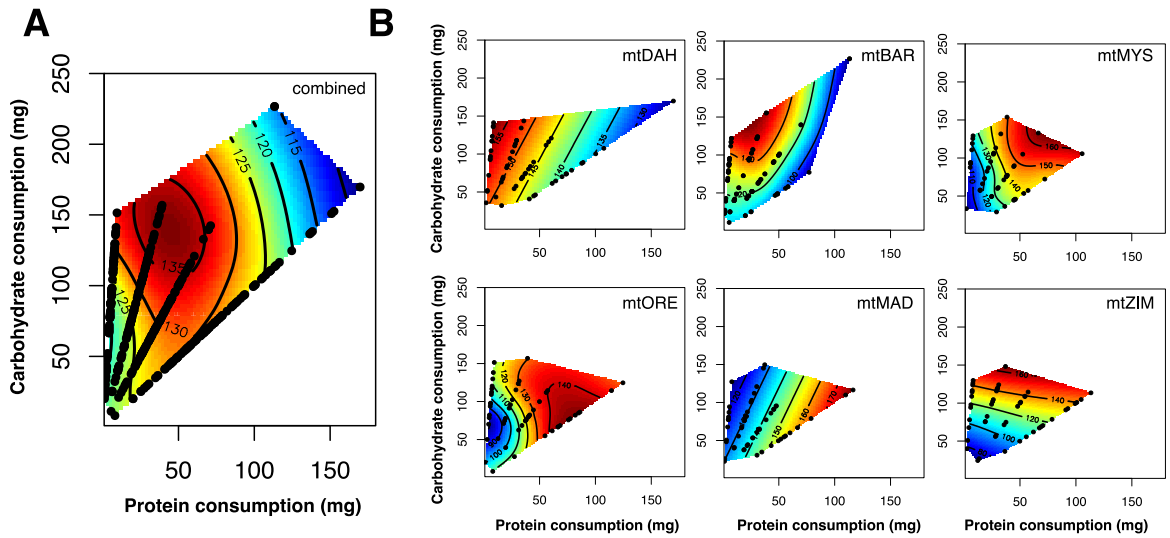

Figure 2: (A) Nutritional landscapes illustrating the effects of protein and carbohydrate intake on the expression of male traits. Black dots are individual data points. **(B)** Exemplary haplotype-specific nutritional fitness landscapes.

Table 1: Full model of the nutritional effects on (**A**) diet consumption and (**B**) fitness. We include results from the parametric bootstrap model comparison, including the value of the test statistic (the log-likelihood ratio, LLR), degrees of freedom, P-value and percentage of the overall variance explained by each model, as well as the percentage of variance attributable to mitochondrial and diet-specific mitochondrial effects (Δ variance).

A. Diet consumption

	F	Df	Resid. Df	P-value
(Intercept)	187.074	1	1.68	0.0102
diet	18.482	3	356.16	> 0.001

Model comparison

	LLR	df	P-value	variance	Δ variance
base model				19.21%	–
mito	2.461	1	0.04811	20.59%	1.38%
diet-specific mito	15.941	9	0.00101	24.83%	4.24%

B. Fitness

	F	Df	Resid. Df	P-value
(Intercept)	58.5258	1	4.27	0.0011
protein	6.5259	1	357.46	0.01104
carbohydrate	4.6687	1	354.85	0.0313
protein^2	6.8662	1	355.42	0.0091
carbohydrate^2	2.9447	1	352.75	0.0870
protein×carbohydrate	0.0865	1	356.07	0.7688

Model comparison

	LLR	df	P-value	variance	Δ variance
base model				16.62%	–
mito	9.8427	1	0.0018	20.29%	3.67%
diet-specific mito	9.7451	14	0.0495	26.40%	6.11%