Report

Sex-Specific Fitness Effects of Nutrient Intake on Reproduction and Lifespan

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Summary

Diet affects both lifespan and reproduction [1–9], leading to the prediction that the contrasting reproductive strategies of the sexes should result in sex-specific effects of nutrition on fitness and longevity [6, 10] and favor different patterns of nutrient intake in males and females. However, males and females share most of their genome and intralocus sexual conflict may prevent sex-specific diet optimization. We show that both male and female longevity were maximized on a high-carbohydrate low-protein diet in field crickets _Teleogryllus commodus_, but male and female lifetime reproductive performances were maximized in markedly different parts of the nutrient intake landscape. Given a choice, crickets exhibited sex-specific dietary preference in the direction that increases reproductive performance, but this sexual dimorphism in preference was incomplete, with both sexes displaced from the optimum diet for lifetime reproduction. Sexes are, therefore, constrained in their ability to reach their sex-specific dietary optima by the shared biology of diet choice. Our data suggest that sex-specific selection has thus far failed fully to resolve intralocus sexual conflict over diet optimization. Such conflict may be an important factor linking nutrition and reproduction to lifespan and aging.

Results and Discussion

Males and females achieve reproductive success in very different ways, resulting in conspicuous differences in morphology, behavior, lifespan, and aging [11–15]. Both theory and

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empirical evidence suggest that trade-offs between life history traits might be resolved differently by males and females [6, 15–17]. Given the well-established connections between diet and lifespan [1–5, 10], between lifespan and reproductive performance [18–20], and between diet and reproductive performance [2, 4, 6], we predict that the sexes should be under selection for different dietary preference and resource utilization that maximize their sex-specific fitness. This issue is significant because of the key influences of nutrition and reproduction on fitness, health, and aging [2, 9, 21].

Exceptional progress toward understanding the effects of diet and reproduction on lifespan has been made by studying female flies [3–5, 8, 20–23] in which it is possible to measure female reproductive effort by counting eggs. Furthermore, nutrient consumption affects lifespan differently in male and female _Drosophila melanogaster_ [10]. However, sex differences in the effects of diet on both major components of fitness—reproduction and lifespan—have remained largely unexplored. We tested for sex-specific effects of diet on life-long reproductive performance and lifespan in _Teleogryllus commodus,_ a species of field cricket amenable to diet manipulation in which we are able to measure the major components of both male and female reproductive performance under laboratory and field conditions: advertisement calling and egg laying, respectively [6]. In a no-choice experiment (Experiment 1), we varied four nutrient concentrations on each of six different protein:carbohydrate ratios (24 diets altogether, Table S1 available online), thereby creating six "nutritional rails" along which our study animals could "move" by eating more or less food (sensu the Geometric Framework [see 24, 25, 26]). As discussed in recent studies stressing the importance of assessing the effects of both nutrient composition and the amount of food actually ingested [26, 27], we measured the amount of food eaten by crickets over their lifetime. We measured reproductive performance—fecundity for mated females and calling effort for males—on a weekly basis from eclosion to adulthood until death. We then used multivariate response surface analysis [28, 29] to estimate and compare the effects of actual protein and carbohydrate (P:C) intake on lifespan and reproductive performance in both sexes.

The nutritional landscapes (Figure 1) show that any effects of dietary restriction on longevity in _T. commodus_ are both nutrient and sex specific. Both sexes maximized lifespan on high-concentration diets containing a low P:C ratio (Figures 1A and 1B). However, the lifespan response surfaces for the sexes differed significantly (partial _F_-test: $F_{5,429} = 4.45$, p = 0.0005), because of the fact that male but not female longevity declined with very high carbohydrate intake (Table S2). In both sexes, lifespan decreased as the P:C ratio of the diet increased, incidentally providing further evidence that the effects of dietary restriction on longevity are not due to caloric restriction [4, 8].

Male nightly and life-long calling peaks in the same region of the P:C intake surface as male lifespan (Figures 1D and 1F). In sharp contrast, female egg-laying rate and lifetime egg output are maximized on a 1:1 P:C intake (Figures 1C and 1E). The response surfaces for male and female reproductive performance differed significantly (nightly calling versus daily egg production, $F_{5,429} = 5.23$, p = 0.0001; lifetime calling versus
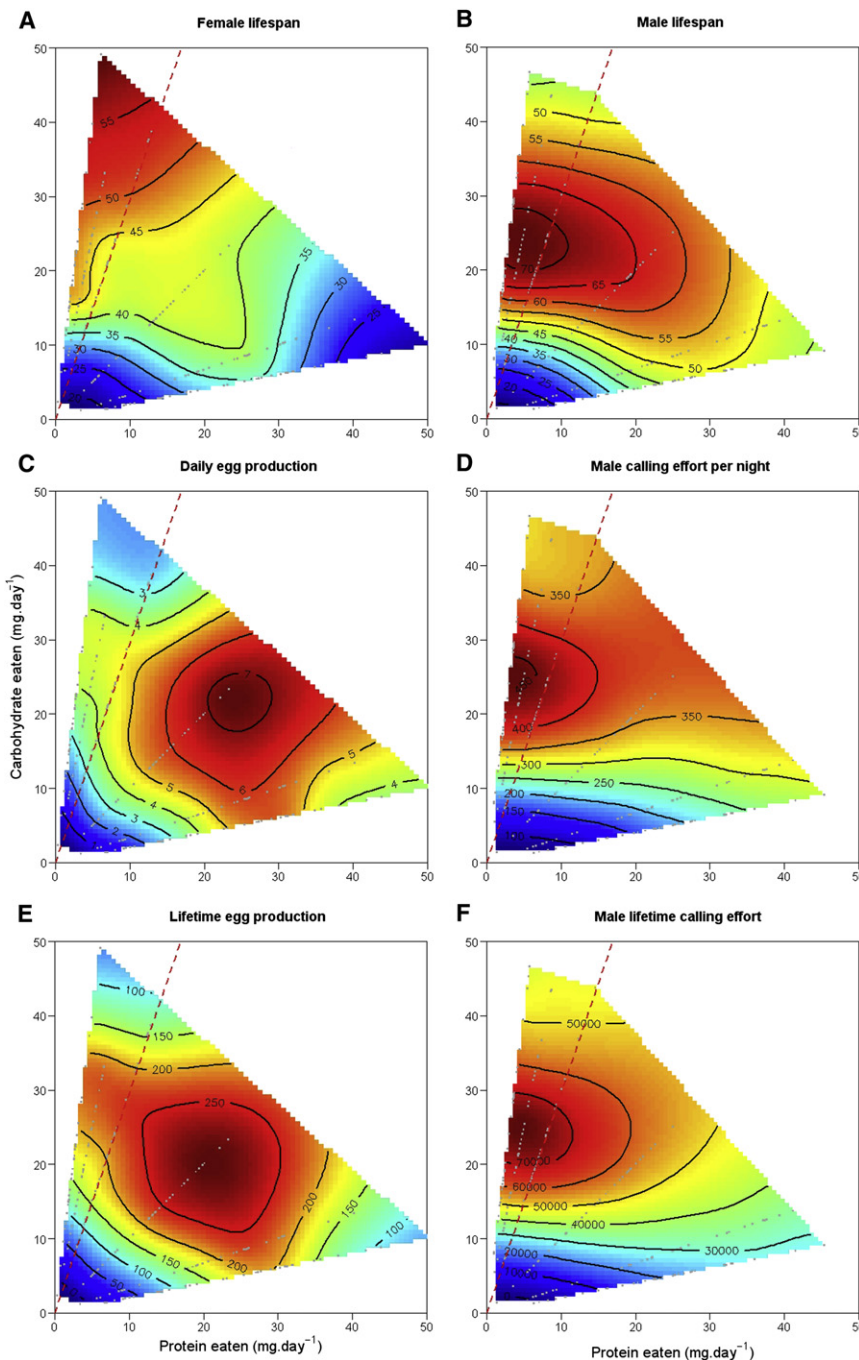
Figure 1. Nutrient Effects on Fitness and Lifespan in Males and Females: Experiment 1

Nonparametric thin-plate spline contour plot visualizations of the response surfaces describing the effects of protein and carbohydrate intake on (A) female lifespan, (B) male lifespan, (C) female egg production rate, (D) male hourly calling performance, (E) female lifetime egg number, and (F) male total lifetime calling performance. High values are red and low values in blue. Gray dots are individual crickets. Red dotted lines represent target intake ratio of 1:2.96, the regulated intake target estimate from Experiment 2.

lifetime egg output, $F_{5,429} = 2.70$, p = 0.020). Detailed examination of the linear models (Tables S3 and S4) implicates sex differences in the effects of protein intake on reproductive performance.

Diet influences the trade-off between longevity and reproduction in females because the nutritional intake that maximizes lifespan results in poor reproductive performance and vice versa. However, male lifespan and reproductive performance can both be maximized on a high-carbohydrate, low-protein diet. This difference between the sexes presumably reflects the different nutritional requirements for reproduction in the sexes: protein for eggs in females [30] and carbohydrate energy for calling in males. Although the

evidence from females in this experiment and from a published diet manipulation in males [6] suggests a trade-off between longevity and reproductive performance, such a trade-off does not explain the effects of overconsuming nutrients, especially carbohydrates, on male longevity. Males accumulate more fat per unit carbohydrate eaten than females, especially on high-carbohydrate diets (Experiment 4 in Supplemental Data). Such fat storage would be adaptive for males in most circumstances because of the energetic demands of calling, but may impose costs of obesity on the highest carbohydrate diets [31]. In two further experiments, we let crickets choose the protein and carbohydrate content of their diet by offering them nutritionally complementary foods. In Experiment 2, we provided males and females with both 42% protein, 0% carbohydrate (i.e., 42:0) and 0:42 foods simultaneously. There was no difference between male and female protein or carbohydrate intake, with both sexes selecting a P:C ratio close to 1:3 (Figure 2A). This selected intake ratio falls between the sex-specific reproductive performance peaks in Experiment 1 (Figures 1C–1F). Two questions remain, however: was the P:C ratio that was chosen actively regulated by the animals, or was it the outcome of some fixed sensory preference for the 0:42 food over the 42:0 food? And, does the total nutrient content of each food alter the outcome of such choice trials? To answer these questions, in Experiment 3 we measured protein and carbohydrate intake over the first 10 days after eclosion to adulthood under three further diet pairings that also differed in total nutrient content (Figures 2B–2D). In combination with Experiment 2, these results indicate that both sexes regulated their intake of protein and carbohydrate, but that this regulation is constrained by a shared inability of males and females to compensate fully for dietary dilution. Whereas in Experiment 2, with identical concentration of nutrients (42%) in the two diets, animals chose a 1:3 P:C ratio, in Experiment 3, the P:C intake trajectory was biased from 1:3 toward the food that was richer in total nutrients (more protein
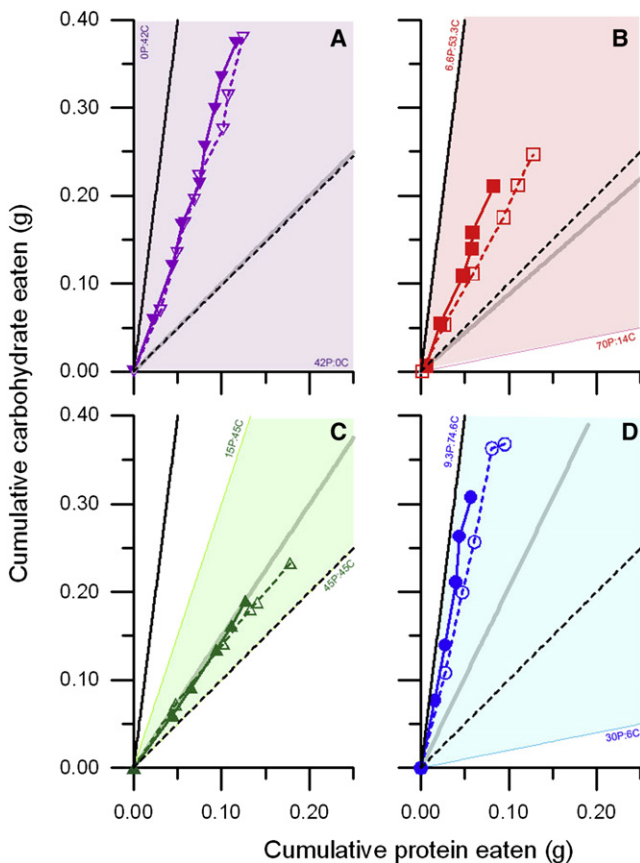
Figure 2. Diet Choice in Males and Females: Experiments 2 and 3

Female (open symbols, dotted colored lines) and male (closed symbols, solid colored lines) protein and carbohydrate intake in choice trials presenting four different combinations of diet. The pale colored areas represent the possible range of values between higher-protein and higher-carbohydrate diet. Diet compositions as percent protein:percent carbohydrate are given in colored text at edges of colored area. Gray lines represent the null expectations if crickets ate equal weights of the two diets. Black dashed lines are the intake trajectory that would maximize female lifetime egg number, and black solid lines are the intake trajectory that would maximize male lifetime calling performance (based on results of Experiment 1).

(A) In Experiment 2, we measured intake of males and females over 32 days after adult eclosion.

(B–D) In Experiment 3, we measured intake over the first 10 days after eclosion on three different diet combinations.

in Figures 2B and 2C, more carbohydrate in Figure 2D) with the magnitude of this bias proportional to the disparity in total nutrient concentration of the two available diets. These results suggest that diet choice is actively regulated and affected by total nutrient content.

Females consumed slightly, but significantly, more protein relative to carbohydrate than males (Figures 2B–2D; $F_{1,31}$ = 4.92, p = 0.034), indicating sex-specific dietary preference in the direction that maximizes sex-specific reproductive performance.

In Experiments 2 and 3, male and female crickets show strikingly similar dietary preference, and although females tend to eat more protein than males, sexual dimorphism in diet choice is limited. Chosen diets fall between the sex-specific peaks for reproductive performance, suggesting that females in particular are pulled away from their sex-specific optimum (Figures 1C and 1D) and that the preference exhibited may represent

a compromise between the sex-specific optima. Furthermore, neither sex is able to compensate completely for the dilution of the total nutrient content of foods, and this can further constrain animals from reaching their optimum dietary intake even when choice is possible.

One caveat is that male calling effort is not the only nutritional demand on males, and thus including other aspects of male reproductive effort such as nutritional requirements for spermatophore production may shift the reproductive response surface toward a higher dietary protein ratio. More generally, the relative contributions of different traits to fitness may differ from natural populations. However, field data from this population suggest that female early mortality is high and that adult females live only 22 days on average [32]. The contours of the response surfaces (Figure 1C) suggest that a wild female of average longevity would produce only ~99 eggs in her lifetime on 1:3 P:C diet, compared to ~154 eggs on a 1:1 P:C diet, a considerable fitness cost.

When traits have sex-specific optima, natural selection may be constrained from reaching one or both optima because many of the same genes are responsible for trait expression in both sexes. This is intralocus sexual conflict (ISC), and it can favor alleles that have sexually antagonistic effects on fitness, or alleles that result in a phenotype intermediate to the two optima [33–36]. Although several studies have provided evidence that intralocus sexual conflict occurs in laboratory and natural populations in different taxa [34–37], very little is known about the specific traits mediating this conflict [38]. Demonstrating ISC requires the demonstration both of sex-specific optima for a particular trait and that the common biology of the sexes constrains one or both sexes from reaching their optimum [38]. Here we have presented the first experimental analysis of sex-specific fitness surfaces to show that sex differences in optimum nutrient intakes generate the potential for sexual conflict over diet choice. We then provided evidence that sexes are indeed constrained in their ability to reach their sex-specific dietary optima, with both male and female preferences displaced toward an intermediate diet. Resolving the genetic basis of traits that underlie dietary preference in both sexes remains an important challenge.

In the only previous attempt to use the Geometric Framework to study the effects of diet on reproduction, longevity, and aging, female *Drosophila melanogaster* showed a similar pattern of maximizing longevity on low P:C ratios and reproductive rate on 1:2 P:C ratios [4]. Given a choice of diet, these females ate an intermediate ratio that maximized lifetime reproductive success. By contrast, in our experiment, female crickets prefer a lower P:C ratio that results in suboptimal net lifetime fecundity. The extent to which *Drosophila* and other organisms exhibit sex-specific dietary optima and ISC remains an interesting topic for further study.

Although evolutionary studies of lifespan and senescence have begun to consider the importance of sex-specific reproductive performance [10, 16, 23, 39], our results indicate a need to couple this with an understanding of sex-specific optimization of nutrition. Lifespan, reproduction, and fitness are subject to complex, sex-specific, and nonlinear relationships with the quantity and ratio of macronutrients, and these relationships involve a combination of factors, including reproductive performance and postingestive processing of nutrients consumed to excess. The intriguing possibility exists that a more thorough understanding of sex differences in the relationships between nutrition, reproduction, and longevity may even offer insights into sex differences in the nutritional

challenges facing human populations and into sex differences in aging-related disease.

Experimental Procedures

Experimental Diets and Estimating Food Intake
We fed animals artificial, dry, granular foods that varied in protein and carbohydrate content based on an established protocol [40]. Full details of diet contents are in Supplemental Experimental Procedures.

Experimental animals were provided food and water ad libitum and checked daily. Animals were weighed and pronotum width measured on the day they eclosed as adults. Each animal was given one (no-choice trials in Experiment 1) or two (choice trials in Experiments 2 and 3) containers of experimental food of measured dry weight on the first day of adulthood, and food was changed regularly (every 3 days during the first 3 weeks, as needed thereafter). When food was changed, or when an animal died, the old container was removed, dried, and weighed. Food was always kept in the drying oven at 30°C for at least 48 hr to remove moisture prior to weighing.

Control containers of each food were kept in the same room and weighed to control for spurious weight loss or gain. If controls gained weight (e.g., if inadequately dried), all samples were returned to the drying oven for a further 24 hr. Consumption was calculated as the difference in dry weight before and after feeding and converted to weight of protein and carbohydrate consumed (e.g., 5 mg of 15P:45C dry food eaten equals 0.75 mg of protein and 2.25 mg of carbohydrates).

Experiment 1: No Choice on Six Nutritional Rails at Four Concentrations
We drew 220 females and 240 males as final instar nymphs from stock and assigned 8–10 of each sex at random to each of the 24 diets. Animals were excluded from the analysis if they died prior to adult eclosion or escaped during the experiment, resulting in a final sample of 213 females and 228 males.

Male *Teleogryllus commodus* start calling in the first week of adulthood. We measured each male's calling effort overnight on the 3rd and 7th nights after eclosion and then every 7 nights thereafter.

We measured female reproductive effort as weekly and lifetime egg production. Females were mated overnight once a week to different randomly assigned stock males. Food was removed during this period. The next day each female was provided a fresh, moist sand pad into which she laid her eggs over the week. Eggs were stored in 95% ethanol before counting.

Statistical Analysis
We used a multivariate response-surface approach [28, 29, 41] to estimate and visualize the linear and nonlinear effects of protein and carbohydrate consumption on longevity, male hourly and total lifetime calling effort, and female weekly and total lifetime egg number. Surfaces were fitted in SPSS13 and visualized via nonparametric thin-plate splines in R (version 2.5.1). To statistically compare male and female response surfaces, we used a partial F-test to assess whether the fitting of sex-specific response surface coefficients (i.e., adding interactions between sex and the diet components) significantly improved the fit of the response surface [28].

Experiment 2: Measuring Intake under Choice
In this experiment, 12 male and 12 female crickets were presented with a choice of two different pure diets of 0:42 and 42:0 P:C. The food intake was measured every 2 days over a period from eclosion to adulthood until 32 days after eclosion.

Experiment 3: Measuring Intake under Choice when Total Nutrient Content Varies
Here we applied three further choice treatments in which crickets were presented with a choice of two foods that differed in their P:C and total nutrient content: (1) 9.3P:74.6C and 30P:6C; (2) 6.6P:53.3C and 70P:14C; and (3) 15P:45C and 45P:45C. We used seven crickets per treatment per sex (n = 42). Food intake was measured every 2 days for the first 10 days after eclosion.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, one figure, and four tables and are available at http://www.current-biology.com/cgi/content/full/18/14/1062/DC1/.

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