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How dietary protein and carbohydrate influence field cricket development, size and mate attraction signalling

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Researchers examining how nutrition impacts fitness traits usually examine one nutrient at a time, ignoring potential interactions. When researchers simultaneously examine multiple nutrients, their studies often focus on adults, ignoring potential changes in nutritional needs that occur during the transition between juvenile and adult stages. To address these issues, we quantified how dietary nutrient balance (relative amounts of protein and carbohydrate) during development and into adulthood influenced life history and sexually selected traits using male Jamaican field crickets, *Gryllus assimilis*. Our findings revealed that male crickets developed significantly faster and grew larger when they were reared on a protein-rich diet, but their average daily time spent signalling for mates was significantly higher when they were reared on a carbohydrate-rich diet. Furthermore, while the probability of signalling and daily time spent signalling increased with age, time spent signalling for mates tended to increase at a higher rate with age when males consumed a carbohydrate-rich diet in adulthood. Together our findings suggest that traits may differ in their nutrient requirements, resulting in diet influencing a possible trade-off between traits across different life stages. The ability to locate and consume foods rich in protein during development should impact adult male fitness, as protein availability results in larger males, and larger males typically produce more attractive signals and are preferred by females. Conversely, the ability to locate and consume foods rich in carbohydrate should also impact a male's fitness, as males signal with higher effort when fed carbohydrate-rich diets, and higher signalling effort can directly impact a male's ability to attract a female.

Directional and stabilizing selection should erode variation in traits that enhance fitness, but extensive variation persists (Houle, 1992; Pomiankowski & Møller, 1995). What maintains this variation? Variation in traits that enhance fitness may be partially maintained by variation in the ability to locate, consume and retain nutrients required for growth, development and energetically demanding mating activities (e.g. Clark, Zera, & Behmer, 2015; Evenden, Whitehouse, & Jones, 2015; Fox & Macauley, 1977; Huberty & Denno, 2006; Mattson, 1980; Perkins, Woods, Harrison, & Elser, 2004; Saastamoinen & Rantala, 2013; Schwab & Moczek, 2016; Sedinger, Flint, & Lindberg, 1995; Sterner & Schulz, 1998). In support of this argument, a host of different studies have suggested that protein availability is important to traits that enhance fitness. For example, when fed diets rich in protein, male neotropical fruit flies (*Anastrepha* spp.) signalled more frequently for mates (Aluja, Jácome, & Macías-Ordóñez, 2001), male black field crickets, *Teleogryllus commodus*, started signalling for mates earlier in adulthood (Hunt et al., 2004) and male bush-crickets (*Requena verticalis*) experienced more mating attempts (Schatral, 1993) compared to males fed diets low in protein. A protein-rich diet may be important in mate attraction signalling, as it should provide the amino acids required to repair damage in metabolically active signalling tissue. However, these studies coarsely manipulated protein content without regard to the amounts of other dietary components (e.g. carbohydrate, lipid, sucrose, calcium, salts), making it difficult to ascertain what nutrient(s) influenced variation in these fitness-enhancing traits.

Recent studies from the fields of nutritional geometry have precisely manipulated the nutrient ratio of key dietary components and have shown an impact on fitness-enhancing traits (e.g. protein:lipid ratio in mink: Mayntz et al., 2009; carbon:nitrogen:phosphorus ratio in crickets: Harrison, Raubenheimer, Simpson, Godin, & Bertram, 2014; macronutrient:calcium ratio in kakapo: Raubenheimer & Simpson, 2006). For example, several studies have manipulated the ratio of protein to carbohydrate

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availability and have shown that carbohydrate availability is more important than protein availability in its influence on insect mate signalling. Male field crickets fed a carbohydrate-rich diet signalled with significantly higher effort than males fed a protein-rich diet (Maklakov et al., 2008). Similarly, carbohydrate availability was more important than protein availability in its influence on male sex pheromone expression and attractiveness in male cockroaches (South, House, Moore, Simpson, & Hunt, 2011). The expression of all three components of the male sexual pheromones was maximized on diets containing a low ratio of protein to carbohydrate. Overall, these studies suggest that carbohydrate availability is important for maximized mate attraction signalling, likely because carbohydrate acts as a source of fuel for energetically costly signalling (Hack, 1997; Thomson, Darveau, & Bertram, 2014).

Many of the aforementioned nutrient-specific studies only manipulated nutrients during the adult phase of life. However, numerous studies have shown that protein availability strongly influences juvenile development (e.g. caterpillars: Roeder et al., 2014; nematodes: Hansen, Ashley, & Chung, 2015; spiders: Mayntz & Toft, 2001; tadpoles: Venesky et al., 2012; fish: Kpundeh, Qiang, He, Yang, & Xu, 2015; birds: Adeyemo, Abioye, & Aderemi, 2012; Searcy, Peters, & Nowicki, 2004). For example, when Hunt et al. (2004) manipulated protein availability throughout *T. commodus* development, protein availability was positively associated with faster development, greater survival to eclosion and larger body size (Hunt et al., 2004). Given the apparent significance of protein for juvenile development and carbohydrate availability for adult mate signalling, there is a need to investigate the importance of protein availability relative to carbohydrate availability in both juveniles and adults using a more nutrient-specific approach. Fitness-enhancing traits should have different optimal diets depending on whether they are more related to growth and development or to reproduction. Variation in fitness-enhancing traits could, therefore, be partially maintained if males differ in their ability to switch from the physiological/metabolic need for protein to build tissues and maximize their growth to the need for carbohydrate to fuel their energetically demanding mate attraction behaviours.

We quantified how the dietary nutrient balance (the relative amounts of protein and carbohydrate) during development and into adulthood influenced field cricket life history and sexually selected traits. Wild crickets are omnivorous, predominately consuming organic debris, leaf materials, grasses, seeds, animal remains, fungi and grains (Criddle, 1925; Gangwere, 1958, 1961). Given that the diet of wild crickets can be highly variable, resulting in substantial differences in the nutritional content, we explored how dietary nutrient balance influenced male Jamaican field cricket, *Gryllus assimilis*, development time, adult body size, adult residual mass at eclosion, the onset of acoustic mate attraction signalling, average daily time spent signalling, and several fine-scale traits associated with mate attraction signalling. In addition, we also examined how the probability of signalling and daily signalling time relates to male age, and whether this relationship is influenced by diet. We focused on development time because males that reach adulthood quickly, and thus become flight capable, should experience reduced predation attempts. Males that reach adulthood quickly should also have earlier opportunities to signal for mates. We focused on body size at adulthood and mate attraction signalling because female *G. assimilis* often preferentially mate with larger males that signal louder and with higher effort (Bertram et al., 2016; Loranger & Bertram, 2016; Pacheco & Bertram, 2014). Females in other field crickets species also show similar preferences, with females preferring males that signal louder and/or with higher effort (*Gryllus texensis*: Cade & Cade, 1992; *Gryllus firmus*: Crnokrak & Roff, 1995; *Gryllus campestris*: Holzer, Jacot, & Brinkhof, 2003; *T. commodus*: Hunt et al., 2004; *Gryllus pennsylvanicus*: Judge, Ting, & Gwynne, 2014), at higher chirp rates (*Gryllus lineaticeps*: Wagner & Basolo, 2007; *Acheta domestica*: Stout & McGhee, 1988), with longer chirp durations (*G. lineaticeps*: Wagner, 1996; Wagner & Reiser, 2000) and/or higher pulses per chirp/trill (*Gryllus bimaculatus*: Popov & Shuvalov, 1977; *G. texensis*: Wagner et al., 1995).

We hypothesized that during development, the ratio of protein (P) to carbohydrate (C) in the diet would influence nymphal survival to adult eclosion, development time, adult body size and adult residual mass. We predicted that juvenile males fed a protein-rich diet would develop faster, experience greater survival to adulthood and be larger and relatively heavier at eclosion than juvenile males fed a carbohydrate-rich diet. To test these predictions we reared crickets from initial wing bud stage to adult eclosion on two different diets: one rich in protein (protein:carbohydrate ratio (P:C) = 3:1) and one rich in carbohydrate (P:C = 1:3). We also hypothesized that the ratio of protein to carbohydrate in the diet would influence acoustic mate attraction signalling. We predicted that males fed a protein-rich diet during development would start signalling for mates earlier in adulthood and would signal more attractively because they would be larger. We also predicted that males fed a carbohydrate-rich diet in adulthood would signal with higher effort (more often, louder and at higher chirp rates) over the course of their adult lives. To test these predictions we used 2×2 factorial design, switching the diets of half the males once they eclosed to adulthood while maintaining the other adult males on the same diet as they experienced during the juvenile stage. We examined how mate signalling displays were influenced by protein-rich versus carbohydrate-rich diets overall and through time as males aged.

METHODS

We conducted our study in accordance with the guidelines of the Canadian Council on Animal Care. Our research was not reviewed by an animal care committee as the Canadian Council on Animal Care does not require research on insects to be reviewed. While no field-collecting or import permits were required to collect or house *G. assimilis*, the facility we used to house this population had Plant Pest Containment Level 1 rating (PPC1 permit no. PC-2014-053).

Subjects and Rearing

All 142 male *G. assimilis* used in our study were descendants of adults collected near the Stengl Lost Pines Biological Station (University of Texas at Austin; Bastrop County, TX, U.S.A.; ~30°17′N, ~97°46′W, elevation ~145 m) in September 2008. We housed our cricket population in a greenhouse located at Carleton University in Ottawa, Ontario, Canada.

We raised mixed-sex juvenile crickets communally in plastic bins (64×40 cm and 42 cm high) with cardboard egg carton for shelter, ad libitum water and food (powdered Harlan Teklad Rodent diet no. 8604 composed of 24.3% protein, 40.2% carbohydrate, 1.1% phosphorus and 12.4% indigestible fibre; Harlan Laboratories, Indianapolis, IN, U.S.A.). As soon as crickets completed the moult that revealed their sex (initial wing bud development), we weighed and photographed the males (described below) and then transferred them into individual 500 ml clear plastic containers with a screened lid, shelter, ad libitum water and their experimental diet (described below). All male *G. assimilis* were housed in the greenhouse at 26 ± 2 °C, under a 14:10 h light:dark cycle throughout the experiment.