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Household and Structural Insects

Comparison of Diet Preferences of Laboratory-Reared and Apartment-Collected German Cockroaches

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Abstract

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae), is a common pest of human-built structures worldwide. German cockroaches are generalist omnivores able to survive on a wide variety of foods. A number of studies have concluded that laboratory-reared *B. germanica* self-select diets with an approximate 1P:3C (protein-to-carbohydrate) ratio. We predicted that field-collected insects would exhibit more variable dietary preferences, related to the wide-ranging quality, quantity, and patchiness of foods available to them. We compared diet self-selection of *B. germanica* within apartments and in the laboratory by offering them a choice of two complementary diets with 1P:1C and 1P:11C ratios. We observed high variation in the population-level self-selection of these diets among individual apartment sites as well as among various life stages tested in laboratory-based assays. Significant differences between populations in various apartments as well as between populations maintained in the laboratory suggested that factors beyond temporary food scarcity influence diet choice. Nevertheless, we found significant correlations between the amounts of diets ingested by cockroaches in apartments and cockroaches from the same populations assayed in the laboratory, as well as between males, females, and nymphs from these populations. These findings suggest that females, males, and nymphs within apartments adapt to the local conditions and convergently prefer similar amounts of food of similar dietary protein content.

Key words: German cockroach, nutrition, urban, ecology

The German cockroach, *Blattella germanica* (L.) (Blattodea: Ectobiidae) is a major pest in homes and other human-built structures. Infestations produce potent allergens that contribute to the high prevalence of asthma in infested communities (Rosenstreich et al. 1997, Gore and Schal 2007, Pomés and Schal 2020). These cockroaches also can harbor and transport pathogenic microorganisms in their gut and feces, as well as mechanically transport pathogens on their bodies (Brenner and Kramer 2018, Donkor 2019, Schal and Devries 2021).

Cockroaches are generalist omnivores able to survive on a variety of foods. There are numerous studies with laboratory-reared cockroaches focused on the effects of diets on development and reproduction (Gordon 1959, Hamilton and Schal 1988, Cooper and Schal 1992, Raubenheimer and Jones 2006, Jensen et al. 2015a, Jensen et al. 2015b, Jensen et al. 2016). German cockroaches depend on sufficient food and nutrients for survival and optimal growth and fecundity (Cooper and Schal 1992, Jensen and Silverman 2018, Appel 2021). Generally, high protein levels have deleterious effects on development rate, oocyte growth, and longevity (Noland and Baumann 1951, Hamilton and Schal 1988, Schal et al. 1993). However, low amounts of protein can also slow down nymphal development and female reproduction (Cooper and Schal 1992, Schal et al. 1997). The self-selected protein-to-carbohydrate ratio (P:C) for developing cockroaches is generally reported as 1P:2C to 1P:3C (Jones and Raubenheimer 2001).

Diet choices of specific field-collected populations of *B. germanica* have also been investigated in the laboratory (Hamilton and Schal 1988, Cooper and Schal 1992, Raubenheimer and Jones 2006, Jensen et al. 2015a, Jensen et al. 2015b, Jensen et al. 2016). Surprisingly, only one study focused on what *B. germanica* might eat in occupied apartments (Kells et al. 1999). This study inferred food intake from indirect measures, including

body composition and respiratory quotient, and it focused exclusively on field-collected nymphs. These indirect measures suggested that nymphs had consumed higher levels of fats and lower levels of protein and carbohydrates than laboratory-reared nymphs (Kells et al. 1999). Although studies in the laboratory have shown negative effects of too much or too little protein, they also found that cockroaches eating low protein diets were still able to grow and reproduce successfully, especially if they could compensate by consuming a greater amount of diet, or could choose between multiple diets for additive effects (Hamilton and Schal 1988, Cooper and Schal 1992, Jones and Raubenheimer 2001, Raubenheimer and Jones 2006). German cockroaches were also able to complete nymphal development on a diet of 7.5% protein, and oocyte maturation was supported on 10–15% protein (Schal et al. 1993). *B. germanica* are able to store excess nitrogen in the form of urates that can be mobilized during times of low protein availability (Valovage and Brooks 1979, Mullins et al. 1992).

The goal of our study was to compare the diet preferences of cockroaches in the field (infested apartments) with those of field-collected cockroaches assayed in the laboratory. The latter also enabled us to differentiate the performance of different life stages. We also aimed to compare multiple apartment-collected cockroaches to two long-established laboratory cultures. By offering cockroaches complementary high-protein and high-carbohydrate diets, they could balance nutrients by eating a preferred combination of the two. We hypothesized, based on previous work, that relative to laboratory cockroaches, field-collected insects would eat higher levels of protein (relative to carbohydrates), which would ensure developmental and reproductive success. Because *B. germanica* are able to self-select between diets for optimal nutrition (Raubenheimer and Jones 2006), and given their nitrogen-storing capabilities, it would be advantageous for them to consume more protein when it is available. This strategy would not only compensate for chronic protein deficits but may also buffer against future protein scarcity. We hypothesized that, in contrast, laboratory-reared cockroaches with ad libitum access to a complete diet would already have sufficient dietary and stored protein.

The geometric framework of nutrition (Simpson and Raubenheimer 2012) is an effective approach to visualize and analyze how animals adjust how much they eat of single or multiple foods, and how they cope with nutritional imbalance, to reach a target that optimally supports growth, development and reproduction. When cockroaches are offered two imbalanced diets, the geometric framework of nutrition can identify how foragers prioritize their intake of these diets and hence specific nutrients. We applied this approach in assays of laboratory-reared and apartment-collected cockroaches.

Materials and Methods

Laboratory Insects

Two laboratory-reared German cockroach strains were assessed for preference with complementary diets in choice assays. Both strains were maintained on ad libitum water and rodent diet (Purina 5001 Rodent Diet, PMI Nutrition International, St. Louis, MO) at approximately 27°C and 35% RH on a 12:12 h L:D cycle. The Orlando Normal strain was collected in 1947 (nearly 400 generations in culture) and is susceptible to insecticides. The PR-712 strain was collected from a single apartment in Puerto Rico in 2012 (about 30 generations in culture). A variety of bait products had failed to control this population (Ko et al. 2016), and it has been maintained in the laboratory with no insecticide selection pressure. Cockroaches were reared in groups of mixed life stages and randomly selected for assays.

Diet Choices

Two diets were modified from Jones and Raubenheimer (2001); see Table 1 for ingredients. Both diets were prepared identically but had differing proportions of protein and carbohydrate ingredients. First, linoleic acid and cholesterol were dissolved in 40 ml chloroform, to which we added cellulose and casein. Chloroform was then completely evaporated in a fume hood for 24 h with occasional stirring. Separately, we dissolved the vitamins in 4 ml of ethanol and added them and all other ingredients, except agar, to the cellulose and casein mixture. Agar was brought to a boil in a microwave oven in 400 ml water, cooled to 60°C, and mixed thoroughly with the other ingredients. The diet mixture (100 g) was poured into six Petri dishes (90 × 15 mm) and lyophilized. Diets were stored at −20°C, and slowly re-dried at a low temperature of 30°C prior to pre-weighing for assays.

Collection of Cockroaches and Diet Choice in Apartments

We collected cockroaches from apartment kitchens using a Eureka Mighty-Mite vacuum cleaner modified with a screen lined plastic tube at the distal end of the extension tube (DeVries et al. 2019) and transferred them to holding cages. Cockroaches were collected only from apartments with large cockroach populations. We sampled a total of 13 apartments, and the sample size of cockroaches assayed ranged from 14 to 33 per apartment (replicates for each apartment are shown in Figs. 1–4). We then placed three dried and pre-weighed diet stations in areas of the kitchen observed to have high densities of cockroaches; thus, there were three replicates of population-wide diet choice within each of the 13 apartments. We used clear plastic Maxforce Refillable Buffet Station (Bayer Crop Science, RTP, NC) with two entrances (inset Fig. 1A); one of each diet type was placed at each entrance to ensure insects encountered both. The stations contained sufficient diet to ensure they would not be depleted overnight. We collected the stations the next day, redried the diets at 50°C to shorten drying time, and reweighed them. Diets were not reused.

Table 1. Compositions of the two synthetic diets (by percentage of total mass) used in self-selection assays[a]

Ingredients[b]	1P:1C[c]	1P:11C
Casein (g)	15	2.5
Peptone (g)	7.5	1.25
Albumin (g)	7.5	1.25
Sucrose (g)	30	55
Cellulose (g)	27	27
Agar (g)	9	9
Cholesterol (g)	0.55	0.55
Linoleic acid (ml)	0.55	0.55
Wesson salt mix (g)	2.5	2.5
Vanderzant vitamins (g)	0.46	0.46

[a]Diets were freeze-dried after preparation, and slowly re-dried at 30°C before pre-weighing for experiments.

[b]Diets were modified from Raubenheimer and Jones (2006).

[c]Ratio of protein (P) to carbohydrate (C). The protein in each diet was made up of casein, peptone, and albumin. Sucrose provided the carbohydrate.

Laboratory Feeding Assays for Individual Insects

We tested diet preferences of the two laboratory strains and apartment-collected cockroaches in controlled conditions in the laboratory, using individual cockroaches. The field-collected cockroaches were assayed within 3 h of collection. The environment was identical to the rearing conditions for the laboratory cultures, and the 12:12 L:D cycle was roughly aligned with local time so as not to disrupt the circadian rhythm of the apartment-collected cockroaches. Cockroaches were placed singly in cylindrical glass jars (10 cm ID × 10 cm high). We treated the top inner walls of each jar with petroleum jelly to prevent escape. Each jar had an egg carton harborage, a cotton-stoppered test tube containing water, and a choice of two pre-weighed diets placed into small vial caps for easy retrieval. Three life stages were included in these assays: adult males, adult females (non-gravid), and unsexed large nymphs. Initially, gravid (oothecae-bearing) females were also included, but their food consumption was too low for meaningful analysis. Smaller nymphs (instars 1–3) were likewise excluded. After 24 h, we removed the diets, redried them at 50°C, and reweighed them. The numbers of replications per stage and per population are indicated in Figs. 1–4.

Statistical Analysis

The combined total mass of diet consumed in laboratory assays was calculated. The amount of each diet consumed by cockroaches in the laboratory or field assays was used to calculate the percentage of dietary protein consumed. Percentage protein was calculated as the mass of protein consumed divided by the total mass of protein and carbohydrate consumed; this ranged from 8.3% (only 1P:11C diet consumed) to 50% (only 1P:1C diet consumed).

One-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post hoc test at a significance level of 0.05 were used to compare the amounts of diet consumed and percentage protein consumed based on apartment, sex, and origin (laboratory or field). The diet, protein consumption, sex, population, and origin were analyzed as factors, with each individual population (13 from apartments and two laboratory strains) serving as an experimental unit. In laboratory assays, each individual insect was a replicate within a population, whereas in the field consumption assays each of three diet stations was a replicate of the population-wide consumption. Data for the total mass of diet consumed were less normally distributed than percentage protein, so we transformed total mass-consumed using the Box-Cox method prior to the analysis. Spearman's correlation ($\alpha = 0.05$) was used to determine whether consumption in the field correlated with consumption of freshly collected cockroaches in the laboratory, and if consumption by females, males, and nymphs correlated within populations. All data were analyzed using R (R Core Team 2020).

Results

Total Diet Consumption: Field and Laboratory Assays

We used Box-Cox transformations to normalize the total consumption data. The ANOVA results for population-level total diet consumption were significant for both field and laboratory assays ($F = 5.12$, df = 7, 16, $P = 0.003$ and $F = 10.84$, df = 14, 480, $P < 0.001$, respectively). The ANOVA was followed up with Tukey's HSD comparison of means (Fig. 1). The field assays varied extensively, from ~30 mg (apartments B2245 and C319) to 614 mg (A20) consumed overnight at the population level (Fig. 1A). Even within an apartment complex (complex A), consumption ranged from 128 mg (A31) to 614 mg (A20). Although the laboratory assays of freshly collected cockroaches appear to show greater differences between apartments in different complexes than within complexes, statistical analysis revealed no effect of the apartment complex on diet consumption. Individual cockroaches of the two laboratory strains consumed significantly different amounts of diet; PR-712, the more recently cultured strain, was more similar to the field populations than the older Orlando Normal, suggesting that time in culture might affect consumption. The Orlando Normal cockroaches consumed more than 3-fold the average consumption of all field-collected populations (Fig. 1B).

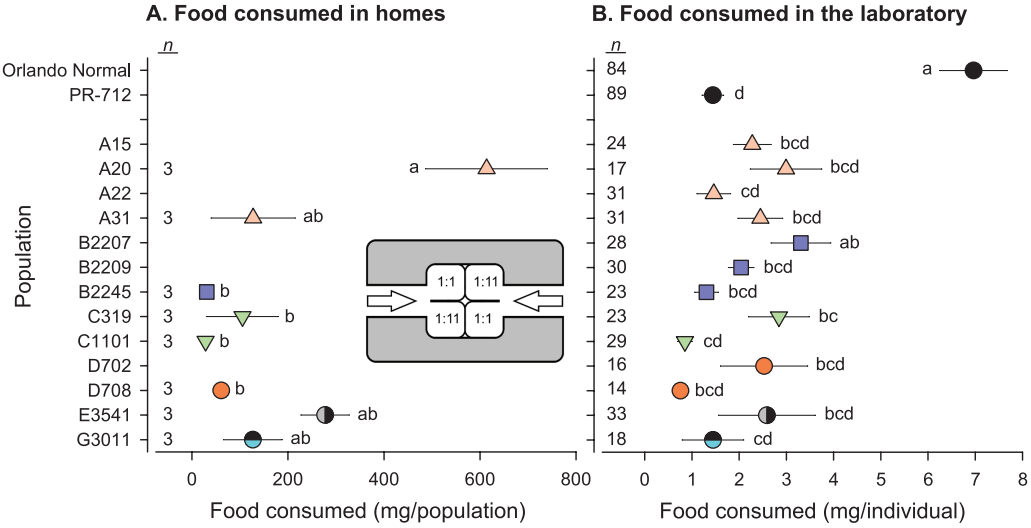

Fig. 1. The total mass of diets consumed (mean ± SEM) within each apartment (A) and in laboratory assays with individual *B. germanica* cockroaches (B). Apartment complex (or laboratory origin) is indicated by the letters in the ID of each population, as well as different colors (online only) and symbols. The number of diet stations per apartment ($n = 3$) and in population assays in the laboratory ($n = 14$–89) are indicated in the figure. The diet station used to assess consumption in homes is shown schematically as an inset in (A). Each diet station used in the field (A) had two entrances, each with one high- and one low-protein diet blocks to ensure the cockroaches entering were exposed to both. Diets in laboratory assays (B) were placed adjacent to each other. Means followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Tukey's honestly significant difference test).

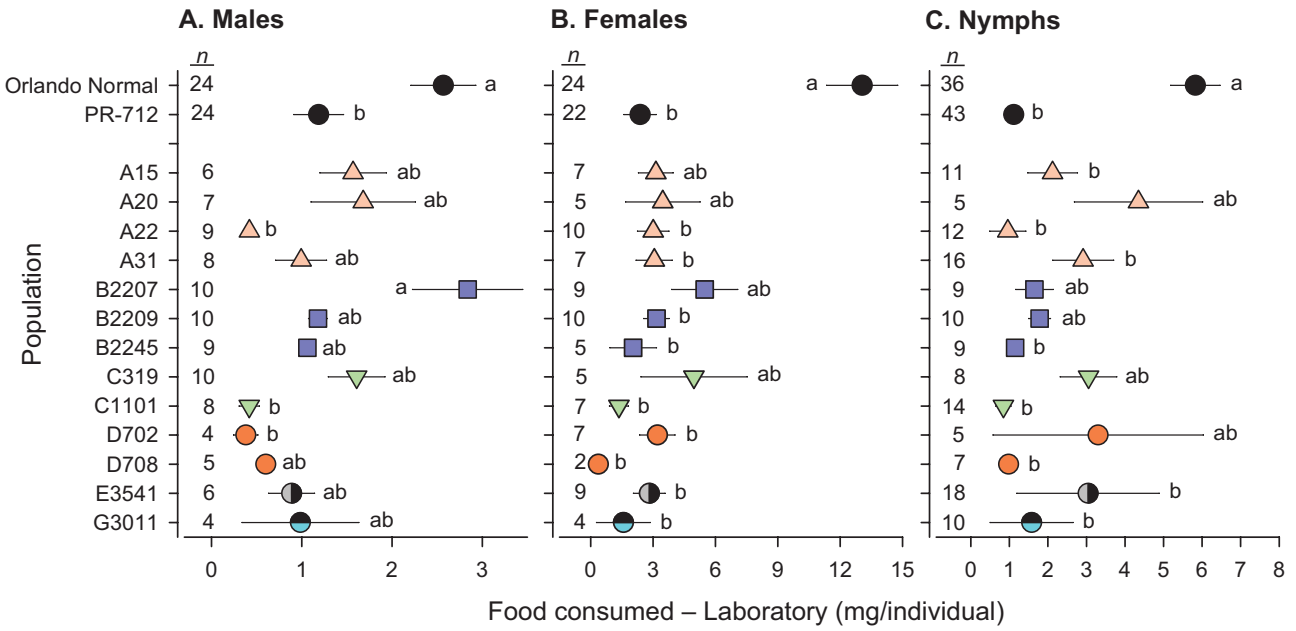

Fig. 2. Total mass of diet consumed (mean ± SEM) in laboratory assays with individual *B. germanica*, separated by life stage. (A) adult males, (B) adult non-gravid females, and (C) large nymphs. Apartment complex (or laboratory origin) is indicated by the letters in the ID of each population, as well as different colors (online only) and symbols. The number of replicates (*n*) for each population is indicated on each figure. Means followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Tukey's honestly significant difference test).

We found a highly significant correlation between population level field consumption in homes and individual consumption in the laboratory by the field-collected cockroaches (Spearman rho = 0.786, $n = 8$, $P = 0.0149$).

Data from the laboratory assays were further separated by stage (i.e., adult males, adult females, and large nymphs) (Fig. 2). The ANOVA results for males, females, and nymphs were all significant ($F = 4.86$, df = 14, 129, $P < 0.001$; $F = 5.00$, df = 15, 123 $P < 0.001$; $F = 6.05$, df = 14, 198, $P < 0.001$, respectively). The consumption patterns were similar to those shown in Fig. 1B, with mean individual consumption varying by stage and by apartment. Males ate the least (Fig. 2A) and females ate the most (Fig. 2B). Again, the Orlando Normal cockroaches consistently ate more than most apartment-collected cockroaches.

We detected a strong correlation between female and male consumption for the 13 apartment-collected populations and two laboratory strains (Spearman rho = 0.668, $n = 15$, $P = 0.0061$), as well as between female and nymph consumption (Spearman rho = 0.764, $n = 15$, $P = 0.0004$). These patterns suggest that all three life stages experience similar nutritional needs in the respective homes they infest.

Relative Protein Consumption: Field and Laboratory Assays

The data for percentage protein consumption were normally distributed and did not require transformation. There were no significant differences in percentage protein consumption among the eight populations that we assayed in the field ($F = 1.068$, df = 7, 16, $P = 0.427$) (Fig. 3A), likely related to small sample size and large variation across replicates. The laboratory assays, however, demonstrated significant differences among the 15 populations ($F = 13.56$, df = 14, 480, $P < 0.001$) (Fig. 3B). Overall, feral cockroaches consumed a higher relative percentage of protein than the two laboratory-reared strains ($F = 268.06$, df = 1, 14, $P < 0.001$). Although protein consumption appears to cluster by apartment complex in Fig. 3B, as does total diet consumption, we again found no significant complex-related effect.

The correlation between protein consumption in the field and laboratory was strong but not statistically significant (Spearman rho = 0.667, $n = 8$, $P = 0.0588$), likely due to the small number of replicates available from the field assays. This pattern nevertheless suggests that protein-seeking cockroaches from specific apartments in the field continue to express this preference in the laboratory.

ANOVA results were also significant when field-collected males, females, and nymphs were singly assayed for protein intake in the laboratory ($F = 12.93$, df = 14, 129 $P < 0.001$; $F = 12.49$, df = 15, 123, $P < 0.001$; $F = 15.24$, df = 14, 198, $P < 0.001$, respectively). Again, all life stages of the two laboratory-reared strains preferred low protein/high carbohydrate consumption, whereas freshly collected cockroaches self-selected across a broad range of protein contents (Fig. 4). Protein intakes by feral cockroaches collected in various apartments within the same apartment complex were not significantly different, but significant differences were evident across different complexes (geographic locations), although again, the complex-related effect was not statistically significant.

As with total diet consumption, we detected a significant correlation between female and male protein consumption for the 13 apartments and two laboratory strains, but this time with the laboratory strains at the lower end of the correlation (Spearman rho = 0.757, $n = 15$, $P = 0.0006$). A significant correlation in protein intake was also evident between females and nymphs (Spearman rho = 0.425, $n = 15$, $P = 0.0004$). These patterns suggest that all three life stages experience similar levels of protein requirement within their respective homes.

Geometric Analysis of Diet Consumption in Homes and the Laboratory

We applied the geometric framework of the nutrition approach (Simpson and Raubenheimer 2012) in dual-choice assays of laboratory-reared and apartment-collected cockroaches. These

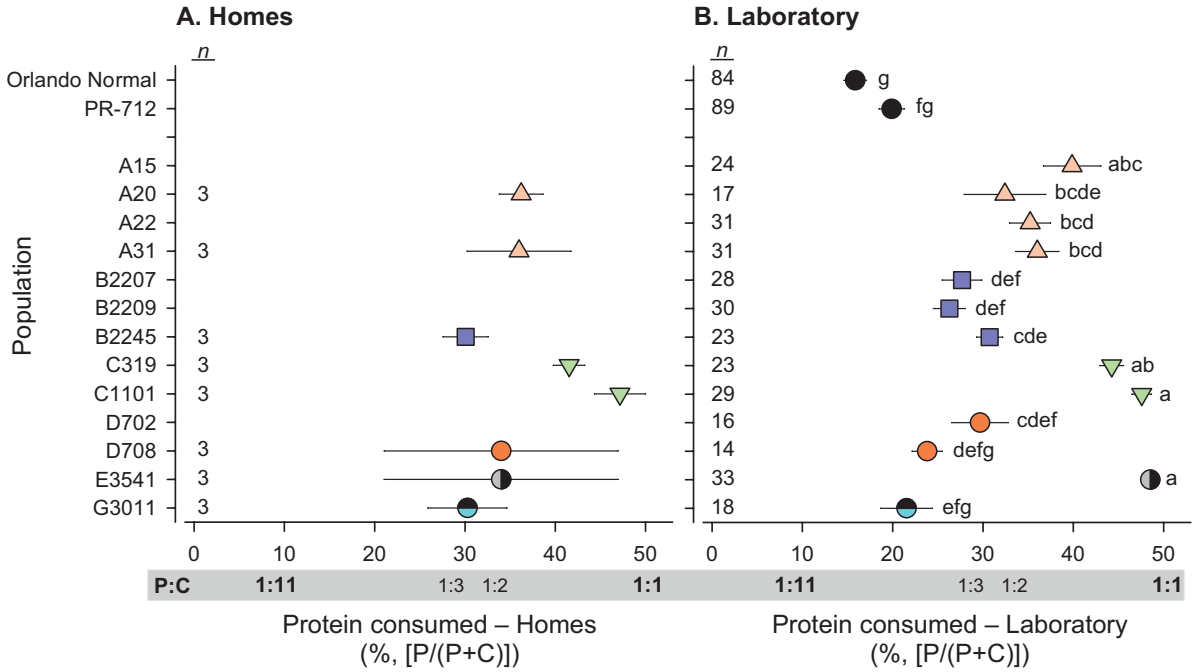

Fig. 3. Percentage of protein consumed (mean ± SEM) at the population level from field stations (A) and by individual *B. germanica* in the laboratory (B). Percentage protein was calculated as the mass of protein consumed divided by the total mass of protein and carbohydrate consumed; this ranged from 8.3% (only 1P:11C diet consumed) to 50% (only 1P:1C diet consumed). Apartment complex (or laboratory origin) is indicated by the letters in the ID of each population, as well as different colors (online only) and symbols. The number of replicates (*n*) for each population assayed in the laboratory is indicated on each figure. Means followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Tukey's honestly significant difference test).

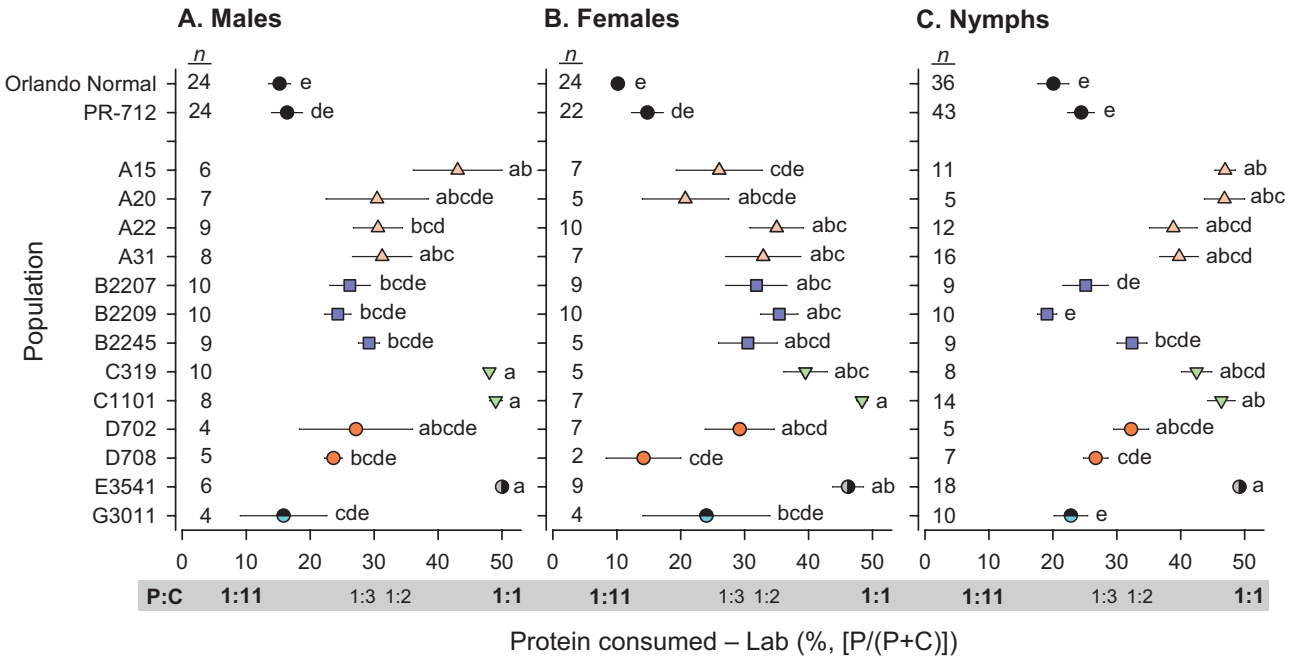

Fig. 4. Percentage of protein consumed (mean ± SEM) by individual *B. germanica* in laboratory assays. (A) Adult males, (B) adult non-gravid females, and (C) large nymphs. Percentage protein was calculated as the mass of protein consumed divided by the total mass of protein and carbohydrate consumed; this ranged from 8.3% (only 1P:11C diet consumed) to 50% (only 1P:1C diet consumed). Apartment complex (or laboratory origin) is indicated by the letters in the ID of each population, as well as different colors (online only) and symbols. The number of replicates (*n*) for each population is indicated on each figure. Means followed by the same letter are not significantly different ($P > 0.05$, ANOVA and Tukey's honestly significant difference test).

assays produced a two-dimensional array, where one axis represents protein intake and the other carbohydrate intake (Fig. 5). Each of the two diets represents a 'nutritional rail' of constant nutrient ratio (P:C) in relation to the amount of diet consumed. We used the amount of each diet consumed to calculate the total mass of protein and carbohydrate consumed. The slopes of these intake arrays

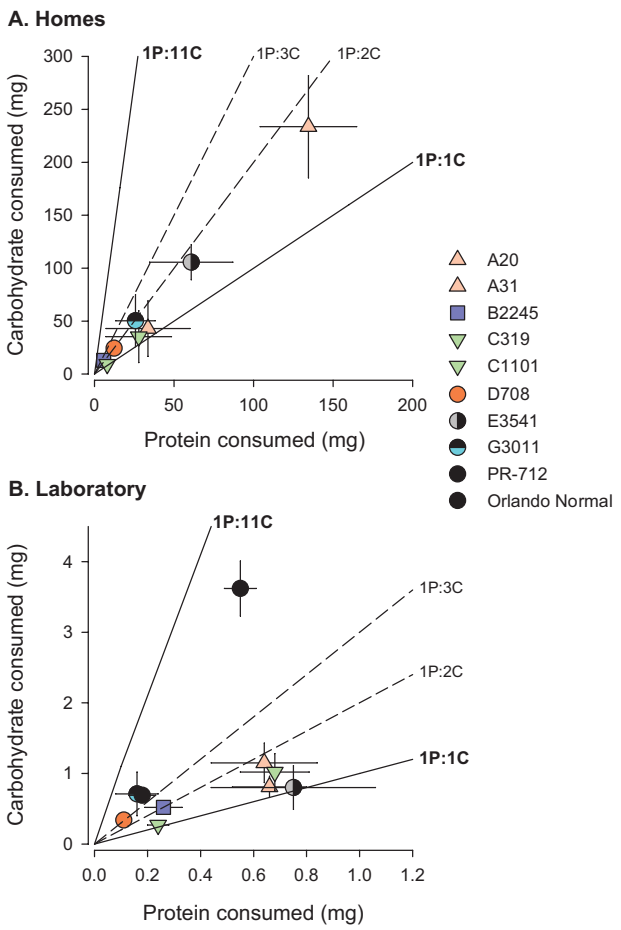

Fig. 5. Nutritional geometry plot of *B. germanica* feeding in homes (A) and the corresponding laboratory results (B); (B) also includes points for the two laboratory-cultured strains. Diet consumption in each apartment population is plotted as mean ± SEM protein eaten (mg), and mean ± SEM carbohydrate eaten (mg). Sample sizes are the same as indicated in Fig. 3, with 3 replicates within each apartment and 14–89 replicates for each population in the laboratory assayed. Four nutritional rails are shown: the solid lines represent 1P:1C and 1P:11C, the protein-to-carbohydrate ratios of the two complementary diets offered in the choice assays; the dotted lines show 1P:2C and 1P:3C, two ratios that are frequently referenced in the literature.

indicate the ratio of nutrients prioritized by populations, each life stage, or individuals. For this analysis, we used only the populations of apartment-collected cockroaches for which we were able to obtain data for both assays. For comparison, we also plotted consumption of the two laboratory-cultured strains. In both apartments and laboratory assays, most cockroaches selected the high protein diet over the high carbohydrate diet (Fig. 5A, B), with most populations self-selecting a nutrient ratio between a 1P:2C and 1P:1C.

Discussion

Food Consumption

Despite feeding on identical diets for seven years, the two laboratory strains were not as similar as we expected. Orlando Normal, a long-term laboratory strain, ate more total diet than any other cockroaches, whereas PR-712, a more recently collected strain, consumed similar amounts of total diet to the freshly field-collected cockroaches (Fig. 1B). Within each of these two strains, all life stages (adult females, adult males, and large nymphs) expressed the same pattern reflected overall (Fig. 2). This pattern suggests that the quantity of ingested food may be positively related to time in culture, and that genetic differences may also be involved.

The field-based assessments of diet consumption revealed large variation in intake across the eight apartments we sampled (Fig. 1A). This variation in *ad libitum* feeding could have been caused by differences in population sizes or demographic differences in populations, as non-gravid adult females feed most and small nymphs and gravid females feed the least (Cochran 1983, Hamilton and Schal 1988, DeMark and Bennett 1994). However, the 24 hr diet intake of freshly collected individual cockroaches from these homes (Fig. 1B) highly correlated with the pattern seen in the field. Because a significant correlation was found for each of the three life stages we examined (Fig. 2), it suggests that cockroaches in some homes feed more than cockroaches in other homes, either due to genetic differences or differences in their nutritional status.

Using one feral population and one related laboratory-cultured strain of the American cockroach (*Periplaneta americana* L. [Blattodea: Blattidae]), Mira and Raubenheimer (2002) demonstrated that the feral insects were heavier and more resilient to starvation, and suggested that the feral cockroaches had experienced directional selection in their harsh environment. Larger insects would be expected to consume more food than laboratory-cultured insects, which would be opposite to our findings that laboratory cultured cockroaches ate more.

It would seem that lower food consumption by feral cockroaches would be at odds with overcoming food scarcity in homes. Whereas laboratory cultures are provided diet in a central predictable location close to the shelters, resources in the field are more variable and widely scattered. An obvious prediction is that feral cockroaches should maximize food intake when they find a rich food source. However, eating less in an apartment setting may adaptively limit their exposure to insecticides, pathogens, and various xenobiotics that may contaminate food sources in the field.

Other factors might have contributed to the pattern we observed. Although heritable diet preferences have been shown in *B. germanica* populations (Silverman and Bieman 1993, Wada-Katsumata et al. 2013), we are not aware of any evidence of genetically biased food intake in this species. Additionally, diet affects the gut microbial community of *B. germanica* (Pérez-Cobas et al. 2015), and apartment-collected and laboratory-reared cockroaches differ in their gut microbiota (Kakumanu et al. 2018). However, it is unknown if the gut microbiota affects diet choice in *B. germanica*. It is possible that feral cockroaches disliked the texture or specific components of our synthetic diet. Food neophobia (reluctance to eat new foods) coupled with their transport from the field to the laboratory might have suppressed the feeding of feral cockroaches in the laboratory. As suggested by Kells et al. (1999), fats may make up a high proportion of the German cockroach diet in apartment settings, and our diets were deficient in lipids, increasing their unfamiliarity. We observed fats in high abundance within apartment kitchens, especially near stovetops and on counters. In addition to neophobia, if the insects are calorically sated by fats this could lead to lower consumption of other foods.

Another possibility, discussed below, is that cockroaches sought a target protein intake. Previous research has shown that *B. germanica* has a preferred target ratio of 1P:2C to 1P:3C (Jones and Raubenheimer 2001, Raubenheimer and Jones 2006, Jensen et al. 2015b). As protein-deficient feral cockroaches seek to ingest more protein, they would take more of the protein-rich 1P:1C diet and require less of the carbohydrate-rich 1P:11C diet. This would result in less overall dietary intake. Related to this consideration, the

laboratory-reared cockroaches were previously restricted to a single diet and having a dietary choice for the first time resulted in greater overall intake as they rebalanced nutrients, as demonstrated by Raubenheimer and Jones (2006). Conversely, the feral cockroaches normally experienced food choices in the field, and their rebalancing of nutrients required lower amounts of the protein-rich diet.

Protein and Carbohydrate Intake

The Blattodea lineage, in general, has evolved multiple adaptive strategies that mitigate nitrogen-deficient foods, including symbiotic association with mycetocyte-housed *Blattabacterium* (Sabree et al. 2009), recycling of exuviae (Mira 2000), storage and recycling of urates (Cochran 1985), coprophagy (Kopanic Jr et al. 2001), and various protein-rich nuptial secretions, including tergal secretions (Brossut and Roth 1977), large spermatophores, and urates (Mullins and Keil 1980, Schal and Bell 1982). The self-selected protein intake of the laboratory-cultured cockroaches was in the 10–12% range, substantially below the expected 1P:2C to 1P:3C protein-to-carbohydrate ratio previously reported for *B. germanica* (Jones and Raubenheimer 2001, Jensen et al. 2015b). This pattern of lower than expected protein consumption was expressed by all three life stages, with even females consuming only about 10% protein (Fig. 4B). These results suggest that the protein content of the rodent chow (29% protein of the total protein and carbohydrate ingredients, equivalent to a 1P:2.5C) fed to these cockroaches exceeded their protein needs; they compensated in our assays by eating more of the 1P:11C carbohydrate-rich diet, rebalancing their nutritional needs. This idea is consistent with the results of Raubenheimer and Jones (2006), who showed that when *B. germanica* nymphs were pretreated with an imbalanced protein-rich diet, they almost immediately (within 4 h) initiated compensatory self-selection, eating more of the complementary carbohydrate-rich diet. Self-selection then directs the cockroaches in the trajectory of their preferred 1P:2C ratio of nutrients.

Conversely, household populations of *B. germanica* were previously found to have lower uric acid stores relative to laboratory cultures, which suggested that they consumed protein-deficient foods (Kells et al. 1999). Therefore, we expected to find greater protein intake in feral cockroaches than in laboratory-cultured cockroaches. Indeed, both laboratory strains were at the lower end of the overall range of protein intake, consuming 1P:5.7C to 1P:4C (Fig. 3B). Feral cockroaches, on the other hand, ate in the range of approximately 20 to 50% protein (1P:4C to 1P:1C) at both the population level in apartments (Fig. 3A) and when cockroaches were individually assayed in the laboratory (Fig. 3B). The correlation between these two measures was strong (Spearman rho = 0.667), but marginally insignificant (P = 0.0588), suggesting a high level of population-specific self-selection to meet protein-to-carbohydrate targets. The patterns expressed by adult females, adult males, and large nymphs in the laboratory assays support this assertion, as they are significantly correlated with each other.

Thus, the pattern of greater protein intake by feral cockroaches versus laboratory-cultured cockroaches confirms the predictions of Kells et al. (1999) from indirect measures of body composition and respiration metrics that feral German cockroaches consumed diets of 7–9% protein. Again, our results also are consistent with the findings of Raubenheimer and Jones (2006) that protein-deficient *B. germanica* nymphs rapidly compensated by self-selecting a protein-rich diet to reach their preferred 1P:2C ratio. They also partly concur with the findings of Clarebrough et al. (2000) who used the same two *P. americana* populations as Mira and Raubenheimer (2002) – this study found that feral males ate more protein in laboratory assays than cultured males, but this pattern was not apparent in females.

Clarebrough et al. (2000) suggested that *P. americana* males differentially allocate ingested protein to their accessory reproductive glands, whereas females have more endosymbionts that aid them in nitrogen metabolism. Likewise, frequently mated *B. germanica* males prefer to ingest more protein-rich diets, presumably to replenish sperm and accessory reproductive gland reserves (Jensen and Silverman 2018). We found that all life stages of feral *B. germanica* consumed more protein than cultured cockroaches. All stages require protein for growth and development (nymphs); vitellogenesis, oocyte maturation and ootheca production (females); and sperm and accessory reproductive gland production (males). However, these life stages vary their protein intake based on their developmental, physiological and gonotrophic stages (Haydak 1953, Schal et al. 1997, Jensen et al. 2016). We selected only non-gravid females (which tend to eat more food than other stages) for inclusion in our 24 hr self-selection assays but could not discern other details of their physiological status, nor those of nymphs and adult males. Unlike *Periplaneta americana* females that have short gestation and produce small oothecae in rapid succession, *B. germanica* females invest in much larger oothecae (relative to body mass) and have a protracted gestation (21 d at 27°C) during which they eat sparingly. Perhaps it is not surprising, therefore, that non-gravid females self-selected more protein in support of vitellogenesis, ootheca production, and the long gestation period.

Finally, various papers on dietary choice in the German cockroach have promulgated an expectation of a 1P:3C preference, likely due to the influential work of Jones and Raubenheimer (2001). It is important to note, however, that the 1P:3C expectation was based on unreported preliminary data, and the empirical results reported by Jones and Raubenheimer (2001) showed that nymphs ate closer to a 1P:2C ratio. The 1P:2C ratio is also supported by other geometric framework analyses, including by Jensen et al. (2015a and b) and our results.

Limitations and Follow-Up Research and Application

We already mentioned some of the limitations of this work, including only two laboratory-cultured strains and a relatively small number of apartments with unknown infestation levels. The stress associated with being captured and transported to the laboratory might also affect consumption 24 h later.

Follow-up work on diet choice in field-collected cockroaches could focus on how dietary preferences change with time in culture, as examined with *P. americana* (Mira and Raubenheimer 2002). Our study focused on freshly collected cockroaches with the aim of representing their field-based preference, which might be shaped by the effects of nutrient scarcity in their feral environment. Culturing field-collected cockroaches on various diets and re-testing them periodically could uncouple the effects of nutrient availability and genetic responses to their initial feral environment. Interestingly, PR-712, the strain that had been in culture for less time than the Orlando Normal cockroaches, behaved more like the field populations, with a wider range of and greater relative amount of protein consumption than the older culture (Orlando Normal). It is possible that with more time away from the pressure of food scarcity, the insects adjust the amount and type of diet they consume, as in the case of glucose-averse cockroaches allowed to proliferate without selection from glucose-containing baits (Jensen et al 2017).

Another consideration for future research is to use various diet choices in self-selection experiments, as done by Jones and Raubenheimer (2001) and Raubenheimer and Jones (2006) with nymphs, to determine whether feral cockroaches consistently converge to the same protein-to-carbohydrate target independently of the diet choices available to them. Notably, the preferred 1P:2C ratio of feral cockroaches approximately coincides with the expected outcome of 1P:2.4C if the insects in our studies randomly ate approximately equal amounts of both 1P:1C and 1P:11C diets. Therefore, it is important to acknowledge that our two diets made it difficult to distinguish between random consumption of the two diets and their self-selection. Nevertheless, we were still able to observe that feral cockroaches self-selected in favor of the more protein-rich diet, while laboratory-reared cockroaches self-selected in favor of the carbohydrate-rich diet.

The most obvious potential application of cockroach dietary preferences is in cockroach control with insecticidal baits. Baits have several advantages over less targeted methods, such as residual sprays. Field populations of cockroaches are less resistant to the active ingredients in baits than to pyrethroids in sprays and aerosols (Wei et al. 2001, DeVries et al. 2019). Additionally, highly palatable bait products can deliver a lethal dose even to resistant insects, which avoids sub-lethal doses of insecticide and the associated issues of selection for insecticide resistance (Gressel 2011). Finally, baits do not leave pesticide residues in non-target areas (Wang et al. 2019). However, optimization of bait palatability and efficacy requires a clear understanding of the odor, taste and nutritional preferences of feral cockroach populations, as well as the effects of bait composition on learning and horizontal transfer of the bait.

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