

Research

Generational variation in nutrient regulation for an outbreking herbivore

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Multivoltine insects can produce multiple generations in one year. Favorable conditions support more generations, leading to serious outbreaks. For herbivores, plant nutrient availability is a major environmental factor affecting fitness and it can shift substantially throughout seasons. In a stochastic environment, organisms can adopt several strategies to regulate their nutrient intake and maximize performance. However, data regarding nutrient regulation of wild herbivores are scarce, and even more so regarding potential intergenerational plasticity. To bridge this gap, we measured nutritional regulation and performance of an outbreking multivoltine herbivore – one of the most serious agricultural pests in the Sahel: *Oedaleus senegalensis*. We surveyed a field population in Senegal and measured its nutritional preference and regulation across two generations (G1 and G3) using artificial diets and plant choice experiments. In the field, G1 locusts were five to ten times more abundant than G3 locusts. We found that G1 and G3 locusts selected different protein:carbohydrate ratios but also that the strength of regulation was different. G1 locusts regulated their nutrient target more tightly than G3 locusts. In contrast, studies with laboratory populations demonstrate strong regulation for grasshoppers, appearing less plastic than field populations. Both generations selected a carbohydrate-biased nutrient ratio, although it was more carbohydrate-biased for G3 locusts. In both cases, plant nutrient contents in the field were more protein biased than their preferred diet. Therefore, choices by locusts were likely influenced by other ecological variables such as leaf toughness or plant defenses. G1 females were heavier and laid more eggs than G3 females. However, G3 locusts survived longer during the experiment than G1 locusts, suggesting a potential generational tradeoff between reproduction and survival. Our data highlight the importance of studying nutritional regulation in situ and incorporating field and lab data to better understand foraging decisions and nutritional tradeoffs.

Keywords: carbohydrates, herbivory, insect outbreak, locust, protein, multivoltine, nutrient regulation, plant nutrients

Introduction

All organisms eat to acquire nutrients to fuel growth, survival and reproduction. For each of these functions there is a blend of nutrient that is optimal and sometimes different from one another. Understanding these nutritional tradeoffs is a key goal in ecology and physiology. In recent years with the development of the Geometric Framework, a physiologically explicit approach to nutrition that emphasizes nutrients that are tasted and selected by animals, much progress has been made in the field. For instance, it was shown that when given the choice, fruit flies select a diet that favors reproduction over survival (Jensen et al. 2015), and that protein is a key nutrient for insect egg production (Barry and Wilder 2013, Clark et al. 2013, Smykal and Raikhel 2015) while carbohydrates are important for migration via lipid production (Weis-Fogh and Uvarov 1952, Jutsum and Goldsworthy 1976, Casey 1981, Hunter et al. 1981, Beenackers et al. 1984, Clark et al. 2013). However, most of those tradeoffs were studied in the laboratory with animals from colonies so we still know very little about how nutritional tradeoffs affect animals in the field. To our knowledge, this study is the first to measure the regulatory capacities of a wild herbivore across generations to understand the mechanisms by which populations can persist in different nutritional habitats and support outbreaks.

For wild herbivores, there is often a mismatch between plant nutrient content and optimal nutrient intake (Toure et al. 2013, Word et al. 2019, Le Gall et al. 2020a). In the field, nutrient availability is highly stochastic because plants chemical composition is dynamic. Plant protein and carbohydrate content vary across space and time, among species (Yeoh and Wee 1994), and also among tissues (Mattson 1980). In contrast, animal body composition is relatively homeostatic (Schoonhoven et al. 2005, Clissold et al. 2006, Behmer and Joern 2012). To solve this problem, herbivores can rely on different strategies. Sessile herbivores can address nutritional deficits by manipulating their host plant nutrient content and/or using post ingestive mechanisms to secure an optimal nutrient intake. For example, the leaf miner *Phyllonorycter blancardella* completes its development within a restricted area of a single leaf and displays both strategies (Kaiser et al. 2010, Body et al. 2013). On the other hand, mobile herbivores can ingest different amounts of various plant tissues and/or plant species to regulate their nutrient intake through foraging (Bernays and Chapman 1994, Lee et al. 2003, Behmer 2009). Grazing animals such as cattle or mobile invertebrates often exhibit strong behavioral nutrient regulation capacities (Provenza and Balph 1987, Behmer 2009). For instance, grasshoppers from a given population will ingest similar quantities of protein and carbohydrates when given the choice between different artificial food pairings (Behmer 2009). In the laboratory, it has been repeatedly shown that the chosen ratio of protein and carbohydrates maximizes performance for one or several traits (Behmer 2009, Simpson and Raubenheimer 2012).

As such, the optimal protein:carbohydrate ratio chosen by an animal can change with its physiological status (i.e. age (Paoli et al. 2014), sex (Lee 2010, South et al. 2011, Reddix et al. 2013, Jensen et al. 2015), migratory status (Cease et al. 2017)). It also appears that different populations of the same species can select different protein:carbohydrate ratios. For example, laboratory populations of final instar differential grasshoppers *Melanoplus differentialis* originally collected from Texas and Nebraska, selected different protein:carbohydrate ratios in two separate studies (Behmer and Joern 2008, Le Gall and Behmer 2014). There are also data on field populations of *Melanoplus femurrubrum* showing those grasshoppers selected different protein:carbohydrate ratios along a latitudinal gradient, suggesting that environmental conditions may be important for shaping the optimal ratio of wild-caught animals (Parsons 2011). However, data regarding nutrient regulation of wild herbivores are scarce, and even more so regarding intraspecific variation by multivoltine species that produce several generations within a year. This is likely because measuring nutritional regulation in the field is more challenging.

To tackle these issues, we used a non-model locust species, the Senegalese grasshopper *Oedaleus senegalensis*. Locusts are swarming grasshoppers that can cause major crop loss leading to famine and outbreaks are often referred to as plagues (COPR 1982). Optimal environmental conditions and/or gregarization is associated with the production of extra generations for several economically important locust species. While extra generations within a growing season are not essential for outbreaks to occur for all locust species, they are reported as a significant contributing factor. For instance, the desert locust *Schistocerca gregaria* can produce two to five generations per year and produces more generations when reared in crowded conditions (Schmidt and Albütz 1999) or after heavy rainfall (Maxwell-Darling 1936). This is the case for the current desert locust outbreak; following two cyclones in the Arabian Peninsula, locusts were able to produce three generations over a nine-month period (Meynard et al. 2020). Similarly, field-caught migratory locusts *Locusta migratoria* seasonally change age of first reproduction, and the species has different numbers of generations as a function of their geographic region (Tanaka et al. 1993). The South American locust *Schistocerca cancellata* also produces a variable number of generations, and additional generations are tied to outbreaks (Hunter and Cosenzo 1990).

These various generations encounter different habitats and are faced with different physiological constraints (Tanaka et al. 1993). This is particularly true for *O. senegalensis* as it inhabits the Sahel, an area where most of the vegetation is only present several months of the year during the rainy season. The Senegalese grasshopper can produce three generations during that relatively short period of time. The first generation (G1) hatches with the first rain and is considered the most important pest of seedling millet, a rain-fed subsistence crop essential to the region. Both first (G1) and second generation (G2) locusts migrate north following the Intertropical Convergence zone (Cheke 1990, Maiga et al.

2008). By contrast, the third generation (G3) migrates south at the end of the rainy season and is a serious pest of millet grains when their return coincides with the milky stage of millet at which the maturing grains are still tender and can be chewed by the grasshoppers. By feeding directly on the grains, the third generation of Senegalese grasshoppers cause direct yield damage (Cheke et al. 1990, Coop and Croft 1993, Maiga et al. 2008). This last generation will then lay eggs that undergo diapause during the dry season (Maiga et al. 2010).

Despite their status as millet pests, the first generation of Senegalese grasshoppers have been reported to be more abundant in fallow fields (fields that are left uncultivated) than in millet fields (Toure et al. 2013, Word et al. 2019, Le Gall et al. 2020a). In previous work, we showed that at the beginning of the rainy season, corresponding to G1 locusts, fallow fields harbor grasses that are more closely matched to *O. senegalensis*' optimal nutrient ratio than millet fields. We now know that the first generation favors a carbohydrate-biased intake (Le Gall et al. 2020b), with female selecting for food that contain slightly more protein than males do (Le Gall et al. 2020b), perhaps for egg production. However, little is known about the nutritional preferences of the third generation.

Thus, in this system, the same species encounters plants that are extremely variable from a nutritional standpoint (from seedling leaves during G1 to grains during G3). Thus, these generations likely face different physiological constraints, allowing us to measure intraspecific variation in the face of environmental change in a relatively short amount of time. To do this, we measured protein and carbohydrate preferences of the first (G1) and third generations (G3) of Senegalese grasshoppers (as estimated by the time they were encountered in the field, G1 at the beginning of the rainy season, and G3 at the end of it) using artificial diets as well as plant choice experiments. We then examined how preference may affect performance in the context of life history tradeoffs between survival and reproduction.

Methods

Oedaleus senegalensis: a multivoltine and migratory species

The Senegalese grasshopper *Oedaleus senegalensis* is a grass-feeder and a major pest of millet and other cereal crops of subsistence agriculture in the Sahel zone of West Africa. *Oedaleus senegalensis* typically produces three generations throughout the rainy season, although when conditions are unfavorable, only two generations are produced (Batten 1969). This species is considered a non-model locust (Song 2011) and locust phase polyphenism (Pener and Simpson 2009, Cullen et al. 2017) is typically poorly understood in non-model species (Song 2011). However, Senegalese grasshoppers, like other locust species, are migratory. They are nocturnal fliers that can travel hundreds of kilometers in one night (Riley and Reynolds 1983, Cheke 1990, Maiga et al. 2008). G1

and G2 travel from south to north along the Intertropical Convergence Front, ahead of the heavy rains. G3 migrates back south at the end of the rainy season (Maiga et al. 2008).

Field site, host plants and locust abundance

We conducted these studies in the summer of 2018 in the Kaffrine region of Senegal. The regional woody shrubland savanna landscape is topographically flat and marked by agriculture expansion that has replaced native dry forests (Mbow et al. 2008). Precipitation ranges from an average of 2 mm in the dry season (November–April) to an average of 737 mm during the rainy season (May–October). Dry season temperatures average ~ 27°C compared to rainy season average of ~ 29°C (D'Alessandro et al. 2015). The early rainy season is marked by quick onset of sprouting vegetation and concomitant emergence or increased activity of associated animals, fungi and bacteria. By the end of the rainy season, annual plants including crops are reaching maturity with thick leaves and seeds (Fig. 1), and natural enemies of grasshoppers are well established and ubiquitous.

The Kaffrine region, along with Kaolack and Fatick regions, is known as the 'West Central Agricultural Region', or Peanut Basin (Tappan et al. 2004) and produces most of the country's millet and peanut. The two crops are typically grown in rotation. pearl millet *Pennisetum glaucum* is a rain-fed crop with excellent tolerance to drought, sandy soil, low nutrient availability and high temperatures. While *O. senegalensis* is considered the main pest of millet, these grasshoppers are typically found to be more abundant in fallow fields containing weed species (Toure et al. 2013, Word et al. 2019, Le Gall et al. 2020a). We decided to contrast millet with one of those weed grasses. We selected *Paspalum scrobiculatum*, also called kodo millet or dugubupicc locally ('dugub' is millet and 'picc' is bird in Wolof), a wild native grass because it is readily eaten by *O. senegalensis* and is preferred over other known host grass species like *Cenchrus biflorus* (Maiga et al. 2008) as shown by a cafeteria experiment that we ran in 2016 (Supporting information).

We measured locust abundance and collected locusts for our experiments in the village of Gniby in fallow and millet fields. We selected Gniby because it consistently supports high density *O. senegalensis* populations (Word et al. 2019, Le Gall et al. 2020a). We collected adult G1 on 4 and 5 August 2018 at three locations 1) 14°43N, 15°67W; 2) 14°42N, 15°67W; 3) 14°25N, 15°40W. On 20 and 22 September we recorded abundance and collected adult G3 at two locations: 1) 14°41N, 15°66W and 2) 14°41N, 15°67W. To measure abundance, for G1 we counted locusts in twenty plots of one square meter each. Each plot was separated by at least ten meters. For G3, the millet reached above eight feet high, so we used a transect method and recorded abundance along ten transects that were ten meters long, one meters wide and separated by five meters each. Results were standardized to locusts per square meter. At both time points, locusts were transported to the Direction de la Protection des Végétaux (DPV) field station in Nganda and kept in wire mesh cages



Figure 1. Millet field at the beginning and end of the growing season. Millet field at the beginning of the rainy season in the Peanut Basin of Senegal (left picture, photo credit Mamour Touré) and at the end of the growing season (right picture; photo credit Marion Le Gall). On the right picture, Idrissa Biaye from the Senegalese Plant Protection Directorate (Direction de la Protection des Végétaux) is seen selecting millet ears at the milky stage of growth, which is the stage where seeds are vulnerable to locust damage because of their softness.

with ambient local vegetation before being used for subsequent experiments.

Artificial diet choice experiment

The aim of this experiment was to compare the protein:carbohydrate ratios selected by G1 and G3 adults when provided with artificial diets. We initiated the choice experiments in August after the start of the rainy season (G1) and in September (G3) towards the end of the rainy season within a day or two of locust collection. For this, we weighed and put locusts in individual aerated plastic containers (15 × 10 cm). Each cage contained a water tube, a perch for roosting and two dishes containing artificial diets. We prepared and dried the food in our laboratory at Arizona State University (United States) following the method developed by Simpson and Abisgold (1985). In total, we made three diets varying in protein to carbohydrate ratios but otherwise isocaloric: p35:c7, p28:c14 and p35:c7. For each diet, 'p' stands for percent of protein in the diet, and 'c' stands for percent of carbohydrates in the diet, by dry mass. The protein component of all foods was a 3:1:1 mix of casein, peptone and albumen, while the digestible carbohydrate (henceforth carbohydrates) component was a 1:1 mix of sucrose and dextrin. All foods contained similar amounts of Wesson's salt (2.4%), cholesterol (0.5%), linoleic acid (0.5%), ascorbic acid (0.3%) and vitamin mix (0.2%) (Dadd 1961). The remainder of the diet was cellulose, a non-nutritive bulking agent.

We gave the locusts one of two treatments of pre-weighed diet pairings: p7:c35 and p35:c7 or p7:c35 and p28:c14 and used 20–25 locusts per treatment (approximately half males and half females). After three days, the diets were removed

and dried for 24–36 h at 60°C (drying oven Memmert and Kowall C1-1) and then re-weighed to record consumption at the nearest 0.1 mg. Locusts were weighed at the beginning and the end of the experiment to calculate mass gain.

Plant choice experiment

The aim of this experiment was to contrast nutrient selection results from the artificial diet choice experiment with nutrient selection from a choice experiment conducted with local plants. For G1, fresh plants were collected in Nganda on the day the experiment started and kept bundled with their stems soaked in water. The millet *P. glaucum* was collected at the seedling stage (~ 25 cm tall, 1–4 leaves sprouted) in a local collaborator farmer's field. The weed *P. scrobiculatum* was collected on the side of the road, also at seedling stage (~ 15 cm tall). For G3, we followed the same protocol but this time we collected mature weed leaves, mature millet leaves and millet seeds at the milky stage (Fig. 1) which are also consumed by locusts (Boys 1978). The first plant choice experiment was started on 5 August; the second one involved an additional treatment (millet seeds) and therefore had to be divided in two experimental blocks to be manageable. These experimental blocks took place on 21 and 27 September.

For each experiment, we put 12 male and 12 female locusts (n = 24) in individual plastic cages (14 × 8 × 4 cm) fitted with a perch for roosting. The leaf stems were maintained in water in a plastic tube sealed with cotton. We cut the millet ears in pieces of roughly 4–5 cm and they were given as is. The experiments each lasted 24 h. At the end of each experiment, we dried all plant material. We estimated dry consumption from regression equations. For this we weighed 20–32 fresh

leaves in August, and again in September; we then dried them at 60°C for 48–72 h and re-weighed them. Regression equations for transforming between plant fresh and dried mass can be found in the supplementary information (Supporting information).

For G3 locusts, we added millet ears. However, despite cutting the millet ears, each piece was several folds heavier than grasshoppers and thus fluctuation in water content made it impossible to collect an accurate estimate of consumption with the same method used for leaves. Thus, we used visual observation to establish preference, for this an observer recorded which food item (weed leaves or millet seeds) had the most grasshopper feeding damage after 30 min.

Plant no-choice

For the plant no-choice experiments, we used the same set up as for the plant choice experiment except that we gave the grasshoppers one plant item only: young millet or weed leaves in August (start date 6 August), and mature millet leaves, millet kernel or mature weed leaves in September (start date 24 September). We used 24 grasshoppers per treatment, half males and half females for G1. We had difficulties collecting enough females for G3 so we used 24 grasshoppers per treatment: 9–10 females and 14–15 males. Each time, the experiment lasted a week and we changed millet and weed leaves every other day and every three days for the kernels. For each grasshopper, we recorded consumption and egg production (for females). We reported consumption for day 0–2 when most locusts were still alive. The cages were checked daily for mortality and the presence of parasites at the time of death was recorded (unidentified dipterans made up the vast majority of parasites and about 25% of the grasshoppers were parasitized).

Chemical analysis

We ran the chemical assays on the dried plant material in our laboratory at Arizona State University (United States). For this, we ground plant samples for 30 s at 200 rpm using a ball mill. We measured plant protein content with a Bradford assay and non-structural carbohydrate content using the phenol–sulphuric acid method (Deans et al. 2018).

Statistical analysis

Locust abundance in the field and locust mass in the plant no-choice experiment were analyzed using ANOVAs. (Although the data were not normally distributed, ANOVA is robust against the violation of normality when group sizes are equal (Donaldson 1968).) Other analysis met the assumptions of parametric tests. The protein:carbohydrate ratios selected in the artificial diet and plant choice experiments were analyzed with MANCOVAs using start mass as a covariate to account for size differences. The dry amount of plant eaten in the choice experiment and plant no-choice experiment, the protein and carbohydrate intakes from the artificial diet and

the plant choice experiments were analyzed using ANCOVAs with start mass as a covariate. Sex was included as cofactor. Visual preference (locusts that did not eat after 30 min were not included in the analysis) in the plant choice experiment, egg laying and parasitism in the plant no-choice experiment, were analyzed by nominal logistic fit. Survival in the plant no-choice experiment was analyzed by survival analysis with Weibull distribution.

Results

Locust abundance

In rural areas surrounding the village of Gniby (Senegal), we found that locusts were more abundant in August (corresponding to the beginning of the rainy season) than September (corresponding to the end of the rainy season). There were ≈ 8 locust per m^2 for G1 and ≈ 0.2 locust per m^2 for G3. When we contrasted land type uses, we found they were more abundant in fallow fields than in millet fields at both time points (August and September) (Fig. 2, Supporting information).

Artificial diet choice experiment

When given the choice between two artificial diet pairings (p35:c7 and p14:c28 or p35:c7 and p7:c35), G1 locusts tightly regulated for a carbohydrate-biased nutrient intake (p1:c1.33) (Fig. 3, Table 2). This ratio was the same for males and females (Table 1). G3 locusts did not regulate nutrient balance as tightly as G1 locusts, with p:c ratios ranging from p1:c1.28 for the p35:c7 and p7:c35 treatment group to p1:c1.52 for the p35:c7 and p14:c28 treatment group (Table 1, Fig. 3). There were also differences between males and females; G3 females ate more carbohydrates than G3 males (Table 1). On average, G3 locusts selected a lower p:c ratio than G1 locusts (roughly p1:c2) (Table 1). Within a generation, the quantity of protein consumed was similar across diet pairings, but it was almost twice as high for G1 as it was for G3 (Table 1, Fig. 3). On the other hand, carbohydrate intake was similar across generations, but higher for locusts eating the p35:c7 and p7:c35 pairing than the p35:c7 and p28:c7 pairing (Fig. 3) and higher for G3 females versus G3 males (Table 1).

Plant choice experiment

When given the choice between different plant species (millet or weed), G1 locusts did not show a preference (Table 3, Fig. 4, 5A). In contrast, G3 locusts consumed more of the mature millet leaves than of the mature weed leaves (Table 3, Fig. 4, 5B). For G3, we also contrasted plant parts by adding a choice with millet ears. Locusts did not distinguish between mature weed leaves or millet seeds based on a 30-minute trial where we recorded plant choice (Table 3, Fig. 4, 5C). For the leaf pairings, the protein: carbohydrate ratio of the nutrients

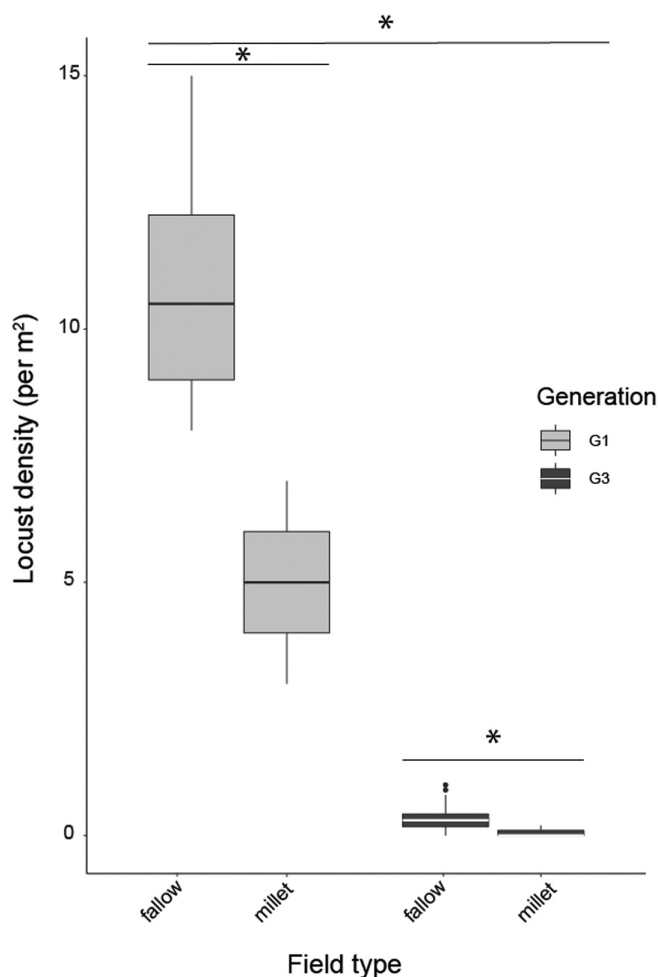


Figure 2. Locust abundance. Locust density (locust per m²) for G1 (4 and 5 August 2018, beginning of the rainy season) and G3 (20 and 22 September, end of the rainy season) in fallow and millet fields. $p < 0.05$.

ingested was protein-biased both for G1 locusts ($p1:c0.51$) and G3 locusts ($p1:c0.77$) (Fig. 4), mirroring the protein-biased content of the leaves (Table 2). G3 locusts ate about three times more plant material overall (Table 3, Fig. 4), and as for the artificial diet choice experiment, their nutrient intake was more carbohydrate-biased than that of G1 locusts (Table 3, Fig. 4).

Plant no-choice experiment

When we restricted the locusts to a single plant species (millet versus weed), or plant part (leaf versus seed), we found that G1 locusts consumed the same amount of young millet and weed leaves, similarly G3 locusts consumed the same amount of mature millet leaves and mature weed leaves. Similar to what we found for the plant choice experiment, locusts from G3 ate more food overall than locusts from G1 (Table 4). We did not find an effect of plant treatment on egg laying, parasitism rate or survival. However, we found differences between the two generations (Table 4). G1 locusts were heavier, but

G3 locusts survived longer (Fig. 5A, D). We found a positive correlation between female start mass and egg laying and G1 locusts laid eggs more frequently than female G3 locusts (Table 4, Fig. 6B). Females were more likely to have parasites at the time of death than males, regardless of the generation (Fig. 6C).

Discussion

Understanding how foraging behavior and resources shape population dynamics is a key question in ecology. Optimal environmental conditions can lead to an increased number of annual generations for multivoltine species, potentially leading to serious outbreaks. For locusts, plant nutritional content has been linked to field density (White 1976, Cease et al. 2012, 2017, Word et al. 2019, Le Gall et al. 2020a); however, no study has compared how changes in the nutritional landscape over time may affect different locust generations. Here, we found consistent differences in nutrient regulation strategies and physiological variables between early and late generations of *O. senegalensis* locusts, suggesting that multivoltine insects can vary nutrient regulation strategies which may allow persistence in stochastic environments.

In the field, we observed that locusts collected at the beginning of the rainy season (G1), were five to ten times more abundant than insects collected toward the end of the rainy season (G3) (Fig. 1). *Oedaleus senegalensis*' migration pattern can lend insight to this result: the first generation hatches with the first rain event during the rainy season and then migrates north following the Inter Tropical Convergence Front (Maiga et al. 2008). A similar pattern is described for the second generation. On the other hand, the third generation is thought to occur in abundance only when conditions are favorable (Batten 1969), supporting higher numbers to migrate south at the end of the rainy season (Cheke 1990, Maiga et al. 2008). While major outbreaks were reported in the previous year (2017) throughout the region, this was not the case during the year of this study. In 2018, the Senegalese Plant Protection Directorate (DPV) reported only a few calls for areas needing treatment (Mamadou Diallo, head of the phytosanitary base of Nganda, pers. comm.). Therefore, the adults we found early in the season (G1) were likely to have hatched locally and had not migrated yet, while adults we found at the end of the rainy season (G3) were possibly migrants, although we have no way of verifying the migratory status of the grasshoppers we caught.

An important feature of grasshopper and locust nutritional ecology is their capacity to tightly regulate for a specific protein:carbohydrate ratio. This regulatory behavior has been highlighted for numerous species over many laboratory studies (Simpson and Raubenheimer 1993, 2000, Chambers et al. 1995, Raubenheimer and Simpson 1999, reviewed in Behmer 2009). In fact, when we measured nutritional preferences with artificial diets, we found that G1 locusts regulated tightly for a moderately carbohydrate-biased nutrient ratio ($p1:c1.33$) as shown by the lack of statistical differences

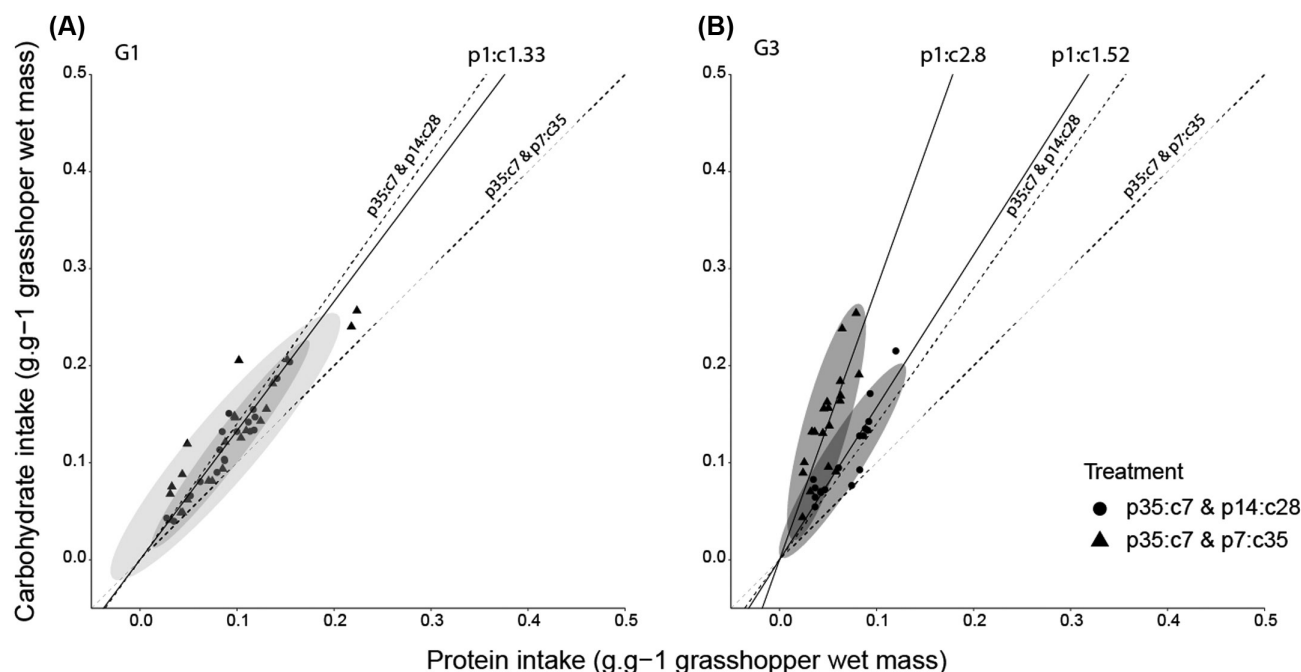


Figure 3. Artificial diet choice experiment: p:c intake. Protein:carbohydrate intake for each artificial diet pairing for G1 locusts ((A); light grey, beginning of the rainy season) and for G3 locusts ((B); dark grey, end of the rainy season). The ellipses represent the confidence intervals. The dashed line illustrates the protein:carbohydrate ratio for random consumption, the solid line illustrates the protein: carbohydrate ratio consumed. For each treatment, we used 24 grasshoppers, $p < 0.05$.

between protein:carbohydrate intake between the two different diets pairings (Table 1). However, for G3 locusts, we found differences in the protein:carbohydrate ratio selected between the two treatments (p1:c2.8 and p1:c1.52). These results showed that the intake was more carbohydrate-biased but also that locusts did not regulate as tightly for a specific nutrient ratio, although nutritional intake was not completely random either (Fig. 3). In a previous study that also used field caught *O. senegalensis* we found that younger final instar juveniles regulated more tightly than older final instar juveniles (Le Gall et al. 2020b). Compared with the extensive lab research on grasshoppers and locusts (Raubenheimer and Simpson 1993, Simpson and Raubenheimer 1993, 2000, Chambers et al. 1995, Simpson et al. 2002, Clissold et al. 2006, Behmer 2009, Le Gall and Behmer 2014), these results suggest that field-caught and laboratory-reared insects exhibit different nutritional regulatory strategies, which could have profound implications for nutritional-based management programs and highlight the need for field verification of lab patterns.

Similarly, a large body of work on nutrient regulation is conducted with artificial diets (Behmer 2009) and few studies use plants. Here, we contrasted the results obtained with artificial diets to the plant choice experiment for each generation. Importantly, all leaves were protein-biased meaning it was not possible for locusts to reach the same carbohydrate-biased intake target that they selected when given artificial diets. Though, surprisingly, we found that G1 locusts ate an equal amount of young millet and young weed leaves, despite young millet leaves being more protein biased than the young

weeds. On the other hand, G3 locusts ate more mature millet leaves than they consumed mature weed leaves, although these two plants were relatively similar in their macronutrient content (Fig. 7). A potential explanation lies in nutrient extraction, between young and tender seedling plants versus mature and tougher leaves. Plant cell walls can prevent herbivore access to both protein and carbohydrates. However, it appears this pattern may be more marked for carbohydrates. For example, a study with the Australian plague locust *Chortoicetes terminifera*, showed that plant toughness reduced carbohydrate assimilation to 40% of the total carbohydrates ingested – versus 80% for protein – for Mitchell grass *Astrebula lappacea* (Clissold et al. 2009). This could explain why host plant selection did not match predictions based on macronutrient contents, as leaf toughness is likely to be species-specific (Lowman and Box 1983, Raupp 1985). Plants belonging to the grass family (Poaceae) often rely on physical properties like silica which is abrasive for insect mandibles and reduces digestibility (Massey et al. 2006, Hunt et al. 2008, Massey and Hartley 2009, Reynolds et al. 2009). Nevertheless, there are some significant exceptions to this, and locusts can be deterred by grass chemicals. For example, the production of cyanoglycoside in *Sorghum* can make seedling plants unpalatable to *Locusta migratoria*, at least during the seedling stage (Woodhead and Bernays 1978). Although responses to macronutrients are typically stronger than responses to micronutrients, grasshoppers can respond to elemental differences as well (Joern et al. 2012). For instance, dietary sodium levels can affect both growth and performance (Peterson et al. 2021), and in mice sodium levels can negatively affect

Table 1. Artificial diet choice experiment: results of MANCOVA for protein and carbohydrate intake and ANCOVAs for protein and carbohydrate intakes as single variables.

Variable	Level	Source	df	F-ratio	p-value
Protein and carbohydrates	whole model	Treatment	2	9.57	< 0.001*
		Generation	2	20.98	< 0.0001*
		Sex	2	2.85	0.06
		Locust wet start mass	2	2.33	0.10
		Treatment	2	0.48	0.62
		Sex	2	0.32	0.73
		Locust wet start mass	2	1.84	0.17
		Treatment	2	20.77	< 0.0001*
		Sex	2	4.64	0.02*
		Locust wet start mass	2	2.85	0.07
	G1	Treatment	1	0.55	0.46
		Generation	1	11.79	< 0.001*
		Sex	1	2.35	0.13
		Locust wet start mass	1	0.65	0.42
		Treatment	1	2.13	0.15
		Sex	1	1.88	0.18
		Locust wet start mass	1	< 0.01	0.96
		Treatment	1	3.09	0.09
		Sex	1	3.58	0.07
Protein	whole model	Treatment	1	7.60	< 0.01*
		Generation	1	0.26	0.61
		Sex	1	5.35	0.02*
		Locust wet start mass	1	0.05	0.82
	G1	Treatment	1	2.99	0.09
		Sex	1	1.41	0.24
		Locust wet start mass	1	0.20	0.66
	G3	Treatment	1	4.29	0.05*
		Sex	1	8.63	0.01*
		Locust wet start mass	1	1.37	0.25
Carbohydrates	whole model	Treatment	1	7.60	< 0.01*
		Generation	1	0.26	0.61
		Sex	1	5.35	0.02*
		Locust wet start mass	1	0.05	0.82
	G1	Treatment	1	2.99	0.09
		Sex	1	1.41	0.24
		Locust wet start mass	1	0.20	0.66
	G3	Treatment	1	4.29	0.05*
		Sex	1	8.63	0.01*
		Locust wet start mass	1	1.37	0.25

(A) reports overall F values for MANCOVA (Pillai's Trace). (B) reports overall F-values for ANCOVAs (Tukey HSD). Treatment refers to the food pairing; locust wet start mass was used as a covariate to adjust for size differences among insects. * $p < 0.05$.

digestion (Wang et al. 2017). Therefore, it is possible that there were differences in sodium levels or other micronutrient variations for plants of different ages.

Despite these differences, overall patterns remained similar for the artificial diet and plant choice experiments and may help explain locust distribution in the field. We found that both for artificial diets and plants, the p:c ratio selected by G1 locusts was less carbohydrate biased than it was for G3 locusts. These results mirrored the nutrient contents of plants accessible to the different generations: older plants contained less protein than younger plants, this was particularly true

Table 2. Plant protein and carbohydrate content.

Plant	Protein (% dry mass)	Carbohydrates (% dry mass)	p:c ratio
(A) August (G1)			
Young millet leaves	27.9	9.8	p1:c0.35
Young weed leaves	15.2	12.3	p1:c0.81
(B) September (G3)			
Mature millet leaves	12.5	9.0	p1:c0.72
Millet seeds	6.8	38.9	p1:c5.8
Mature weed leaves	11.4	10.3	p1:c0.90

Millet = *P. glaucum* and weed = *P. scrobiculatum*.

for young millet that contained almost two to three time as much protein as the other plants (Table 2), and is on par with the nutrient content of millet reported in other studies (Mahalakshmi et al. 1985). We also found that G3 locusts preferred millet seeds which are extremely carbohydrate biased and nutrient dense. This result is in accordance with the existing literature on *O. senegalensis* that describes it as being a pest of seedling millet and soft seeds (Boys 1978) and as preferring carbohydrate biased artificial diets (Le Gall et al. 2020a, b) and plants (Le Gall et al. 2020c). While the weed *Paspalum scrobiculatum* was not readily preferred in the choice experiment to millet, previous work (Toure et al. 2013, Word et al. 2019, Le Gall et al. 2020a) and our current survey data, all showed that at the beginning of the rainy season locusts are more abundant in fallow fields containing mainly weeds of many species than in millet fields. There are several potential (non-mutually exclusive) explanations for this observation: 1) fallow fields harbor a higher diversity of plants that are carbohydrate-biased and therefore provide a more optimal environment for diet mixing, 2) predation is higher in millet fields, 3) fallow fields are better suited for oviposition. While the first hypothesis has received some support (Le Gall et al. 2020a), the two latter still remain to be tested.

For most animals, physiological status (i.e. age, sex, morph, disease, etc.) plays an important role in nutrient regulation (Maklakov et al. 2008, Lee 2010, South et al. 2011, Clark et al. 2013, Reddiex et al. 2013, Paoli et al. 2014, Jensen et al. 2015, Le Gall et al. 2020b). In this study, we compared adult males and females and found no differences in nutrient regulation for G1 locusts. However, G3 females selected a more carbohydrate-biased intake than males. In a previous study, we found that last instar nymphs collected at the beginning of the rainy season (corresponding to nymphs of G1 locusts) selected for different protein:carbohydrate intakes based on sex. Both male and female intakes were carbohydrate-biased, which appear to be a consistent feature for the species regardless of age, generation, and/or sex. However, last instar female nymphs consumed slightly more protein than males (Le Gall et al. 2020b). It is possible that protein acquisition for egg production is more prevalent before the adult molt.

A potential explanation for the importance of carbohydrates in this species is energetic needs linked to migration (Weis-Fogh and Uvarov 1952, Weis-Fogh 1956, Jutsum

Table 3. Plant choice experiment. Results of ANCOVAs for the dry amount of plant material eaten (day 0–2), and for protein and carbohydrate intake by grasshoppers for leaf-only (i.e. treatments involving millet kernels are not included), MANCOVA for protein:carbohydrate intake for leaf-only (i.e. treatments involving millet kernels are not included), and results of nominal logistic fit for visual observation (i.e. treatment involving millet kernels).

Variable	Level	Source	df	F-ratio/ χ^2	p-value
Plant consumption (dry mass)	whole model	Generation	1	6.50	0.01*
		Sex	1	0.11	0.74
		Locust wet start mass	1	0.43	0.52
	G1	Choice	1	3.33	0.08
		Sex	1	0.14	0.71
		Locust wet start mass	1	0.27	0.60
	G3	Choice	1	29.92	< 0.0001*
		Sex	1	4.03	0.05
		Locust wet start mass	1	10.28	< 0.01*
Visual preference	G3	Choice	1	0.66	0.4164
Protein and carbohydrates	whole model	Generation	2	21.05	< 0.0001*
		Sex	2	0.40	0.67
		Locust wet start mass	2	3.66	0.03*
Protein	whole model	Generation	1	9.36	< 0.01*
		Sex	1	0.49	0.49
		Locust wet start mass	1	0.33	0.57
Carbohydrates	whole model	Generation	1	21.08	< 0.0001*
		Sex	1	0.25	0.61
		Locust wet start mass	1	0.67	0.42

Choice refers to a preference within the pairings offered to the locusts, for G1 young millet leaves (*P. glaucum*) versus young weed leaves (*P. scrobiculatum*) and for G3 mature millet leaves versus mature weed leaves and mature weed leaves versus millet seeds. Locust wet start mass was used as a covariate to adjust for size differences among insects. * $p < 0.05$.

and Goldsworthy 1976, Hunter et al. 1981, Talal et al. 2020). Insect migrations are energetically costly and, for locusts, sustained flights are fueled with lipid reserves that are built via carbohydrate consumption (Weis-Fogh and Uvarov 1952, Jutsum and Goldsworthy 1976, Hunter et al. 1981). G3 females may need more carbohydrates to migrate because their southern migration is longer than G1 and

G2 northern migrations (Launois and Launois-Luong 1989, Cheke 1990). Additionally, there are often tradeoffs between migration and reproduction in insects (Zera and Brink 2000, Clark et al. 2013, 2015). This may explain why G1 females were heavier and laid more eggs than G3 females that likely had a longer migration (Fig. 6, Table 4). Therefore, it appears that G1 insects experience an environment that

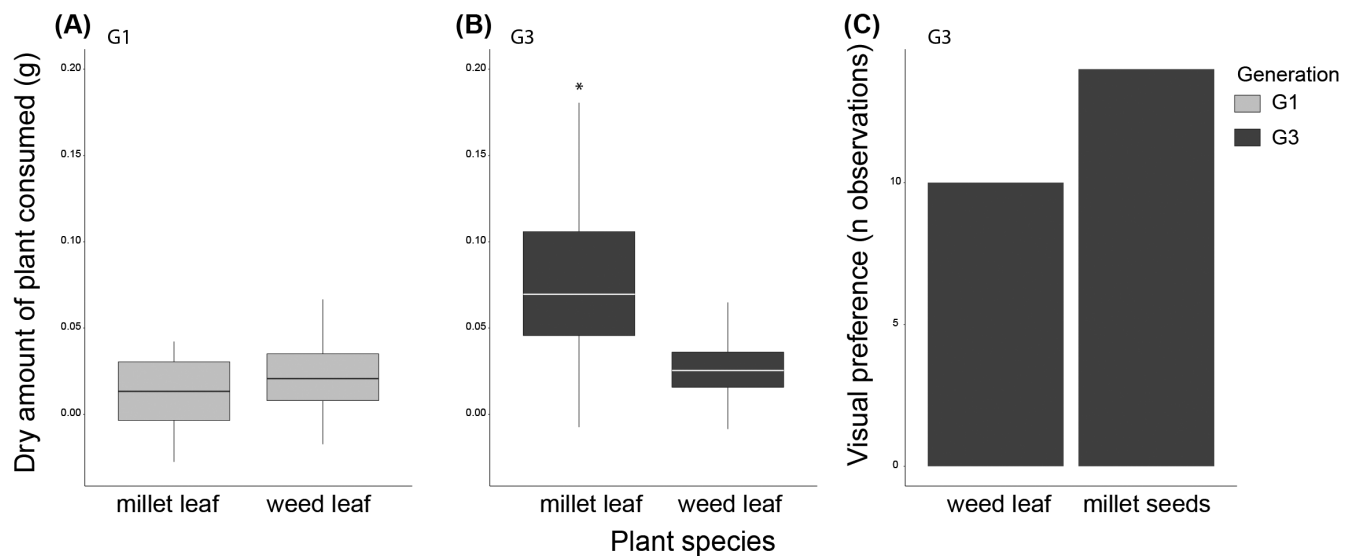


Figure 4. Plant choice experiment: preference. Dry amount of plant consumed in the plant choice experiment (weed = *Paspalum scrobiculatum* and millet = *Pennisetum glaucum*) for G1 locusts (panel (A); light grey, beginning of the rainy season) and for G3 locusts (panel (B); dark grey, end of the rainy season). Panel (C) represents the additional treatment for G3 locusts using millet ears and analyzed using visual preference between weed leaf and millet seeds (end of the rainy season). For each treatment, we used 24 grasshoppers, $p < 0.05$.

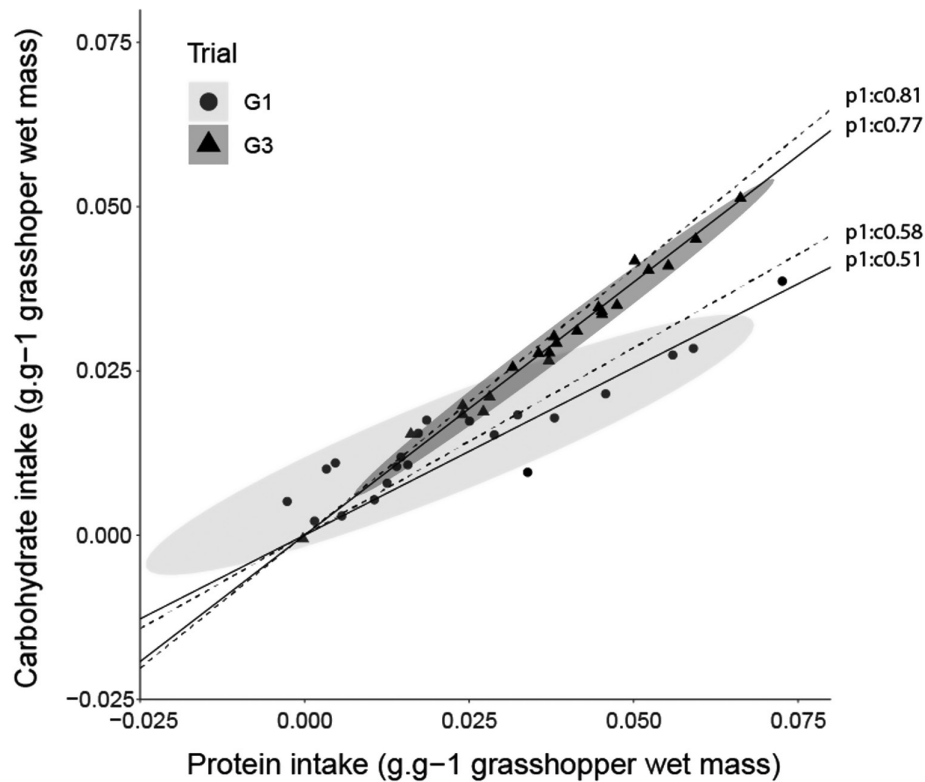


Figure 5. Plant choice experiment: nutrient intakes. Protein:carbohydrate intake from the plant choice experiment for G1 locusts (young millet leaves versus young weed leaves; light grey, beginning of the rainy season) and for G3 locusts (mature millet leaves versus mature weed leaves; dark grey, end of the rainy season). The ellipses represent the confidence intervals. The dashed line illustrates the protein:carbohydrate ratio the locusts would have reached had they eaten randomly (equally) from both plants; the solid line illustrates the protein: carbohydrate ratio consumed. For each treatment, we used 24 grasshoppers.

may be more favorable with plants that are younger and softer, and potentially fewer pathogens and predators. They were more numerous, more nutritionally selective, females were heavier and laid more eggs than G3 locusts, suggesting they allocated more resources to reproduction. On the other hand, the third generation faces more mature and tougher leaves and perhaps more natural enemies. Accordingly, G3 locusts were less nutritionally selective and laid fewer eggs, suggesting they needed to prioritize survival and migration over reproduction. Nonetheless, further research examining the reproductive and migratory potential of females collected across the migration zone during the rainy season is needed to validate this hypothesis. We also noted that females were more frequently parasitized than males. This could be due to their larger size. It is possible that parasitism plays a role in shaping nutritional tradeoff as it negatively affects physiological functions (Hayes and DeCoursey 1938, Danyk et al. 2000, Lomer et al. 2001, Branson 2003, Maiga et al. 2009). However, since we did not observe generational differences, our study does not allow for a more specific hypothesis on the role that it may play in this particular system.

To conclude, we found that the nutritional regulation strategies and performance were substantially different between generations for an outbreaking herbivore (Fig. 7).

Table 4. Plant no-choice experiment. Results of ANCOVA for plant leaf-only consumption from day 0 to 2 (millet kernel treatment not included), ANOVA for start mass, nominal logistic fit for egg laying and parasitism, and survival analysis for survival rate of locusts.

Variable	Source	df	χ^2 /F-ratio	p-value
Plant consumption (dry mass)	Treatment	1	3.19	0.08
	Generation	1	18.99	< 0.0001*
	Sex	1	1.39	0.24
	Start mass	1	0.48	0.49
Start mass	Generation	1	2.23	0.139
	Sex	1	501.80	< 0.001*
Egg laying	Generation × Sex	1	9.75	0.002*
	Treatment	2	2.94	0.231
	Generation	1	4.73	0.03**
	Start mass	1	8.33	0.004*
Parasitism	Treatment	2	1.47	0.479
	Generation	1	0.34	0.558
	Sex	1	7.31	0.007*
Survival	Treatment	2	1.37	0.504
	Generation	1	7.04	0.008*
	Sex	1	0.38	0.538

Generation refers to locusts from G1 or locusts from G3. Treatment refers to the plants offered to the locusts (G1) young millet leaves (*P. glaucum*) or young weed leaves (*P. scrobiculatum*) and G3) mature millet leaves or mature weed leaves or millet seeds. * $p < 0.05$.

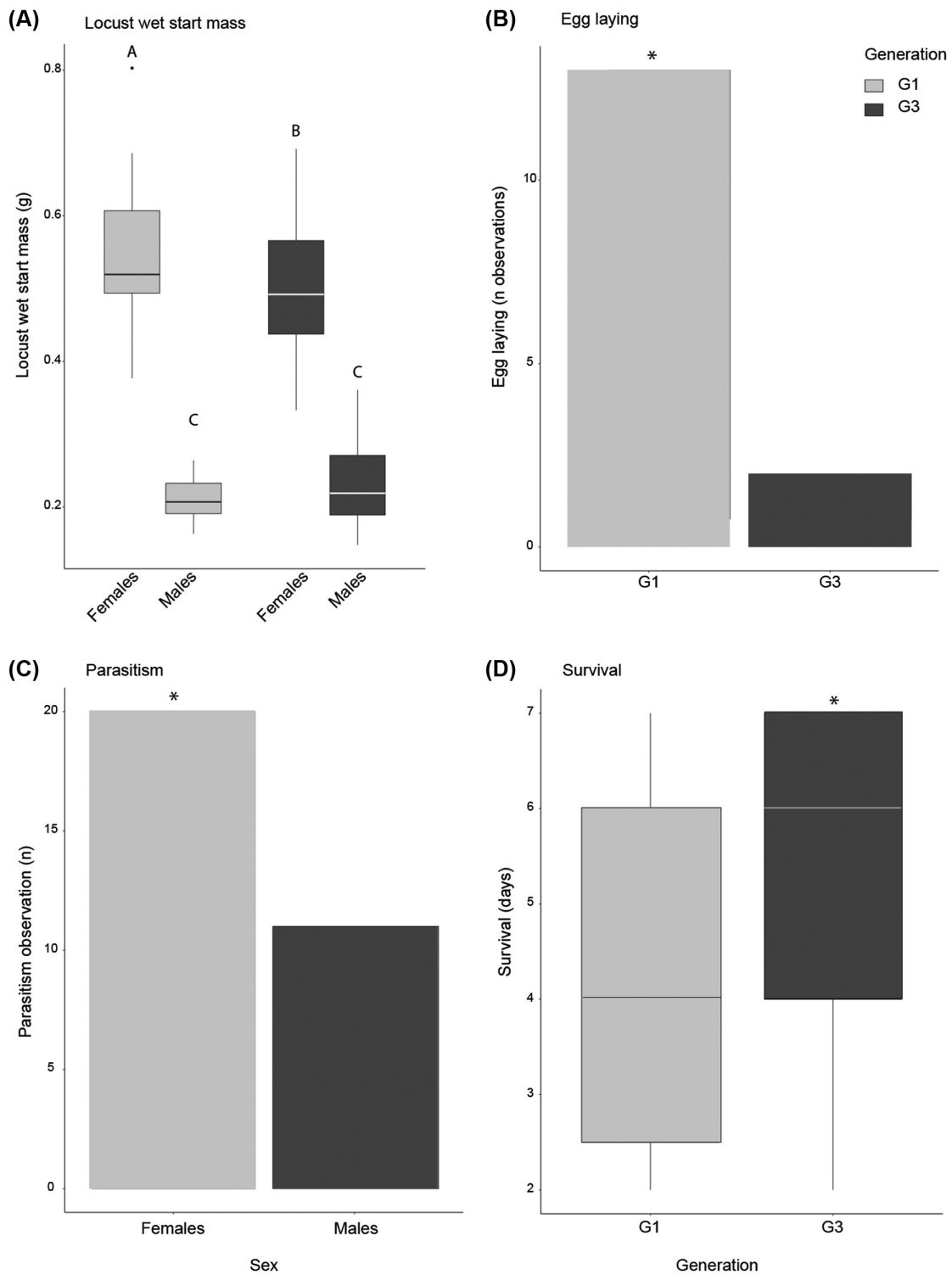


Figure 6. Plant no-choice experiment: performance. Locust wet start mass (A), egg laying (B), parasitism at death (C) and survival (D) on the plant no-choice experiment. For G1 (beginning of the rainy season) we used 48 grasshoppers and 72 for G3 (end of the rainy season), $p < 0.05$.

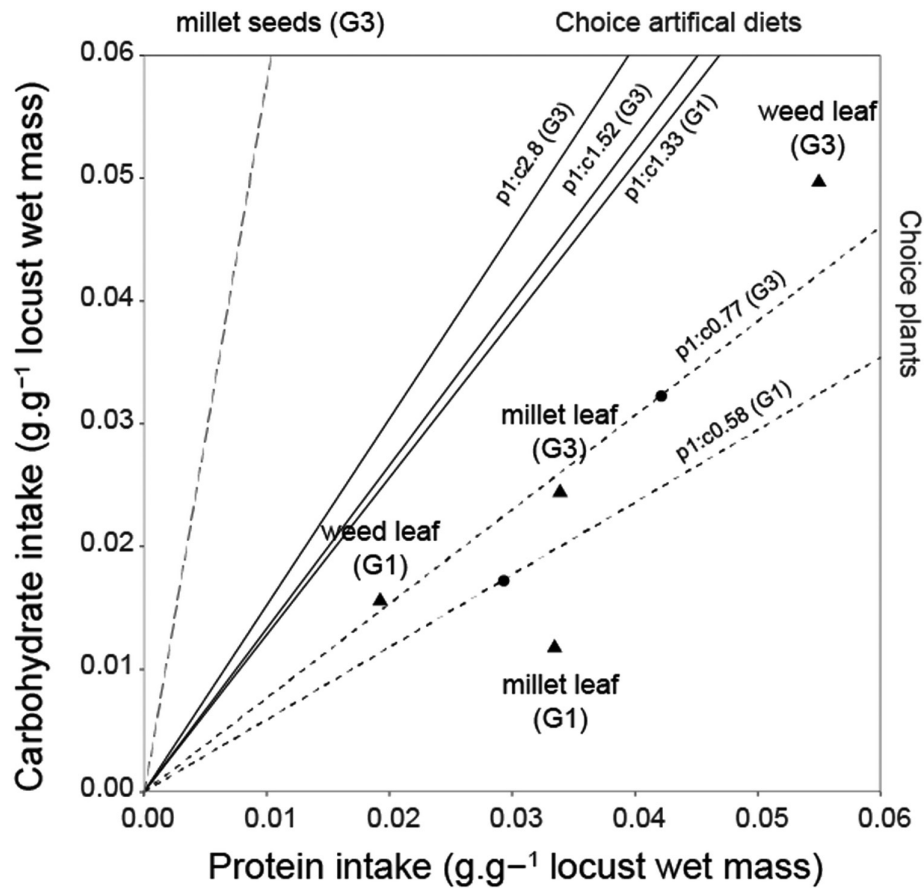


Figure 7. Summary of nutrient intakes by the grasshoppers. Protein:carbohydrate intakes from the artificial diet choice experiment (solid lines), plant choice experiment (round symbol, dashed lines), and the no-choice experiment (triangles) (because we could not measure consumption, the protein:carbohydrate content of millet seeds is illustrated by a grey dashed line). G1 grasshoppers occur at the beginning of the rainy season and G3 at the end of the rainy season. For each treatment, we used 24 grasshoppers.

Unlike grasshoppers and locusts from laboratory colonies, which exhibit highly consistent nutritional regulation, wild population of locusts showed plasticity not only for foraging behavior but also in life history traits such as survival and reproduction. Therefore, plasticity in terms of nutrient intake and allocation may be a key feature allowing persistence of multivoltine species in stochastic environments. Mixing laboratory experiments with field components and using wild populations is challenging for obvious logistical reasons, nevertheless our results illustrate how important this work is to merge the gap with laboratory results to test hypotheses regarding plant–insect interactions and herbivore population dynamics.

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Author contributions

Marion Le Gall: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (supporting); Writing – original draft (lead). **Alioune Beye:** Data curation (supporting); Methodology (supporting); Project administration (supporting); Resources (lead); Validation (supporting). **Mamadou Diallo:** Data curation (supporting); Methodology (supporting); Project administration (supporting); Resources (supporting); Validation (supporting). **Arianne J. Cease:** Conceptualization (supporting);

Data curation (supporting); Funding acquisition (lead); Methodology (equal); Project administration (lead); Resources (lead); Supervision (lead); Writing – review and editing (lead).

Data availability statement

Data are available from the Dryad Digital Repository: <<https://doi.org/10.5061/dryad.v15dv41xp>> (Le Gall et al. 2022).

Supporting information

The supporting information associated with this article is available from the online version.

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