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Gut physicochemistry of grassland grasshoppers

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Abstract

We examined the pH and Eh of the digestive tract of 23 species of mixed-grass prairie grasshoppers, and asked whether these traits were associated with the species breadth and forb composition of their diets. We report that the gut lumen of all grasshoppers was oxidizing and ranged from slightly acid to neutral depending on the gut region and species. Although gut physicochemical conditions differed among species, the differences were of small magnitude. Conditions were fairly uniform along the digestive tract, which suggests little or no regulation of pH or Eh. Gut conditions were independent of diet breadth and the percentage of forbs in the diet. These results suggest that physicochemical conditions of grasshopper guts are not highly regulated and are not influenced by their most recent meal or by broad scale patterns of host-plant use.

Keywords: Grasshopper; Midgut physiology; Midgut pH; Midgut Eh; Diet breadth

1. Introduction

Herbivorous insects employ a number of biochemical mechanisms in the gut lumen to digest plant material and limit the activity of potentially harmful plant allelochemicals and pathogens. Mechanisms identified thus far from one or more insect species include acid lysis, alkaline lysis, enzymatic lysis and microbial fermentation (Appel, 1994; Martin, 1987; Terra, 1990). Many of these involve redox reactions and are thus sensitive to the availability of electrons (Eh) (Johnson and Felton, 1996b) and protons (pH). As few, if any, of these reactions are likely to be at equilibrium in the gut lumen, their specific dynamics are difficult to predict. However, physicochemical parameters, such as Eh and pH, can be readily measured to indicate the general nature of digestion and allelochemical detoxification that occurs in the gut lumen. Comparisons among gut regions in physicochemistry can also provide circumstantial evidence for the presence of pH or Eh buffering systems. In addition, such measurements provide the biochemical context necessary to the design of physiologically appropriate *in vitro* assays of gut function and ingested microbial pesticide action, and for commercial production systems for ingested microbial pathogens.

The first goal of this work was to characterize the physicochemical conditions along the digestive tract of grasshoppers. Although gut conditions of many species of larval Lepidoptera (caterpillars) have been characterized (Appel and Martin, 1990; Berenbaum, 1980), those of Orthoptera (grasshoppers, katydids and crickets) are comparatively poorly known. A survey of midgut pH in seven species of grasshoppers revealed a pH neutral midgut lumen (Bomar et al., 1991), but other gut regions were not examined. A recent study reported mildly oxidizing and pH neutral guts of *Melanoplus sanguinipes* and *Phoetaliotes nebrascensis* (Barbehenn et al., 1996).

The second goal of this work was to begin to characterize the relationship between diet and grasshopper gut physicochemistry. Gut conditions may be determined by ecological and/or evolutionary factors, i.e. by the chemical composition of the most recent meal, by broad patterns of host-plant use, or by a combination of the two. For example, all caterpillar species examined thus far maintain alkaline midguts, and there is some evidence that those species feeding on woody plant foliage have more alkaline midguts than those feeding on forb foliage (Berenbaum, 1980). However, the Eh of caterpillar midguts is determined, in large part, by the most recent meal (Appel and Maines, 1995); Redox properties of

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individual leaf allelochemicals interact with the alkaline pH of their midguts, giving rise to intra- and interspecific differences in midgut Eh on different host plants (Johnson and Felton, 1996b). Thus, in caterpillars, alkaline midgut pH is an evolved trait that is influenced by the host-plant growth form that, which, in turn, determines the impact of food on midgut Eh.

North American acridid grasshoppers are an ideal group of herbivores in which to examine the impact of host-plant growth form and diet breadth on gut physicochemistry. Some species feed exclusively on forbs, some eat only grasses, and some eat both forbs and grasses (Chapman, 1990; Joern, 1979, 1983, 1985). Furthermore, diet mixing by individuals has been well documented for several species (Bernays and Bright, 1991; Bernays et al., 1991). Grasses and forbs usually differ in their water and nitrogen contents and allelochemistry (Bernays and Barbehenn, 1987; Mole and Joern, 1993). If variation in gut physicochemistry is important to handling this variation in host-plant chemistry, then gut conditions of acridid grasshoppers may be associated with the percentage of forbs versus grasses in their diet.

In this study we examined the pH and Eh of the digestive tract of 23 grasshopper species from mixed-grass prairie, and asked whether these traits were influenced by the breadth and forb composition of their diets. We report that the gut lumen of all grasshoppers was oxidizing and ranged from slightly acid to neutral depending on the gut region. Although gut physicochemical conditions differed among species, they were independent of diet breadth and the percentage of forbs in the diet.

2. Materials and methods

2.1. Grasshopper collection

Adult grasshoppers and hostplants were collected May–August of 1994 from Arapaho Prairie, a research site in the sandhills of Arthur County, Nebraska. Individuals were placed in portable cages containing plants that had been put immediately into water after cutting. At the Cedar Point Biological Station laboratory, host-plants were reclipped and placed in water picks and placed with grasshoppers in large Petri dishes for overnight shipment to Pennsylvania. Grasshoppers arrived in good condition, and measurements were made the following afternoon on all individuals with full guts.

2.2. Gut measurements

Live grasshoppers were immobilized briefly on ice and their legs quickly removed by scissors. Guts were exposed with a ventral longitudinal dissection and the body wall pinned back for ready access by microprobes. Measurements were made in four major regions of the grasshopper gut: foregut, caeca (attached at the beginning of the midgut), midgut and ileum (hindgut). The grinding organ (proventriculus) at the junction of the foregut and midgut contained insufficient fluid for measurement. Measurements commenced within 30 s of the start of dissection, and were completed within 1 min. Eh and pH were measured with a micro-needle pH electrode (Sam Agulian, Hamden CT), a 0.02-inch platinum electrode (Microelectrodes, Inc.), and liquid junction Ag–AgCl reference electrodes (Mere-1, WPI) on Accumet (Model 291) and WPI (Model px-250) meters. Because size has been shown to influence gut pH in caterpillars, body lengths were also recorded. To standardize measurements to the hydrogen electrode, 200 mV were added to the Eh readings. Eh is sometimes expressed as its log equivalent pE (similar to pH) by the divisor Eh/59.2, and redox state is sometimes expressed as the sum of pH + pE, but we found no biological or statistical justification to do so in this study.

Although Bomar et al. (1991) concluded that measurement of midgut pH following fast freezing of grasshoppers in propane or liquid nitrogen is superior to live dissections because it yielded lower pH values and lower variance among replicates, we felt that measurement of live grasshoppers was superior for two reasons. First, a lower pH is not necessarily a truer measure of midgut conditions because acidic foliage (pH 4–5) is buffered to a neutral pH in the midgut; in fact, a higher value could be more representative. Second, although biological and experimental sources of variance are impossible to distinguish in this study, there is no *a priori* reason to predict that low variance represents reality better.

2.3. Diet breadth and composition

Values for diet breadth and forb composition were taken directly from previously published work at this site (Otte and Joern, 1977; Joern, 1979, 1983, 1985) for all species except *Aulocara elliotti*, *Hadrotettrix trifasciatus* and *Melanoplus lakinus*. For these species, diet composition and breadth were calculated from published data (Mulkern et al., 1969) on grasshoppers collected at North Platte, Nebraska, a site 30 miles to the south of Arapaho Prairie. Diet breadth (B) was calculated based on a standard index that weights the proportion (pi) of each food plant species taken: $B = \exp(H')$, where $H' = -\sum pi \ln pi$. The percentage of forbs in the diet was determined as the percentage of the total diet that was forbs (i.e. not grasses and sedges). In addition to descriptions of diet use by grasshoppers based on generalized indices, diets of most species consist of unique combinations of host plants that range from essentially no overlap with other species to a fairly high degree of diet similarity. Such variation in diets was observed, even though grasshoppers species employed in this study basically encountered the same range of plant species.

2.4. Statistics

The relationship between grasshopper species and gut conditions was examined using General Linear Models (GLM; SAS Institute) with species, sex, body length and their interactions as main effects. Significant differences among species were determined using the Tukey Studentized range test (HSD), which controls the maximum experiment-wise error rate in pairwise comparisons. The relationship between gut conditions and diet characteristics (breadth and percentage of forbs) was examined by stepwise linear regression (REG; SAS Institute) using diet breadth and percentage of forbs in diet (arcsin transformed) as the independent variables.

3. Results

The average pH of each region of the digestive tract for each species by sex is provided in Table 1. All species examined possessed guts that were slightly acid to neutral. In general, the pH of the gut lumen was lowest in the crop, and ranged from 5.6–7.3 depending on the species. The pH increased slightly in the caeca (6.0–7.4), midgut (5.9–7.3) and hindgut (6.5–7.5).

The average Eh of each region of the digestive tract for each species by sex is shown in Table 2. All species examined possessed guts that were mildly oxidizing. In general, the Eh of the gut lumen was highest in the crop (192–346 mV). The Eh decreased slightly going from the caeca (200–335 mV), to midgut (179–327 mV) and hindgut (172–298 mV).

The relationship between species, sex, and their interaction on gut pH and Eh was examined using general linear models, and these results are shown in Table 4. Body length (Table 3) was not related to sex or species or their interactions, and was dropped from the model.

There were significant differences among species in pH and Eh of all gut regions (Table 4). However, most species were very similar, and the statistically significant differences in pH were due to only a few species and were inconsistent across gut regions. For example, the midgut pH of *Melanoplus foedus* females was lower than most, the midgut and hindgut pH of *Ageneotettix deorum* and *Hesperotettix speciousus* was higher than most, and foregut and caecal pH of *Pardalophora haldemanii* males and females was lower than that of most other species (Table 1). Statistically significant differences in Eh were also due to only a few species, but in contrast to pH the differences were consistent across gut regions. Thus, *H. speciosus* females had a lower Eh and *Melanoplus sanguinipes* males had a higher Eh in all gut regions (Table 2).

There was no direct relationship between sex and gut pH and Eh, but there were significant interactions between species and sex in Eh of all gut regions. This was largely because of *H. speciosus* females and *M. sanguinipes* males described above (Table 2).

The relationship between gut conditions and diet characteristics (breadth and percentage of forbs; Table 3) was examined by stepwise linear regression (REG; SAS Institute) using diet breadth and the percentage of forbs in the diet (arcsin transformed) as independent variables. Neither diet breadth or the percentage of forbs in the diet was a significant predictor of gut pH or Eh.

4. Discussion

The lack of dramatic variation in gut physicochemistry among so many grasshopper species was surprising because we expected at least gut Eh to vary, simply because it was measured against a background of different plants, as has been reported for caterpillars. Our results could arise because: (i) grasshoppers gut conditions are independent of diet, (ii) grasshoppers have different gut conditions when fed on plants other than those on which they were measured, or (iii) our assay conditions interfered in some manner with the detection of differences. Although this experiment was not designed to distinguish between the first two alternatives, we suspect that all grasshopper guts are similar for a number of reasons. First, others (Bomar et al., 1991) found no differences in midgut pH among six field-collected species. Second, the species in this study were measured on a diverse background of plant species (nine species of grasses and composites) that should have produced differences in gut conditions, if diet can do so. Third, the neutral pHs reported here may preclude large differences in gut Eh. Differences in gut Eh of caterpillars on different diets arise, in large part, from individual redox properties of allelochemicals at the alkaline pH of caterpillar guts (Johnson and Felton, 1996a). Last, we think our assay conditions were likely to detect differences if they existed because grasshoppers arrived, and were measured, in good health with guts full of freshly consumed food.

Given the lack of interspecific variation, it is not surprising that we were unable to associate differences among species with the breadth and percent composition of forbs in their diet. Chemical differences among the prairie host-plants of these grasshoppers may be insufficient to select for differences in gut physicochemistry at near neutral pHs. In contrast, at the alkaline pHs of caterpillar midguts, structural variation in nutrients or allelochemicals may lead to large differences in redox chemistry (Johnson and Felton, 1996b) and may actually select for differences in gut Eh.

The lack of dramatic variation between gut regions suggests that pH and Eh are either unregulated or only slightly regulated in grasshoppers. An appropriate test of this would be to compare the pH and Eh of macerated

Table 1
Average pH (mean ± sd) by sex of each region of the digestive tract of grasshoppers. The sample size is 4 individuals per sex

Species	Digestive Tract Region			
	Foregut	Caeca	Midgut	Hindgut
Ageneotettix deorum				
Female	6.80 ± 0.33^{abcd}	7.15 ± 0.06^{ab}	7.33 ± 0.22^{a}	7.48 ± 0.10^{a}
Male	6.63 ± 0.40^{abcde}	6.88 ± 0.38^{abc}	7.05 ± 0.31^{ab}	7.13 ± 0.13^{abcd}
Aulocara elliotti				
Female	6.78 ± 0.17^{abcd}	6.45 ± 0.39^{bcd}	6.80 ± 0.47^{abc}	6.78 ± 0.10^{abcd}
Cordillacris occipitalis				
Female	6.70 ± 0.41^{abcd}	6.93 ± 0.10^{abc}	6.98 ± 0.21^{ab}	7.10 ± 0.23^{abcd}
Male	6.75 ± 0.25^{abcd}	6.73 ± 0.15^{abcd}	6.85 ± 0.31^{abc}	7.13 ± 0.10^{abcd}
Hadrotettix trifasciatus				
Female	6.28 ± 0.17^{abcde}	6.35 ± 0.13^{bcd}	6.35 ± 0.24^{bc}	6.70 ± 0.08^{bcd}
Hesperotettix speciosus				
Female	6.53 ± 0.49^{abcde}	6.80 ± 0.08^{abcd}	7.33 ± 0.19^{a}	7.40 ± 0.14^{ab}
Male	6.65 ± 0.24^{abcde}	6.75 ± 0.38^{abcd}	7.20 ± 0.79^{ab}	7.18 ± 0.39^{abc}
Hesperotettix viridis				
Female	6.70 ± 0.41^{abcd}	6.80 ± 0.14^{abcd}	7.00 ± 0.22^{ab}	7.08 ± 0.21^{abcd}
Male	6.40 ± 0.41^{abcde}	6.73 ± 0.19^{abcd}	6.78 ± 0.28^{abc}	7.03 ± 0.21^{abcd}
Hypochlora alba				
Female	6.53 ± 0.34^{abcde}	6.80 ± 0.38^{abcd}	7.10 ± 0.16^{ab}	7.18 ± 0.15^{abcd}
Male	6.73 ± 0.10^{abcd}	6.88 ± 0.10^{abc}	6.98 ± 0.10^{ab}	7.10 ± 0.14^{abcd}
Melanoplus angustipennis				
Female	6.05 ± 0.13^{cde}	6.48 ± 0.13^{bcd}	6.78 ± 0.33^{abc}	6.73 ± 0.17^{bcd}
Male	6.68 ± 0.25^{abcde}	6.55 ± 0.35^{abcd}	6.63 ± 0.21^{abd}	6.90 ± 0.18^{abcd}
Melanoplus bivittatus				
Female	6.68 ± 0.15^{abcde}	6.80 ± 0.18^{ab}	6.98 ± 0.26^{ab}	7.13 ± 0.17^{abcd}
Male	6.90 ± 0.20^{ab}	7.00 ± 0.14^{ab}	7.10 ± 0.22^{ab}	6.98 ± 0.10^{abcd}
Melanoplus confusus				
Female	6.75 ± 0.29^{abcd}	6.83 ± 0.33^{abcd}	7.25 ± 0.24^{ab}	7.20 ± 0.14^{abc}
Male	6.13 ± 0.39^{bcde}	6.60 ± 0.34^{abcd}	7.08 ± 0.19^{ab}	7.03 ± 0.10^{abcd}
Melanoplus differentialis				
Female	7.20 ± 0.08^{a}	7.03 ± 0.38^{ab}	6.90 ± 0.41^{ab}	7.13 ± 0.29^{abcd}
Male	7.23 ± 0.37^{a}	7.38 ± 0.15^{a}	7.00 ± 0.93^{ab}	7.23 ± 0.43^{abc}
Melanoplus femurrubrum				
Female	7.00 ± 0.41^{abc}	7.08 ± 0.28^{ab}	7.13 ± 0.35^{ab}	7.25 ± 0.24^{abc}
Male	7.05 ± 0.34^{abc}	7.00 ± 0.27^{ab}	7.08 ± 0.46^{ab}	7.18 ± 0.22^{abc}
Melanoplus flavidus				
Female	6.15 ± 0.41^{bcde}	6.78 ± 0.17^{abcd}	6.90 ± 0.22^{ab}	7.00 ± 0.14^{abcd}
Male	6.58 ± 0.17^{abcde}	6.83 ± 0.13^{abcd}	6.93 ± 0.19^{ab}	7.15 ± 0.13^{abcd}
Melenoplus foedus				
Female	6.50 ± 0.37^{abcde}	6.48 ± 0.25^{bcd}	5.90 ± 0.32^{c}	6.63 ± 0.63^{cd}
Male	6.65 ± 0.78^{abcde}	6.95 ± 0.31^{abc}	6.45 ± 0.13^{abc}	7.13 ± 0.21^{abcd}
Melanoplus gladstoni				
Female	6.65 ± 0.31^{abcde}	7.08 ± 0.32^{ab}	7.18 ± 0.17^{ab}	7.15 ± 0.13^{abcd}
Male	6.65 ± 0.13^{abcde}	7.00 ± 0.29^{ab}	7.08 ± 0.10^{ab}	7.33 ± 0.50^{abc}
Melanoplus lakinus				
Female	6.55 ± 0.39^{abcde}	7.13 ± 0.05^{ab}	6.58 ± 0.17^{abc}	7.48 ± 0.13^{a}
Male	6.58 ± 0.10^{abcde}	6.65 ± 0.17^{abcd}	6.48 ± 0.31^{abc}	6.83 ± 0.21^{abcd}
Melanoplus sanguinipes				
Female	7.30 ± 0.18^{a}	7.10 ± 0.24^{ab}	6.40 ± 0.42^{ab}	6.93 ± 0.46^{abcd}
Male	6.65 ± 0.64^{abcde}	6.73 ± 0.49^{abcd}	6.90 ± 0.22^{ab}	7.18 ± 0.21^{abc}
Mermiria bivitatta				
Female	7.10 ± 0.55^{ab}	7.13 ± 0.30^{abcd}	7.18 ± 0.38^{ab}	7.03 ± 0.36^{abce}
Opeia obscura				
Female	7.15 ± 0.66^{ab}	6.80 ± 0.63^{abcd}	6.93 ± 0.38^{ab}	7.18 ± 0.15^{abc}
Pardalophora haldemanii				
Female	5.65 ± 0.40^{de}	6.13 ± 0.49^{cd}	6.70 ± 0.39^{abc}	6.45 ± 0.44^{d}
Male	5.90 ± 0.27^{de}	6.00 ± 0.42^{d}	7.03 ± 0.46^{ab}	7.10 ± 0.37^{abcd}
Phlibostroma quadrimaculatum				
Female	6.80 ± 0.42^{abcd}	6.68 ± 0.31^{abcd}	6.90 ± 0.32^{ab}	7.05 ± 0.24^{abcd}
Phoetaliotes nebrascensis				
Female	6.70 ± 0.37^{abcde}	6.86 ± 0.3^{abcd}	6.93 ± 0.29^{abc}	6.86 ± 0.31^{abcd}
Spharageman collare				
Female	6.90 ± 0.61^{abcd}	6.90 ± 0.26^{abc}	6.83 ± 0.42^{abc}	7.15 ± 0.42^{abcd}
Male	7.00 ± 0.18^{abc}	7.13 ± 0.05^{ab}	6.68 ± 0.17^{abc}	7.02 ± 0.15^{abcd}

Means (within columns) significantly different at the $p < 0.05$ level are indicated by different letters.

Table 2
Average E_h (mean ± sd) by sex of each region of the digestive tract of grasshoppers. The sample size is 4 individuals per sex

Species	Digestive Tract Region			
	Foregut	Caeca	Midgut	Hindgut
Agenotettix deorum				
Female	269 ± 23^{abcd}	266 ± 15^{bcd}	255 ± 17^{abcde}	250 ± 17^{abcdef}
Male	260 ± 18^{abcd}	257 ± 17^{abcd}	254 ± 19^{abcde}	260 ± 13^{abcde}
Aulocara elliotti				
Female	192 ± 39^{d}	222 ± 49^{abc}	204 ± 39^{bcde}	190 ± 28^{efg}
Cordillacris occipitalis				
Female	279 ± 20^{abcd}	266 ± 08^{abc}	251 ± 17^{abcde}	251 ± 22^{abcdef}
Male	246 ± 21^{bcd}	239 ± 28^{bcd}	238 ± 28^{bcde}	234 ± 21^{abcdefg}
Hadrotettix trifasciatus				
Female	249 ± 05^{bcd}	241 ± 17^{bcd}	241 ± 15^{bcde}	238 ± 13^{abcdefg}
Hesperotettix speciosus				
Female	192 ± 29^{d}	184 ± 22^{d}	179 ± 18de	172 ± 09^{g}
Male	295 ± 37^{ab}	294 ± 20^{ab}	263 ± 23^{abc}	261 ± 11^{abcde}
Hesperotettix viridis				
Female	245 ± 21^{bcd}	255 ± 13^{bcd}	233 ± 21^{bcde}	234 ± 21^{abcdefg}
Male	249 ± 27^{bcd}	233 ± 25^{bcd}	230 ± 18^{bcde}	225 ± 11^{bcdefg}
Hypochlora alba				
Female	265 ± 19^{abcd}	248 ± 13^{bcd}	235 ± 11^{bcde}	228 ± 19^{abcdefg}
Male	256 ± 14^{abcd}	240 ± 14^{bcd}	231 ± 10^{bcde}	233 ± 18^{abcdefg}
Melanoplus angustipennis				
Female	255 ± 16^{bcd}	251 ± 18^{bcd}	258 ± 15^{abcd}	256 ± 16^{abcdef}
Male	246 ± 16^{bcd}	243 ± 19^{bcd}	241 ± 25^{bcde}	249 ± 22^{abcdef}
Melanoplus bivittatus				
Female	245 ± 15^{bcd}	230 ± 15^{bcd}	235 ± 17^{bcde}	231 ± 23^{abcdefg}
Male	243 ± 12^{bcd}	231 ± 17^{bcd}	223 ± 10^{bcde}	223 ± 21^{bcdefg}
Melanoplus confusus				
Female	200 ± 38^{cd}	200 ± 28^{cd}	193 ± 21^{cde}	201 ± 21^{defg}
Male	233 ± 21^{bcd}	228 ± 10^{bcd}	204 ± 21^{bcde}	203 ± 12^{cdefg}
Melanoplus differentialis				
Female	259 ± 30^{abcd}	254 ± 25^{bcd}	241 ± 18^{bcde}	240 ± 14^{abcdefg}
Male	261 ± 82^{abcd}	265 ± 72^{abc}	256 ± 75^{abcde}	237 ± 64^{abcdefg}
Melanoplus femurrubrum				
Female	249 ± 03^{bcd}	244 ± 17^{bcd}	244 ± 05^{bc}	231 ± 05^{abcdefg}
Male	300 ± 24^{ab}	287 ± 33^{ab}	274 ± 18^{ab}	274 ± 19^{abc}
Melanoplus flavidus				
Female	278 ± 05^{abcd}	261 ± 25^{abcd}	254 ± 21^{abcde}	246 ± 14^{abcdef}
Male	244 ± 14^{bcd}	236 ± 15^{bcd}	234 ± 29^{bcde}	243 ± 09^{abcdefg}
Melanoplus foedus				
Female	283 ± 25^{abc}	261 ± 21^{abcd}	261 ± 11^{abc}	271 ± 06^{abcd}
Male	260 ± 28^{abcd}	250 ± 17^{bcd}	234 ± 20^{bcde}	248 ± 19^{abcdef}
Melanoplus gladstoni				
Female	253 ± 12^{bcd}	253 ± 16^{bcd}	245 ± 09^{bcde}	251 ± 09^{abcdef}
Male	276 ± 11^{abcd}	265 ± 08^{abc}	271 ± 02^{abc}	271 ± 06^{abcd}
Melanoplus lakinus				
Female	284 ± 39^{abc}	266 ± 45^{abc}	269 ± 44^{abc}	230 ± 42^{abcdefg}
Male	220 ± 16^{bcd}	216 ± 09^{bcd}	223 ± 24^{bcde}	201 ± 21^{defg}
Melanoplus sanguinipes				
Female	270 ± 53^{abcd}	255 ± 40^{bcd}	259 ± 38^{abcd}	258 ± 40^{abcdef}
Male	346 ± 34^{a}	335 ± 25^{a}	327 ± 25^{a}	298 ± 35^{a}
Mermiria bivitatta				
Female	273 ± 71^{abcd}	248 ± 41^{bcd}	223 ± 30^{bcde}	218 ± 23^{bcdefg}
Opeia obscura				
Female	245 ± 36^{bcd}	224 ± 40^{bcd}	213 ± 36^{bcde}	220 ± 40^{bcdefg}
Pardalophora haldemanii				
Female	258 ± 27^{abcd}	259 ± 09^{abcd}	254 ± 41^{abcde}	284 ± 21^{ab}
Male	260 ± 41^{abcd}	260 ± 27^{abcd}	263 ± 05^{abc}	256 ± 25^{abcdef}
Phlibostroma quadrimaculatum				
Female	254 ± 23^{bcd}	248 ± 38^{bcd}	250 ± 36^{abcde}	244 ± 34^{abcdefg}
Phoetaliotes nebrascensis				
Female	218 ± 23^{bcd}	203 ± 23^{bcd}	196 ± 21^{bcde}	204 ± 19^{bcdefg}
Spharageman collare				
Female	231 ± 57^{bcd}	232 ± 67^{bcd}	226 ± 87^{bcde}	246 ± 75^{abcdef}
Male	263 ± 48^{abcd}	268 ± 26^{abc}	273 ± 22^{abc}	270 ± 12^{abcd}

Means (within columns) significantly different at the $p < 0.05$ level are indicated by different letters.

Table 3
Body length (BL), diet breadth (DB), % forbs in diet (%F) and hostplant on which gut measurements were taken

Species	BL (cm)	Hostplant	DB	%F
Ageneotettix deorum				
Female	2.28 ± 0.17	*Bouteloua gracilis*	5.8	1.0
Male	1.75 ± 0.17		5.8	1.0
Aulocara elliotti				
Female	2.75 ± 0.21	*Bouteloua gracilis*	5.0	4.0
Cordillacris occipitalis				
Female	2.65 ± 0.24	*Bouteloua gracilis, Stipa comata*	6.2	0
Male	1.95 ± 0.19		6.2	0
Hadrotettix trifasciatus				
Female	3.98 ± 0.13	*Bouteloua gracilis, Stipa comata*	7.7	78.0
Hesperotettix speciosus				
Female	3.38 ± 0.22	*Iva xanthifolia*	7.3	98.0
Male	2.50 ± 0.22		7.3	98.0
Hesperotettix viridis				
Female	2.53 ± 0.22	*Solidago mollis*	4.4	98.0
Male	2.20 ± 0.16		4.4	98.0
Hypochlora alba				
Female	2.45 ± 0.25	*Artemisia ludoviciana*	1.5	99.0
Male	1.88 ± 0.26		1.5	99.0
Melanoplus angustipennis				
Female	2.63 ± 0.10	*Helianthus petiolaris*	16.5	39.0
Male	2.48 ± 0.15		16.5	39.0
Melanoplus bivittatus				
Female	4.08 ± 0.10	*Helianthus petiolaris*	19.3	75.0
Male	3.40 ± 0.22		19.3	75.0
Melanoplus confusus				
Female	2.73 ± 0.32	*Secale cereale*	15.3	90.0
Male	2.43 ± 0.17		15.3	90.0
Melanoplus differentialis				
Female	3.35 ± 0.24	*Iva xanthifolia*	9.2	46.0
Male	3.38 ± 0.41		9.2	46.0
Melanoplus femurrubrum				
Female	2.73 ± 0.38	*Helianthus petiolaris*	7.3	81.0
Male	2.53 ± 0.05		7.3	81.0
Melanoplus flavidus				
Female	3.20 ± 0.22	*Helianthus petiolaris*	9.4	92.0
Male	2.73 ± 0.26		9.4	92.0
Melanoplus foedus				
Female	3.05 ± 0.06	*Helianthus petiolaris*	22.4	79.0
Male	3.13 ± 0.10		22.4	79.0
Melanoplus gladstoni				
Female	2.98 ± 0.05	*Bouteloua gracilis*	13.9	73.0
Male	2.73 ± 0.22		13.9	73.0
Melanoplus lakinus				
Female	2.38 ± 0.29	*Amaranthus retroflexum*	1.2	98.0
Male	2.18 ± 0.17		1.2	98.0
Melanoplus sanguinipes				
Female	2.60 ± 0.42	*Helianthus petiolaris, Agropyron smithii*	16.8	50.0
Male	2.75 ± 0.19		16.8	50.0
Mermiria bivitatta				
Female	4.00 ± 0.28	*Bouteloua gracilis*	7.4	3.0
Opeia obscura				
Female	2.35 ± 0.13	*Bouteloua gracilis*	2.7	0
Pardalophora haldemanii				
Female	4.98 ± 0.39	*Secale cereale*	3.1	8.0
Male	3.33 ± 0.13		3.1	8.0
Phlibostroma quadrimaculatum				
Female	2.60 ± 0.23	*Bouteloua gracilis*	3.6	2.0
Phoetaliotes nebrascensis				
Female	2.90 ± 0.26	*Bouteloua gracilis*	11.0	11.0
Spharageman collare				
Female	3.40 ± 0.29	*Bouteloua gracilis, Agropyron smithii, Stipa comata*	10.1	11.0
Male	2.68 ± 0.10		10.1	11.0

The sample size for body length is 4 individuals per sex. See text for source of DB and % forbs data.

Table 4
General Linear Model Analysis of pH and Eh of each region of the digestive tract of grasshoppers

Digestive Tract Region	Source	df	MS	*F*-ratio	*P*
Foregut pH	Species	22	0.727	5.33	0.0001
	Sex	1	0.029	0.22	0.6433
	Species*Sex	16	0.212	1.56	0.0912
	Error	124	–	–	–
Foregut E_h	Species	22	3141	3.12	0.0001
	Sex	1	2431	2.41	0.1229
	Species*Sex	16	3421	3.39	0.0001
	Error	124	–	–	–
Caeca pH	Species	22	0.461	5.44	0.0001
	Sex	1	0.029	0.35	0.5570
	Species*Sex	16	0.125	1.48	0.1169
	Error	124	–	–	–
Caeca E_h	Species	22	2460	3.04	0.0001
	Sex	1	3087	3.82	0.0530
	Species*Sex	16	3231	3.99	0.0001
	Error	124	–	–	–
Midgut pH	Species	22	0.509	4.37	0.0001
	Sex	1	0.000	0.00	0.9800
	Species*Sex	16	0.119	1.03	0.4334
	Error	124	–	–	–
Midgut E_h	Species	22	3291	3.96	0.0001
	Sex	1	2465	2.97	0.0874
	Species*Sex	16	2308	2.78	0.0008
	Error	124	–	–	–
Hindgut pH	Species	22	0.186	2.84	0.0001
	Sex	1	0.003	0.04	0.8409
	Species*Sex	16	0.188	2.88	0.0005
	Error	124	–	–	–
Hindgut E_h	Species	22	3404	5.11	0.0001
	Sex	1	1200	1.80	0.1821
	Species*Sex	16	1829	2.74	0.0009
	Error	124	–	–	–

Body length was unrelated to any of the measures, and was dropped from the model.

foliage with that of the gut, not undertaken by this study. In species that do regulate, however, there are one or more gut regions that are very different than others and different than foliage. In crickets, for example, food is acidified in the foregut and then titrated to mild alkalinity in the midgut (Teo and Woodring, 1994). Although pH tended to increase slightly from foregut to hindgut in this study, the effect was not statistically significant, and there was no consistent change in Eh along the length of the gut. Thus, grasshoppers appear to differ markedly from crickets, caterpillars and beetles who regulate pH in one or more gut regions. To mimic *in vivo* conditions, *in vitro* studies of physiological processes that occur within the gut lumen of grasshoppers, including digestive enzyme function and the action of ingested microbial pesticides, should be buffered at neutral pHs under mildly oxidizing conditions.

Compared with the highly alkaline or acid conditions in some herbivore guts, grasshopper guts appear relatively moderate for an herbivore. This may be because some grasshoppers are not strict folivores, but will supplement a largely leaf diet with flower parts or even dead animal material in an opportunistic fashion. Thus, the need of strict herbivores to maximize protein extraction from refractile plant material by maintenance of highly alkaline or acid guts may be non-existent in facultative omnivores, such as many grasshoppers.

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