

Weakness in the band: nutrient-mediated trade-offs between migration and immunity of Mormon crickets, *Anabrus simplex*

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Mormon crickets, *Anabrus simplex*, form large migratory bands that march over rangeland in the western United States in search of nutrients. Immune defence is particularly relevant to survival in migratory bands, but little is known about the role of nutrition in insect immunity, particularly in nature. We hypothesized that immune defences are compromised in Mormon cricket bands because of nutrient limitations. Members of a migratory band in Nevada, U.S.A., preferred carbohydrate diets over proteins. After feeding on the carbohydrate diet, migratory velocity was less and the ability to encapsulate foreign particles and lyse bacteria was greater than for Mormon crickets consuming protein. Less locomotory demand for lipids may result in greater antibacterial activity. Total phenoloxidase (PO) activity also increased following feeding on carbohydrates, whereas spontaneously active PO was not different between the two diets. These results were very different from those of a band in Utah, U.S.A., that preferred the protein diet and that had enhanced spontaneous PO activity after protein supplementation. Haemolymph of Mormon crickets from the Nevada band was sampled 18 h after the diet treatments, whereas that from the Utah band was drawn 4 h after treatment. Either the difference in immune measures was due to the difference in sampling time, or spontaneous PO activity was protein-limited whereas encapsulation and antibacterial activity required carbohydrates. Currencies for the generalized immunity of insects may differ, and constraints on immunity in a given environment depend on which macronutrients are in short supply.

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There is empirical and theoretical evidence that resistance to disease is costly (Sheldon & Verhulst 1998; Zuk & Stoehr 2002; Rolff & Siva-Jothy 2003; Elder et al. 2008). However, in some instances, laboratory animals must be under nutritional stress for the cost of immunity to be observable (e.g. Moret & Schmid-Hempel 2000). Migration is often associated with a scarcity of nutrients or other resources, and yet migration itself is energetically costly. Animals in nutrient-poor habitats may redirect resources from other life history traits to fuel migration.

The flightless Mormon cricket (*Anabrus simplex* Haldeman, Orthoptera: Tettigoniidae) forms dense bands of late-instar nymphs and adults in late spring and early summer. Mormon crickets march across rangeland in the western United States in search of nourishment. Food supplementation has been shown to

have immediate effects on the locomotor behaviour of individual Mormon crickets. For example, Mormon crickets fed protein demonstrated less locomotor activity relative to those fed carbohydrates both in captivity (Simpson et al. 2006) and when released back into the migratory band in the field (Srygley et al. 2009).

Studies of insects in the laboratory have shown that nutrition is important to immune function. In both choice and no-choice diet experiments, *Spodoptera* moth caterpillars fed protein-rich diets had enhanced immunity to viral and bacterial pathogens (Lee et al. 2006; Povey et al. 2009). Dietary antioxidants influenced the ability of *Parasemia* moths to encapsulate foreign objects (Ojala et al. 2005), whereas *Epiritta* moth caterpillars fed low-quality food had higher immune response than those fed high-quality food (Klemola et al. 2007). Knowledge of nutritional effects on immune defence is particularly lacking in field populations (Adamo 2004; Boggs 2009), and even more so in migrating animals (Weber & Stilianakis 2007). When migrating Mormon crickets from a band in Utah, U.S.A., were fed protein, they had enhanced spontaneous PO activity relative to those fed carbohydrates (Srygley et al. 2009). In replicating this study, we recently observed a migrating Mormon cricket band from Nevada, U.S.A., that fed more on carbohydrates

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than on protein. Here we assessed the immediate effects of protein and carbohydrate diets on migratory activity and immunocompetence of Mormon crickets in a marching band in northeast Nevada and compare the results with those from a band in northeast Utah (Srygley et al. 2009). We found that the populations contrasted sharply in their dietary requirements and in their immune response to dietary supplements.

METHODS

Study Site and the Mormon Cricket Band

During 27–30 June 2008, we studied a band of prereproductive adult Mormon crickets near Eagle Mountain in the Independence Range (41°13'00"N; 116°00'25"W; 2002 m elevation) in Elko County approximately 50 km northwest of Elko, Nevada. The valley floor is dominated by sagebrush (*Artemisia tridentata*), which is typical vegetation for high Great Basin desert. Mormon crickets eat a variety of foods including broad-leaved plants, invertebrates and fungi (Ueckert & Hansen 1970). They also scavenge road kills and cannibalize other crickets.

Intake Trials

To characterize intake diets of field-collected Mormon crickets, we followed the methods of Simpson et al. (2006). We prepared a 42% protein diet consisting of a 3:1:1 mix of casein, peptone and albumen; and a 42% carbohydrate diet consisting of equal parts of sucrose and dextrin. Both diets contained 54% cellulose and 1.8% Wesson's salt mixture and 2.2% vitamins, linoleic acid and cholesterol. In a free-choice experiment, six male and six female Mormon crickets were collected from the migratory band, housed individually with free access to water and 0.5 g of each diet for 24 h. After 24 h, the diets were removed and replaced with fresh diet, which remained with the insects for an additional 24 h. The dry masses consumed from each diet is a measure of the relative intake of carbohydrates:protein over the first and second 24 h period. Diet consumption during the second 24 h period lies closer to the ideal intake target of the band. A vector drawn from the mean intake during the first 24 h period and that for the second 24 h period indicates the dietary needs of members drawn from the migrating band relative to the same insects in a free-choice dietary environment. A migrating band is protein (or carbohydrate) deficient when its members prefer to feed on proteins (or carbohydrates) when first captured and then switch to a more even ratio of proteins and carbohydrates (Simpson et al. 2006). We replicated the experiment with Mormon crickets taken from a different location within the band to provide a better graphical estimate of their preferred intake target as indicated by vector convergence (5 females and 6 males).

Diet Manipulation, Radiotracking and Haemolymph Collection

We captured 20 male and 23 female crickets over 3 days and held each in a 1 litre translucent container. Approximately half of the crickets captured each day were fed a 42% protein diet, and the others were fed a 42% carbohydrate diet (as described above). The insects were shaded beneath a tarp and allowed to feed from this diet for 1 h at mid-day. To measure consumption, diets were weighed to the nearest milligram on an Acculab field-portable balance (model PP-2060D) before and after presentation to the crickets.

To follow each Mormon cricket's migratory path, a 0.4 g radio-transmitter (Biotrack, Ltd., Dorset, U.K.) was glued to the pronotum. The added mass of the radio did not affect locomotion in the laboratory (Lorch et al. 2005). Sex, treatment and the unique

radiofrequency that identified each insect were recorded. Using a Trimble GPS datalogger to record location and time for each cricket, we released the insects back into the band, with each cricket initially separated by about 8 m on a linear transect perpendicular to the general direction of band movement at that time. Crickets were released between 1530 and 1700 hours and retrieved the following day between 0900 and 1200 hours (Pacific Daylight Time). The location and time of recapture were recorded, and the cricket was placed in a 50 ml Corning plastic centrifuge tube. Velocity (mm/s) was calculated as the straight-line distance between release and recapture over time.

Returning to our field laboratory in Elko in the early afternoon, we measured body mass of each cricket to the nearest milligram with an Ohaus field-portable microbalance (model AV53) and measured femur length with calipers. We drew haemolymph by puncturing the arthroal membrane at the base of each insect's hindleg with a 26-gauge hypodermic needle. In Orthoptera, prophenoloxidase (proPO) is primarily held in circulating haemocytes until wounding or infection (Kanost & Gorman 2008). Hence lysis of haemocytes at the wound may cause local elevation of proPO and initiation of the proPO cascade to yield spontaneously active PO. We collected 25 µl of haemolymph into a capillary tube, puncturing again if necessary. The haemolymph was diluted 1:50 with phosphate buffered saline (PBS) solution to be used in assays of PO activity and total haemolymph protein. An additional capillary tube with 4 µl of haemolymph diluted 1:10 with PBS was collected to be used to assay lysozyme activity. We immediately froze the haemolymph samples at –18 °C in a field-portable Engel freezer (model MT45F-U1). Then we inserted two quartz glass rods (National Scientific Co., 1 mm diameter × 2 mm) dorsally between the first and second abdominal segments. The crickets were given water and tropical fish flakes (composed of at least 45% protein, 10% fat). Twenty-four hours later (±8 min), we froze the crickets to halt their encapsulation of the rods.

Immunocompetence Assays

We followed the protocols detailed in Srygley et al. (2009). Briefly, samples of thawed haemolymph diluted in PBS were centrifuged and activated with 10 mM dopamine solution to measure spontaneous PO activity. The plate was loaded into a temperature-controlled Biotek microplate reader (25 °C), and absorbance at 492 nm was read between 5 and 15 min. If sample absorbance was linearly related with time, we calculated mean *V* (change in absorbance/min). One unit of PO activity per millilitre of haemolymph is defined as the amount of enzyme resulting in a 0.001 increase in absorbance.

To measure total PO activity (both PO and proPO), we adapted the protocol of Goldsworthy et al. (2002). We dissolved 1 mg of alpha-chymotrypsin from bovine pancreas (Sigma) in 1 ml of PBS, combined an equal volume of this solution with centrifuged haemolymph in PBS (1:50), and incubated it for 30 min. In the plate wells, we added 5 µl of the incubated solution to 195 µl of 10 mM dopamine. As above, mean *V* was calculated from plate readings between 5 and 15 min to measure total PO activity in units/ml of haemolymph.

To measure encapsulation response, rods were dissected from the Mormon crickets, dried and weighed. Weight of the cleaned rod was subtracted. The encapsulation mass was normally distributed following log transformation.

To measure lysozyme-like antibacterial activity, a turbidimetric method was used. Thawed and PBS-diluted haemolymph (1:50) was added to a well with suspended Gram-positive bacteria cells *Micrococcus lysodeikticus* (Worthington). Clearing of the well was compared to a serial dilution of egg-white lysozyme (Sigma) added to the bacteria suspension. The plate was loaded into a temperature-

controlled Biotek microplate reader (25 °C), and absorbance was read at 450 nm between 10 and 30 min. If the sample absorbance was linearly related with time, we calculated mean V . When sample activity fell below 6.5 $\mu\text{g/ml}$, the sample was excluded because the standards showed that the data were unreliable when sample activity was this weak.

We measured total haemolymph protein in milligrams of protein/millilitres of haemolymph with a Total Protein Kit, Micro (Sigma) compared to a serial dilution of the human albumin standard.

Statistical Analyses

To analyse effects on marching velocity, we conducted an ANCOVA using JMP 6.0.2 (SAS Institute, Cary, NC, U.S.A.) with square-root transformed velocity (root velocity) as the dependent variable, body mass and femur length as covariates, and sex and diet as independent factors. All interactions were evaluated in building the model. We used forward stepwise regression analysis ($\alpha = 0.15$ to enter), adding each feature with the lowest P value. We report the model for which the Akaike information criterion (AIC) was minimized (Draper & Smith 1998). When we analysed immunity measures to see how they were affected by sex, diet and body mass, not all immunity assays were conducted on all insects, so we ran ANCOVA on each measure of immunity separately. Encapsulation mass and total PO activity were log transformed to normalize these dependent variables. In building our models, body mass was the covariate and sex and diet were independent factors with all interactions evaluated. We used forward stepwise regression as with root velocity above, reporting the model that minimized the AIC. Because the statistical methods are identical to those applied to the Utah population (Srygley et al. 2009), we are able to directly compare the results from the two populations.

RESULTS

On the first day, Mormon crickets ate two to four times more carbohydrates (C) than protein (P), on average (Fig. 1). Over the course of the second day, this ratio of C:P intake fell to approximately 1.5. Although females ate more diet than males, they ate the same proportion of the two diets. For those Mormon crickets with access to a single diet and subsequently released back into the band, the amount that the Mormon crickets consumed in 1 h was independent of body mass ($P = 0.249$) and dependent on the type of diet presented ($P = 0.0016$), with those given a carbohydrate diet also eating much more than those with access to protein.

Adult body mass was not proportional to femur length ($r = 0.07$). On average, females were not significantly heavier than males (ANOVA: $F_{1,40} = 0.78$, $P = 0.381$). In contrast to body mass, femur length is fixed after the final moult, and relates directly to structural size. Females had significantly longer femurs than males (means \pm SD: $N = 22$ females 26.0 ± 1.0 mm; $N = 19$ males 24.1 ± 1.0 mm; ANOVA: $F_{1,39} = 39.02$, $P < 0.0001$).

Velocities ranged from 0.4 to 12.6 mm/s (1.4–45.4 m/h). Diet was the only variable selected to explain variation in square-root transformed velocity (ANOVA: $R^2 = 0.174$, $F_{1,39} = 8.2$, $P = 0.0066$). Mormon crickets fed carbohydrates were slower than those fed protein (Fig. 2a).

Diet and sex were selected, in that order, to explain total PO activity ($N = 10$ carbohydrate-fed 'C' females, $N = 10$ protein-fed 'P' females, $N = 10$ C males, $N = 9$ P males; two-way ANOVA: $R^2 = 0.258$, $F_{2,36} = 6.3$, $P = 0.0046$). Insects fed a carbohydrate diet had greater total PO than those fed proteins ($F_{1,36} = 9.9$, $P = 0.0033$; adjusted log-transformed means: carbohydrate = 3.91 units/ml; protein = 3.82 units/ml; Fig. 2b). Males had slightly greater total PO activity than females ($F_{1,36} = 2.3$, $P = 0.136$; adjusted log-transformed

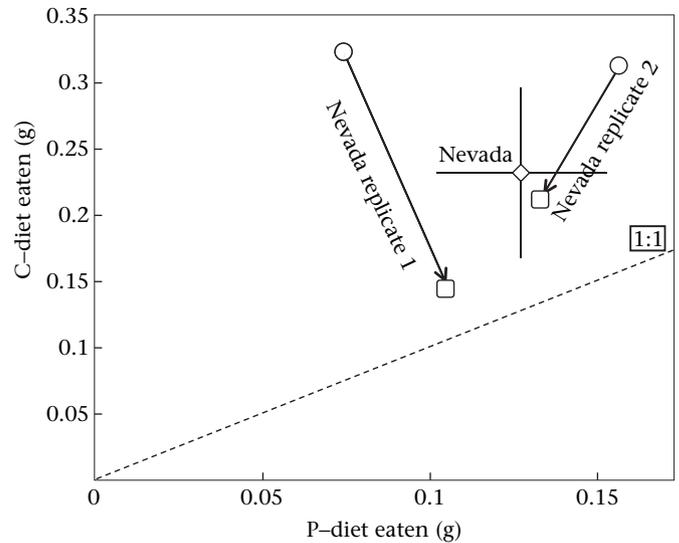


Figure 1. Mean dietary intake for Mormon crickets given access to either a carbohydrate (C) or protein (P) diet for 1 h (open diamond with bars representing SE), and mean consumption of C and P diets by migrating Mormon crickets over 2 days. Vectors drawn from day 1 (open circles) to day 2 (open squares) show consumption of C relative to P for the two replicates. Dashed line = expected consumption of balanced C:P ratio in nature (Simpson et al. 2006).

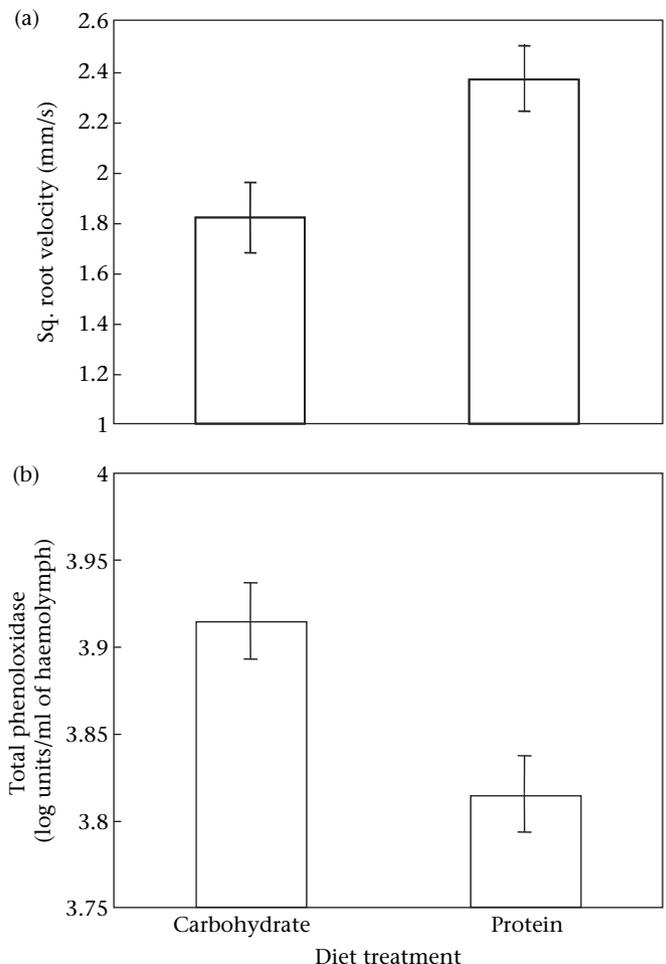


Figure 2. Mean \pm SE (a) migratory velocity and (b) lysozyme-like activity of migrating Mormon crickets fed a carbohydrate diet or a protein diet.

means: males = 3.89 units/ml of haemolymph; females = 3.84 units/ml of haemolymph). Spontaneous PO activity did not differ significantly with body mass, sex, diet or their interactions. No variables were selected in the stepwise regression analysis.

Body mass, diet and sex were selected, in that order, to explain variation in log-transformed encapsulation mass (ANCOVA: $N = 11$ C females, $N = 10$ P females, $N = 10$ C males, $N = 9$ P males; $R^2 = 0.369$, $F_{3,36} = 7.0$, $P = 0.0008$). The encapsulation response was directly proportional to body mass ($F_{1,36} = 17.5$, $P = 0.0002$; Fig. 3a), and crickets fed a carbohydrate diet had significantly greater encapsulation response than those fed proteins ($F_{1,36} = 4.5$, $P = 0.041$; adjusted log-transformed means: carbohydrate = -0.95 mg; protein = -1.09 mg). Males had slightly greater encapsulation response than females ($F_{1,36} = 2.8$, $P = 0.099$; adjusted log-transformed means: males = -0.97 mg; females = -1.07 mg). Inclusion of the clean rod mass as a covariate for encapsulation mass did not change the variables selected, nor did it change the best model qualitatively ($R^2 = 0.373$, $F_{4,35} = 5.2$, $P = 0.0021$). Controlling for body mass, log encapsulation mass was significantly and positively correlated with spontaneous PO activity (partial $r = 0.415$, $N = 38$, $P = 0.010$), but not total PO activity (partial $r = 0.035$, $N = 37$, $P = 0.84$; Fig. 3b). In contrast to the effect of diet on the encapsulation response, there was no significant effect of diet on spontaneous PO activity.

The interaction between body mass and diet and the associated first-order variables, body mass and diet, were the only ones

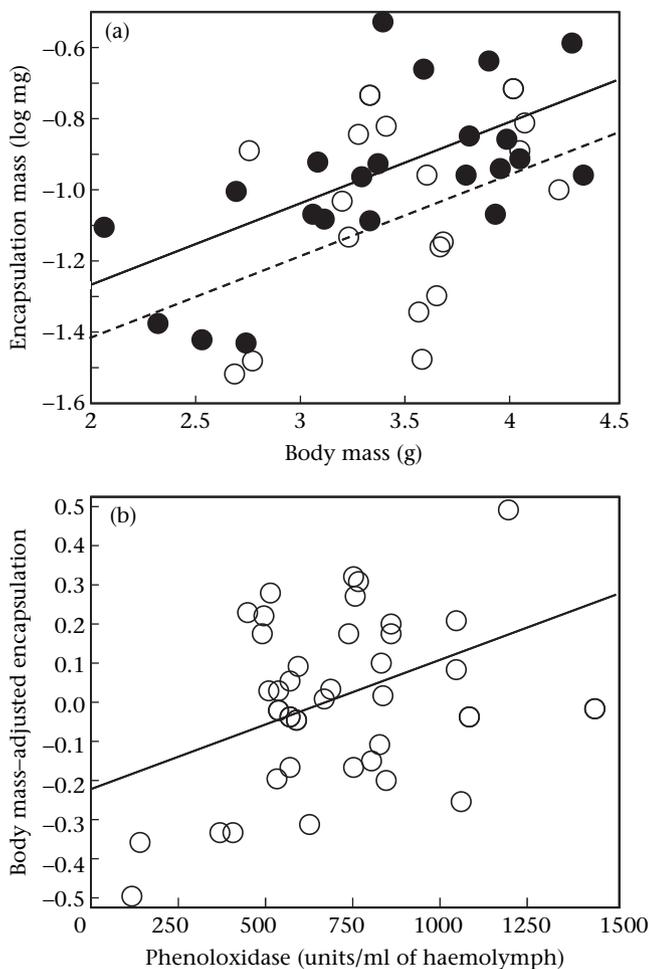


Figure 3. Encapsulation responses of migrating Mormon crickets in relation to (a) body mass and (b) spontaneously active PO, after adjusting for body mass. In (a), solid circles, solid line = carbohydrate diet; open circles, dashed line = protein diet. Diet treatments are not shown in (b).

selected to explain variation in lysozyme-like activity (ANCOVA: $N = 5$ C females, $N = 8$ P females, $N = 7$ C males, $N = 6$ P males; $R^2 = 0.392$, $F_{3,22} = 4.7$, $P = 0.0108$). Lysozyme-like activity was directly proportional to body mass in carbohydrate-fed crickets but not in protein-fed crickets, and it was greater in crickets fed a carbohydrate diet relative to those fed a protein diet ($F_{1,22} = 7.7$, $P = 0.0109$; adjusted means: C-fed = 4494 units/ml of haemolymph; P-fed = 3952 units/ml of haemolymph).

The best model for total circulating protein included body mass, sex and diet, selected in that order ($N = 13$ C females, $N = 12$ P females, $N = 11$ C males, $N = 13$ P males; ANCOVA: $R^2 = 0.388$, $F_{3,37} = 7.8$, $P = 0.0004$). Total protein was directly proportional to body mass ($F_{1,37} = 18.3$, $P = 0.0001$), and males had marginally greater circulating protein than females ($F_{1,37} = 2.8$, $P = 0.099$; adjusted means: males = 40.4 mg/ml of haemolymph, females = 33.8 mg/ml of haemolymph). Insects fed a protein diet had marginally greater circulating protein than those fed carbohydrates ($F_{1,37} = 2.6$, $P = 0.112$; adjusted means: protein = 40.3 mg/ml, carbohydrate = 34.0 mg/ml). Those fed a protein diet may have manufactured more protein (see Srygley et al. 2009). Alternatively, they may have shown signs of dehydration, which could affect concentrations of protein and enzymes in the haemolymph. To control for dehydration, we added total circulating protein as a covariate with the dependent variables in the regression models selected for log proPO and lysozyme activities. Adjusting for protein did not qualitatively alter their associations with diet ($P = 0.0008$ and $P = 0.012$, respectively), nor did it change the lack of association of diet with spontaneous PO activity ($P = 0.69$).

DISCUSSION

We found that diet had important effects on migratory velocity and immunity to disease. Crickets taken from the migratory band ate more carbohydrates than protein. After returning to the band, they moved more slowly after feeding on a carbohydrate diet compared to those fed protein, and they had greater total PO and lysozyme-like activities than those fed protein. They also encapsulated foreign bodies more rapidly than protein-fed crickets.

The reduction in movement with a carbohydrate diet broadens the hypothesis of Simpson et al. (2006) that Mormon crickets migrate in search of protein. Both protein and carbohydrates are growth-limiting macronutrients that are frequently studied in the generalized framework of nutrition (Simpson & Raubenheimer 1993; Behmer 2009), especially in migratory locusts. Thus, Mormon cricket migrations can be more generally perceived as being motivated by a search for limited nutrients. Moreover, the difference between populations in the nutrients that are limiting can be useful for contrasting the populations' immune responses to pathogen attack.

The sharp contrast between the effect of diet on movement in the Nevada population and the effect seen in Utah and Idaho populations, where crickets fed protein moved more slowly than those fed carbohydrates (Simpson et al. 2006; Srygley et al. 2009), can be used to reject the alternative hypothesis that migration is slowed by a lack of carbohydrate fuel. According to this alternative, since carbohydrates are an important migratory fuel, the reduction in locomotion recorded in Utah and Idaho following access to a protein diet might also be explained as a reduction in velocity due to reduced access to carbohydrate fuel. Our results in Nevada nicely eliminate this alternative explanation because when members of the carbohydrate-starved Nevada band were given access to carbohydrates, they too reduced their velocity. With this additional evidence from Nevada, we have greater confidence that one reason why individual Mormon crickets migrate is to satisfy nutritional requirements.

Crickets fed carbohydrates in Nevada had greater lysozyme-like activity relative to those fed protein. Antibacterial lysozyme is

secreted from cells in the fat body and circulating haemocytes (Gillespie et al. 1997). Food limitation during immature stages of the cricket *Gryllus campestris* reduced lysozyme activity in the adult stage, but it did not influence spontaneous PO activity (Jacot et al. 2005). We think it unlikely that the carbohydrate diet increased secretion of lysozyme by the fat body over the short-term. Instead, other haemolymph components that are sensitive to carbohydrate concentration in the blood may also affect the enzymatic ability to lyse bacterial cells. For example, apolipoprotein III is typically a water-soluble, lipid-free protein, but when blood sugar is low and energy requirements are high, it associates reversibly with high-density lipoprotein protein transporting lipids from the fat body (Weisner et al. 1997; Goldsworthy & Joyce 2001). In its lipid-free state, apolipoprotein III increases lytic activity by increasing the susceptibility of bacteria to lysozyme (Weisner et al. 1997; Halwani & Dunphy 1999). As a result, a trade-off between immune activity and locomotion may exist (Adamo et al. 2008). Mormon crickets given access to carbohydrates subsequently moved less, which reduces the transportation of lipid for fuel. In addition, the carbohydrates can be metabolized directly and further reduce the need to transport lipid. Both are likely to increase the availability of lipid-free apolipoprotein III to interact with bacteria and facilitate lysozyme activity as we have observed.

Crickets fed carbohydrates had greater total PO activity compared to those fed protein, but they were not different in spontaneous PO activity. PO also occurs in an inactive zymogen called prophenoloxidase (proPO), which is assayed along with spontaneously active PO in our measure of total PO activity. On average, circulating proPO was an order of magnitude more concentrated than PO in the Nevada band. However, there was no relationship between the amount of active PO and the amount of proPO in the haemolymph ($r = -0.14$).

Crickets fed carbohydrates had a greater response to invasion by a foreign object than those fed protein. The first stage of encapsulation of a glass rod is recognition of the foreign object by haemocytes and haemocyte adhesion to the object (Gillespie et al. 1997). Cell motility, in particular, that of the amoeboid plasmotocytes, is likely to increase in direct proportion with carbohydrates in the blood. The PO cascade is an important part of encapsulation, particularly in the latter stages, because melanization causes the cell mass to harden around the foreign body by cross-linkages. Thus, it is interesting that the mass adhering to the rod over 24 h is more closely associated with the concentration of spontaneously active PO and is not associated with the concentration of proPO. This may result from the tight regulation of the proPO cascade to avoid excessive activation (Cerenius & Söderhäll 2004). Although confining the Mormon crickets with a protein-rich diet for 24 h may have affected the encapsulation response, the fact that the response was directly proportional to the spontaneously active PO activity prior to inserting the rods suggests that we did not systematically bias the encapsulation response in accordance with the diet treatments. Because encapsulation was measured after blood was drawn, differences in encapsulation response might also reflect dietary effects on coagulation. However, Mormon crickets do not typically bleed excessively in the field, and so we do not think that one treatment group bled systematically more than another.

PO is generally in its inactive proPO form in the haemolymph, which is activated when wounded or infected (reviewed in Kanost & Gorman 2008). After wounding for the blood draws or insertion of the rods, it is clear that only a fraction of the proPO converts to the active form of PO. A magnitude of concentration greater of the PO remains inactive over the short-term. Only by activation of proPO with chymotrypsin does the proPO convert to active PO. This low activation results in little correlation between circulating proPO and spontaneous PO concentrations, but the association of

Table 1

Macronutrient consumption and effects of diet on movement and immunity in Mormon crickets from Nevada and Utah

	Elko County, Nevada	Daggett County, Utah*
Diet consumption	C>>P	P>C
Movement	C<<P	P<C
Spontaneous PO activity	No effect	P>C
Total PO activity	C>P	No data
Encapsulation	C>P	No effect
Lysozyme activity	C>P	No effect

* From Srygley et al. (2009).

spontaneously active PO titres with the Mormon crickets' response to invasion by the glass rods indicates that spontaneous PO is an important measure of immune defence (see Cerenius et al. 2008). In Mormon crickets, spontaneously active PO titres increased with infection by the entomopathogenic fungus *Beauveria bassiana* and were associated with attempted clearing of *B. bassiana* blastospores and hyphae from the haemolymph (Srygley & Jaronski 2011).

Comparison of Migratory Bands in Nevada and Utah

Effects of diet on cricket immunity contrasted sharply between Nevada and Utah populations (Table 1). PO activity did not vary with treatment in the carbohydrate-limited population in Nevada; whereas in the protein-limited band in Utah, insects fed a protein diet had greater PO activity than those fed carbohydrates (Srygley et al. 2009). Encapsulation mass was proportional to body mass in both locations, but only insects in Nevada responded to the diet treatments, with those fed carbohydrates having greater response to a foreign object than those fed protein. Finally, those fed carbohydrates in Nevada also had greater lysozyme-like activity relative to those fed protein, whereas there was no detectable response to the diet treatments in Utah.

The Nevada crickets were much larger and heavier. For each sex on average, femurs were 4 mm (18–20%) longer than those in the Utah band (Table 2). In the Utah population, body mass had major effects on locomotion and immunocompetence in migrating Mormon crickets. Migratory velocity, total circulating proteins, encapsulation response and lysozyme-like activity all increased with body mass. In Nevada, only the encapsulation response and total circulating proteins were proportional to body mass.

Within each diet treatment, total circulating protein titres were greater in Nevada compared to Utah. For those fed a protein diet, mass-adjusted protein for the Nevada band was 8% greater than that for the Utah crickets, whereas for those fed a carbohydrate

Table 2

Comparison of body size, migratory movement and immunity in Mormon crickets from Nevada and Utah

	Elko County, Nevada		Daggett County, Utah	
Sex*	Female	Male	Female	Male
Femur length (mm)	26.0±1.0	24.1±1.0	22.0±0.7	20.3±0.6
Mass (g)	3.52±0.5	3.37±0.6	2.95±0.8	2.64±0.5
Diet treatment†	C-fed	P-fed	C-fed	P-fed
Velocity (mm/s)	3.3	5.6	5.1	4.2
Circulating proteins (µl/ml)	34.0	40.3	28.2	37.2
Total PO (units/ml)	8128	6607	9120	8192
Spontaneous PO (units/ml)	728	663	426	661
Lysozyme (units/ml)	4406	3940	4158	4019
Encapsulation response (mg)	0.11	0.08	0.10	0.12

* Means ± SD.

† Where applicable, transformed means have been converted to untransformed means.

diet, mass-adjusted protein was 17% more concentrated in Nevada than in Utah. These results are consistent with those from the feeding trials showing Utah Mormon crickets were more starved for protein than were Nevada crickets (Fig. 1): individuals in the Utah band preferred the protein diet over carbohydrates, whereas those in the Nevada band preferred carbohydrates over protein. Daily intake targets for Mormon crickets in Nevada were similar to those in Utah and Idaho in that the C:P ratio was near 1 (Simpson et al. 2006), but consistent with their larger body size, the bulk ingested was approximately twice that measured at the other sites.

We assume that the difference in dietary requirements between the two populations reflects differences in the availability of macronutrients in the two sites. However, the habitats are grossly similar. Both the Nevada and Utah bands comprised pre-reproductive adults, and both sites are similar in latitude and elevation. The flora of the locations is very similar, with sagebrush and other vegetation typical of high Great Basin desert. Seeds, flowers and invertebrates are sources of protein for Mormon crickets, but it is not known whether these dietary constituents were less abundant in Utah. Certainly, the band tracked in Utah was less dense than that in Nevada, and less dense than other bands tracked previously (Lorch & Gwynne 2000; Lorch et al. 2005; Sword et al. 2008). As a result, opportunities to contact and cannibalize other band members may have occurred less frequently. Broad-leaved plants and fungi are carbohydrate sources (Ueckert & Hansen 1970), but whether they were less rich in Nevada is not known.

One major difference between the two locations was methodological: the Utah crickets were loose approximately 4 h and recaptured the same day, whereas the Nevada crickets were loose overnight for approximately 18 h. This methodological difference may have allowed crickets in Nevada more time to compensate for their diet treatments by feeding on protein-rich or carbohydrate-rich sources in the field. However, if the insects could compensate for dietary deficiencies so readily, they would not wait until after they were held captive and fed a single macronutrient ad libitum. If the effect of diet on lysozyme activity lags behind that on PO activity, then this methodological difference might also explain differences between the two locales.

Shortly after feeding on protein or carbohydrates, Mormon crickets showed measurable effects on the immune system. Future research on diet and immunity of bands with comparable differences in nutritional status will give us further insight into nutritional costs of immunity. At present, the difference in components of the immune system that are enhanced by the contrasting dietary constituents suggests that PO activity is limited by lack of protein, whereas encapsulation and antibacterial activity are limited by lack of carbohydrate fuels. However, it is also possible that there is a difference in immune response to macronutrient ingestion, with circulating PO increasing within hours but lysozyme activity requiring a longer response time. If differences in protein and carbohydrate availability for an insect's diet limit the insect's ability to develop different components of its immune system as suggested by this study, then there may not be a common currency for the generalized immunity of insects. Constraints on immunity in a given environment may depend on which nutrients are in short supply.

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