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Signalling and phosphorus: correlations between mate signalling effort and body elemental composition in crickets

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Recent findings in the field of ecological stoichiometry indicate that the relationships among key macronutrient elements (e.g. carbon, nitrogen and phosphorus) of organisms and their resources may underlie variation in fitness-conferring behaviours. The amount of phosphorus in an individual's body is often correlated with its rate of growth and reproduction, and low-phosphorus diets are known to reduce growth in a number of insect and crustacean herbivores. These findings suggest that the stoichiometric imbalance between organismal biomass requirements and the relative scarcity of nutrients in nature may underlie variation in condition-dependent behaviours. Here we investigate relationships between body elemental composition and long-distance mate attraction signals produced by male Texas field crickets, *Gryllus texensis*. Signalling was strongly and positively correlated with the percentage of phosphorus present in the body, but was not correlated with the percentage of carbon or nitrogen present. We also found evidence suggesting that callers and noncalling satellites differ in their elemental composition. To our knowledge, our data are the first to indicate that there may be a relationship between total body phosphorus content and a sexually selected trait. We present a preliminary evaluation of proximate hypotheses to account for the observed patterns. Our results indicate that a stoichiometric perspective may help us to understand the causes of variation in behaviour.

Understanding how variation is maintained in fitness-conferring sexually selected traits remains a fundamental problem in animal behaviour (Pomiankowski & Iwasa 1995). We propose that variation in such traits may be linked to trade-offs in the demand for multiple essential resources that limit key physiological processes affecting condition. Our proposal stems from a surge of recent research on ecological stoichiometry, the study of the balance of energy and multiple chemical elements in ecological interactions (Sterner & Elser 2002). Essential elements must be obtained in sufficient quantities from food because they cannot be synthesized. Plant biomass tends to have low concentrations of essential elements. For example, concentrations of nitrogen and phosphorus tend to be 10–20 times lower in autotrophs than in herbivores (Mattson 1980; Elser et al. 2000a). This mismatch in nitrogen and phosphorus content between animals and plants appears to be a critical factor influencing the physiological state and fitness of many herbivores (Mattson 1980; Strong et al. 1984; Elser et al. 2000a).

Most studies of insect nutrition have concentrated on proteins and lipids (Mattson 1980; Strong et al. 1984; Elser et al. 2000a). Proteins are fairly rich in nitrogen (~17%) and rarely contain phosphorus, while most lipids are rich in carbon and contain little nitrogen or phosphorus (Sterner & Elser 2002). Organismal responses to variation in dietary protein include changes in growth rate and compensatory feeding (Raubenheimer & Simpson 2003). Likewise, research suggests that sexually selected traits respond directly to variation in dietary protein. For example, Hunt et al. (2004) found that male crickets reared on high-protein diets (45% protein) showed significantly greater nymphal survival, faster development, were heavier and larger at eclosion, had elevated calling effort early in life, and called more often throughout their lives than did males reared on medium- (36.75% protein) and low-protein (28.5% protein) diets. These results suggest that the ability to obtain nitrogen or nitrogen-rich protein constituents may influence condition and its dependent life-history and sexually selected traits.

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Although nitrogen has traditionally been considered the essential element limiting production in many contexts, phosphorus also limits production in a number of ecosystems (Redfield 1958; Hecky & Kilham 1988; Vitousek et al. 1993; Verhoeven et al. 1996; Schindler & Eby 1997; Karl 1999). Body phosphorus and its abundance relative to nitrogen and carbon appear to correlate positively with growth and reproduction in several invertebrate taxa (Quraishi et al. 1966; Elser et al. 2000a, 2003; Eskelinen 2002; Fagan et al. 2002; Schade et al. 2003; Perkins et al. 2004). Furthermore, experimental reduction of phosphorus content of food in an ecologically relevant range appears to reduce invertebrate growth rate and reproduction (Urabe & Sterner 2001) while supplementation of phosphorus appears to stimulate invertebrate growth (Elser et al. 2001; Perkins et al. 2004). For example, when the crustacean *Daphnia* is fed phosphorus-limited food, fewer and smaller eggs are produced and 15–32% of the eggs do not develop (Urabe & Sterner 2001). Furthermore, phosphorus-limited food appears to retard oogenesis in *Drosophila* (Markow et al. 2001). These results corroborate other studies suggesting that low dietary phosphorus in the natural range can reduce growth rate and reproduction (Sterner 1993; Sterner et al. 1993; Weers & Gulati 1997; Sterner & Schulz 1998; Schulz & Sterner 1999; Sterner & Elser 2002; Acharya et al. 2004a, b).

Until now, stoichiometric approaches have received little attention in animal behaviour. The one exception involves the use of a stoichiometric perspective to consider possible effects of food biochemistry and elemental composition on feeding behaviours. *Daphnia*, for example, increase feeding activity when fed a diet low in phosphorus (Plath & Boersma 2001). Furthermore, mobile amphipods preferentially feed on high-quality food when given the choice between diets that have different amounts of protein (Cruz-Rivera & Hay 2000). Likewise, fifth-instar locusts (*Locusta migratoria*) preferentially consumed protein-rich food when given the choice between a diet rich in protein and a diet rich in carbohydrates (Behmer et al. 2003). Invertebrate predators also selectively forage for proteins and lipids when they experience nutritional imbalances (Mayntz et al. 2005), so compensatory foraging is not limited to herbivores.

Here we use a stoichiometric approach to evaluate possible relationships between variation in fitness-conferring behaviours and the elemental composition of cricket body tissue. Field crickets are an ideal organism to examine whether key macronutrient elements influence variation in fitness-conferring behaviours because males show extensive variation in their mate attraction behaviour. Texas field crickets show two alternative mating behaviours: callers produce long-range acoustic trills to attract females and then switch to short-range courtship signals once a female has been attracted; satellites wait silently near callers and attempt to intercept attracted females (Alexander 1961; Cade 1979). Callers show extensive variation in mate attraction signals: individuals vary in their total nightly calling time (Cade 1981; Bertram & Johnson 1998; Bertram 2000), the amplitude at which they trill (Bertram & Warren 2005), their number of pulses per trill (Wagner et al. 1995; Gray & Cade 1999a, b), average trilling bout duration (trills are concatenated into bouts) (Bertram & Warren 2005) and average trilling bout rate (number of trilling bouts per hour) (Bertram & Warren 2005).

Dietary manipulations have revealed that sexually selected traits of crickets are condition dependent (reviewed by Cotton et al. 2004). Wagner & Hoback (1999), for example, found that full sibling adult brothers (*Gryllus lineaticeps*) on high- and low-nutrition feeding regimes (produced by diluting normal food with non-nutritional cellulose) did not differ in weight change, but males on the low-nutrient diet called less than one-third as often and at half the chirp rate as males on the high-nutrient diet (Wagner & Hoback 1999). Scheuber et al. (2003a) also limited food intake for male *G. campestris* and found that well-fed adults gained significantly more weight, and called significantly more often at significantly higher chirp rates than adults fed limited quantities of food. While chirp duration, syllable number, chirp intensity and carrier frequency were not affected by the quantity of food provided to adults (Scheuber et al. 2003a), food restriction during the nymphal stage strongly influenced carrier frequency (Scheuber et al. 2003b). Overall (and not surprisingly), condition and condition-dependent traits tend to decline when food supply is limited.

Many organisms (especially herbivores and primary consumers of plant detritus) have unlimited access to food and are instead limited by food quality. Fewer studies have examined how food quality influences condition and its dependent sexually selected traits (reviewed by Cotton et al. 2004), and most of these have focused on varying the protein content of the diet. Holzer et al. (2003), for example, provided supplemental protein-rich food (45% protein content) to a subset of adult male *G. campestris* at their field burrows and found that males with protein supplements showed a significant increase in body condition, called more frequently and attracted more females compared to control males. Furthermore, Mallard & Barnard (2004) fed *G. bimaculatus* and *Gryllodes sigillatus* two diets that differed in protein (15% versus 12%), fat (2.3% versus 1.8%), fibre (7.5% versus 7.2%) and calcium content and found that crickets reared on high-quality diets had hind femur that were more symmetrical, and they stridulated at a higher rate, took less time to mate, produced more sperm and were more active. These studies strongly suggest that food quality influences cricket condition and its dependent sexually selected traits.

Here we use a stoichiometric approach to evaluate possible relationships between variation in fitness-conferring behaviours and the elemental content of cricket body tissue. We investigate whether body elemental content is correlated with sexually selected acoustic mating displays used in long-distance mate attraction by male Texas field crickets, *Gryllus texensis*. Specifically, we investigated the correlative relationships between body macronutrient composition (percentage of phosphorus, nitrogen and carbon) and total signalling time, average trilling bout duration and trilling bout rate. We also determined whether males classified as low-effort signallers (satellite males) differ from high-effort signallers (callers) in their body

macronutrient composition. We included body size, development time and growth rate to determine whether correlations between signalling and body stoichiometry are associated with these morphological and developmental parameters. Although studies that manipulate protein or lipid in the diet are implicitly altering food stoichiometry, we believe that our study is the first to explicitly examine correlations between condition-dependent behaviours and body stoichiometry.

METHODS

Crickets were derived from 3054 female and 1690 male macropterous crickets field-captured in September 2002 from Austin, Texas, U.S.A. Collected females mated multiply with males and laid their eggs in moist soil. Soil and eggs were transported to Arizona State University, Tempe, Arizona, U.S.A.

Crickets were reared in the laboratory for two generations under a controlled temperature and photoperiod: $\overline{X} \pm \mathrm{SD} = 26 \pm 2^{\circ}\mathrm{C}$, 14:10 h light:dark. They were given unlimited access to a uniform diet (Harlan's Teklad Rodent Diet (W) 8604, Indianapolis, Indiana, U.S.A.). Food and water were replenished as needed (usually twice weekly).

In the first generation, crickets were reared in 36-litre plastic rearing containers ($36 \times 28 \times 23$ cm). Each 36-litre container held several shelters (paper egg cartons) for the crickets. The containers were checked weekly for individuals that had reached nymphal stadium 4. Juvenile crickets at stadium 4 and beyond were housed individually in 500-ml plastic-coated paper bowls (7×11 cm) with a shelter (one cell of a paper egg carton) and unlimited access to food and water. Individuals were checked daily to obtain the date that they moulted to adulthood.

At 17 days post final moult, each virgin male cricket ($N = 484$) was mated to a randomly selected virgin dam. The male and female were housed together for 3 days and allowed to mate freely. After this 72-h mating period, the male was removed from the container. A small (1.5 ounce, 44 ml) plastic cup containing moist soil was added to each container housing the mated female. Females laid eggs in the containers for a period of 5 days. The female was then removed and the eggs were given time to develop. These developing crickets became the second generation and made up our study population.

The second-generation crickets were reared under the same controlled temperature and photoperiod as their parents. Newly hatched individuals were reared in 500-ml plastic-coated paper bowls with their full siblings, a shelter, and unlimited access to food and water. When siblings reached nymphal stadium 2 or 3, they were subdivided into identical containers so that family group size was no more than six individuals per container. Food (Harlan's Teklad Rodent Diet (W) 8604) and water was changed as necessary, usually twice weekly. All containers were checked weekly for individuals that had reached nymphal stadium 4. They were then housed individually in 500-ml containers with a shelter, food and water. Individuals were checked daily to obtain the date of final moult.

Second-generation males ($N = 1292$) were placed in an electronic acoustic recording system (EARs) to electronically monitor each male's long-distance mate attraction signals. During this acoustic monitoring, males were housed individually in 500-ml containers with food and water. Each male's signalling behaviour was monitored for one week, from 1800 hours until 1000 hours each night, when the males were 10–16 days post final moult. A microphone was hung within each male's container approximately 5 cm above the cricket. The EAR reads the microphone and assesses the container's noise level. It then amplifies the signal, converts it from AC to DC, filters it, and records it to disk. This methodology is described in detail in Bertram et al. (1996, 2004). Each microphone is sampled eight times per second, allowing detailed signalling information to be collected for all monitored individuals. Seven centimetres of acoustic foam separated crickets from one another to minimize the effects of neighbouring calls on both the crickets and the EARs. We extracted from these data each male's total nightly signalling time, average bout duration and hourly bout number. Bouts were separated from each other by at least one minute. The EAR sampling rate does not enable determination of dominant frequency, number of pulses per trill, interpulse interval, trill duration, or trill rate.

All 1292 males were weighed to 1 mg on a Denver Instrument Company XE Series Model 400 balance. Males were then placed in labelled plastic vials, snap-frozen in liquid nitrogen, and then stored at -80°C. At a later date, we measured each male's head width to 1 μm using electronic callipers (Mitutoyo Corporation Absolute Digimatic Calipers Model No, CD-4″C). We estimated each male's development time by determining the duration of time (in days) from egg laying to final moult. Each female had 5 days during which she could lay her eggs, so for the development time analyses, we assumed all eggs were laid on day 3. Therefore, we estimated development time by calculating the number of days from day 3 of egg laying to final moult. Development time therefore is accurate within ± 2 days, because the female could have laid the egg 2 days earlier or 2 days later than day 3. We used this development time to estimate cricket growth rate (mg/day): we calculated growth rate by dividing total body wet mass by development time.

We used male calling effort, body size, development time and growth rate data to select a subset of the males for stoichiometric analysis. The process we used to select the subset was not entirely random. Instead, our goal was to capture the entire range of variation that existed among males in their signalling behaviour, body size, development time and growth rate. For body size, development time and growth rate, we subdivided all the males into four groups (low quartile, low–medium quartile, medium–high quartile and high quartile). We then randomly selected four individuals from each quartile for each of these three traits. For calling behaviour, we initially subdivided callers into four groups based on their average nightly calling time. However, four of the males never signalled acoustically during the week-long monitoring period, so we placed these males together in a separate (fifth) group. Therefore, of the 68 males

selected, 16 males each spanned the full range of body size (4 males × 4 quartiles), development time (4 males × 4 quartiles), and growth rate (4 males × 4 quartiles), and 20 males spanned the range of calling behaviour (4 males × 5 groups).

Our sample size of males on which we ran stoichiometric analyses was reduced from 68 to 55 because a few of the individuals were selected for more than one trait, and several had a body part (usually a hind leg) missing upon further inspection. Ten of the 55 selected males were noncallers (four from the noncaller group and six others that spanned the ranges of body size, development time and growth rate). Of the 45 males that signalled acoustically, nightly total signalling time ranged from 1 min to 8.5 h, and averaged 2.33 h. The 55 selected males showed a two-fold difference in development time, a two-fold difference in head width and a four-fold difference in growth rate.

The 55 males that were selected for stoichiometric analysis were dried at 60°C for 96 h, then weighed (±0.01 mg) using a Mettler MX5 electrobalance. We recalculated growth rate using dry mass (dry mass divided by development time = mg/day) to control for variation in cricket hydration in our original wet-mass-based growth rates. Wet-mass-based growth rates were used only to select males for the stoichiometric analyses. The dry-mass-based growth rates were used in all subsequent analyses.

We pulverized dried crickets to a fine powder using a Spex Certiprep 8000D ball mill and used approximately 1 mg of powder from each individual to assess body carbon and nitrogen contents. We used a Perkin–Elmer 2400 CHN elemental analyser to quantify carbon and nitrogen contents (percentage of dry mass). We also determined body phosphorus content for each individual using approximately 1 mg of dried powder from each male. We used the persulfate oxidation technique followed by analysis of orthophosphate using the acid molybdate technique (APHA 1992).

We classified selected males as high-effort signallers (callers; $N = 19$) or low-effort signallers (satellites; $N = 36$) based on whether their total signalling was above or below a 84-min threshold (following Bertram & Warren 2005). We classified males in this way because males were isolated from each other during acoustic monitoring. When satellite males are isolated from callers they often signal acoustically, but spend significantly less time signalling than callers (callers: $\overline{X} \pm \mathrm{SD} = 252 \pm 168$ min; satellites: $\overline{X} \pm \mathrm{SD} = 126 \pm 20$ min; Kruskal–Wallis ANOVA: $H_1 = 9.1$, $P = 0.01$; Cade 1991). We therefore identified low-effort 'satellite' males as those males signalling 84 min or less on average across nights, and high-effort 'callers' as those males signalling more than 84 min per night. The 84-min threshold lies one standard deviation below the callers' average total signalling time (252 min−168 min = 84 min; Cade 1991). This caller and satellite classification approach has been used in other studies exploring potential differences between callers and satellites when they are monitored acoustically in the laboratory (i.e. Bertram & Warren 2005). We used this classification strategy to ascertain whether callers and satellites might differ in their carbon:nitrogen:phosphorus stoichiometry.

Statistical Analysis

Statistical analyses were conducted using JMP software (SAS Institute, Cary, North Carolina, U.S.A.). The Shapiro–Wilk goodness-of-fit test was used to ensure that the data did not differ significantly from a normal distribution. Total signalling time, hourly bout number and bout duration data were log-transformed to meet assumptions of normality.

We used correlation analysis to determine the relationships between signalling, body elemental content, size, development time and dry-mass-based growth rate. To account for the 46 correlations performed, we adjusted the significance probability to 0.004 using Sidak's method. We used ANOVA to determine whether high-effort signallers (callers) differed from low-effort signallers (satellites) in their signalling behaviours, size, development time, dry-mass-based growth rate, elemental composition (percentage of carbon, nitrogen and phosphorus). We adjusted the significance probability to 0.01 using Sidak's method to account for the 10 ANOVAs performed.

RESULTS

Body carbon, nitrogen and phosphorus contents of laboratory-reared male Texas field crickets showed extensive variation (Table 1). Carbon made up 47–57% of the total body mass, nitrogen 9–13% and phosphorus showed a two-fold difference, ranging from 0.45 to 0.9%. Across males, the percentage of nitrogen (%N) and phosphorus (%P) were highly positively correlated (Pearson's correlation: $r_{53} = 0.65$, $P < 0.0001$; Fig. 1) and

Table 1. Variation in signalling behaviour, body size, development time, growth rate and stoichiometric balance of laboratory male crickets reared on identical diets

Parameters	Mean	SE	Range	CV
Total signalling time on all nights (min)	132.27	25.20	0.43–511.06	122.01
Total signalling time only on nights signalling (min)	140.26	26.05	1.00–511.06	140.26
Average number of calls per night	6.04	0.68	1.00–20.43	72.08
Trilling bout duration (min)	19.45	3.62	0.27–97.62	119.11
Hourly bout number	0.38	0.04	0.06–1.26	72.08
Development time (days)	108.61	2.62	79–152	15.43
Dry weight (g)	0.13	0.01	0.06–0.23	29.83
Growth rate (mg/day)	1.20	0.05	0.06–1.98	28.78
% Carbon (dry weight)	52.51	0.33	46.49–56.85	4.05
% Nitrogen (dry weight)	10.99	0.15	9.04–12.66	8.71
% Phosphorus (dry weight)	0.66	0.02	0.47–0.87	14.98

Acoustic mating signals were monitored electronically on days 10–17 post final moult. Males were then killed and analysed for body size, dry weight and stoichiometric balance.

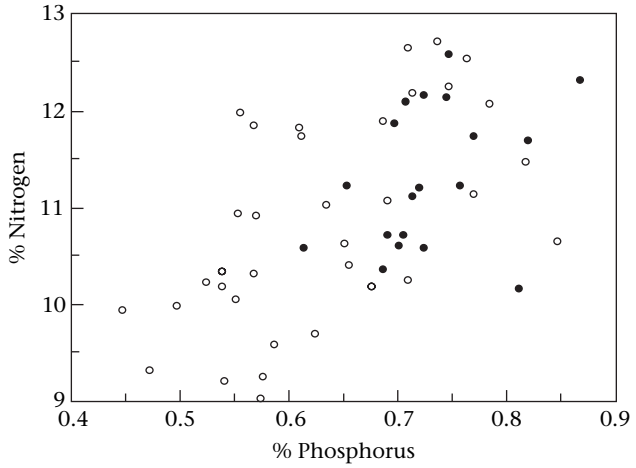

Figure 1. Correlation between the percentage of nitrogen and phosphorus in laboratory-reared male Texas field crickets, *Gryllus texensis*. Each data point represents an individual. ●: males classified as high-effort signallers (callers); ○: males classified as low-effort signallers (satellites).

negatively correlated with the percentage of carbon (%N: $r_{53} = -0.86$, $P < 0.0001$; %P: $r_{53} = -0.64$, $P < 0.0001$, $N = 55$).

Acoustic mate attraction displays were significantly correlated with several aspects of body elemental composition (Table 2). Specifically, %P was positively correlated with total signalling time and trilling bout duration and showed a nonsignificant tendency to correlate with hourly bout number ($r_{53} = 0.31$, $P = 0.0374$).

Significant correlations also existed between body size and elemental composition (Table 2). Cricket dry weight and growth rate were significantly positively correlated with the percentage of carbon (%C) and significantly negatively correlated with the percentage of nitrogen (%N) and phosphorous (%P). Head width also showed many of the same significant correlations with the elemental composition parameters, although the %C correlation was nonsignificant (Table 2). Development time was not correlated with any of the elemental composition parameters. We found no correlation between any aspect of acoustic mate signalling behaviour and body size, development time, or growth rate (Table 2).

High- and low-effort signallers differed significantly in elemental composition (Table 3, Fig. 1). High-effort signallers had significantly higher %P than did low-effort signallers. There was also a nonsignificant tendency for high-effort signallers to have higher %N and lower %C than did low-effort signallers. Although high- and low-effort signallers did not differ in body size or growth rates, there was a nonsignificant tendency for high-effort signallers to develop more rapidly than low-effort signallers.

DISCUSSION

Our results reveal a relationship between body phosphorus content and fitness-conferring behaviours in crickets. Crickets in our study showed extensive variation in body phosphorus content (Table 1), and this variation correlated strongly with observed variation in mate signalling effort (Table 2, Fig. 1). In general, males that had elevated body phosphorus content appeared to invest significantly more effort into acoustic mating displays. Our results also indicate that callers and satellites may differ in elemental composition, because males classified as high-effort signallers had significantly higher body phosphorus content than did males classified as low-effort signallers (Table 3, Fig. 1). In contrast to phosphorus-related parameters, body content of carbon and nitrogen, size, mass, development time and growth rate did not correlate with signalling behaviour, nor did these parameters differ between high- and low-effort signallers. Our findings suggest that condition-dependent signalling may depend in part on phosphorus acquisition. Below we discuss why phosphorus acquisition may affect signalling behaviour.

Why Might Males Differ in their Phosphorus Content?

Although crickets were raised on an identical diet, we found two-fold differences in male body phosphorus

Table 2. Correlations between signalling, body size, development time, growth rate and stoichiometric balance

	Total signalling time	Trilling bout duration	Hourly bout number	Head width	Dry weight	Development time	Growth rate	%Carbon	%Nitrogen
Trilling bout duration	**0.96**								
Hourly bout number	**0.74**	**0.54**							
Head width	0.15	0.15	0.08						
Dry weight	0.10	0.08	0.11	**0.87**					
Development time	−0.14	−0.12	−0.20	0.14	0.14				
Growth rate	0.12	0.11	0.15	**0.73**	**0.87**	−0.34			
% Carbon	−0.10	−0.17	0.11	**0.40**	**0.48**	−0.05	**0.43**		
% Nitrogen	0.16	0.21	−0.04	**−0.36**	**−0.47**	0.07	**−0.45**	**−0.88**	
% Phosphorus	**0.52**	**0.52**	0.31	−0.26	**−0.41**	−0.02	**−0.40**	**−0.59**	**0.57**

Statistically significant correlations are given in bold. Statistical significance was reduced to $P < 0.004$ using Sidak's method to account for the 46 correlations.

Table 3. Differences between high- and low-effort signallers in their signalling behaviour, size, development and stoichiometric balance

	High-effort signallers (callers)	Low-effort signallers (satellites)	*F*	*P*	R^2
Signalling parameters					
Total signalling time*	**314.47 (23.65)**	**11.73 (3.12)**	**148.03**	**<0.0001**	**0.77**
Trilling bout duration*	**42.24 (5.11)**	**3.56 (0.86)**	**84.00**	**<0.0001**	**0.66**
Hourly number of trilling bouts*	**0.60 (0.06)**	**0.21 (0.02)**	**49.06**	**<0.0001**	**0.53**
Size and development					
Head width	4.89 (0.05)	4.87 (0.10)	0.20	0.6550	0.00
Dry weight	0.13 (0.00)	0.13 (0.01)	0.00	0.9957	0.00
Development time	102.68 (2.22)	113.11 (3.57)	4.13	0.0473	0.07
Growth rate	1.26 (0.04)	1.17 (0.08)	0.60	0.4413	0.01
Stoichiometry					
% Carbon	51.71 (0.46)	52.81 (0.41)	2.89	0.0949	0.05
% Nitrogen	11.33 (0.17)	10.84 (0.18)	3.10	0.0837	0.06
% Phosphorus*	**0.73 (0.01)**	**0.62 (0.02)**	**19.56**	**<0.0001**	**0.27**

*Mean values for each mating strategy and parameter are presented; standard errors are in parentheses. Parameters that differed significantly between high- and low-effort signallers are given in bold. Statistical significance was reduced to $P < 0.01$ using Sidak's method to account for the 10 ANOVAs.

content. Variation in body phosphorus content may be a consequence of variation in the efficiency with which males absorb phosphorus-rich nutrients. It may also result from differences between males in the amount of time they spend foraging, or in their ability to retain this essential limiting resource.

What Might Drive the Correlation between Signalling and Phosphorus Content?

Total body phosphorus content was strongly and positively correlated with long-distance mate attraction signalling behaviour. One hypothesis for this correlation is that the evolution of signalling behaviour has placed a constraint on the organism that has lead to a high phosphorus requirement. If crickets differ in their ability to acquire and retain the necessary phosphorus, this difference could drive some of the variation in these sexually selected traits. Is there a biochemical basis for such a hypothesis? To address this question, we follow the approach taken by Sterner & Elser (2002) by considering the elemental composition of major biochemicals associated with different structures and functions in crickets. By ascertaining the elemental composition of biochemicals, how they contribute to organismal biomass, and how they vary among crickets, we can identify candidate biochemicals that may provide the proximate basis for the correlation between body stoichiometry and signalling in the Texas field cricket. Furthermore, by ascertaining the proximate biochemical determinants of observed differences in elemental composition, we might be better able to suggest and evaluate the possible evolutionary significance of these patterns.

The major classes of ubiquitous biological materials that contain carbon, nitrogen and/or phosphorus in crickets are proteins, lipids, energetic nucleotides, carbohydrates, and nucleic acids (Sterner & Elser 2002). Proteins are mostly phosphorus-free (Sterner & Elser 2002) and are therefore unlikely to drive the observed correlations. Storage lipids and triglycerides such as triacylglycerol, which is used to fuel flight and may be used to power signalling, are also unlikely to drive the observed correlations because they do not contain nitrogen or phosphorus (Sterner & Elser 2002). In contrast, phospholipids, energetic nucleotides and nucleic acids all contain phosphorus (Sterner & Elser 2002). On average, phospholipids contain 1.6% nitrogen and 4.2% phosphorus, adenosine 5′-triphosphate (ATP) contains 14% nitrogen and 18% phosphorus, while nucleic acids (RNA and DNA) contain 14.5% nitrogen and 9.6% phosphorus. Therefore, increases in relative contributions of any of these compounds to biomass would tend to raise organismal body phosphorus content (Sterner & Elser 2002).

Phospholipids are unlikely to drive the observed correlations between signalling and body phosphorus content because they do not contribute enough to biomass. Zera & Larsen (2001) examined phospholipids in the wing-polymorphic congener, *G. firmus*, and they found that phospholipids make up only 3–4% of total biomass. At this allocation, the phosphorus contributed by phospholipids would represent only 0.1% of total dry mass, an amount too small to explain the extensive variation in body phosphorus content that we observed. Therefore, provided that *G. texensis* phospholipid allocation does not differ too much from that of *G. firmus*, male phospholipid differences are unlikely to drive the observed correlations between signalling and body phosphorus content.

Differences between males in energetic turnover of phosphorus-containing molecules are also unlikely to explain the observed correlation between signalling and body phosphorus content because these molecules do not contribute enough to biomass. Although signalling is an energetically demanding activity and males that signal frequently turn over substantial amounts of ATP (Prestwich & Walker 1981; Prestwich 1994; Hoback & Wagner 1997; Reinhold 1999; Wagner & Hoback 1999), ATP makes up only a small proportion of organismal biomass in terrestrial insects, averaging 0.05% dry mass (0.02–2%; de Zwann & van den Thillart 1985). At this level of ATP

allocation, ATP-P would be only a modest fraction of body mass (~0.1%, small relative to the overall phosphorus content of ~0.75%).

While body DNA content is usually too low to appreciably affect whole organism phosphorus content (1% of dry weight or less; Sterner & Elser 2002), total body RNA content could explain the correlations between signalling and body phosphorus content. RNA content contributes substantially to whole organism biomass, ranging from 0.5% to 15% of dry mass in various arthropod taxa (Dagg & Littlepage 1972; Bämstedt 1986; Elser et al. 2003). Individuals and species also vary extensively in their RNA content, suggesting that RNA variation may account for variation in body phosphorus content. If the RNA content of Texas field crickets lies in the middle of this range (~6%), then RNA-P would contribute ca. 0.5%, a significant value given that overall phosphorus content is ca. 0.75%.

Variation in RNA allocation may also reflect differences in growth rate because higher levels of ribosomal RNA are needed to synthesize proteins during rapid growth (Kita et al. 1996; Elser et al. 2000b, 2003; Bush et al. 2003; Storey & Storey 2004). However, growth rate differences in our study were not associated with differences in cricket phosphorus content and calling level. Thus, it is possible that the lack of a relationship between growth and phosphorus content in our study was due to excess phosphorus in the rearing diet (Elser et al. 2003), or to the way in which we estimated growth rate. We measured growth rate using the cricket's entire development time (from egg to final moult), but measured phosphorus content 17 days later (after measuring cricket acoustic mate attraction behaviours). A more appropriate way to examine phosphorus–RNA–growth correlations would have been to examine growth rate during a shorter time interval and then immediately measure organismal phosphorus and RNA content.

Irrespective of the RNA–growth relationship, RNA content could contribute to an organism's ability to signal extensively because enhanced protein synthesis may also be required for high metabolic activity (Welle & Nair 1990; Quigg & Beardall 2003). This is because high metabolic rates generate excess free radicals, increasing protein damage and necessitating its replacement (Welle & Nair 1990; Quigg & Beardall 2003). Given that acoustic signalling is a highly energetically demanding activity (Prestwich & Walker 1981; Prestwich 1994; Hoback & Wagner 1997; Reinhold 1999; Wagner & Hoback 1999), the muscle and glandular tissue involved should show high metabolic activity, and therefore require high allocation to RNA in support of protein turnover and repair. Under this scenario we would predict that individuals that have more RNA are able to call more often and for longer durations, because they can use this phosphorus-rich RNA to replace the muscle and glandular tissue damaged by the generation of excess free radicals. We would also predict that callers would differ from satellites in the amount of RNA that they have, and that low dietary phosphorus may drive an organism to adopt satellite behaviour.

The possible associations between calling effort, protein turnover, RNA allocation and phosphorus content raise an interesting question of cause and effect. That is, if animals that signal often also have high-protein turnover, as a result of frequent signalling, then they might need a high RNA allocation for protein synthesis, resulting in a correlation between body phosphorus content and signalling. Establishing the nature of the cause and effect relationship in these observations will require direct dietary manipulations of phosphorus.

Diet is well known to affect condition-dependent acoustic mate attraction signalling in gryllids (Wagner & Hoback 1999; Holzer et al. 2003; Scheuber et al. 2003a, b; Mallard & Barnard 2004). However, until now, the condition-dependent nature of these sexually selected traits had not been hypothesized to relate to the demand for key macronutrient elements. Our finding that body phosphorus composition is correlated with mate signalling in crickets is commensurate with research showing a correlation between body phosphorus content and growth rate and reproduction in other invertebrates (Quraishi et al. 1966; Elser et al. 2000a, 2003; Eskelinen 2002; Fagan et al. 2002; Schade et al. 2003; Perkins et al. 2004). Together these findings suggest that a stoichiometric imbalance between an organism's nutrient requirements and the relative scarcity of nutrients in nature may underlie variation in condition-dependent traits. Given the limiting nature of phosphorus in many ecosystems (Redfield 1958; Hecky & Kilham 1988; Vitousek et al. 1993; Verhoeven et al. 1996; Schindler & Eby 1997; Karl 1999), variation in its availability to consumers may frequently influence condition and its dependent traits.

Ecological stoichiometry provides us with a new set of tools for addressing the causes of variation in behaviour. An important first step to addressing whether organismal stoichiometry plays a role in explaining the extensive variation that we observe in behaviours will be careful experimental studies that manipulate dietary nitrogen and phosphorus content. If dietary studies reveal that organismal stoichiometry influences condition-dependent behaviour and other condition-dependent traits, then cross-taxa comparative studies designed to test the role of stoichiometric constraints in shaping behavioural traits and in influencing fitness may prove highly informative.

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References

Acharya, K., Kyle, M. & Elser, J. J. 2004a. Biological stoichiometry of *Daphnia* growth: an ecophysiological test of the growth rate hypothesis. *Limnology and Oceanography*, **49**, 656–665.

Acharya, K., Kyle, M. & Elser, J. J. 2004b. Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction. *Oecologia*, **138**, 333–340.

Alexander, R. D. 1961. Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour*, **17**, 130–223.

APHA 1992. *Standard Methods for the Examination of Water and Wastewater*. Washington, D.C: American Public Health Association.

Bämstedt, U. 1986. Chemical composition and energy content. In: *The Biological Chemistry of Marine Copepods* (Ed. by E. D. S. Corner & S. C. M. O'Hara), pp. 1–58. Oxford: Oxford University Press.

Behmer, S. T., Cox, E., Raubenheimer, D. & Simpson, S. J. 2003. Food distance and its effect on nutrient balancing in a mobile insect herbivore. *Animal Behaviour*, **66**, 665–675.

Bertram, S. M. 2000. The influence of age and size on temporal mate signalling behaviour. *Animal Behaviour*, **60**, 333–339.

Bertram, S. M., Berrill, M. & Nol, E. 1996. Male mating success and variation in chorus attendance within and among breeding seasons in the gray treefrog (*Hyla versicolor*). *Copeia*, **1996**, 729–734.

Bertram, S. M. & Johnson, L. 1998. An electronic technique for monitoring the temporal aspects of acoustic signals of captive organisms. *Bioacoustics*, **9**, 107–118.

Bertram, S. M. & Warren, P. 2005. Trade-offs in signalling components differ with signalling effort. *Animal Behaviour*, **70**, 477–484.

Bertram, S. M., Johnson, L., Clark, J. & Chief, C. 2004. An electronic acoustic recorder (EAR) for quantifying when, how much, and how loud individuals signal acoustically. *Technical Acoustics*, **20**, 1–15.

Bush, J. A., Kimball, S. R., O'Connor, P. M. J., Suryawan, A., Orellana, R. A., Nguyen, H. V., Jefferson, L. S. & Davis, T. A. 2003. Translational control of protein synthesis in muscle and liver of growth hormone-treated pigs. *Endocrinology*, **144**, 1273–1283.

Cade, W. H. 1979. The evolution of alternative male reproductive strategies in field crickets. In: *Sexual Selection and Reproductive Competition in Insects* (Ed. by M. S. Blum & N. A. Blum), pp. 343–378. New York: Academic Press.

Cade, W. H. 1981. Alternative male strategies: genetic differences in crickets. *Science*, **212**, 563–565.

Cade, W. H. 1991. Inter- and intraspecific variation in nightly calling duration in field crickets, *Gryllus integer* and *G. rubens* (Orthoptera: Gryllidae). *Journal of Insect Behavior*, **4**, 185–194.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society of London, Series B*, **271**, 771–783.

Cruz-Rivera, E. & Hay, M. E. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness in marine mesograzers. *Ecology*, **81**, 201–219.

Dagg, M. J. & Littlepage, J. L. 1972. Relationships between growth rate and RNA, DNA, protein, and dry weight in *Artemia salina* and *Euchaeta elongata*. *Marine Biology*, **17**, 162–170.

Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D., Folarin, A., Huberty, A., Interlandi, S., Kilham, S. S., McCauley, E., Schulz, K. L., Siemann, E. H. & Sterner, R. W. 2000a. Nutritional constraints in terrestrial and freshwater food webs. *Nature*, **408**, 578–580.

Elser, J. J., Sterner, R. W., Gorokhova, E., Fagan, W. F., Markow, T. A., Cotner, J. B., Harrison, J. F., Hobbie, S. E., Odell, G. M. & Weider, L. J. 2000b. Biological stoichiometry from genes to ecosystems. *Ecology Letters*, **3**, 540–550.

Elser, J. J., Hayakawa, H. & Urabe, J. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology*, **82**, 898–903.

Elser, J. J., Acharya, K., Kyle, M., Cotner, J. B., Makino, W., Markow, T. A., Watts, T. D., Hobbie, S. E., Fagan, W. F., Schade, J., Hood, J. & Sterner, R. W. 2003. Growth rate: stoichiometry couplings in diverse biota. *Ecology Letters*, **6**, 936–943.

Eskelinen, O. 2002. Diet of the wood lemming *Myopus schisticolor*. *Annales Zoologici Fennici*, **39**, 49–57.

Fagan, W. F., Siemann, E. H., Denno, R. F., Mitter, C., Huberty, A., Woods, H. A. & Elser, J. J. 2002. Nitrogen in insects: implications for trophic complexity and species diversification. *American Naturalist*, **160**, 784–802.

Gray, D. A. & Cade, W. H. 1999a. Quantitative genetics of sexual selection in the field cricket, *Gryllus integer*. *Evolution*, **53**, 848–854.

Gray, D. A. & Cade, W. H. 1999b. Sex, death and genetic variation: natural and sexual selection on cricket song. *Proceedings of the Royal Society of London, Series B*, **266**, 707–709.

Hecky, R. E. & Kilham, P. 1988. Nutrient limitation of phytoplankton in fresh-water and marine environments: a review of recent evidence on the effects of enrichment. *Limnology and Oceanography*, **33**, 796–822.

Hoback, W. W. & Wagner, W. E. 1997. The energetic cost of calling in the variable field cricket, *Gryllus lineaticeps*. *Physiological Entomology*, **22**, 286–290.

Holzer, B., Jacot, A. & Brinkhof, M. W. G. 2003. Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. *Behavioral Ecology*, **14**, 353–359.

Hunt, J., Brooks, R., Jennions, M. D., Smith, M. J., Bentsen, C. L. & Bussiere, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature*, **432**, 1024–1027.

Karl, D. M. 1999. A sea of change: biogeochemical variability in the North Pacific Subtropical Gyre. *Ecosystems*, **2**, 181–214.

Kita, K., Matsunami, S. & Okumura, J. I. 1996. Relationship of protein synthesis to mRNA levels in the muscle of chicks under various nutritional conditions. *Journal of Nutrition*, **126**, 1827–1832.

Mallard, S. & Barnard, C. 2004. Food stress, fluctuating asymmetry and reproductive performance in the gryllid crickets *Gryllus bimaculatus* and *Gryllodes sigillatus*. *Behavior*, **141**, 219–232.

Markow, T. A., Coppola, A. & Watts, T. D. 2001. How *Drosophila* males make eggs: it is elemental. *Proceedings of the Royal Society of London, Series B*, **268**, 1527–1532.

Mattson, J. J. J. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, **11**, 119–161.

Mayntz, D. L., Raubenheimer, D., Salomon, M., Toft, S. & Simpson, S. J. 2005. Nutrient-specific foraging in invertebrate predators. *Science*, **307**, 111–113.

Perkins, M. C., Woods, H. A., Harrison, J. F. & Elser, J. J. 2004. Dietary phosphorus affects the growth of larval *Manduca sexta*. *Archives of Insect Biochemistry and Physiology*, **55**, 153–168.

Plath, K. & Boersma, M. 2001. Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. *Ecology*, **82**, 1260–1269.

Pomiankowski, A. & Iwasa, Y. 1995. What causes diversity in male sexual characters? *Revue Suisse de Zoologie*, **102**, 883–893.

Prestwich, K. N. 1994. The energetics of acoustic signaling in anurans and insects. *American Zoologist*, **34**, 625–643.

Prestwich, K. N. & Walker, T. J. 1981. Energetics of singing in crickets: effects of temperature in three trilling species (Orthoptera: Gryllidae). *Journal of Comparative Physiology*, **143**, 199–212.

Quigg, A. & Beardall, J. 2003. Protein turnover in relation to maintenance metabolism at low photon flux in two marine microalgae. *Plant Cell and Environment*, **26**, 693–703.

Quraishi, M. S., Brust, R. A. & Lefkovitch, L. P. 1966. Uptake, transfer, and loss of ^{32}P during metamorphosis, mating, and oviposition in *Aedes vexans*. *Journal of Economic Entomology*, **59**, 1331–1333.

Raubenheimer, D. & Simpson, S. J. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology*, **206**, 1669–1681.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. *American Scientist*, **46**, 205–221.

Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology*, **13**, 217–224.

Schade, J., Kyle, M., Hobbie, S. E., Fagan, W. F. & Elser, J. J. 2003. Stoichiometric tracking of soil nutrients by a desert insect herbivore. *Ecology Letters*, **6**, 96–101.

Scheuber, H., Jacot, A. & Brinkhof, M. W. G. 2003a. Condition dependence of a multicomponent sexual signal in the field cricket, *Gryllus campestris*. *Animal Behaviour*, **65**, 721–727.

Scheuber, H., Jacot, A. & Brinkhof, M. W. G. 2003b. The effect of past condition on a multicomponent sexual signal. *Proceedings of the Royal Society of London, Series B*, **270**, 1779–1784.

Schindler, D. E. & Eby, L. A. 1997. Stoichiometry of fishes and their prey: implications for nutrient recycling. *Ecology*, **78**, 1816–1831.

Schulz, K. L. & Sterner, R. W. 1999. Phytoplankton phosphorus limitation and food quality for *Bosmina*. *Limnology and Oceanography*, **44**, 1549–1556.

Sterner, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. *Ecology*, **74**, 2351–2360.

Sterner, R. W. & Elser, J. J. 2002. *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*. Princeton, New Jersey: Princeton University Press.

Sterner, R. W., Hagemeier, D. D., Smith, W. L. & Smith, R. F. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia*. *Limnology and Oceanography*, **38**, 857–871.

Sterner, R. W. & Schulz, K. L. 1998. Zooplankton nutrition: recent progress and a reality check. *Aquatic Ecology*, **32**, 261–279.

Storey, K. B. & Storey, J. M. 2004. Metabolic rate depression in animals: transcriptional and translational controls. *Biological Reviews*, **79**, 207–233.

Strong, D. R., Lawton, J. H. & Southwood, R. 1984. *Insects on Plants: Community Patterns and Mechanisms*. London: Blackwell Scientific.

Urabe, J. & Sterner, R. W. 2001. Contrasting effects of different types of resource depletion on life-history traits in *Daphnia*. *Functional Ecology*, **15**, 165–174.

Verhoeven, J., Koerselman, W. & Meuleman, A. 1996. Nitrogen- or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *Trends in Ecology and Evolution*, **11**, 494–497.

Vitousek, P., Walker, L., Whiteaker, L. & Matson, P. 1993. Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry*, **23**, 197–215.

Wagner, W. E. & Hoback, W. W. 1999. Nutritional effects on male calling behaviour in the variable field cricket. *Animal Behaviour*, **57**, 89–95.

Wagner, W. E., Murray, A. M. & Cade, W. H. 1995. Phenotypic variation in the mating preferences of female field crickets, *Gryllus integer*. *Animal Behaviour*, **49**, 1269–1281.

Weers, P. M. M. & Gulati, R. D. 1997. Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. *Limnology and Oceanography*, **42**, 1584–1589.

Welle, S. & Nair, K. S. 1990. Relationship of resting metabolic rate to body composition and protein turnover. *American Journal of Physiology*, **258**, E990–E998.

Zera, A. J. & Larsen, A. 2001. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, *Gryllus firmus*. *Journal of Insect Physiology*, **47**, 1147–1160.

de Zwann, A. & van den Thillart, G. 1985. Low and high power output modes of anaerobic metabolism: invertebrate and vertebrate strategies. In: *Circulation, Respiration, and Metabolism* (Ed. by R. Gilles), pp. 166–192. Berlin: Springer-Verlag.