Applying Biogeochemistry to Identify the Geographic Origins of Insects-A Model Using *Prostephanus truncatus*

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Abstract

The application of geochemical screening for tracking insects and other organisms is poorly understood. We explored multi-elemental profiles of an insect pest and its food from different locations in order to identify intrinsic markers of geographic origin.

ICP-AES screening was completed for soil, maize grains and *Prostephanus truncatus* of different geographic origin, with insects collected in the field or reared on maize under controlled conditions. Background level geochemical data are presented with concentrations of Cu, S and Sr in maize, and Al, Ba, Ca, Fe, S, Si, Sr, Ti and Zn in insects differing according to their origin, or that of their food. Cu, Fe and Zn determinations of insects were 20, 6.5 and 11.5 times the concentrations of the maize they were reared on.

A Linear Discriminant Analysis (LDA) based on Al, Fe, Sr and Zn successfully assigned 78% of laboratory-reared *P. truncatus* and 94% of field-caught insects to their origin (68% and 75% respectively after jacknifing). There was excellent discrimination between insects reared on maize from Guanajuato and Nuevo Léon, and Guanajuato and Michoacán, good separation between Sonora and Nuevo Léon but overlap for Sonora, Guanajuato and Michoacán, and Michoacán and Nuevo Léon. The same LDA for field-caught insects gave complete separation between Michoacán and Sonora, but some overlap between Michoacán and Nuevo Léon.

This robust ICP-AES technique successfully identified a combination of elements able to classify insects according to their origin. The model was restricted to elements showing a normal distribution but non-parametric tests suggest other elements may act as intrinsic markers. This shows the potential of atomic spectroscopy for remotely tracing insects and other biological materials, with applications including providing provenance data for species routinely captured for surveillance and to track natural enemies and wildlife.

Abbreviations

ICP-AES: Inductively Coupled Plasma Atomic Emission Spectrometry; LGB: Larger Grain Borer; RBPH: Rice Brown Plant Hopper; PCA: Principal Components Analysis; LDA: Linear Discriminate Analysis; ICP-MS: Inductively Coupled Plasma Mass Spectrometry

Introduction

Many pests are routinely monitored for surveillance and to gain information about their distribution and range, as well as their population density and temporal fluctuations, in order to develop effective pest management models [1]. However, in most cases the origin of the individuals captured is not known [2,3] and since adult insects generally disperse away from their natal origin, more precise location data about the sources of invasion of mobile species would improve management decisions and risk analyses, with potential applications for many other taxa and scenarios including migrant species of conservation interest that cross international boundaries.

Local and regional variations in background geochemistry result in distinct conditions which may profoundly influence flora and fauna [4]. Terrestrial invertebrates gain most of their elemental loading from food, with the highest chemical mobility into food webs occurring in nutrient-rich soils that typical occur in arable production systems [5]. Where geochemical data for insects exist, they suggest that the elemental profile of insects reflects the geochemistry of their environment, with anthropogenic activity likely to increase levels of toxic elements including heavy metals, and acting to reduce both invertebrate abundance and diversity [6,7]. Whilst many studies explore the fate of elevated levels of chemicals in invertebrate food webs [8-11] few have considered naturally-occurring, background level elemental loadings [12,13] or their application as intrinsic markers [3]. This is in contrast to the more widespread adoption of stable isotopes as assimilated markers obtained from background levels in food, that can be used to remotely trace animal dispersal and migration [14,15].

There is some evidence that individual chemical elements and species interact in different ways although most studies consider the fate and behaviour of artificially elevated levels of heavy metals which are toxic. For example, some herbivorous insect larvae accumulate excess Cd, Cu and Zn which are eliminated in their pupal exuviae, whilst elevated Cd levels slow down the developmental rate of predatory ground beetle larvae [7,16]. Rates of uptake and excretion in ground beetles suggest that Ni and Cd are rapidly accumulated, and then partially eliminated before reaching equilibrium at lower concentrations [17]. Specific effects caused by life-stage and sex have been shown in a few species and taxa, however most studies suggest that the chemical profile of insects achieves a steady state related to background elemental concentrations [18]. Therefore elements that can be detected via analytical screening methods are potential

markers for tracing the origin of insects, particularly where rarer elements including heavy metals and trace elements show distinct geographic patterns of distribution.

Various atomic spectroscopy techniques are used to chemically screen biological materials, and their application to studies of pest dispersal and the human food chain were reviewed by Akey and Burns [19] and Beavington [20] respectively. These authors discuss the many factors influencing the choice of analytical technique, including specific elements, concentrations and material(s) to be tested, sensitivity and cost, as well as availability and personal preference. The range of atomic spectroscopy techniques currently available, provide powerful tools to reveal how environmental contamination moves between trophic levels and into ecosystems [21-24], and such methods are increasingly used to authenticate the provenance of high value foods such as honey, tea, rice and meat products based on the background geochemistry of their unique production systems [25-28], as well as for forensic investigations [29,30]. Entomological applications have included making multi-elemental chemoprints for a range of agricultural pests such as cereal aphids and moths associated with the soil chemistry of their larval food plant [31-35]. However, those elements best describing geographic origin were not fixed, and varied both with species and life stage for aphids [32,33,35], whilst polyphagous moths had highly variable elemental compositions suggesting that the identity of their larval host-plant was as important as soil geochemistry [31,34]. Dispersal by cotton boll weevils was traced by chemically characterising them and using trace elements as independent variables in multivariate models which assigned100% weevils to one of three locations where they had been collected [36].

Recent studies show renewed and emerging interest in natural background variations in insect geochemistry and its applications. Such methods have benefitted from improved detection, sensitivity and computing power, and include examples based on trace elements and stable isotopes for wildlife management [37] and insect pest dispersal [3,38]. It has been suggested that multi-elemental and isotopic profiling could trace movement between natural reservoirs and infestations of *Prostephanus truncatus* [39], and for a closely related pest, *Rhizopertha dominica,* Ca, Na, S and Zn concentrations were associated with specific larval hosts and natal environments [40]. A model based on V, Mn, Fe, Mo, Se, Cd, Ce and Nd classified the majority of Rice Brown Plant Hoppers (RBPH) to their correct geographic origin [18] and multivariate models using combinations of trace elements and stable isotopes gave better determination of the origin of *Helicoverpa armigera* than models using the same factors individually [3].

This study explores background levels of elements and aims to identify a suite of markers to determine the geochemical origin of *P. truncatus*, a beetle which is considered to be highly invasive outside its native range [41]. The objective was to collect insects and their food from places that are geographically isolated and likely to exhibit a different geochemistry, maximising the likelihood of finding intrinsic markers for location. Insects were either collected in the field or reared under controlled conditions from egg to adult on maize collected from different locations, allowing the transfer of elements in a simple food chain to be followed.

ICP-AES was used to synchronously screen samples and chemically characterise soil, maize and *P. truncatus* for 20 elements: Al, B, Ba, Cd, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Si, Sr, Ti, V, Zn and Zr. A multivariate Linear Discriminate Analysis (LDA) was used to classify laboratory-reared *P. truncatus* according to the origin of the maize they were reared on, and the model was applied to field-caught insects from some of the same locations.

We present a robust technique to identify a combination of elements able to determine the origin of this insect, and discuss those elements that are appropriate candidates for intrinsic markers. In addition to remotely tracking dispersal by species of economic interest particularly migratory species of biosecurity concern, multielemental profiling of biological materials has wider applications in ecology, environmental chemistry and natural resources management. This includes studying the movement of rare migratory species of conservation interest, and forensic investigations where the provenance of biological materials is unknown.

Methods

Sample collection

Mexican maize of known provenance was collected direct from producers in four federal states of Mexico: Nuevo Léon, Sonora, Michoacán and Guanajuato, with additional maize of unknown provenance purchased from two commercial suppliers to increase sources of elemental variation (the sources of maize are listed in S1). A total of 22 maize samples were subjected to chemical screening to characterize and examine differences in geochemistry.

Limited soil samples were collected from the top 5 cm of areas where the maize collected had been cultivated to investigate background soil geochemistry and to explore elemental transfer into a food web. Soil samples were bulked from around sampling locations as previously described [42] and were assumed to represent regularly turned over soil, therefore smoothing out any variability and reflecting the soil exposed to root systems. Data for soils were not sufficient for the same statistical scrutiny as maize and *P. truncatus* and are presented to allow comparison with other data on Mexican soils, supporting recent publications and interest in background plant and soil geochemistry [43].

Adult *P. truncatus* were caught in the field close to where maize was collected, although in some areas very few were captured. They were caught in flight traps baited with synthetic analogues of their aggregation pheromone (supplied by AgriSense BCS Ltd, Taffs Mead Rd, Treforest Industrial Estate, Glamorgan CF37 5SU, UK). Traps were emptied after 48 hrs to catch those insects flying close by [44] which are most likely to be young adults dispersing from their natal origin nearby [45]. Insects were euthanized by freezing at -5 ºC.

Elemental transfer from soil to maize, and maize to insects

Maize from 12 sources was used in controlled feeding trials with *P. truncatus*, seven of these were paired with the soil in which they were grown in order to investigate elemental transfer between soil, maize and *P. truncatus* (see S1). All glassware used for insect cultures was decontaminated by soaking in Decon 90 (Fisher Scientific UK Ltd, Bishop Meadow Road, Loughborough, LE11 5RG, UK) for ≥6 h, and then rinsed in Ultra High Purity (UHP) water (45 Ohm) and oven-dried. Stainless steel lids and mesh gauzes were hand-checked

for loose metal, then heated in UHP water in an ultrasonic bath, oven-dried and sealed in plastic bags until required.

Three replicate honey jars containing 50 g of maize (types 1 to 12, S1) were equilibrated overnight at 25 ± 0.5 ᵒC and 75 ± 1.5% rh. The next day, 20 *P. truncatus* adults were added and maintained at the same constant conditions throughout the experiment. After two weeks the founder beetles were extracted by hand, to ensure that all adult insects emerging 6 weeks later had been reared exclusively from egg to adult on maize from a single source. Insects were manually removed and euthanized by freezing at -5 ᵒC prior to chemical screening.

The soil samples provide background information on geochemistry and an insight into elemental transfer from soil to maize, and maize to *P. truncatus* reared on maize. The accumulation in terms of the increase in concentration between each trophic level for elements above DL in all three materials was expressed as a Mobility Index (MI) [46] where:

$$\text{Mobility Index} = \frac{\text{Concentration of element (mg kg}-1) \text{ in the receiving level}}{\text{Concentration of element (mg kg}-1) \text{ in the source level}}$$

Sample preparation and ICP-AES assays

The preparation and digestion of beetles, maize and soil follow previously described procedures for invertebrates, plants and soil [24], and included preparing bulk reference samples for all materials tested. Such methods provide results which have proven robust and sensitive enough for area wide surveys even where invertebrate

concentrations were very low. Detection Limits (DL) were estimated from bulk reference samples of biological materials [24] ensuring we selected a population of positive data and elements that were consistently in that range, so minimising zero value influence.

To ensure reliability of determinations a pooled sample of 10 beetles was used for each analysis. Two replicates of each sample were
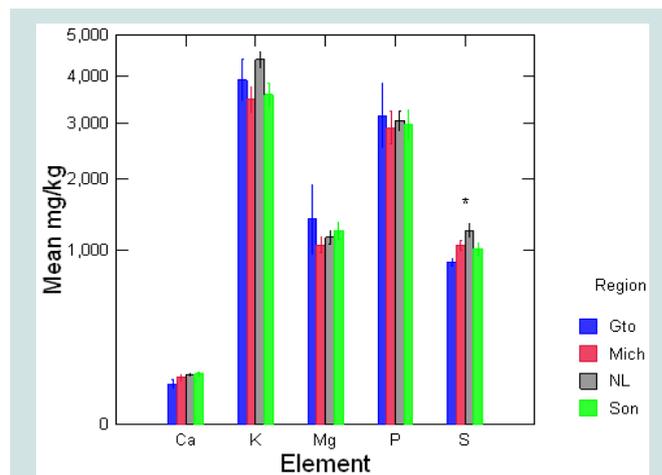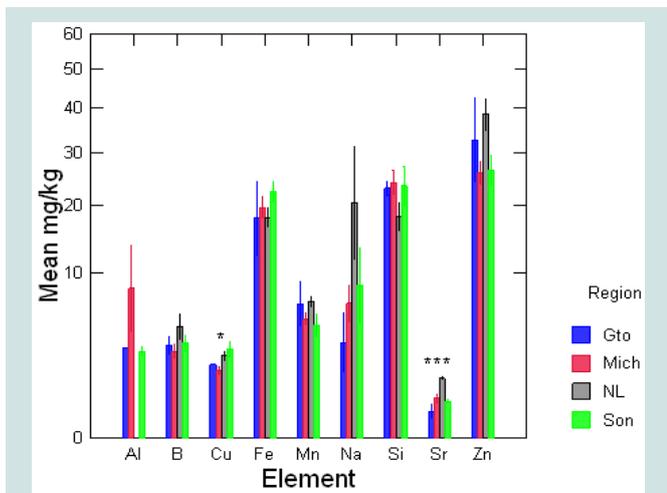

Figure 1: Mean mg/kg of Ca, K, Mg, P and S in maize from different regions (elements at high concentrations). Error bars are standard error around the mean at 0.68. Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora.

Table 1: Mean mg/kg and SD of elemental determinations for soil, maize and *P. truncatus* (DL indicates at or below Detection Limit).

Element	Soil (n=13)		Maize (n=24)		Lab *P. truncatus* (n=46)		Field *P. truncatus* (n=18)	
	Mean mg/kg	SD	Mean mg/kg	SD	Mean mg/kg	SD	Mean mg/kg	SD
Al	41108	28303	22.17	15.11	95.33	63.37	41.67	7.37
B	56.54	28.92	8.27	5.34	8.77	3.92	13.67	5.51
Ba	203.00	65.11	8.67	19.18	7.92	16.36	26.33	43.02
Ca	41173	32801	656.46	141.21	1268.06	622.71	1140.00	285.78
Cr	37.15	25.76	DL	DL	DL	DL	DL	DL
Cu	15.54	10.34	36.61	92.67	28.83	18.60	33.67	6.66
Fe	26639	15860	104.15	35.15	168.67	63.32	126.67	11.59
K	7436.85	5858.14	6721.30	943.68	6058.89	1549.05	6503.33	629.63
Mg	7549.62	3580.26	1072.33	113.77	1174.78	223.42	760.00	51.96
Mn	425.31	291.70	26.65	4.82	33.33	12.80	20.00	0.00
Na	762.77	635.17	NA	NA	NA	NA	NA	NA
Ni	21.15	20.64	24.39	52.08	8.23	6.09	6.67	0.58
P	989.00	426.48	5373.91	552.84	5442.22	1043.25	4246.67	435.47
S	968.15	533.17	2861.52	272.27	3405.56	606.25	3513.33	320.36
Si	1155.83	1073.93	293.17	149.69	445.33	208.39	337.33	18.61
Sr	195.39	102.57	1.71	0.82	4.42	4.36	3.17	0.64
Ti	816.54	1409.21	DL	DL	3.44	3.75	1.43	0.40
V	62.15	46.82	DL	DL	DL	DL	DL	DL
Zn	63.85	25.55	333.76	56.60	429.33	110.35	270.67	36.91
Zr	18.54	18.74	DL	DL	DL	DL	DL	DL

Figure 2: Mean mg/kg of Al, B, Cu, Fe, Mn, Na, Si, Si, and Zn in maize from different regions (elements at low concentrations). Error bars are standard error around the mean at 0.68. Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora.
Where * and *** indicate p≤0.05 and p≤0.005 respectively Kruskal-Wallis test.
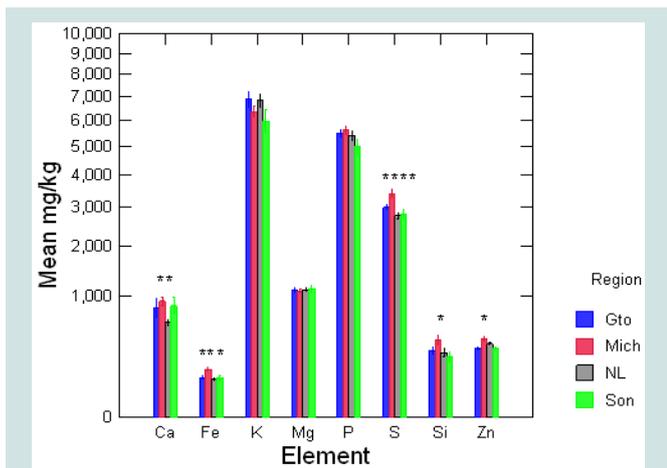
Figure 3: Mean concentration (mg/kg) of Ca, Fe, K, Mg, P, S, Si and Zn, elements detected in *P. truncatus* (high concentrations) reared on known maize in the lab by region, error bars are standard errors around the mean at 0.68. Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora. Where *, **, *** and *** indicate p≤0.05, p≤0.01, p≤0.005 and p≤0.001 respectively Kruskal-Wallis test.

chemically screened in a Perkin-Elmer Optima 3000 ICP Spectrometer operating at default conditions, alongside reference samples and laboratory standards to ensure consistency of determination. Mean concentrations of analytes were taken from four readings off the calibration curve for each element.

Sample interpretation and analysis

Most elemental determinations of maize and beetles were skewed towards low concentrations and many remained non-normally distributed even after transformation (tested via a Shapiro Wilk's test where p>0.005 indicates normality, S2). Therefore to identify which multiple-elements varied significantly by location for each material tested, all elemental concentrations were analysed using a conservative non-parametric Kruskal-Wallis (Mann-Whitney U).

Analyses were performed using SYSTAT V13.1 with exact tests [47] to account for uneven sample sizes and occasional missing data points for samples where rarer elements were below DLs.

An LDA was used to classify *P. truncatus* and maize according to the geochemical origin of capture [18] or the origin of the maize that laboratory-reared insects were reared on. The region of capture or collection was used as the dependent categorical variable (insects reared on two commercially brought grains were excluded as they lack provenance data, S1). LDA assumes a normal distribution of data, therefore only elements above DLs showing significantly different concentrations according to origin in a Kruskal-Wallis test (S3) and a normal distribution after transformation (p>0.005 Shapiro Wilk's test, S2) were used as independent variables in the model. Stepwise LDA classification matrices were calculated with and without jackknifing, as the latter can adjust for overestimation when the case being classified is in the same classification matrix [48].

Results

Elemental profiles of different materials

Mean determinations (mg/kg) were highest in soils followed by insects, with maize having the lowest determinations of most elements (Table 1), with 14 elements above DL in all materials: Al, B, Ba, Ca, Fe, K, Mg, Mg, Mn, P, S, Si, Sr and Zn. The high variation evident in Table 1 supports the assumption that soil, grain and insect background geochemistry show variations, and that the concentrations of different elements may vary according to the location or origin.

For ease of interpretation elements were grouped into high or low concentrations for each material tested. Ca, Fe, K, Mg, P, S and Si were at high concentrations (>1000 mg/kg) in all materials, with high concentrations of Al, Na and Ti in soils and of Zn in insects (Table 1). Elements at low concentrations fit the general definition of trace elements, occurring at <100 mg/kg in the biological materials tested.
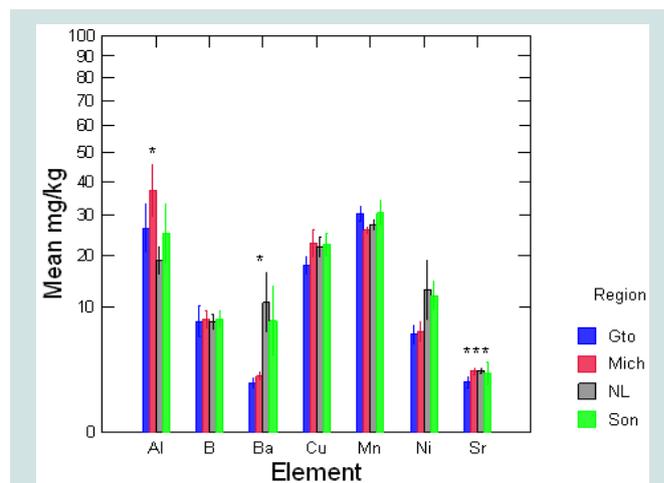
Figure 4: Mean concentration (mg/kg) of Ca, Fe, K, Mg, P, S, Si and Zn, elements detected in *P. truncatus* (high concentrations) reared on known maize in the lab by region, error bars are standard errors around the mean at 0.68. Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora.
Where * and *** indicate p≤0.05 and p≤0.005 respectively Kruskal-Wallis test.

Table 2: Transfer of selected elements from soil to plant product to insects with mean (Standard Deviation, SD) values from ICP-AES determinations of soil, maize and *P. truncatus* in mg/kg, and calculated mobility index (MI, shown in bold) for these elements between each trophic level.

Element	Soil mean (SD)	Maize mean (SD)	MI soil to maize	*P. truncatus* mean (SD)	MI maize to *P. truncatus*
Cu	15.1 (9.7)	2.2 (0.4)	**0.2 (0.1)**	41.9 (55.8)	**20.1 (28.2)**
Fe	26248 (14727)	18.3 (5.8)	**0.0 (0.0)**	107.3 (12.6)	**6.5 (2.4)**
Zn	63.2 (22.7)	32.9 (10.4)	**0.7(0.4)**	343.2 (30.6)	**11.5 (3.7)**

Table 3: Results from the LDA classification matrix for percentage of correctly assigned lab-reared *P. truncatus* to four regions before and after Jackknifing using Al, Fe, Sr and Zn as independent variables.

Region	Classification Matrix % correctly classified	Jackknifed Classification Matrix % correctly classified
Guanajuato	82	73
Michoacán	60	40
Nuevo Léon	92	85
Sonora	63	50
Total	78	68

Elemental characterization of soil

The mean and standard error of soil determinations of elements detected at high and low concentrations are shown in S3 and S4 respectively. The results seen in Table 1 suggest differences between samples collected in different regions particularly for Al, Ca, K, Sr and V. As soil data were scant (n=13) they only provide baseline information on Mexican soil profiles and allow the trophic transfer of elements above DL in all three materials to be traced from soil to grain and into an insect.

Exploration of maize by origin

Elemental profiles of maize by region are shown in Figures 1 and 2 (high and low concentrations respectively). A Kruskal-Wallis comparison between elemental concentrations in maize by region showed significant differences for Cu, S and Sr according to region of origin (S5).

Exploration elemental profiles for *P. truncatus* by origin

Elemental profiles of *P. truncatus* of known origin or raised on maize of known origin were explored by region, with results at high and low concentration shown in Figures 3 and 4. A Kruskal-Wallis test showed significant differences between concentrations of Al, Ba, Ca, Fe, S, Si, Sr, Ti and Zn according to origin (S6).

Only two elements, S and Sr, were significantly different according to their origins in the Kruskal-Wallis tests for *P. truncatus* and for maize (S5 & S6, Figures 2-4).

Elemental transfer from soils to maize to insects

A MI was estimated for Cu, Fe and Zn which were the only elements above DLs in all materials tested. These were followed up the food chain into *P. truncatus* (Table 2). Relative concentrations of elements were lower in maize than soil whilst they were higher for insects than the maize they had consumed, with values for Cu, Fe and Zn at 20, 6.5 and 11.5 times (respectively) the concentration of maize. These are typical of the differences between crop plants and soils, and crop plants and insects [42,43,46] Relative Fe concentrations were highest in insects, and all three elements are potential intrinsic markers.

Linear Discriminate Analysis to classify the origin of *P.truncatus* and maize using elemental profiling

Following the Kruskal-Wallis (S6) and Shapiro Wilk's tests (S2) on elemental determinations of *P. truncatus* four elements, Al, Fe, Sr and Zn were identified as independent variables with a normal distribution. These were used in an LDA to classify insects according to their region of capture or of the maize they were reared on. The first LDA used data from laboratory-reared beetles with origin of maize indicating region, and the second LDA used field-captured beetles collected from a limited set of locations (regions) because some pheromone-baited flight traps did not catch any insects.

The classification matrix for lab-reared *P. truncatus* correctly assigned 78% of the beetles to the region where maize (their exclusive diet) had been collected (Table 3). This was highest for Nuevo Léon (92%) and Guanajuato (82%) and lowest for Sonora (63%) and Michoacán (60%). More conservative classification estimates obtained after jackknifing lowered the overall success rate to 68% (Table 3) with similarly lower success rates when assigning beetles by region, and this was particularly poor for Michoacán (40%). Canonical score 1 explained >89% of the correlations and >93% of the data dispersal, increasing to >98% with the addition of Factor 2 (Table 4). The Eigen value for canonical score 1 was 4.169, with scores 2 and 3 contributing little compared with score 1. Overall results show a Wilk's λ of 0.146 (with 12, 79 df) with an approximate F ratio of 7.096 at P<0.001 (Table 4).

Canonical scores 1 and 2 are plotted in Figure 5 and show
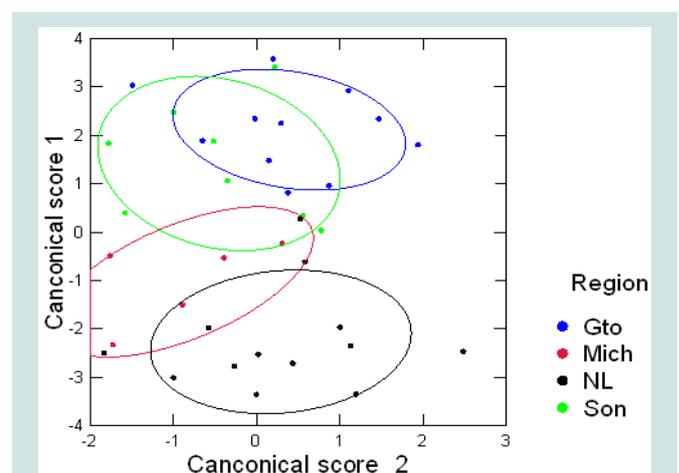
Figure 5: Plot of canonical scores 1 and 2 classifying laboratory-reared *P. truncatus* by region. The canconical scores arose from an LDA model using Al, Fe, Sr and Zn as predictor variables for region and explain >98 % of the data dispersal. The colours of the ellipses outline the distribution around 68% of the data points, empathizing the correctly classified data points for each region and the separation or overlap between regions.
Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora.

Table 4: LDA canconical scores and Eigen values using Al, Fe, Sr and Zn to classify lab-reared *P. truncatus* by region.

Canconical score	Eigenvalues	Canonical Correlations	Cumulative Proportion of Total Dispersion
1	4.169	0.898	0.931
2	0.252	0.449	0.987
3	0.057	0.233	1.000

Table 5: Results from the LDA classification matrix for percentage of correctly assigned field-captured *P. truncatus* to three regions before and after Jackknifing using Al, Fe, Sr and Zn as independent variables.

Region	Classification Matrix % correctly classified	Jackknifed Classification Matrix % correctly classified
Michoacán	100	85
Nuevo Léon	0	0
Sonora	100	67
Total	94	76

Table 6: LDA results using Al, Fe, Sr and Zn to classify field-captured *P. truncatus* by region.

Factor	Eigenvalues	Canonical Correlations	Cumulative Proportion of Total Dispersion
1	3.083	0.869	0.966
2	0.108	0.312	1.000

Note: Samples were limited by availability, in some areas very few were captured.

that the LDA for laboratory-reared *P. truncatus* gave complete separation between beetles from two regions, Guanajuato and Nuevo Léon, as well as Guanajuato and Michoacán, and almost complete separation between beetles from Sonora and Nuevo Léon. However, the distribution of Sonoran beetles overlapped with those from Guanajuato and some of the Michoacán distributions, with further overlap between beetles from Michoacán and Nuevo Léon.

Only limited data were obtained for field-captured insects, none were captured from Guanajuato and only a couple from Nuevo Léon limiting the regions in the model. The classification correctly assigned 94% of the beetles to one of the three regions where they had been collected (Table 5) with 100% success for both Michoacán and Sonora, but zero for Nuevo Léon. After jackknifing the overall success rate was 76% with slightly lower success rates for assigning beetles to the two regions mentioned above.

The canonical score 1 for field-captured *P. truncatus* explained 86% of the correlations and 96% of the data dispersal (Table 6). Data for scores 1 and 2 are plotted in Figure 6 and show complete separation between beetles from Michoacán and Sonora reflecting their 100% classification rates. The Nuevo Léon samples overlapped with the distribution of Michoacán beetles. The Eigen value for Canonical score 1 was 4.169, with lower contributions from scores 2 and 3. Wilk's λ was 0.221 (with 8, 22 df) with an approximate F ratio of 3.098 at P=0.017.

A similar LDA was carried out on the maize *P. truncatus* was reared on for comparison. However, Al was excluded as it was below DL in many maize samples. The classification correctly assigned 77%

of the maize to the geochemical origin where it had been collected (Table 7) with 100% success for both Guanajuato and Nuevo Léon Sonora, 80% for Sonora and lower for Michoacán. After jackknifing the overall success rate was 64% with reduced rates for assigning maize to region except for Nuevo Léon.

The canonical score 1 for maize explained 89% of the correlations and 74% of the data dispersal (Table 8). Data for scores 1 and 2 are plotted in Figure 7 and show complete separation between maize from Guanajuato, Nuevo Léon and Sonora reflecting their high classification rates. The Michoacán data show a wider spread and completely contain the Sonoran data within them. The Eigen value for Canonical score 1 was 3.731, with lower contributions from scores 2 and 3.

Discussion

We present a robust technique based on ICP-AES that identified a combination of elements able to determine the geographic origin of an insect or the maize it was reared on with a high degree of success. The methodology was sensitive enough to detect background levels of a range of elements including some trace elements in insects, grain and soil, and added to knowledge of natural background geochemistry of animals and plants. It produced multi-elemental profiles that could distinguish between materials collected from different locations, although the elements selected varied according to the material analysed. This is to be expected since factors including background concentration and sample weight can influence whether or not a particular element is detected. In addition, the ease of extraction and mobility of elements are likely to vary between materials [5,19,20,42,43].

For our study species Al, Fe, Sr and Zn concentrations correctly classified most laboratory-reared and field-caught *P. truncatus* according to the geochemistry of the location where they or their food had been collected. Bi-plots of canonical scores from this LDA
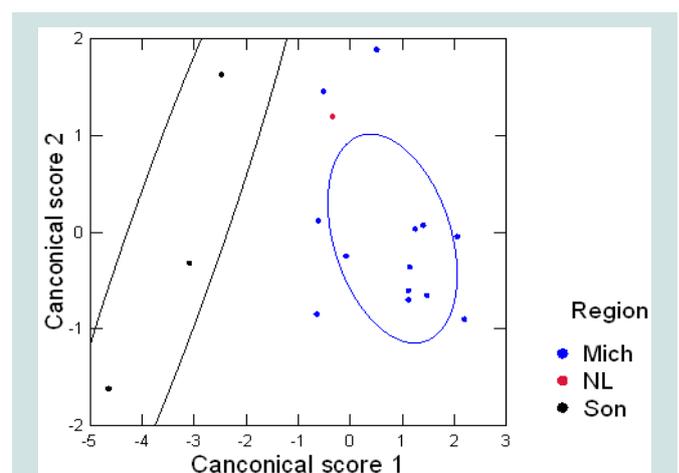
Figure 6: Plot of canonical scores 1 and 2 classifying field-captured *P. truncatus* by region. The canconical scores arose from an LDA model using Al, Fe, Sr and Zn as predictor variables for region and explain 100% of the data dispersal. The colour of the ellipses outlines the distribution around 68% of the data points (end not visible for Sonoran data), empathizing the clustering of correctly classified data points for each region and the separation between regions.

Mich: Michoacán; NL: Nuevo Léon; Son: Sonora.

Table 7: Results from the LDA classification matrix for percentage of correctly assigned maize to four regions before and after Jackknifing using Fe, Sr and Zn as independent variables.

Region	Classification Matrix % correctly classified	Jackknifed Classification Matrix % correctly classified
Guanajuato	100	75
Michoacán	43	29
Nuevo Léon	100	100
Sonora	80	60
Total	77	64

Table 8: LDA results using Fe, Sr and Zn to classify maize by region.

Factor	Eigenvalues	Canonical Correlations	Cumulative Proportion of Total Dispersion
1	3.731	0.888	0.743
2	1.264	0.747	0.994
3	0.029	0.169	1.000

showed complete separation between some locations, and where these overlapped this mirrored the lower percentage of correct classifications, particularly for Michoacán. Similar patterns were seen in the LDA for maize, which showed complete separation for three regions although those from Sonora were entirely within those of Michoacán. LDAs based on elemental concentrations have been used to determine the geolocation of other invertebrates [18,49] with V, Mn, Fe, Mo, Se, Cd, Ce and Nd concentrations classifying >92% of the migratory pest RBPH to one of seven Chinese locations [18], and Cd, Cu, Fe, Pb and Zn concentrations classifying fresh water invertebrates according to the elevated levels they had accumulated in contaminated lakes [48].

The LDA for *P. truncatus* was restricted to independent variables with a normal distribution, although the non-parametric tests suggest that other elements may be intrinsic markers linked to the origin of an organism including Ba, Ca, S, and Si for insects, and Cu, S and Sr for maize. Older studies suggest that markers can vary with study species and host-specificity, as well as life-stage. A Principal Components Analysis (PCA) for the aphids *R. padi* and *Metapolodium dirhodum* used Al, Fe and Mn, and Al, Ca and Cl respectively to distinguish whether they had been reared on oats or wheat [32], whilst S, K and Cl distinguished between *M. dirhodum* alates (winged, sexually reproductive adults) and apterae (wingless asexually reproductive adults), suggesting that apterae were more readily categorised by host-plant soil geochemistry than were alates [33]. Whilst for polyphagous moths, distinctive chemoprints based on Zn, Si, Al, Fe, Cu, Mn, Ni and Cr showed better association with the choice of larval host-plant than the soil chemistry of plants [34]. The LDA for laboratory-reared *P. truncatus* classified insects according to their geochemical profile when reared exclusively on maize, eliminating variation due to alternative natal hosts. Whilst the LDA for field-captured insects suggests that the short sampling period helped restrict capture to young adults dispersing from their natal hosts in nearby agricultural areas [44,45].

Holder et al. used multivariate models to determine the origin of an invasive insect, *H. armigera*. Concentrations of trace elements (Sr, Cs, Ba and Pb) combined with stable isotopes (δ^2H, ^{87}Sr/^{86}Sr, ^{208}Pb/^{206}Pb

and ^{208}Pb/^{207}Pb) gave the most accurate models and determined origin of about 73% insects [3]. They also report differences between years and sex, and emphasise the importance of the controlled rearing of mobile herbivores on known host-plants, grown in soils of known and differing geochemistry, in order to validate multi-elemental models used to classify field samples of unknown origin.

There are some limitations to our study. Firstly, it was necessary to use a pooled sample of 10 insects for each determination which may lower the classification rate, particularly where geochemistry shows high local variation. However, multivariate models have the capacity to reveal where the majority of a population originates from, as demonstrated by the LDA for RBPH which required 350 insects per determination whilst producing sufficient resolution to distinguish between insects captured at seven geographical locations where this highly mobile species had been sampled [18].

Using 10 beetles per determination helped to maximise the number of samples we could analyse consistently and since it was a pooled value this reduced the influence of the occasional field insect from different food sources or from a host grown on a locally varying geochemistry. It is also possible that some elements detected are involved in metabolic processes, although the exact role of trace elements and minerals is poorly known in most insects [50,51]. However, using multivariate models which are not dependent on single elements in isolation helps to reduce the effect of each element including those involved in regulatory processes, which are likely to differ between insects and their foods. Future work would also benefit from further investigating soil-plant relationships based on background elemental levels, since these often regulate uptake for trace elements and transfer factors work as consistent values even when soil content varies [5].

In common with other studies that validate field data on elemental loads with controlled rearing experiments [3,31-35], we reared
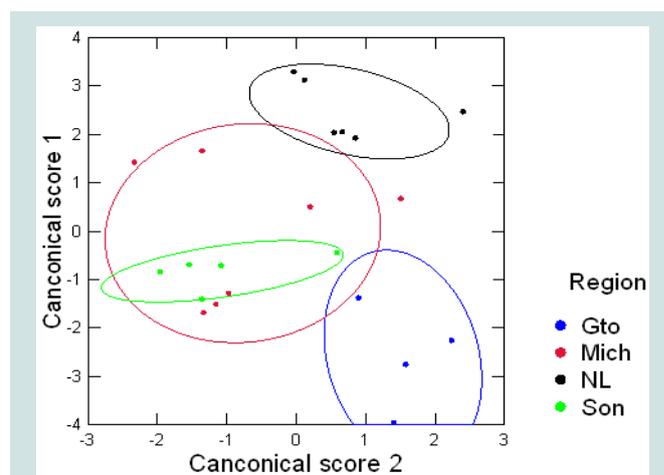
Figure 7: Plot of canonical scores 1 and 2 classifying maize by region. The canonical scores arose from an LDA model using Fe, Sr and Zn as predictor variables for region and explain 99% of the data dispersal. The colour of the ellipses outlines the distribution around 68% of the data points, empathizing the clustering of correctly classified data points for each region and the separation between regions.
Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora Gto: Guanajuato; Mich: Michoacán; NL: Nuevo Léon; Son: Sonora.

P. truncatus on maize of different origins for one generation. This reflects the assimilation of background levels of elements up the food chain, from egg to adult. It assumes that elemental profiles are not likely to be influenced by changes in geochemistry resulting from adult feeding activities. This is a poorly known topic but may explain differences between the regional elemental loads of laboratory reared and field-caught insects, and is a topic which would benefit from further research. In addition, collecting maize direct from farmers meant we could not control for the variety of maize, so any unknown differences in the assimilation of elements between maize varieties was not accounted for. It is also possible that profiles of field beetles differed if they had fed on alternative food sources from maize.

The soil samples were of limited value but give data on background variations in Mexico, and support the current interest in regional geochemical surveys such as those of agricultural soils [52] and the transfer of elements into human food plants including maize [42,43]. Future studies would benefit from controlled rearing of both insects and their host plants under experimental conditions. This would facilitate more rigorous sampling of soils, host plants and insects, and transfer up the food chain, and help provide data on the transfer of natural levels of multiple elements. Such data also help by enabling GIS modelling approaches where combinations of elemental concentrations detected in biological materials are linked to location data, this continues to be widely applied in the isoscape approach for H and O isotopes [15].

Intrinsic geochemical markers have great potential for tracking animals, particularly invasive species which are an increasing cause for concern, both in terms of agricultural and medical pests, but also destructive species that out compete native species and threaten a wide range of taxa and ecosystems [53,54]. Although detection methods have continued to develop since we completed this study, these results emphasise that meaningful data can be obtained using less sensitive methods such as ICP-AES. As atomic spectroscopy techniques become more widely available and more sensitive; their ability to determine naturally occurring levels of elements will increase. Therefore elemental profiling can be used to identify the origin of organisms that move from one geochemically unique environment to another with recent studies suggesting that resolution improves when specific elemental concentrations, particularly the rarer ones, are combined with other intrinsic markers including stable isotopes and molecular genetics into multivariate models [3,18].

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