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Sweet problems: insect traits defining the limits to dietary sugar utilisation by the pea aphid, *Acyrthosiphon pisum*

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Summary

Plant phloem sap is an extreme diet for animals, partly because of its high and variable sugar content. The physiological and feeding traits of the pea aphid *Acyrthosiphon pisum* that define the upper and lower limits to the range of dietary sucrose concentrations utilised by this insect were determined principally using chemically defined diets containing 0.125–1.5 mol l^{-1} sucrose. On the diets with 0.125 mol l^{-1} and 1.5 mol l^{-1} sucrose, the aphids died as larvae within 8 and 14 days of birth, respectively. On the other diets, 60–96% of aphids developed to adulthood, and the 0.5 mol l^{-1} and 0.75 mol l^{-1} diets supported the highest fecundity. The diet with 0.125 mol l^{-1} sucrose was ingested at 36% of the rate of the 0.25 mol l^{-1} sucrose diet, but >90% of ingested sucrose-carbon was assimilated on both diets. This suggests that the lower limit is dictated by the aphid feeding response, specifically, a requirement for a minimal concentration of sucrose for sustained feeding. The haemolymph osmotic pressure of aphids on diets with 0.125–1.5 mol l^{-1} sucrose was up to 68% higher than on 0.125–1.0 mol l^{-1} sucrose diets, but diet consumption and sucrose-carbon assimilation was not reduced on the very high sucrose diets relative to 1.0 mol l^{-1} sucrose. This suggests that failure of the osmoregulatory capacity of the insects on high sucrose diets may define the upper limit to the range of dietary sucrose utilised by the aphids. The mean haemolymph osmotic pressure of aphids on plants with phloem sap containing 0.37–0.97 mol l^{-1} sucrose was 1.61±0.063 MPa (mean ± s.e.m.), not significantly different from that (1.47±0.059 MPa) on diets with 0.25–1.0 mol l^{-1} sucrose. It is concluded that the osmoregulatory response of aphids to diets and plants are comparable, and, more generally, that the feeding and osmoregulatory capabilities of the aphids are compatible with the phloem sugar levels commonly encountered by aphids feeding on plants.

Key words: aphid, *Acyrthosiphon pisum*, osmoregulation, phagostimulation, phloem sap, sucrose.

Introduction

Organisms live within their environmental limits or, more precisely, within a multidimensional envelope defined by the conditions and resources compatible with their physiological capabilities (Willmer et al., 2000; Begon et al., 2005). Outside of this envelope, they display reduced growth, reproduction and survivorship. A great many studies have determined the environmental limits of organisms, usually with respect to abiotic factors, such as temperature, irradiance, water availability, and to specific nutrients or toxins. These data provide both vital information for studies of physiological mechanism and robust explanations for the ecology of many taxa (e.g. Spicer and Gaston, 1999; Hochachka and Somero, 2002).

In order for environmental limits to have a rational basis and predictive value, research should address the physiological processes defining the environmental limits as well as cataloguing of organismal response to environmental extremes. The physiological breakdown of organisms under extreme conditions commonly involves multiple processes and organ systems, but it is generally underpinned by a single, primary process that breaks down most rapidly or in response to the smallest change in conditions. Deterioration in other processes is secondary; either more extreme conditions are required for their breakdown or their deterioration is a consequence of the failure of the primary process.

This study concerns the nutritional limits for plant phloem sap feeders. Largely because its nutrient content is unbalanced, phloem sap is an ‘extreme’ food source that is used as the dominant or sole diet of very few animals, specifically insects

of the order Hemiptera, including aphids, whitefly, planthoppers and some pentatomid bugs (Douglas, 2003). The focus of this paper is the high and variable sugar content of phloem sap. In many plants, sucrose is the dominant phloem-mobile sugar (Fisher, 2000), and reliable reports of its concentration in phloem sap range from 0.2 to 1.5 mol l^{-1}, varying with environmental conditions, especially temperature and irradiance, but also plant species and developmental age (Winter et al., 1992; Geiger and Servaites, 1994; Kehr et al., 1998). A second nutritional problem posed by phloem sap is its low essential amino acid content which, in aphids, is resolved by symbiotic bacteria of the genus *Buchnera* that provide the insect with supplementary amino acids (Douglas, 1998). Other phloem-feeders possess symbiotic microorganisms (Buchner, 1965) which, by analogy to aphids, are also believed to provide essential amino acids.

The response of phloem-feeding insects to phloem sugars is physiologically complex, involving nutritional, osmoregulatory and behavioural components. Phloem sugars are the principal carbon source and respiratory fuel for these insects (Rhodes et al., 1996; Febvay et al., 1999; Salvucci and Crafts-Brandner, 2000). They are also responsible for the high osmotic pressure of phloem sap, up to five times that of insect body fluids. Phloem-feeding insects assimilate only a proportion of the ingested sugar, after hydrolysis by the gut sucrase to its constituent monosaccharides (Rhodes et al., 1996; Ashford et al., 2000). In aphids, the osmotic pressure of the remaining sugar (which is voided in honeydew) is reduced by a gut transglucosidase, which catalyses the polymerisation of the monosaccharide, especially glucose, into oligosaccharides in the gut (Fisher et al., 1984; Walters and Mullin, 1988; Rhodes et al., 1997; Wilkinson et al., 1997; Ashford et al., 2000). In this way, aphids avoid losing water from their body fluids, especially their haemolymph, to the gut. The behavioural aspect to the insect response to phloem sugars arises from their strong feeding response, such that the amount of sucrose ingested does not vary in a simple fashion with dietary concentration. For example, aphids require a certain minimal concentration of dietary sucrose for sustained feeding (i.e. sucrose is a phagostimulant) but, above this minimal level, aphids compensate for variation in dietary concentration by feeding faster from diets of lower sucrose concentrations (Mittler and Meikle, 1991; Simpson et al., 1995).

The specific purpose of this study was to identify the nutritional, osmoregulatory and behavioural traits of pea aphids, *Acyrthosiphon pisum*, that define the upper and lower limits to the range of dietary sugar concentrations utilised. This research was founded on the excellent understanding of the nutritional physiology of sucrose utilisation at the whole organism level, especially for the pea aphid (reviewed by Douglas, 2003). Most of the experiments were conducted on aphids reared on chemically defined diets, so that the dietary inputs were known and could be manipulated precisely, with a final set of experiments that compared key results of diet-reared aphids to aphids feeding from plants.

Materials and methods

The aphids and plants

Parthenogenetic females of the pea aphid *Acyrthosiphon pisum* (Harris) clone LL01 were maintained routinely on pre-flowering *Vicia faba* cv. The Sutton at 20°C with 18 h:6 h L:D and irradiance of 100 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR). For experiments with aphids on chemically defined diets, the offspring of plant-reared apterae were transferred to sterile diets of formulation A containing 0.15 mol l^{-1} amino acids (Douglas and Prosser, 1992), and maintained under the same conditions as the cultures on plants. The offspring were apterae. Two experimental designs were adopted. For studies of life-time performance, 20 replicate aphids within 12 h of birth were transferred individually to each of seven test diets with 0.125 mol l^{-1}, 0.25 mol l^{-1}, 0.5 mol l^{-1}, 0.75 mol l^{-1}, 1.0 mol l^{-1}, 1.25 mol l^{-1} and 1.5 mol l^{-1} sucrose, and the diet sachets were changed twice weekly. For analysis of short-term responses of aphids to dietary sucrose concentration, 2-day-old aphids were transferred from plants to diets with 0.5 mol l^{-1} sucrose, and then 10 replicate aphids were reared individually on each of the seven test diets, as above, from day 6 to day 8.

For experiments on aphids feeding from plants, pre-flowering *V. faba* (3 weeks after sowing) were exposed to three test conditions: (1) 24 h D at 20°C for 36 h; (2) 18 h L (600 μmol m^{-2} s^{-1}):6 h D at 20°C for 2 days; and (3) 18 h L (600 μmol m^{-2} s^{-1}) at 20°C:6 h D at 12°C for 2 days; with control plants under standard culture conditions of 18 h L (100 μmol m^{-2} s^{-1}):6 h D at 20°C. Aphids reared from day 2 to day 6 on the 0.5 mol l^{-1} sucrose diet were transferred to these plants, which were returned to test conditions for a further 2 days, when the sucrose content of the phloem sap and the osmotic pressure of the aphid haemolymph were quantified (see below).

Aphid performance assays

For the life-time performance experiment, the aphids were checked daily from birth until they died, and the date on which they developed to adulthood and the number of offspring produced per day were scored. Aphid performance between day 6 and day 8 was assessed by relative growth rate [RGR: $\log_e$(day-8 mass/day-6 mass)/2], with each aphid weighed on day 6 and day 8 to the nearest μg on a Mettler MT5 microbalance.

Radiochemical analyses of aphid feeding and sucrose assimilation

Ten replicate 6-day-old aphids were transferred individually to 50 μl of each test diet with 0.146 MBq [^{3}H]inulin and 0.148 MBq [^{14}C]sucrose ml^{-1} diet on a Perspex ring (3.5 cm diameter, 0.5 cm height). Honeydew produced by the feeding aphid was deposited onto a 3.5 cm GF/C filter (Whatman, Maidstone, Kent, UK) placed beneath each ring. On day 8, each filter was added to 4 ml scintillation fluid (Ultima Gold XR, Perkin Elmer, Boston, MA, USA) and the ^{3}H and ^{14}C contents were determined in a scintillation counter (Tri-Carb, Perkin

Elmer, Boston, MA, USA) with preset $^{3}H/^{14}C$ dual windows and quench curve. The mean of counts obtained from filters of two aphids feeding from non-radioactive diets of the same formulation was subtracted from experimental data. Inulin is not degraded or assimilated by pea aphids (unpublished results), and the volume of diet ingested and dietary sucrose-derived carbon ingested and egested were calculated from the ^{3}H and ^{14}C contents of filters, as described by Wright and coworkers (Wright et al., 1985) and Douglas and coworkers (Douglas et al., 2001). The sucrose-carbon assimilated was obtained by subtraction of sucrose-carbon egested from that ingested, and the assimilation efficiency was quantified as the proportion of ingested sucrose-carbon that was assimilated.

Osmotic pressure determinations

Each of 10 replicate groups of five 6-day-old aphids were starved for 2 h to ensure their guts were evacuated and then transferred to either a test diet or the abaxial surface of a leaf of a plant suspended over a 3.5 cm Petri dish filled with 99% *n*-hexadecane (Fisher, Loughborough, Leics, UK). Honeydew droplets released from the aphids sank below the surface of the hexadecane. On day 8, two honeydew samples were collected from each dish into pulled micropipettes, with a small volume of hexadecane either side of the honeydew to prevent evaporation of the sample. In parallel, haemolymph samples were collected from aphids on each test diet. The aphid body was held under water-saturated light white oil (Sigma-Aldrich, Poole, Dorset, UK) to prevent any evaporation, and one hindleg was amputated by a single, sharp pull on the leg with forceps. The haemolymph droplet that exuded from the stump was collected in a pulled micropipette, between two droplets of oil. The honeydew and haemolymph samples were stored at –80°C prior to analysis. The osmolarity of 0.05–0.5 nl samples was determined by freezing point depression (Malone and Tomos, 1992) calibrated against 0–0.6 mol l^{-1} NaCl standard.

Quantification of Buchnera symbiotic bacteria

DNA from 10 individual 8-day-old aphids from each test diet was extracted by the method of Cenis et al. (Cenis et al., 1993), with minor modification for single insect analysis. Real-time PCR (TaqMan®) reactions were set up in 96-well reaction plates. Cycling and data collection were performed using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK).

The primers and probe, designed for a 120 bp region of the *dnaK* gene of *Buchnera* APS (GenBank accession number D88673) with the Primer Express™ software (Applied Biosystems), were: forward primer TGT-AAA-TCC-AGA-TGA-AGC-TGT-AGC-AG; reverse primer ACC-CAT-AGT-TTC-AAT-TCC-TAG-GGA; and probe CAG-GGA-GGA-GTT-CTC-TCT-GGT-GAT-GTT-AAA-GAC-GTC-T. (The nucleotide numbers of sequence D88673 corresponding to 5′ nucleotide of the forward and reverse primers and the probe were 1092, 1132 and 1215, respectively.) For the probe, the 5′ terminal reporter dye was FAM (6-carboxyfluorescein) and the 3′ quencher dye was TAMRA (tetra-methylcarboxy-rhodamine). The probe and primers were supplied by MWG-Biotech AG (Ebersberg, Germany).

The reaction mixture consisted of 1× PCR buffer (10 mmol l^{-1} Tris–HCl pH 8.1 at 25°C, 50 mmol l^{-1} KCl, 0.001% gelatin), 5.5 mmol l^{-1} $MgCl_2$, 0.2 mmol l^{-1} each of dATP, dGTP, dCTP and dUTP, 0.5 μl Rox Reference Dye (Invitrogen Ltd, Paisley, UK), 0.3 μmol l^{-1} each of forward and reverse primers, 0.1 μmol l^{-1} fluorescence-labelled probe, 0.625 i.u. Hot Taq DNA Polymerase (BioGene Ltd, Kimbolton, Cambridgeshire, UK), and template DNA in a total volume of 25 μl. The cycling conditions were: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Values of threshold cycle (C_T: the cycle at which a significant increase in fluorescence occurs) below 40 were taken as a positive result. Standard curves were generated with serial dilutions of the template, synthesised and gel-purified by MWG-Biotech AG, and *dnaK* copy number was estimated from the standard curve. The data were normalised to total DNA content per sample, determined by a NanoDrop ND-1000 spectrophotometer (LabTech International, Ringmer, East Sussex, UK), to control for any variation in efficiency of DNA extraction.

Analysis of free amino acids of aphids

Ten replicate day-8 aphids from each test diet were weighed individually and then hand-homogenised in 0.1 ml ice-cold 80% methanol. The homogenate was centrifuged at 500 ***g*** for 5 min at 4°C, and the supernatant was stored at –20°C. Amino acids were separated by reverse-phase HPLC following derivatisation with *o*-phthaldialdehyde (Jones et al., 1981) using a Hewlett-Packard HP1100 Series autosampling LC system with C_{18} ZORBAX™ Eclipse XDB-C8 column and fluorescence detection. The amino acids were quantified by comparison to the AA-S-18 standard amino acids (Sigma) supplemented with tryptophan, asparagine and glutamine.

Sucrase assay

Ten individual 8-day-old aphids from each test diet were homogenised in 200 μl 50 mmol l^{-1} Hepes pH 7.4 + 1% (v/v) Triton X-100, and then centrifuged at 10 000 ***g*** for 5 min. The sucrase activity of the supernatant was assayed by the method of Dahlqvist (Dahlqvist, 1984) using reagents from the Sigma Diagnostics glucose assay kit with the chromogen *o*-dianisidine at 125 μg ml^{-1}. One unit of activity is defined as the amount of enzyme that releases 1 μmol glucose from sucrose per minute at 37°C, and the activities were normalised to aphid protein, as determined by the BCA protein microassay (Pierce Biotechnology, Rockford, Illinois, USA), according to the manufacturer's instructions, with bovine serum albumin as standard. Total aphid sucrase activity was adopted as an index of gut sucrase activity following preliminary experiments that assigned >98% of the total sucrase activity to the gut (D.R.J.P., unpublished data).

Quantification of phloem sap sucrose

Phloem sap samples of *Vicia faba* were collected by stylectomy of adult apterae using a procedure modified from

the method of Fisher and Frame (Fisher and Frame, 1984). A feeding aphid in a suitable position was selected by examination under a dissecting microscope. The platinum needle of a microcautery unit (Syntech CA-50, Hilversum, Netherlands) was positioned with a micromanipulator, and the stylets were severed by a high frequency pulse from the microcautery unit. A 2 cm diameter Perspex ring with a grease-coated rubber base was clipped to the leaf around the severed stylets and immediately filled with water-saturated light white mineral oil (Sigma), into which the phloem sap exuded from the stylet stump. The phloem sap was collected into a microcapillary tube backfilled with mineral oil. Subsequently, subsamples of known volume were prepared using constriction pipettes, working under oil to prevent evaporation, and then diluted into 10 μl distilled water and stored at –80°C prior to analysis. To determine the sucrose content, each 10 μl sample was hydrolysed to completion with 0.1 i.u. invertase (Sigma I-4504) in 50 mmol l^{-1} sodium acetate buffer, pH 4.5 at 37°C for 30 min, and the glucose produced was determined by the Sigma Diagnostics glucose assay kit, following the manufacturer's instructions, but with *o*-dianisidine at 100 μg ml^{-1}, with glucose standards.

Statistical analysis

Data sets were analysed by parametric tests [*t*-test, analysis of variance (ANOVA) or least squares regression], following confirmation that they were normally distributed (Anderson-Darling test) with homogenous variances (Bartlett's test). This required logarithmic or arcsine-square root transformations where indicated. For analyses of aphid feeding rate and sucrose-carbon assimilation, initial aphid weight was included in the ANOVA as a covariate. Tukey's honestly significant difference method was used for pairwise comparisons contributing to significant ANOVA differences; and mean values that were not significantly different (P>0.05) by this test are indicated by the same superscript letter in figures and tables. Exceptionally, no transformation conducted yielded homogenous variances for aphid lifespan, and this was analysed by the nonparametric Kruskal–Wallis test.

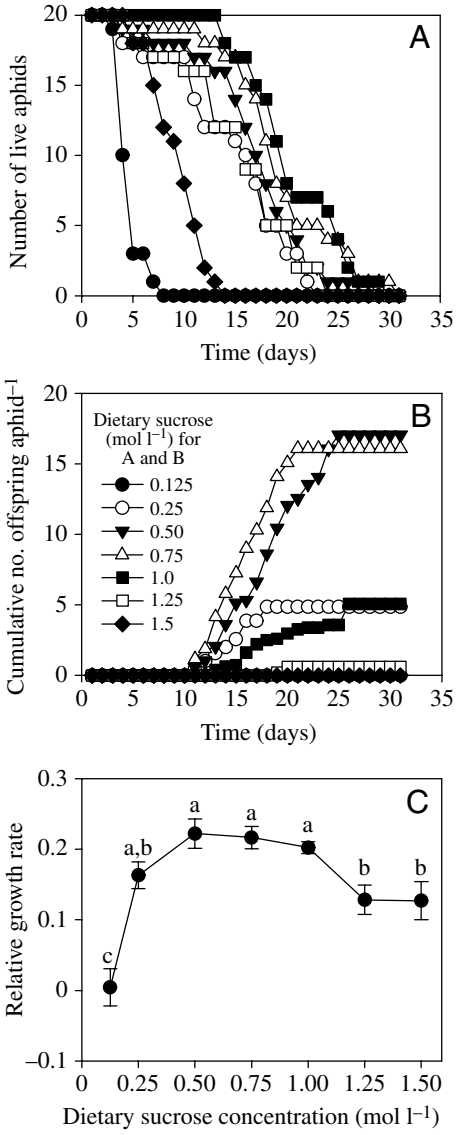

Fig. 1. Aphid performance on diets of different sucrose concentration. (A) Lifetime survivorship. (B) Cumulative reproductive output per live aphid. (C) Relative growth rate of 6- to 8-day-old final instar larvae that had been raised from day 2 to day 6 on a diet with 0.5 mol l^{-1} sucrose (means ± s.e.m.). Values that are not significantly different (P>0.05) are indicated by the same letter.

Results

Aphids on chemically defined diets

The first experiments quantified the lifetime performance of the pea aphids on chemically defined diets of different sucrose concentrations. Aphids lived for between 3 and 31 days (Fig. 1A), and the median lifespan varied significantly with dietary sucrose (Kruskal–Wallis: H_6=73.69, P<0.001). At least half the aphids on diets with sucrose concentrations in the range 0.25–1.25 mol l^{-1} lived for at least 15 days, but aphid lifespan on the diets with lowest and highest sucrose concentrations was severely curtailed, with all the aphids dead by day 8 on the 0.125 mol l^{-1} diet and by day 14 on the 1.5 mol l^{-1} diet. At the daily scoring of this experiment, most of the aphids were observed attached to the diet sachets on all diets except 0.125 mol l^{-1} sucrose, for which many of the aphids were moving around the cage or settled on the cage wall away from the diet sachet.

All the aphids on diets with 0.125 mol l^{-1} and 1.5 mol l^{-1} sucrose died as larvae. Between 12 and 18 of the 20 aphids on each of the other diets developed to adulthood on day 9 to day 16. Larviposition rates were highest on 0.5 mol l^{-1} and 0.75 mol l^{-1} sucrose diets, with total fecundity of 16–17 offspring per live aphid, and were depressed by 60–70% on 0.25 mol l^{-1} and 1.0 mol l^{-1} diets (Fig. 1B). Just two aphids on the 1.25 mol l^{-1} sucrose diet larviposited, producing one and three offspring, respectively.

The second experimental design addressed the impact of dietary sucrose on the performance of aphids that had been raised from day 2 to day 6 on 0.5 mol l^{-1} sucrose. All the aphids were in the final larval stadium on day 6. They survived the 2-day experiment to day 8. Aphid relative growth rate (RGR; Fig. 1C) varied significantly with dietary sucrose ($F_{6,63}$=14.15, P<0.001). The *post hoc* analysis revealed a significantly lower RGR, first, on 0.125 mol l^{-1} sucrose diet than on all the other diets and, second, on the 1.25 mol l^{-1} and 1.5 mol l^{-1} diets compared to 0.5–1.0 mol l^{-1} diets.

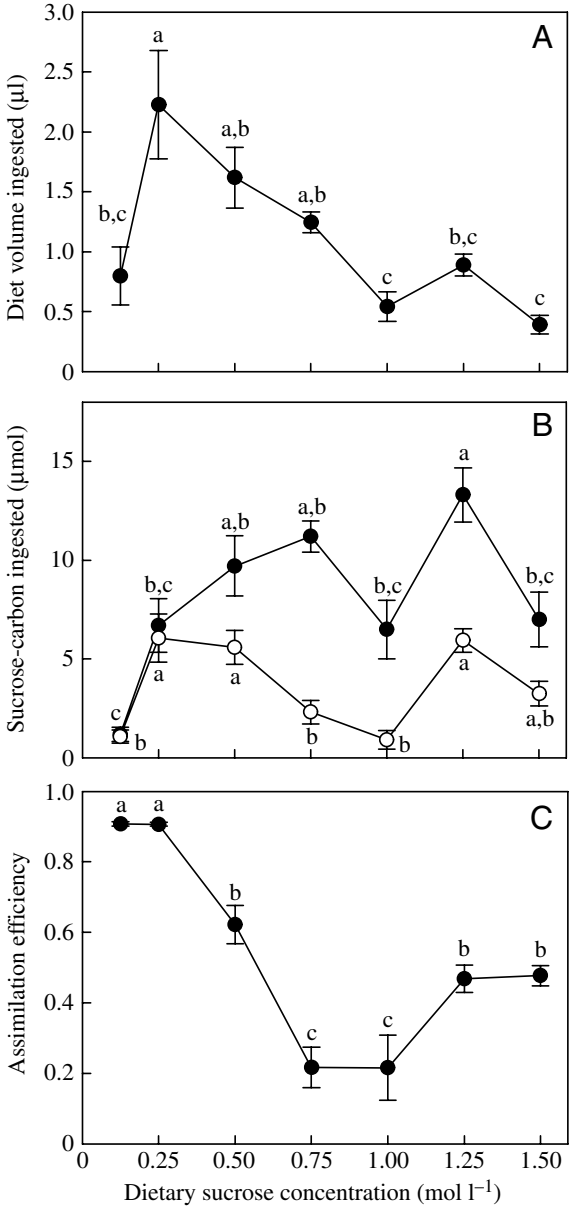

Fig. 2. Diet ingestion and assimilation by 6- to 8-day-old pea aphid clone LL01 on diets of different sucrose concentration. (A) Volume of diet ingested (means ± s.e.m.). (B) Dietary sucrose-carbon ingested (closed symbols) and assimilated (open symbols) (means ± s.e.m.). (C) Assimilation efficiency [100×(moles assimilated/moles ingested]. Values that are not significantly different (P>0.05) are indicated by the same letter.

Diet consumption by the 6- to 8-day-old aphids varied nearly sixfold with dietary sucrose concentration (Fig. 2A), and the variation was statistically significant (ANOVA for log-transformed data: $F_{6,53}$=7.87, P<0.001). The volume ingested increased progressively with decreasing dietary sucrose from 1.0 mol l^{-1} to 0.25 mol l^{-1}, for which the mean volume ingested, 2.23 µl, was the highest mean value obtained. Aphids on the 0.125 mol l^{-1} diet ingested just 36% of the volume consumed by those on the 0.25 mol l^{-1} diets and similar amounts to aphids on 1.0–1.5 mol l^{-1} diets. In a supplementary analysis, the RGR was regressed on the volume of food ingested by each aphid. A significant regression with a positive slope was obtained for the aphids on the 0.125 mol l^{-1} diet ($F_{1,4}$=26.71, P=0.007, r^2=87.0%) (Fig. 3) but for no other diet (data not shown), suggesting that feeding rate is a particularly important determinant of growth rate on the 0.125 mol l^{-1} diet.

Fig. 2B shows the absolute amounts of sucrose-carbon ingested and assimilated; and the assimilation efficiency is shown in Fig. 2C. All three measures of sugar utilisation varied significantly with dietary sucrose concentration [ANOVA for sucrose-carbon ingested: $F_{6,53}$=8.32, P<0.001; sucrose-carbon assimilated: $F_{6,53}$=9.48, P<0.001; assimilation efficiency (arcsin-square root transformed data): $F_{6,53}$=22.31, P<0.001]. The aphids feeding on 0.25 mol l^{-1} and 0.5 mol l^{-1} diets assimilated significantly more sucrose than those on 0.75 and 1.0 mol l^{-1} diets and comparable amounts to the aphids on the 1.25 mol l^{-1} sucrose diet; and this result could be attributed to a combination of high feeding rates and assimilation efficiency on the 0.5 mol l^{-1} and especially 0.25 mol l^{-1} diets. Although the aphids on 0.125 mol l^{-1} diet assimilated more than 90% of the sucrose ingested, their low feeding rates meant that the absolute amount of sucrose they ingested was less than 20% of that for aphids on the 0.25 mol l^{-1} diet. The aphids on 1.25 mol l^{-1} and 1.5 mol l^{-1} diets assimilated more than those on 0.75 mol l^{-1} and 1.0 mol l^{-1} diets.

For aphids on diets with 0.125 mol l^{-1} to 1.0 mol l^{-1} sucrose (and osmotic pressure of 0.9–4.0 MPa), the mean osmotic pressure of the haemolymph ranged between 1.35 and

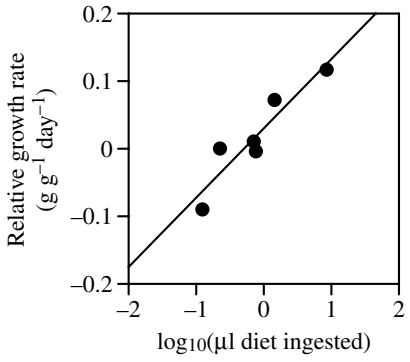

Fig. 3. Regression of aphid relative growth rate on volume of diet ingested on the 0.125 mol l^{-1} sucrose diet.

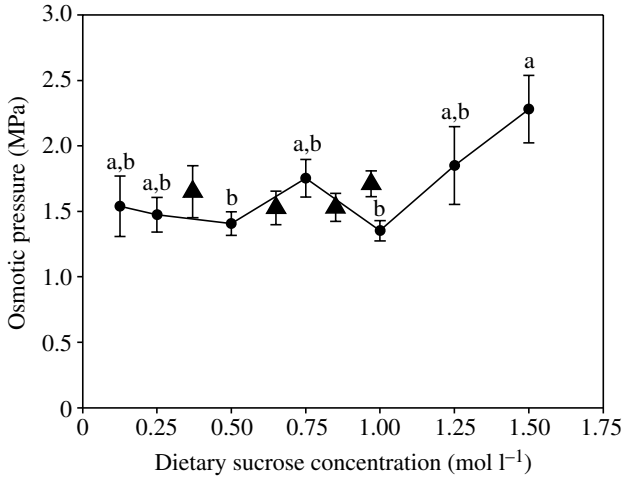

Fig. 4. Osmotic pressure of the haemolymph of 8-day-old aphids reared on either chemically defined diets of different sucrose concentration (circles) or on plants (triangles) with phloem sap sucrose concentration as shown in Table 1. Values are means ± s.e.m. Mean values that are not significantly different (P>0.05) are indicated by the same letter.

1.75 MPa, with no consistent trend in relation to dietary sucrose concentration; but it increased progressively with higher dietary sucrose concentrations to 2.3 MPa on the 1.5 mol l^{-1} sucrose diet (of osmotic pressure 5.8 MPa; Fig. 4). The variation in osmotic pressure with dietary sucrose was statistically significant (ANOVA: $F_{6,63}$=2.86, 0.05>P>0.01), with a significantly elevated mean value on the 1.5 mol l^{-1} diet. The haemolymph osmotic pressure of the aphid clone used here was somewhat greater than values of ~1.0 MPa obtained in our previous and ongoing research on different pea aphid clones (Wilkinson et al., 1997; Karley et al., 2005) (E.J., unpublished data), indicative of intraspecific variation.

Elevated osmotic pressure of the haemolymph of pea aphids has been described previously under two conditions: (a) aposymbiosis, i.e. the experimental elimination of the symbiotic bacteria, *Buchnera* sp., a treatment that causes an increase in free amino acid levels and consequent increase in haemolymph osmotic pressure (Wilkinson et al., 1997); and (b) inhibition of the aphid gut sucrase, which results in inhibition of both oligosaccharide synthesis in the gut and the linked reduction in osmotic pressure of the gut contents, causing the passage of water from body fluids to the gut (Karley et al., 2005). Further experiments were therefore conducted to establish the abundance of *Buchnera*, the concentration of free amino acids and the sucrase activity in the aphids reared on diets of different sucrose concentration.

The copy number of the *Buchnera* gene *dnaK*, an index of *Buchnera* abundance, varied significantly with dietary sucrose (ANOVA: $F_{6,61}$=4.46, P=0.001) and was significantly depressed in aphids on 1.25 and 1.5 mol l^{-1} sucrose relative to aphids on lower dietary sucrose concentrations (Fig. 5A). The free amino acid content of the aphids also varied significantly with dietary sucrose concentration (ANOVA: $F_{6,62}$=7.99,

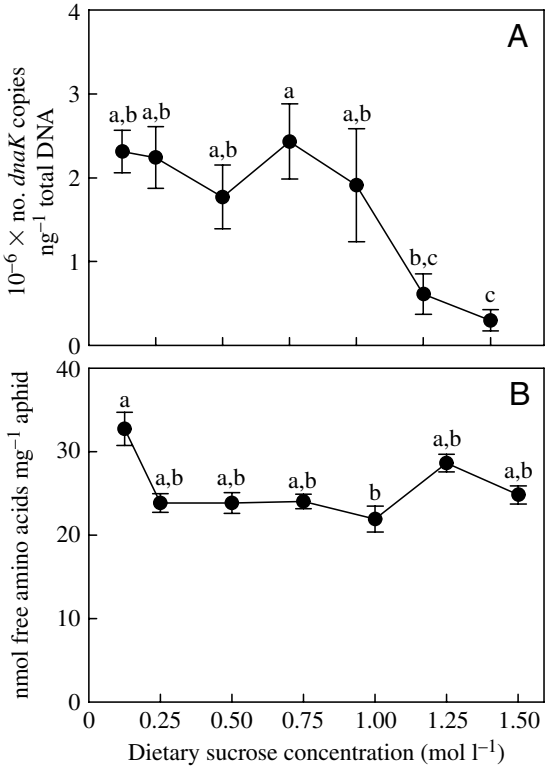

Fig. 5. Abundance and function of symbiotic bacteria *Buchnera* sp. in 8-day-old aphids on diets of different sucrose concentration. (A) Number of copies of *Buchnera* gene *dnaK* normalised to total DNA content. (B) Free amino acid content normalised to aphid mass. Values are means ± s.e.m. Mean values that are not significantly different (P>0.05) are indicated by the same letter.

P<0.001), with levels in the aphids on 0.125 mol l^{-1} sucrose diets significantly greater than on 1.0 mol l^{-1} sucrose (Fig. 5B). The data do not support the prediction (above) that reduced *Buchnera* density on 1.25–1.5 mol l^{-1} diets is accompanied by elevated free amino acid content. A second characteristic of aposymbiotic aphids with high total free amino acid titres is depressed essential amino acid content as a proportion of the total free amino acids (Prosser and Douglas, 1991). The essential amino acids were, on average, 29–33% of the total free amino acids in the diet-reared aphids and were not reduced in aphids on high sucrose diets (data not shown).

The sucrase activity of the aphids varied significantly with dietary sucrose ($F_{6,63}$=8.23, P<0.001), and was elevated on diets containing 1.0 mol l^{-1} and 1.25 mol l^{-1} sucrose relative to lower dietary concentrations (Fig. 6).

Aphids on plants

Phloem sap was collected by stylectomy from *Vica faba* reared under the control and three test treatments given in the Materials and methods. The sucrose concentration in the phloem sap varied significantly across the treatments (Table 1). The 6-day-old aphids transferred to these plants settled readily and fed, producing honeydew. At the end of the experiment, the osmotic pressure of their haemolymph (Fig. 4) did not vary

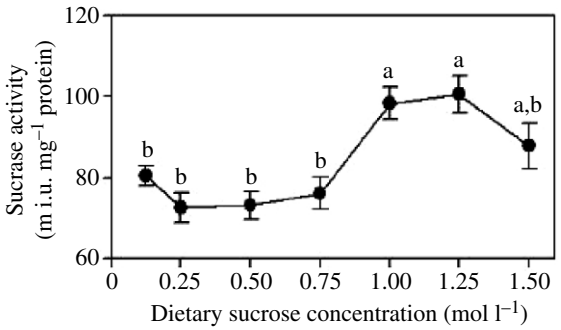

Fig. 6. Sucrase activity of 8-day-old aphids reared on diets of different sucrose concentration. Values are means ± s.e.m. Mean values that are not significantly different (P>0.05) are indicated by the same letter.

significantly with plant treatment ($F_{3,27}$=0.43, P>0.05). The overall mean osmotic pressure of the haemolymph of aphids reared on plants, 1.61±0.063 MPa (mean ± s.e.m., N=31), did not differ significantly for the overall mean value for aphids reared on diets with 0.25–1.0 mol l^{-1} sucrose, at 1.47±0.059 MPa (N=30; t_{58}=1.52, P>0.05).

Discussion

To investigate the limits to the dietary sucrose concentrations utilised by pea aphids, this study focused mainly on the responses of final-instar larvae over 2 days. This experimental design enabled the primary processes underpinning poor performance on diets with extreme sucrose concentrations of 0.125 mol l^{-1} and 1.5 mol l^{-1} (Fig. 1) to be identified without interference from non-specific secondary effects associated with general malaise that are expected to arise over longer time-scales. In the experimental design, only dietary sucrose concentration was varied, resulting in variation of the ratio of sucrose to all other dietary constituents. In particular, the sucrose:amino acid ratio ranged from 0.83:1 (the 0.125 mol l^{-1} sucrose diet) to 10:1 (the 1.5 mol l^{-1} sucrose diet). Other experiments (Douglas et al., in press) had demonstrated that aphid performance was not driven entirely by sucrose:amino acid ratio. Specifically, aphid performance differed between diets of different sucrose content (0.4 and 1.0 mol l^{-1}) but the same sucrose:amino acid ratio (6.4:1). Despite this, the possibility cannot be excluded that variation in the ratio of sucrose to other nutrients may have influenced the responses of aphids in this study.

The central role of sucrose phagostimulation in defining the lower limit of dietary sucrose utilised by aphids has been suggested by early studies, especially of Mittler and Dadd (Mittler and Dadd, 1963) and Srivastava and Auclair (Srivastava and Auclair, 1971). This interpretation is confirmed amply by the evidence from this study that the aphids on the 0.125 mol l^{-1} sucrose diet performed very poorly because they ingested food slowly. Specifically, the volume of diet ingested by these aphids was less than half of that on the 0.25 mol l^{-1} diet (Fig. 2A), and aphid performance was strongly correlated with the volume of diet ingested on the diet with 0.125 mol l^{-1} sucrose but on no other diet (Fig. 3). The low feeding rate on this diet presumably reflects the high energetic cost of maintaining high feeding rates for little nutritional advantage. It is most unlikely that impairment of post-ingestive processes contributed to the poor performance of the aphids on the 0.125 mol l^{-1} diet because, first, the aphids assimilated >90% of the sucrose-carbon ingested (Fig. 2C) and, second, they maintained the osmotic pressure of their haemolymph (1.5 MPa) despite the osmotic challenge of a diet with lower osmotic pressure (0.9 MPa) (Fig. 4).

The aphids also performed poorly on diets with 1.25–1.5 mol l^{-1} sucrose (Fig. 1), even though they fed sufficiently to ingest more sucrose than achieved by the aphids on 1.0 mol l^{-1} sucrose diet and had an assimilation efficiency greater than aphids on 0.75 mol l^{-1} and 1.0 mol l^{-1} diets (Fig. 2). The one potentially deleterious physiological response identified for aphids on the high sucrose diets was the elevated osmotic pressure of their haemolymph (Fig. 4). Increases in haemolymph osmotic pressure have been reported previously, of 30% for aphids with elevated free amino acid contents linked to elimination of the symbiotic bacteria (Wilkinson et al., 1997) and of 50% for aphids lacking gut sucrase activity (Karley et al., 2005). However, further experiments (Figs 5, 6) showed that the aphids on high sucrose diets had neither elevated free amino acid titres nor substantially depressed sucrase activity. It is, therefore, very probable that the osmotic pressure of the aphid haemolymph was very high because the osmotic pressure of the high sucrose diets exceeded the osmoregulatory capability of the aphids. One possibility is that the levels of sucrose-derived monosaccharide in the gut of aphids on the high sucrose diet were greater than the sugar

Table 1. *Concentration of sucrose in phloem sap of Vicia faba plants*

Treatment	Sucrose concentration (mol l^{-1}) of phloem sap
(1) 24 h D at 20°C for 36 h	0.37±0.029^{c}
Control: 18 h L (100 μmol m^{-2} s^{-1}):6 h D at 20°C	0.65±0.038^{b}
(2) 18 h L (600 μmol m^{-2} s^{-1}):6 h D at 20°C for 2 days	0.85±0.067^{a,b}
(3) 18 h L (600 μmol m^{-2} s^{-1}) at 20°C:6 h D at 12°C for 2 days	0.97±0.085^{a}
ANOVA (after logarithmic transformation)	$F_{3,28}$=24.75, P<0.001

Values are mean ± s.e.m., N=8
Mean values that are not significantly different (P>0.05) are indicated by the same letter.

polymerisation capacity of the gut transglucosidase, leaving the osmotic pressure of the gut contents substantially greater than that of the body fluids. This would cause the net movement of water from the aphid body fluids to the gut lumen, a process which can be corrected only by the active transport of water in the reverse direction. The increased assimilation efficiency of aphids on 1.25–1.5 mol l^{-1} sucrose diets may reflect a heightened demand for dietary carbon as a respiratory fuel in response to this osmotic stress.

A second effect of high dietary sucrose was the reduced abundance of copies of the *dnaK* gene of the symbiotic bacterium *Buchnera* (Fig. 5). This index of *Buchnera* abundance provides an accurate measure of the number of bacterial genomes because *dnaK* is a single-copy gene (Shigenobu et al., 2000), but not necessarily of the number of bacterial cells, because the genome copy number per *Buchnera* cell is large and variable (Komaki and Ishikawa, 1999). Although further research is required to establish the full implications of the results in Fig. 5, the consequence of reduced numbers of *Buchnera* genomes could be substantial because of the importance to the aphid of *Buchnera* as a source of essential amino acids, which are in short supply in the aphid diet of phloem sap (Douglas, 1998). Generally, chemically defined diets offer a more balanced mix of amino acids than phloem sap, and budget analysis of clone LL01 on the diet formulation used here indicates that just two amino acids derived from *Buchnera*, methionine and phenylalanine, are required for sustained growth (A.E.D., unpublished data). The reduction in *Buchnera* genome copy number on the high sucrose diets is therefore likely to depress performance. Over the 2-day timescale of the experiment, however, it did not translate into the consequence of impaired *Buchnera* function believed to result in increased haemolymph osmotic pressure: a reduced concentration of limiting essential amino acids in the free amino acid pool, causing depressed protein synthesis and associated accumulation of other non-limiting amino acids in the free amino acid pool (Prosser and Douglas, 1991; Wilkinson et al., 2001).

In summary, the main conclusion of this study is that the lower and upper limits to the dietary sucrose concentrations utilised by pea aphids are shaped primarily by different processes: a behavioural response, specifically reduced feeding reflecting the importance of sucrose as a phagostimulant, for the lower limit; and osmoregulatory failure for the upper limit.

A further issue is the relevance of these results to aphids feeding on their natural diet of plant phloem sap. It is widely recognised that, although aphids perform less well on chemically defined diets than on some plants, their physiological responses, especially in short-term studies, are similar on the two food sources (e.g. Rhodes et al., 1996; Simpson et al., 1995; Febvay et al., 1999; Douglas et al., 2001) so that results can be extrapolated from diets to plants with some confidence. This is illustrated by data in this study. The lifetime reproductive output, up to 17 offspring per aphid, on the diets was much reduced compared to the 60–80 offspring on the standard culture plant *Vicia faba* (unpublished data); but aphids reared on diets with sucrose concentrations of 0.25–1.0 mol l^{-1} maintained broadly uniform haemolymph osmotic pressures that matched the values obtained for aphids reared on plants with similar phloem sucrose contents (Fig. 4).

In this study, the aphids performed well in the short-term on diets with sucrose contents of 0.25–1.0 mol l^{-1}, spanning the full range of phloem sucrose concentrations of 0.37–0.97 mol l^{-1} obtained by environmental perturbations under laboratory conditions in this study (Fig. 1C; Table 1). Furthermore, the osmoregulatory response of aphids to diets and plants were comparable (Fig. 4). This suggests that the feeding and osmoregulatory capabilities of the aphids are compatible with the phloem sugar levels commonly encountered by aphids feeding on plants.

However, it would be premature to conclude that the sucrose concentration in phloem sap is invariably compatible with aphid physiology and behaviour, i.e. that aphids are perfectly adapted to the full range of sucrose content of plants. The phloem sucrose content of plants in the field may be more variable than in the laboratory. In particular, field plants generally experience higher light levels than can be generated under laboratory conditions; and stylectomy samples of some plants exposed for short periods to light intensities approaching 'natural' daylight yielded phloem sap with 1.5 mol l^{-1} sucrose (K.V.P., unpublished results). This raises the possibility that, under certain circumstances individual sieve elements, certain plant parts or even entire plants may challenge the osmoregulatory capacity of aphids, rendering them unsuitable as a food source.

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