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FATE OF DIETARY SUCROSE AND NEOSYNTHESIS OF AMINO ACIDS IN THE PEA APHID, *ACYRTHOSIPHON PISUM*, REARED ON DIFFERENT DIETS

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Summary

The fate of sucrose, the major nutrient of an aphid's natural food, was explored by radiolabeling in the pea aphid *Acyrthosiphon pisum*. To investigate the influence of nitrogen quality of food on amino acid neosynthesis, pea aphids were reared on two artificial diets differing in their amino acid composition. The first (diet A) had an equilibrated amino acid balance, similar to that derived from analysis of aphid carcass, and the other (diet B) had an unbalanced amino acid composition similar to that of legume phloem sap. Aphids grown on either diet expired the same quantity of sucrose carbon as CO_2, amounting to 25–30 % of the ingested sucrose catabolized in oxidation pathways. On diet A, the aphids excreted through honeydew about twice as much sucrose carbon as on diet B (amounting to 12.6 % of the ingested sucrose for diet A and 8.4 % for diet B), while amounts of sucrose carbons incorporated into exuviae were almost identical (1.9 % of the ingested sucrose on diet A and 2.7 % on diet B). There was also no difference in the amounts of sucrose carbon incorporated into the aphid tissues, which represented close to 50 % of the ingested sucrose. Sucrose carbons in the aphid tissues were mainly incorporated into lipids and the quantities involved were the same in aphids reared on either diet. On diet B, we observed neosynthesis of all protein amino acids from sucrose carbons and, for the first time in an aphid, we directly demonstrated the synthesis of the essential amino acids leucine, valine and phenylalanine. Amino acid neosynthesis from sucrose was significantly higher on diet B (11.5 % of ingested sucrose carbons) than on diet A (5.4 %). On diet A, neosynthesis of most of the amino acids was significantly diminished, and synthesis of two of them (histidine and arginine) was completely suppressed. The origin of amino acids egested through honeydew was determined from the specific activity of the free amino acid pool in the aphid. Aphids are able to adjust to variation in dietary amino acids by independent egestion of each amino acid. While more than 80 % of excreted nitrogen was from food amino acids, different amino acids were excreted in honeydew of aphids reared on the two diets. The conversion yields of dietary sucrose into aphid amino acids determined in this study were combined with those obtained previously by studying the fate of amino acids in pea aphids reared on diet A. The origin of all the amino acid carbons in aphid tissues was thus computed, and the metabolic abilities of aphid are discussed from an adaptive point of view, with respect to their symbiotic status.

Key words: Homoptera, Aphididae, *Acyrthosiphon pisum*, pea aphid, nutrition, symbiosis, *Buchnera*, artificial diet, amino acid metabolism, sucrose metabolism, honeydew.

Introduction

Most aphids (Hemiptera: Aphididae), like many homopteran insects, feed on the phloem sap of plants (Pollard, 1973), which is a diet rich in sugars and usually dominated by sucrose (Ziegler, 1975). The main nitrogen sources of aphids are free amino acids of unbalanced composition, with only very low concentrations of most or all of the essential amino acids that insects and other animals cannot synthesize (Douglas, 1993; Girousse et al., 1991; Rahbé et al., 1990). The endosymbiotic bacteria of aphids, assigned to the genus *Buchnera* in the gamma-subdivision of Proteobacteria by Munson et al. (1991), have long been postulated to complement the aphid's diet by supplying it with these essential amino acids (see Houk and Griffiths, 1980). The nutritional dependency of aphids upon *Buchnera* was first studied by maintaining aphids containing their bacteria, or experimentally deprived of their bacteria through the use of antibiotics (called symbiotic and aposymbiotic aphids, respectively), on defined artificial diets from which individual amino acids were omitted (Dadd and Krieger, 1968; Mittler, 1971). In recent years, molecular analyses of the genome of *Buchnera* have permitted the identification of almost 100 genes from these endosymbionts (Baumann et al., 1995, 1998). Among these, nearly 25 % were genes encoding enzymes in amino acid biosynthetic pathways, mostly essential amino acids. There are, however, very few

metabolic studies proving an effective synthesis of essential amino acids in aphids. The first persuasive evidence was for methionine, which was synthesized by the bacteria in the green peach aphid *Myzus persicae* (Douglas, 1988). Measurement of tryptophan synthetase activity in isolated *Buchnera* preparations, and in symbiotic compared with aposymbiotic pea aphids *Acyrthosiphon pisum*, also provided strong evidence for the synthesis of tryptophan by the symbionts of *A. pisum* (Douglas and Prosser, 1992). Aphid symbionts were shown to be directly involved in the transfer of nitrogen from glutamic acid or glutamine to several different amino acids, including some essential ones (Sasaki et al., 1993; Sasaki and Ishikawa, 1995). The synthesis of three essential amino acids (threonine, isoleucine, lysine) from carbons of other common dietary amino acids was demonstrated in *A. pisum* using ^{14}C-labelled precursors (Febvay et al., 1995), as a result of the synthetic capabilities of the symbiont; this was shown with control aposymbiotic aphids (Liadouze et al., 1996).

Carbohydrates are the major nutrients in an aphid's natural food, but their contribution to the syntheses of amino acids in aphids through the glycolytic pathway has never been investigated. The fate of sucrose in aphids has been studied by different authors only to quantify the part oxidized through respiration and used as an energy source (Ehrhardt, 1962; Kunkel and Hertel, 1975; Rhodes et al., 1996). As the high concentration of sucrose in plant phloem sap results in elevated osmotic pressure, and causes problems to aphids in maintaining the water balance and acquiring the necessary levels of other dietary components, all other studies on dietary sucrose in aphids have been focused on oligosaccharide synthesis and osmoregulation (Fisher et al., 1984; Rhodes et al., 1997; Walters and Mullin, 1988; Wilkinson et al., 1997).

The primary purpose of this study was to explore the fate of dietary sucrose in the pea aphid, and to quantify amino acid neosynthesis from this major nutrient. These analyses were achieved using [^{14}C]sucrose, and in order to investigate the influence of nitrogen quality of food on amino acid synthesis, they were conducted with pea aphids reared on two artificial diets differing markedly in their amino acid composition. In addition to analysis of the carbon and nitrogen absorption budget, we sought the origin of the amino acids egested by aphids through honeydew, in order to determine the quantities of each amino acid in honeydew that were from food or of metabolic origin. The conversion yields of dietary sucrose into aphid amino acids obtained in this study were combined with the results of previous studies, obtained by studying the fate of amino acids (Febvay et al., 1995; Liadouze, 1995), which enabled us to determine the precise origin of amino acid carbons in aphid tissues.

Materials and methods

Insects and artificial diets

A parthenogenetic clone of *A. pisum* (Lusignan strain, Ap-LL01) was maintained on broad bean seedlings (*Vicia faba* L. var. Aquadulce) in Plexiglas cages (21 °C, 70 % relative humidity, 16 h:8 h L:D photoperiod). As described previously (Febvay et al., 1995), alate viviparous adults, reared at low density on *V. faba* seedlings for 2–3 days, were allowed to lay progeny for 6 h on young plants. Larvae aged less than 6 h were used for the experiments.

Table 1. *Amino acid compositions of chemically defined artificial diets*

	Diet A		Diet B	
Amino acids	mmol l^{-1}	% of total	mmol l^{-1}	% of total
Alanine	20.1	7.6	5.4	2.1
β-Alanine	4.5*	1.7	0.5	0.2
Arginine	14.1	5.3	3.5	1.4
Asparagine	19.9	7.5	179.0	68.9
Aspartic acid	6.6	2.5	11.9	4.6
Cysteine	2.4	0.9	1.6	0.6
Glutamic acid	10.2	3.8	4.9	1.9
Glutamine	30.5	11.6	5.9	2.3
Glycine	22.2	8.4	1.7	0.7
Histidine	6.5	2.5	1.7	0.7
Isoleucine	12.6	4.8	4.0	1.5
Leucine	17.7	6.7	3.8	1.5
Lysine	19.2	7.3	2.2	0.8
Methionine	4.8	1.8	0.8	0.3
Ornithine	0.6	0.2	0.4	0.2
Phenylalanine	14.0	5.3	3.0	1.1
Proline	11.2	4.3	4.3	1.7
Serine	11.8	4.5	10.4	4.0
Threonine	10.7	4.0	6.8	2.6
Tryptophan	2.1	0.8	1.4	0.5
Tyrosine	5.9*	2.2	1.1	0.4
Valine	16.3	6.2	5.9	2.3

*To overcome the limited solubility of tyrosine, these amino acids were partly added as β-alanyltyrosine (3.75 mmol l^{-1}).

Two diet formulations were used, differing only in their amino acid composition. Diet A was based almost exactly on the total amino acid profile of whole aphid tissues (diet A5 described by Febvay et al., 1988), and diet B simulated the amino acid composition of phloem sap of alfalfa, the original host-plant of our pea aphid clone (Girousse et al., 1990). The amino acid compositions of these diets are reported in Table 1. The sucrose content (584 mmol l^{-1}, 20 % w/v) and the total amino acid concentration (260 mmol l^{-1}) of these diets were the same. Vitamins and oligoelements were supplied as described previously (Febvay et al., 1988). The solutions were filtered (0.45 μm pore size) and stored at –20 °C until used.

A radiolabeled compound (Isotopchim, Ganagobie-Peyruis, France) was aseptically added to these artificial diets just before the beginning of an experiment. For metabolic studies, [U-^{14}C]sucrose (22.2 GBq mmol^{-1}) was added to the diets at about 1 MBq ml^{-1}. For diet uptake measurements, a non-metabolized compound, inulin (hydroxy[^{14}C]methyl inulin; 81.4 kBq mmol^{-1}), was added to the diets at about 47.5 kBq ml^{-1}, as previously used (Febvay et al., 1995; Wright et al., 1985). The initial radioactivity of these diets was counted in triplicate 10 μl samples with 4.5 ml of Ultima Gold scintillation fluid (Packard Instrument SA, Rungis, France).

Experimental designs

For the metabolic analysis, we used the experimental method described earlier (Febvay et al., 1995) with only slight modifications. Neonate larvae collected from plants were deposited in groups of two in small Plexiglas cages sealed with a Parafilm® sachet stretched over the experimental chamber. Artificial diet with [U-^{14}C]sucrose (17 µl) was aseptically enclosed in this sachet and made available to the feeding of larvae. The experiment was conducted at 21±1 °C for 8 days, when aphids reached the fourth instar. 4 days after the beginning of the experiment, the sachets were changed to prevent microbial growth. At the end of the experiment, insects were individually weighed on a Setaram balance (accuracy, 1 µg). The exuviae were picked up with forceps, and the honeydew was recovered by rinsing each cage twice with 300 µl of distilled water. To trap expired CO_2, we used the specific cages previously described (Febvay et al., 1995). Air was drawn through the chamber at a rate of 0.32 ml min^{-1}, and the exhaust was bubbled through a CO_2 trap consisting of 400 µl of Carbo-Sorb (Packard) in a disposable radioactivity counting vessel. To measure diet consumption, a single neonate larva was deposited in a cage sealed with a Parafilm® sachet containing 17 µl of the artificial diet with [^{14}C]inulin. To obtain aphids over a wide range of mass, development was continued for 0, 2, 4, 6 or 8 days. At the end of experiment, the aphid, its honeydew and its exuviae were collected as described.

Sample treatments

The total radioactivity found separately in aphid, honeydew, exuviae and CO_2 was counted for each experimental cage (two aphids). The aphids or their exuviae were crushed and digested for 16 h in 200 µl of Soluene-350 (Packard). These digests were then neutralized with 50 µl of glacial acetic acid, and counted with 4.5 ml of Ultima Gold fluid. The honeydew recovered with distilled water was counted directly (or in two samples when subsequent amino acid analysis was performed) with 4.5 ml of Ultima Gold fluid. The amount of activity in expired CO_2 was determined by counting the radioactivity in the Carbo-Sorb (CO_2 trap) shaken with 4.5 ml Ultima Gold.

For total amino acid analysis, the aphids or the exuviae from an experimental cage were hydrolyzed, under nitrogen, in HCl vapor at 110 °C for 24 h using the Pico-Tag work station (Waters, St-Quentin-Les-Yvelines, France). Along with 2-β-mercaptoethanol (4 %) to preserve sulfur amino acids, 200 µl of 6 mol l^{-1} HCl were placed in the hydrolysis tank. After hydrolysis, 50 nmol (for aphids) or 10 nmol (for exuviae) of glucosaminic acid were added as an internal standard. The samples were dried under vacuum in a SpeedVac apparatus (Savant Instrument Inc., Farmingdale, New York) and taken up in exactly 150 µl of 0.05 mol l^{-1} lithium citrate buffer, pH 2.2. Triplicate samples (10 µl each) were counted for radioactivity with 4.5 ml of Ultima Gold. Another sample of 50 µl was subjected to amino acid analysis.

In preliminary analyses, we showed that amino acids rejected by aphids in honeydew were almost exclusively in the free state. Amino acid analysis of the honeydew of a cage was then performed without hydrolysis. Glucosaminic acid (10 nmol) was added to the cage as an internal standard, and the honeydew was recovered twice with 300 µl of distilled water. After vacuum drying, these samples were taken up in 100 µl of lithium citrate buffer. For aphid analysis, the radioactivity was counted in two 10 µl samples and the amino acids were analyzed in a 50 µl sample.

For the quantification of the radioactivity in the lipids and the analysis of free amino acids, the aphids of six experimental cages were pooled (12 aphids). After addition of 50 nmol of glucosaminic acid, these samples were dried under vacuum. Total lipids were extracted according to the method of Folch et al. (1957). Lipid extracts were dried under vacuum and taken up in exactly 500 µl of chloroform. The radioactivity of these samples was counted in three 50 µl samples with 4.5 ml Ultima Gold, after chloroform evaporation. The 350 µl of remaining lipid extract was fractionated to prepare neutral lipids and phospholipids, as previously described (Febvay et al., 1992). The extract was applied to a silica Sep-Pak cartridge (Waters Associates, Milford, MA) previously conditioned with chloroform. The neutral lipids were eluted successively with chloroform (2.5 ml) and diethyl ether (1.5 ml), and the phospholipids were then eluted with methanol (4.5 ml). These two fractions were dried under vacuum and resuspended in 200 µl of chloroform. Their radioactivity was counted with 4.5 ml Ultima Gold on three 50 µl samples, after chloroform evaporation. After extraction of lipids, the dried aphid samples were used to analyze free amino acids. They were extracted twice by vigorous stirring of the samples in 200 µl of trichloroacetic acid (5 % w/v) followed by centrifugation (5 min at 6000 ***g***). Trichloroacetic acid was then removed by three successive extractions with 400 µl of chloroform and, after vacuum drying, the free amino acid extract was resuspended in 125 µl of lithium citrate buffer. Total radioactivity was counted in two 10 µl samples with 4.5 ml Ultima Gold, and individual amino acids were analyzed in a 50 µl sample.

For each cage used to measure diet consumption, the aphid, its honeydew and its exuviae were pooled and digested for 16 h in 200 µl of Soluene-350. This digest was then neutralized with 50 µl of glacial acetic acid and counted with 4.5 ml of Ultima Gold fluid.

Analytical methods

All radioactive samples were counted using a Packard Tri-Carb 460C Liquid Scintillator System, with a preset ^{14}C-window (efficiency >90 % for ^{14}C). The amino acids were analyzed by ion-exchange chromatography on an automatic amino-acid analyzer (Beckman 6300), in which amino acids were detected by the Ninhydrin reaction, identified by their retention time and wavelength ratio, and quantified by their absorption at 570 nm (440 nm for proline). This analyzer was coupled with an on-line radioactivity flow detector (Flo-one/Beta A500; Packard), which allowed detection and quantification of radiolabeled compounds. A non-gelling liquid

scintillation cocktail (Ultima Flo AP; Packard) was mixed with the chromatography eluent before its passage through the counting cell. The counting efficiency for ^{14}C was 70–85 % along the chromatography elution gradient, and was corrected against that of the Packard Tri-Carb scintillation counter. The flow rate (1.1 ml min^{-1}) and cell volume (0.5 ml) were adapted for optimal detection and resolution. Peak identification, trace detection and minor peak quantification, together with resolution problems, were processed using a mathematical method described elsewhere (Bonnot and Febvay, 1995).

Expression of data and statistical analyses

Regression analyses were performed to determine the relationship between the volume of the ingested diet and the final mass of aphids. To compare regression coefficients obtained with the two diets, a parallelism test with a Student's *t*-distribution was performed (Dagnelie, 1975). For metabolic experiments, because sucrose was the sole radiolabeled tracer in the diets, the radioactivity recovered in the different fractions or in each amino acid was computed in nmol equiv. sucrose. All data are expressed as mg^{-1} fresh mass aphid and a parametric statistical test (Student's *t*-test) was used to compare results between aphids grown on the two artificial diets. To analyse and discuss the fate of all the dietary carbons, the transformation yields of dietary sucrose (this study) and of dietary amino acids (earlier results reported by Febvay et al., 1995; Liadouze, 1995) were calculated using the conditional correlation method (Lefebvre, 1976) as previously presented (Febvay et al., 1995).

Results

Diet uptake and growth of aphids

Whatever the age of aphids (between 0 and 8 days), there was a linear relationship between the ingested volume and final mass of aphids on both diets tested (Fig. 1), with high coefficients of determination. These regressions enabled us to predict the volume of food ingested from aphid mass, and to calculate the quantity of ingested nutrients in all the metabolic experiments performed with [^{14}C]sucrose. This correlation also indicates the nutritional efficiency of the diet, which is inversely proportional to the slope of the linear regression line. Diet B (slope=3.06, S.D.=0.093) was significantly more efficient than diet A (slope=3.52, S.D.=0.088), (t_{59}=3.6, P<0.001): the aphids had to ingest 3.1 µl of diet A to achieve a mass of 1 mg, compared with only 2.7 µl for diet B. However, nutritional efficiency does not predetermine aphid growth rate on this diet. As shown in Fig. 1, 8-day-old insects on diet A were on average larger (fresh mass >1 mg) than those reared on diet B (fresh mass approximately 0.7 mg). This result was confirmed in metabolic experiments with aphids reared for 8 days on these two diets: on diet A, the insects weighed 0.99±0.026 mg (mean ± S.E.M., N=84), and were significantly larger than the diet B aphids, which weighed 0.71±0.029 mg (N=80; t_{162}=7.1, P<0.001).

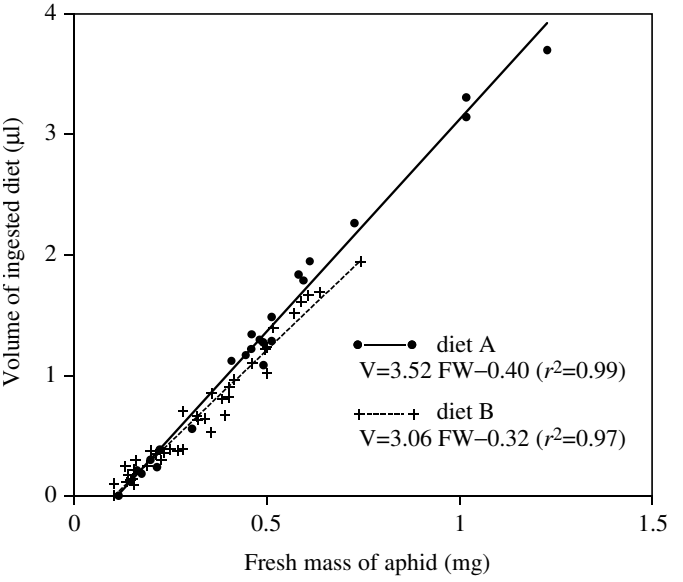

Fig. 1. Relationship between volume of ingested diet, as measured with [^{14}C]inulin, and final fresh mass of pea aphids maintained for 0 to 8 days on the artificial diets A or B.

Amino acid content of aphids, exuviae and honeydew

The amino acid compositions of the aphids, their exuviae and their honeydew are given in Fig. 2 (free or total, expressed as nmol mg^{-1} fresh mass aphid). The free amino acid content of 8-day-old aphids (Fig. 2A) was approximately 45 % lower in aphids reared on diet A (72±3.2 nmol mg^{-1}) than in those reared on diet B (132±5.4 nmol mg^{-1}; means ± S.E.M.), consistent with the inverse relationship between body size and free amino acid concentration that was identified previously in pea aphids reared on the same artificial diets (Liadouze et al., 1995). The total amino acid content of aphid tissues (Fig. 2B) was significantly higher (t_{13}=3.5, P=0.004) in aphids from diet B (681±18.3 nmol mg^{-1}, N=8) than from diet A (603±10.4 nmol mg^{-1}, N=7). This was specifically due to the higher concentrations of three amino acid groups: Asx (aspartic acid and asparagine), Glx (glutamic acid and glutamine) and alanine. Glx excepted, these variations may be attributed to the free pools of these amino acids (wholly for alanine, largely for Asx; Fig. 2A). In addition, these differences in Glx and Asx could be related to two key compounds in aphid amino acid metabolism and transport (γ-glutamylglutamine and γ-glutamylasparagine), identified by Sasaki et al. (1993) but not formally identified here. For all amino acids, the concentration in hydrolyzed exuviae was not significantly different between aphids reared on diet A and those on diet B (Fig. 2C; expressed as nmol mg^{-1} aphid fresh mass). The total amino acid amount lost by aphids in their exuviae was 54±2.1 nmol mg^{-1} for diet A (N=4), and 64±8.8 nmol mg^{-1} for diet B (N=6; t_8=0.9, P=0.4), accounting for approximately 10 % of the total amino acids of adult aphid tissues. Total amino acid concentrations were very significantly different in honeydew collected from the two aphid groups. Aphids raised on diet B excreted twice as much

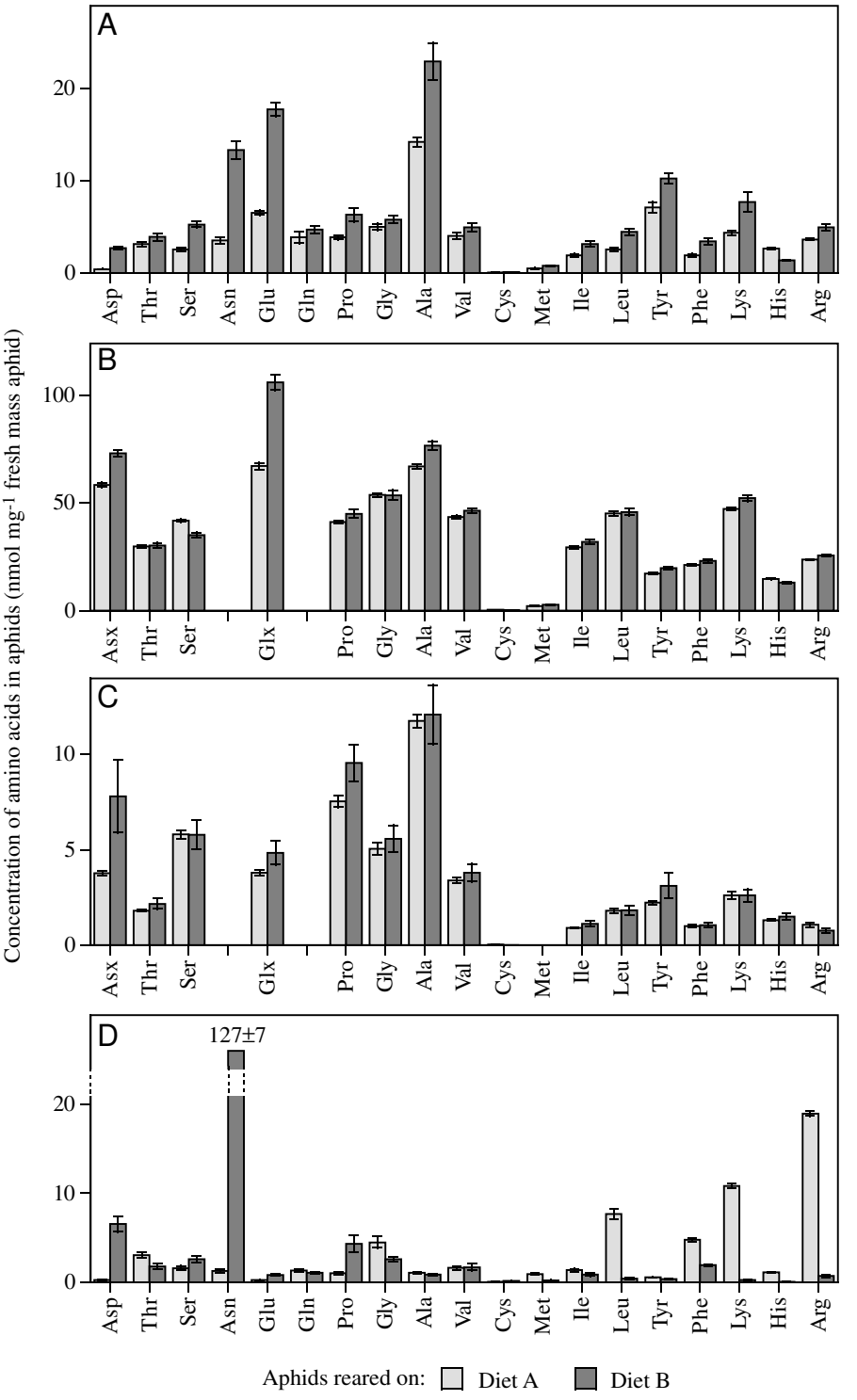

Fig. 2. Amino acid contents of pea aphids grown on diet A or on diet B. Values are expressed in nmol mg^{-1} fresh mass aphid, as means ± S.E.M. (A) Free amino acids in aphid tissues (N=7 for diet A, N=6 for diet B). (B) Total amino acids in aphid tissues (N=7 for diet A, N=8 for diet B). (C) Total amino acids in exuviae (N=4 for diet A, N=6 for diet B). (D) Free amino acids in honeydew (N=4 for diet A, N=6 for diet B).

as those on diet A: means ± S.E.M. were, respectively, 154±9.2 (N=6) and 62±3.3 nmol mg^{-1} fresh mass aphid (N=4) (t_8=7.8, P<0.001). In the honeydew of aphids maintained on diet B (Fig. 2D), asparagine was strongly predominant, amounting to 82 % of the total. In honeydew rejected by diet A aphids, four amino acids were excreted in much greater amounts than from diet B aphids (arginine, lysine, leucine and phenylalanine).

Fate of dietary sucrose carbons

The fate of dietary sucrose is summarized in Table 2 and compared between insects reared on diets A and B. The amount of sucrose ingested by aphids was computed from the regressions between diet uptake and aphid mass, established previously. The number of sucrose carbons expired as CO_2 was not significantly different for the two diets. For diets A and B,

Table 2. *Fate of dietary sucrose in the pea aphid (*A. pisum*) reared on different artificial diets*

	Diet A	Diet B	*P**
Ingested sucrose‡	1828±7.8 (26)	1536±15.1 (21)	<0.001
CO_2	459±18.7 (6)	440±12.6 (15)	0.42
Honeydew (whole)	230±11.9 (26)	129±7.8 (21)	<0.001
Free AA in honeydew	3.5±0.22 (4)	9.3±1.39 (4)	0.005
Exuviae (whole)	34±1.5 (15)	41±2.8 (5)	0.03
Total AA in exuviae	7.6±0.59 (3)	10.1±0.32 (4)	0.01
Aphid (whole)	738±8.8 (7)	731±24.7 (6)	0.78
Total lipids in aphid	324±11.6 (4)	285±13.6 (5)	0.07
Neutral lipids	281±12.8 (4)	258±9.6 (5)	0.18
Phospholipids	26.3±0.33 (4)	20.5±0.67 (5)	<0.001
Total AA in aphid	98±1.9 (7)	175±5.8 (8)	<0.001
Free AA in aphid	16.6±1.13 (4)	37.2±1.19 (4)	<0.001

Values are means ± S.E.M., expressed in sucrose equivalents (nmol mg^{-1} fresh mass aphid).

AA, amino acid.

*Student's *t*-test, comparing means between aphids reared on the two diets.

‡Amounts of ingested sucrose were computed from the previously established regressions between diet uptake and fresh aphid mass (differences between the two diets result from the different slopes).

respectively, 25.1 % and 28.6 % of the ingested sucrose were catabolized in oxidation pathways. However, aphids excreted in honeydew almost twice the quantity of sucrose carbons on diet A as on diet B, i.e. 12.6 and 8.4 % of the sucrose carbons ingested, respectively. The amino acids neosynthesized from sucrose and found in honeydew were in very low quantities, and accounted only for 1.5 % (diet A) and 7.2 % (diet B) of the honeydew ^{14}C rejected by aphids. Similarly, sucrose carbons incorporated into exuviae represented only 1.9 % (diet A) and 2.7 % (diet B) of the ingested sucrose. The slightly higher amount of ^{14}C found in the exuviae of aphids on diet B (compared with diet A) was partly explained by the greater quantity of amino acids neosynthesized from sucrose and rejected in these exuviae.

Most of the ingested sucrose was incorporated into aphid tissues (40.4 % for aphids reared on diet A and 47.6 % on diet B). There was no difference between aphids grown on either diet with respect to the number of sucrose carbons used in the tissues. In aphid tissue, lipids were the main recipients of sucrose carbons; they represented approximately 40 % of the aphid ^{14}C, and the quantities involved in these syntheses were not different between aphids raised on the two diets. About 90 % of the neosynthesized lipids were recovered in the neutral lipids (no difference between diets). In contrast, the neosynthesis of phospholipids from sucrose in aphids grown on diet B was lower than on diet A. Neosynthesis of amino acids from sucrose carbons were not negligible in the pea aphid, and depended largely on the amino acid composition of the diet (5.4–11.5 % of the ingested sucrose carbons were metabolized in this way). While approximately 80 % of these neosynthesized amino acids were ultimately incorporated in peptides and proteins, they also supplied the free amino acid pool, which was accordingly twice as heavily labeled in aphids grown on diet B as in those grown on diet A.

Table 3. *Neosynthesis of amino acids from carbons of sucrose in the tissues of the pea aphid (*A. pisum*) reared on different artificial diets**

Amino acids	Diet A	Diet B	*P*‡
Asx	9.4±0.51	7.6±0.60	0.036
Threonine	3.5±0.27	4.5±0.34	0.041
Serine	6.7±0.16	5.4±0.32	0.005
Glx	18.3±0.31	28.4±1.54	<0.001
Proline	10.9±0.37	14.4±0.81	0.002
Glycine	3.7±0.11	6.7±0.41	<0.001
Alanine	13.0±0.35	14.6±0.57	0.037
Valine	5.7±0.36	13.2±0.52	<0.001
Cysteine	0.4±0.08	0.4±0.17	0.97
Methionine	0.7±0.07	0.5±0.14	0.40
Isoleucine	3.8±0.28	9.9±0.45	<0.001
Leucine	7.7±0.23	17.3±0.66	<0.001
Tyrosine	4.5±0.21	10.3±1.06	<0.001
Phenylalanine	6.6±0.31	14.8±0.99	<0.001
Lysine	3.1±0.21	16.4±0.64	<0.001
Histidine	n.d.	4.8±0.29	<0.001
Arginine	n.d.	5.4±0.37	<0.001

Asx, aspartic acid + asparagine; Glx, glutamic acid + glutamine.

*Values are means ± S.E.M., expressed in sucrose equivalents (nmol mg^{-1} fresh mass aphid).

N=7 (diet A), *N*=8 (diet B).

‡Student's *t*-test, comparing means of each amino acid between aphids reared on the two diets.

n.d., null value or below the detection threshold.

Individual amino acid synthesis from sucrose carbons

Apart from the amount of ingested sucrose carbons recovered in amino acids, we quantified the neosynthesis of each amino acid in aphids (free or total amino acids), in exuviae (total) and in honeydew (free). For aphid tissues, these results, expressed in nmol sucrose equiv. mg^{-1} fresh mass aphid, are reported in Table 3. In aphids grown on diet A, neosynthesis of each amino acid, except for histidine and arginine, was observed. Glx was the most intensively synthesized amino acid and, consistent with our previous results (Febvay et al., 1995; Liadouze et al., 1996), was the source of the neosynthesis of some other amino acids. For some of them, especially the essential amino acids threonine, isoleucine and lysine (but also partly for aspartic acid and proline), this synthesis from sucrose carbons was achieved *via* the glutamate pathway (the intensity of their neosynthesis being in line with our previous findings). For others such as serine, glycine and alanine, however, the more extensive synthesis from labeled sucrose than from glutamate was the result, at least partially, of other standard metabolic pathways (deriving directly from glycolysis). The new biosynthetic results from this study were the syntheses of three other essential amino acids from sucrose: valine, leucine and phenylalanine (also leading to tyrosine, through

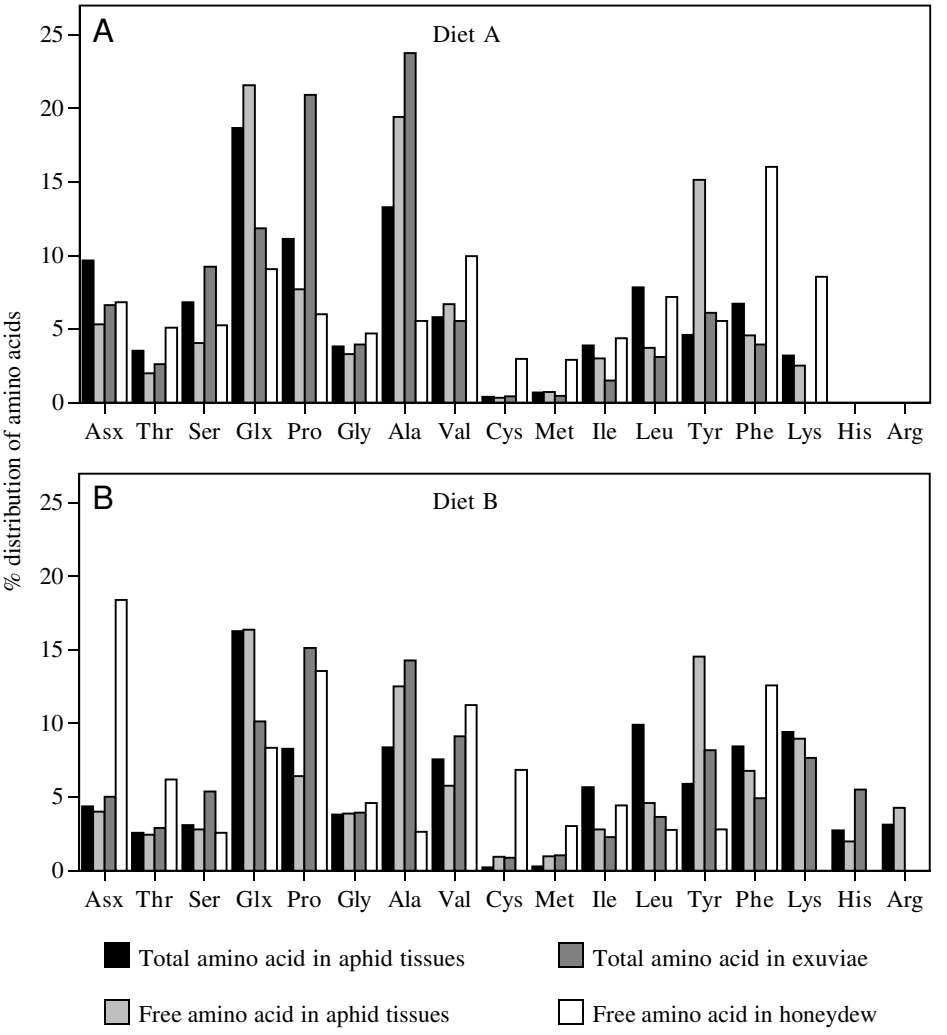

Fig. 3. Percentage distribution of amino acids neosynthesized in the pea aphid from sucrose carbons, recovered in aphid tissues (total amino acids), in the free amino acid pool, in the exuviae or in the honeydew. (A) Aphids reared on diet A; (B) aphids reared on diet B.

hydroxylation). As shown in a preliminary experiment (data not shown), the neosynthesis of these three essential amino acids results from the synthetic abilities of the intracellular symbionts of aphids, and was totally disrupted in aposymbiotic aphids obtained by mild treatment with the antibiotic rifampicine. In aphids reared on diet B, we observed the neosynthesis of all amino acids from sucrose (Table 3), including those of histidine and arginine. With the exceptions of sulfur amino acids, serine and Asx, the extent of neosynthesis was significantly increased in aphids raised on diet B compared with diet A. The increase was low for threonine or alanine, reached nearly 50 % for Glx, proline or glycine, but largely exceeded 100 % for the synthesis of all the other essential amino acids.

As stated previously, the amounts of neosynthesized amino acids in exuviae or honeydew were low (Table 2), and we compared their relative distribution with that of the main neosynthesized aphid pool (Fig. 3). For aphids grown on either diet, the free amino acid pools of aphids were characterized by high proportions of neosynthesized alanine and tyrosine. Neosynthesized alanine was also in high proportions in exuviae, accompanied (on either diet) by relatively large amounts of neosynthesized proline. The main difference between diet treatments was a higher incorporation in exuviae of tyrosine and the two essential basic amino acids, lysine and histidine, for aphids reared on the plant-type food diet B. As for honeydew label, it was extensively modified by the diet treatment, specifically the ratio of neosynthesized and excreted phenylalanine + lysine/Asx. Phenylalanine and lysine were high while asparagine was low in both diet A and honeydew of diet A-reared aphids, and therefore the biosynthesis of these amino acids from dietary sucrose was not tightly regulated by external levels (unlike for histidine and arginine, for example).

Nitrogen, carbon and amino acids absorbed, and origin of amino acids excreted

Because the diets used in this study were fully defined, it was possible to infer complete nitrogen and carbon budgets from our data (Fig. 4A). Neglecting vitamins, the sole nitrogen supply in the diets was the free amino acid pool, and carbon was provided by either amino acids or sucrose. Diets A and B possessed identical molar concentrations of amino acids, but owing to their different amino acid profiles, their total nitrogen

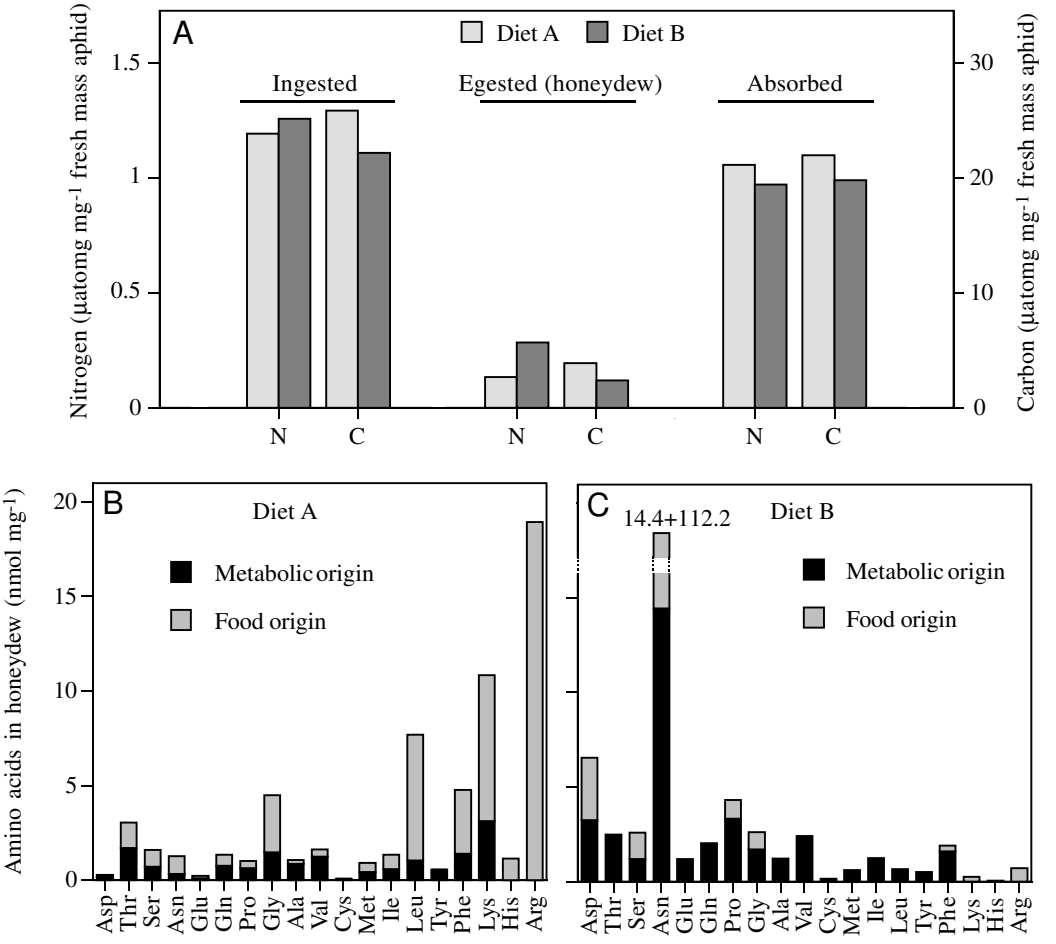

Fig. 4. (A) Global budget of ingested, egested and absorbed nitrogen (N) and carbon (C) by pea aphids grown on diet A or diet B. Values are µatomg mg^{-1} fresh mass aphid. (B,C) Metabolic origin compared with food origin of amino acids recovered in the honeydew of pea aphids reared on diet A (B) or on diet B (C).

levels were different: 391.2 µatomg ml^{-1} for diet A and 462.9 µatomg ml^{-1} for diet B. For carbon, the buffering effect of sucrose led to very similar carbon contents: 8311 µatomg ml^{-1} for diet A and 8112 µatomg ml^{-1} for diet B. Expressed as µatomg mg^{-1} fresh mass aphid, the amounts of ingested nitrogen were similar for aphids grown on either diet (Fig. 4A, difference close to 5 %), while the ingested carbon amount was smaller for aphids grown on diet B. To calculate the egested amounts, we took into account only the amino acids (quantified directly by analysis) and the carbohydrates rejected in the honeydew (taken as a whole, they were calculated from their specific activity measured in the honeydew, and that of sucrose in the diets). This computation is undoubtedly inaccurate, because nitrogen and carbon could be rejected through compounds other than protein amino acids or carbohydrates, as for example γ-aminobutyric acid (Prosser and Douglas, 1991). From our amino acid chromatograms, however, we consider this inaccuracy as minor. As shown in Fig. 4A, aphids modulate independently, through their egested compounds, the differences in ingested nitrogen and carbon resulting from diet compositions. As a consequence, C/N ratios of absorbed nutrients were remarkably constant for the two diets (20.8 for diet A and 20.4 for diet B).

Compounds recovered in honeydew were either unabsorbed compounds in the food that had passed intact through the gut, or were of metabolic origin and subsequently excreted through the gut. Using a simplifying assumption, it was possible to determine the proportionate origin for each of the honeydew amino acids. We assumed here that the specific activity of excreted amino acids of metabolic origin was the same as that of the free amino acid pool in the aphid that was measured in this experiment. Amino acids directly rejected from the food were then calculated as the difference from the total amino acid amounts recovered in the honeydew. In the honeydew of aphids grown on diet A (Fig. 4B), tyrosine was the sole amino acid entirely of metabolic origin (i.e. all dietary tyrosine was absorbed by the aphid). For most other amino acids, more than 50 % of the amounts recovered in the honeydew were dietary amino acids that escaped absorption in the gut. This was particularly true for the most abundantly rejected amino acids, arginine (100 % of food origin), lysine (71 %), leucine (87 %) and phenylalanine (71 %). Globally, 5.9 % of the total ingested amino acid amount was rejected directly in the honeydew, but there was a large variation among amino acids: the amount rejected directly from the food represented 43, 13, 12 and 8 % of the ingested amino acid for arginine, lysine, leucine and phenylalanine, respectively. In contrast with the results for aphids reared on diet A, most of the amino acids recovered in the honeydew of aphids reared on diet B were of exclusive metabolic origin (Fig. 4C), indicating high absorption

efficiency. However, this was not the case for asparagine, or to a lesser extent of the related aspartic acid (88.6 % of the rejected asparagine originated from the food). Diet B, as phloem sap, contained a large excess of asparagine, and the amount that escaped absorption in the gut represented here 23 % of the ingested quantity.

Discussion

Uptake of diet and origin of honeydew amino acids

Simpson and Raubenheimer (1993) have developed a conceptual framework for the study of insect feeding and nutrition, viewing the nutritional needs of an animal as a problem of multidimensional geometry and allowing discrimination among mechanisms regulating the fate of different nutrients (Raubenheimer and Simpson, 1993; Simpson and Raubenheimer, 1993). In terms of this model, aphids were confined in our experiments to the same 'rail' in their multidimensional nutrient space, because the two diets used had the same sucrose:amino acid ratio (2.2:1 expressed in $mmol\,l^{-1}$). However, their *intake target*, as defined by these authors, was markedly different: over 8 days, the aphids consumed 1.7 times more diet A than diet B. Therefore, these data show that the intake target does not simply result from total amino acid or sucrose intake, and that aphids can react to the ingested amount of a particular amino acid or a combination of specific amino acids, or to the level of any particular nutrient in a sensitive compartment. With the data expressed in mass units (as in this study), we reach the *growth target* as defined by the same authors (i.e. the level of nutrients incorporated into growth). To achieve 1 mg of growth, the aphids on diet A must ingest more food than those on diet B. Nitrogen appears to be a major determinant of the aphid growth: a lower concentration of dietary nitrogen was counterbalanced by an increased consumption of food (diet A), leading to amounts of ingested nitrogen per mg growth that were similar for aphids grown on either diet (Fig. 4A).

Independently of ingestion, this growth target may be achieved via post-ingestive processes, as suggested by Abisgold et al. (1994) to explain the clustered range of carbohydrate- and protein-derived growth of pea aphids reared on diets with different amino acid and sucrose concentrations. One of these processes is the rejection of nutrients through honeydew. Our study, limited to two artificial diets, does not permit the formulation of general rules, but aphids seem to regulate for variation in dietary amino acids through differential egestion of nutrients, leading to a remarkably constant C/N ratio of absorbed nutrients. These absorbed nutrients are used for metabolism and growth, and the constant C/N ratio obtained here for both diets represents the *nutritional target* of the pea aphid.

As shown by the computation of the origin of amino acids in the honeydew, such a post-ingestive regulation can be achieved precisely by the aphid relatively independently for every amino acid. This constitutes the first direct data on the origin of rejected amino acids in an aphid. In contrast to the suggestion of Prosser et al. (1992), we show that nitrogen excretion of aphids is not principally of metabolic origin. For the two diets tested, for which nitrogen was supplied through very different amino acid compositions, more than 80 % of the excreted nitrogen was food-borne, and excretion was through amino acids that were in excess in the diets.

Metabolism of dietary sucrose

Pea aphids reared on diet A or B were found to respire the same amount of dietary sucrose, irrespective of the amino acid composition of the food. However, differences in diet uptake led to 25.1 % and 28.6 % of the ingested sucrose respired, respectively. These values are higher than that reported by Rhodes et al. (1996), who found only 14.6 % of the ingested sucrose oxidized in the pea aphid. This difference may be explained by the higher sucrose concentration in the diet used by these authors (25 % w/v compared with 20 % in our study). With the green peach aphid *Myzus persicae*, reared on an artificial diet with 15 % sucrose, Kunkel and Hertel (1975) reported that 24 % of the ingested sugar was oxidized, a proportion similar to ours. Another explanation may be that respiration in pea aphid is influenced by age, as demonstrated by Randolph et al. (1975): after a strong rise in early growth stages, respiration per unit biomass declines slowly. In our experiment, sucrose oxidation was measured during the first 8 days of aphid development, while Rhodes et al. (1996) experimented with adult aphids. The proportion of sucrose oxidized was weak compared with that of some dietary energetic amino acids (25–60 % measured in identical conditions; Febvay et al., 1995). Nevertheless, taking into account the large amount of sucrose ingested, CO_2 production in the pea aphid was essentially of sugar origin. Calculations carried out from conversion yields of all dietary amino acids consumed by pea aphids raised on diet A (Febvay et al., 1995; Liadouze, 1995), and from that of sucrose measured in this study, show that 82 % of the CO_2 carbons (5451 natomg mg^{-1} fresh mass aphid) originated from ingested sucrose, while only 18 % (1195 natomg mg^{-1} fresh mass aphid) had an amino acid origin. This is the first direct study to show that aphids do not use amino acids as their predominant energy source, as has been suggested by different authors (Llewellyn and Qureshi, 1979; Van Hook et al., 1980), but also that even in the presence of normal sucrose level, this sugar was obviously not the only compound respired by the pea aphid, as was claimed for either plant-reared *Megoura viciae* (Ehrhardt, 1962), or diet-reared *Myzus persicae* (Kunkel and Hertel, 1975).

Excretion of dietary sucrose was evaluated in several studies on osmoregulation in aphids. It is now clearly demonstrated that the relatively constant osmolality of the honeydew of aphids reared on plants differing widely in osmotic pressure of phloem sap (Downing, 1978), or on an artificial diet with increasing sucrose concentrations (Fisher et al., 1984) is achieved by the aphid's ability to synthesize oligosaccharides (Fisher et al., 1984; Walters and Mullin, 1988; Wilkinson et al., 1997). In our study, honeydew sugars represented more

than 95 % of the excreted radioactivity, and displayed a nonosmoregulatory response: although the sucrose concentrations of the two diets were the same, the amounts of sucrose carbons rejected in the honeydew varied by a factor of two, and were thus a means for the aphid to regulate the ratio of assimilated carbon and nitrogen (see Fig. 4A). Dietary sucrose carbons excreted through the honeydew amounted to 12.6 % and 8.4 % of the ingested carbons. These values are lower than the 31 % reported for the pea aphid by Rhodes et al. (1996), but consistent with their use of a diet with 25 % of sucrose. The proportion of ingested sucrose which is absorbed by aphids was shown to be directly related to sucrose concentration in the diet, as demonstrated by Mittler and Meikle (1991), who report a proportion of 80 % of ingested sucrose absorbed by the pea aphid on a 20 % sucrose diet.

Apart from oxidation into carbon dioxide, lipids in aphid tissues were the main destination of dietary sucrose. The proportions of sucrose carbons used for the synthesis of neutral lipids and phospholipids (Table 2) are in close agreement with previous analyses showing that neutral lipids and phospholipids accounted for 89 % and 11 %, respectively, of the total lipids of pea aphids raised on an artificial diet (Febvay et al., 1992). As already demonstrated, particular fatty acids of aphid lipids are neither of dietary nor of symbiotic origin (Febvay et al., 1992; Rahbé et al., 1994). In insects, fatty acid biosynthesis proceeds through acetyl-CoA by a pathway similar to that described for bacteria and other animal groups (reviewed by Downer, 1985). The neosynthesis of phospholipids was smaller in aphids reared on diet B than on diet A. Similar variations in total amounts of phospholipids have already been described between aphids reared on artificial diets and on plants, or between aposymbiotic and normal aphids (Febvay et al., 1992, 1993). Because phospholipids are essential components of biological membranes, their diminution was assumed to be linked to lower fecundity, leading to a significant reduction of embryonic tissues. Sasaki et al. (1991) showed that the fecundity of pea aphids was smaller for insects reared on a diet with an amino acid composition derived from a phloem-sap analysis of *Vicia faba* (relatively close to our diet B), than for insects reared on a diet similar to diet A.

Amino acid syntheses from dietary sucrose

This study directly demonstrates that all protein amino acids can be synthesized from carbons of dietary sucrose in an aphid. Only one previous study has focused on amino acid syntheses from dietary sugar in an aphid, the green peach aphid *Myzus persicae* (Strong and Sakamoto, 1963). At that time, the low sensitivity of the methods used led the authors to conclude that only alanine, aspartic acid, serine, glutamic acid, glycine and cystine were neosynthesized from glucose carbons to any appreciable extent. In recent years, the ability of aphids to synthesize amino acids has been investigated using different approaches that were always related to (1) the nutritional imbalance of their natural food, primarily in essential amino acids, and (2) their association with bacterial endosymbionts, which should be the basis of essential amino acid syntheses (for a review see Douglas, 1998). Carbons of common amino acids are utilized by the pea aphid for the synthesis of the C skeleton of other amino acids (Febvay et al., 1995), the synthesis of three essential ones (threonine, isoleucine and lysine) being strictly the result of symbiotic activity (Liadouze et al., 1996). The results reported here complete this scheme, with the demonstration that C skeletons of all amino acids, and especially the essential ones, may be synthesized from sucrose carbons by the symbiotic aphid. The neosynthesis of the essential amino acids valine, leucine, phenylalanine and histidine is demonstrated here for the first time. Experiments with aposymbiotic aphids have shown that these pathways are under the control of aphid symbionts. They were not observed in previous experiments with labeled amino acid precursors because these biosynthetic pathways do not derive from the Krebs cycle, but start from compounds produced during glycolysis. Valine and leucine are synthesized directly from pyruvate, the end product of glycolysis, and the penultimate compound phosphoenolpyruvate is a precursor of phenylalanine biosynthesis. Histidine biosynthesis starts with ribose 5-phosphate, resulting directly from the oxidation of glucose 6-phosphate along the pentose phosphate pathway. It was demonstrated previously that glutamate could be regarded as the main, if not the sole, amino acid supplied from the insect to its symbionts (Febvay et al., 1995; Whitehead and Douglas, 1993). Our results are best explained if glucose itself is also supplied to the symbionts, and effectively utilized as a substrate for the observed biosyntheses (or through other sugars such as trehalose or fructose).

The neosynthesis of amino acids from dietary sucrose in the pea aphid was evaluated with two different nutritional conditions. On diet B, the dietary supply of almost all amino acids was limited, especially the essential ones. This induced increased neosynthesis of all essential amino acids, except the sulfur amino acids (cysteine and methionine), even though their supply in diet A was higher. This increase of neosynthesis of most amino acids (excluding Asx and serine) was most noticeable for histidine and arginine, which were not synthesized at all on diet A. This is not surprising for arginine, which seems to be supplied in excess in diet A, since it was excreted in large amounts in honeydew. For histidine, neosynthesis appears to be strongly inhibited by even low concentrations in the diet. The essential amino acids, like the branched-chain ones (valine, leucine and isoleucine) or the aromatic group amino acids (phenylalanine and tyrosine), were likewise more intensively neosynthesized in aphids grown on diet B. On this diet, as it is probably the case on phloem sap, the supply is certainly not satisfactory for optimal growth of the pea aphid. All these differences clearly underline the abilities of the pea aphid to regulate amino acid synthesis by its endosymbiotic bacteria, in response to food quality. Several studies have recently focused on the *Buchnera* genome, and numerous genes involved in essential amino acid biosynthesis have been described (Baumann et al., 1995). These genes encode enzymes of the common pathway of aromatic amino

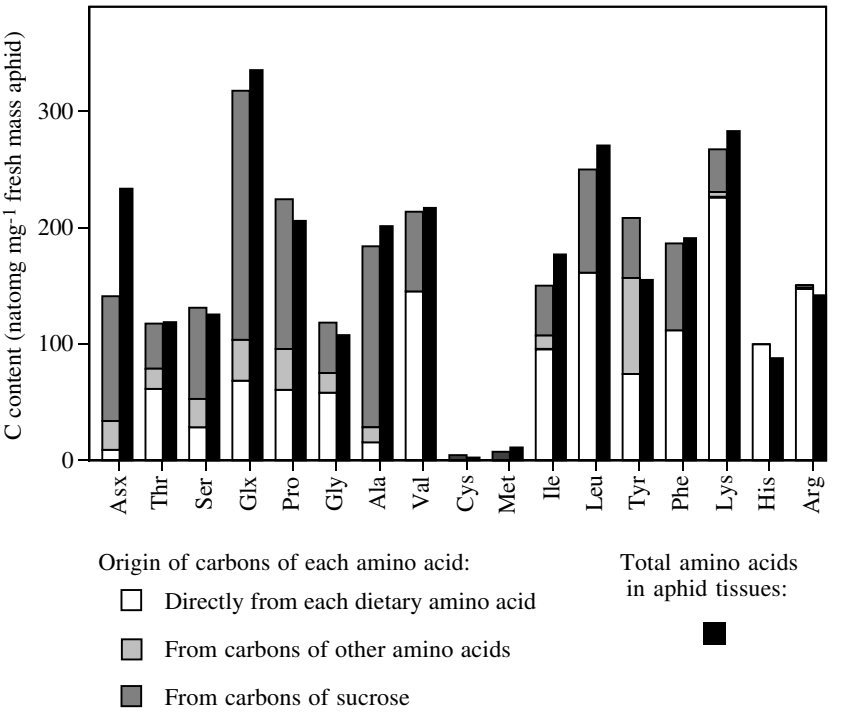

Fig. 5. Origin of the carbons of each amino acid in the pea aphid grown on diet A, divided into those arising directly from the same dietary amino acid without conversion, from other dietary amino acids after conversion, or from dietary sucrose. These calculations were carried out from conversion yields of all dietary amino acids (except for sulfur amino acids and asparagine) consumed by pea aphids raised on diet A. Data were obtained either from previous studies (Febvay et al., 1995; Liadouze, 1995) or from the sucrose data measured in this study. Carbon amounts in each amino acid, deduced from total amino acid analysis, are reported for comparison (black bars). Values are expressed as natomg C mg^{-1} fresh mass aphid.

acid synthesis (Kolibachuk et al., 1995), of the parallel pathway of isoleucine and valine synthesis (Clark and Baumann, 1998; Thao and Baumann, 1998) and of the specific biosynthetic pathways of leucine (Bracho et al., 1995), of tryptophan (Lai et al., 1994; Munson and Baumann, 1993), of lysine (Thao and Baumann, 1998) and of histidine (Clark et al., 1998). In Aphididae, many genes of amino acid biosynthetic pathways display peculiar organization in *Buchnera* and are located on plasmids. Amplification on plasmids is interpreted as a potentially increased capability of *Buchnera* to synthesize or regulate key enzymes of these pathways (Baumann et al., 1998; Douglas, 1997). Nevertheless, physiological regulations are still completely unknown at the molecular level in the aphid/*Buchnera* symbiocosm, and are currently being investigated on artificial diets with *A. pisum* for two biosynthetic pathways (leucine and histidine). Data were recently obtained, however, that demonstrate that two aphid species display different levels of leucine plasmid amplification (Thao et al., 1998), which constitutes a first element in the identification of differential regulatory responses, at evolutionary levels, in amino acid metabolism.

The conversion yields of almost all dietary amino acids into all obtained products were measured in previous studies on the pea aphid reared on diet A, in the same conditions as in this study (Febvay et al., 1995; Liadouze, 1995). Because amino acids and sucrose were the sole source of carbons in the artificial diet consumed by aphids, it was possible to combine previous results with those obtained here with sucrose, and to determine the origins of all the aphid tissular amino acids. The results of this computation are presented in Fig. 5: only the dietary sulfur amino acids (methionine and cysteine, which were in low concentrations in the diet as in aphid tissues) and asparagine (radiolabeled compound not available commercially) were not taken into account in this computation. This processing enabled us to calculate the amount of carbon of each resulting amino acid, coming either directly from the amino acid without conversion, from other dietary amino acids after conversion, or from dietary sucrose. For each amino acid, the sum of its carbons calculated from its different dietary sources is roughly similar to its amount recovered in aphid tissues (Fig. 5), which validates the results obtained previously and the present computation. The discrepancy for Asx is linked to the fact that the fate of dietary asparagine was not studied, and the difference between the calculated carbon amount and that recovered in aphid tissues may be attributed to this dietary asparagine. Another divergence, of opposite sign, is observed for tyrosine. This aromatic amino acid is an important metabolite used in some of the major functional pathways of insects, such as pigment syntheses or sclerotization process, and from which many compounds are synthesized (Rahbé and Bonnot, 1986). To explain the higher calculated amount of tyrosine carbons than that recovered in aphid tissues, we must assume that a metabolite of tyrosine, less or not stainable with Ninhydrin (such as for example dityrosine or other phenolic adducts) was unresolved from tyrosine, and its radioactivity imputed to tyrosine. From the computation presented here, we observe that the fraction of carbons provided directly by the same dietary amino acid ranged widely among amino acids. Although it reached 100 % for histidine, and it was high (more than 50 %) for all essential amino acids, this proportion was generally less than 30 % for other amino acids, and especially for alanine and Asx (less than 10 %). The contribution of other dietary amino acids to the carbon amount of each amino acid was generally

weak, except for tyrosine as the result of the intensive conversion of phenylalanine into tyrosine (Liadouze, 1995). The most interesting result of this reconstruction concerns the high contribution of dietary sucrose to the C skeleton of aphid amino acids. Except for histidine and arginine, as discussed above, the dietary sucrose carbons are used for the synthesis of all aphid amino acids, with a proportion exceeding 50 % for Asx, serine, Glx, proline or alanine. For all combined amino acids, sucrose thus provides 41 % of the amino acid carbons in aphid tissues. We did not perform the same analysis for aphids grown on diet B because the fate of dietary amino acids was not measured as exhaustively in these conditions. Nevertheless, as neosyntheses of amino acids from dietary sucrose were increased in aphids raised on diet B compared with diet A, we can infer that in nutritional conditions close to those prevailing in natural food, a great majority of aphid tissue amino acids originates from dietary sucrose.

Concluding remarks

The extensive survey of the fate of every carbon source given to the aphids shows how these insects adjust their nutritional supplies to their needs. When fed on diet A, one might expect the metabolic processes to be reduced to a minimum: when amino acids are provided to cover the needs for growth as exactly as possible, the aphid should not need to modify anything. Actually, this is far from what is observed, and aphids use a powerful catabolism (dealing with up to 70 % of the dicarboxylic amino acids) and an intense intermediary metabolism, to build up *de novo* the required metabolites from different carbon sources, and especially from the dietary sugars. All these metabolic conversions have high energy costs (but is energy really a limiting factor for aphids?) and delay product availability; they can therefore be considered as remarkably wasteful. However, balanced food such as diet A is not a normal nutritional context for aphids, which encounter saps much closer in composition to diet B, and which are probably also widely variable. In such a context, the aphid's constitutive metabolic equipment is immediately and fully efficient. Some pathways, which remain undetected with rich diets, become available in more restrictive environments, showing further possibilities of adaptive regulation. The large excesses of Asx and/or Glx are readily oxidized to CO_2, the amino groups being transferred to the newly built ketoacids deriving mainly from glycolysis, hence providing effective nitrogen upgrading. This is an active strategy to overcome strongly unbalanced and changing nutritional conditions.

The intensity and the variety of the metabolic flows reported in this and earlier work (Febvay et al., 1995), provide clear evidence that these metabolic abilities are the key factors that enable aphids to use plant sap as its exclusive food source. Many of these metabolic abilities, and especially the most critical ones for animals, such as the biosynthesis of essential amino acids, are provided by symbiotic activity. Symbiont acquisition has obviously been one of the most important events in the adaptive history of aphids.

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