

**Multimodal dynamic response of the *Buchnera aphidicola* pLeu plasmid to variations in leucine demand of its host, the pea aphid *Acyrtosiphon pisum***

José Viñuelas<sup>†</sup>, Gérard Febvay, Gabrielle Duport, Stefano Colella, Jean-Michel Fayard,  
Hubert Charles, Yvan Rahbé and Federica Calevro\*

UMR203 BF2I, Biologie Fonctionnelle Insectes et Interactions, INSA-Lyon, INRA,  
Université de Lyon, Bât. Louis Pasteur, 20 av. Albert Einstein, F-69621 Villeurbanne, France.

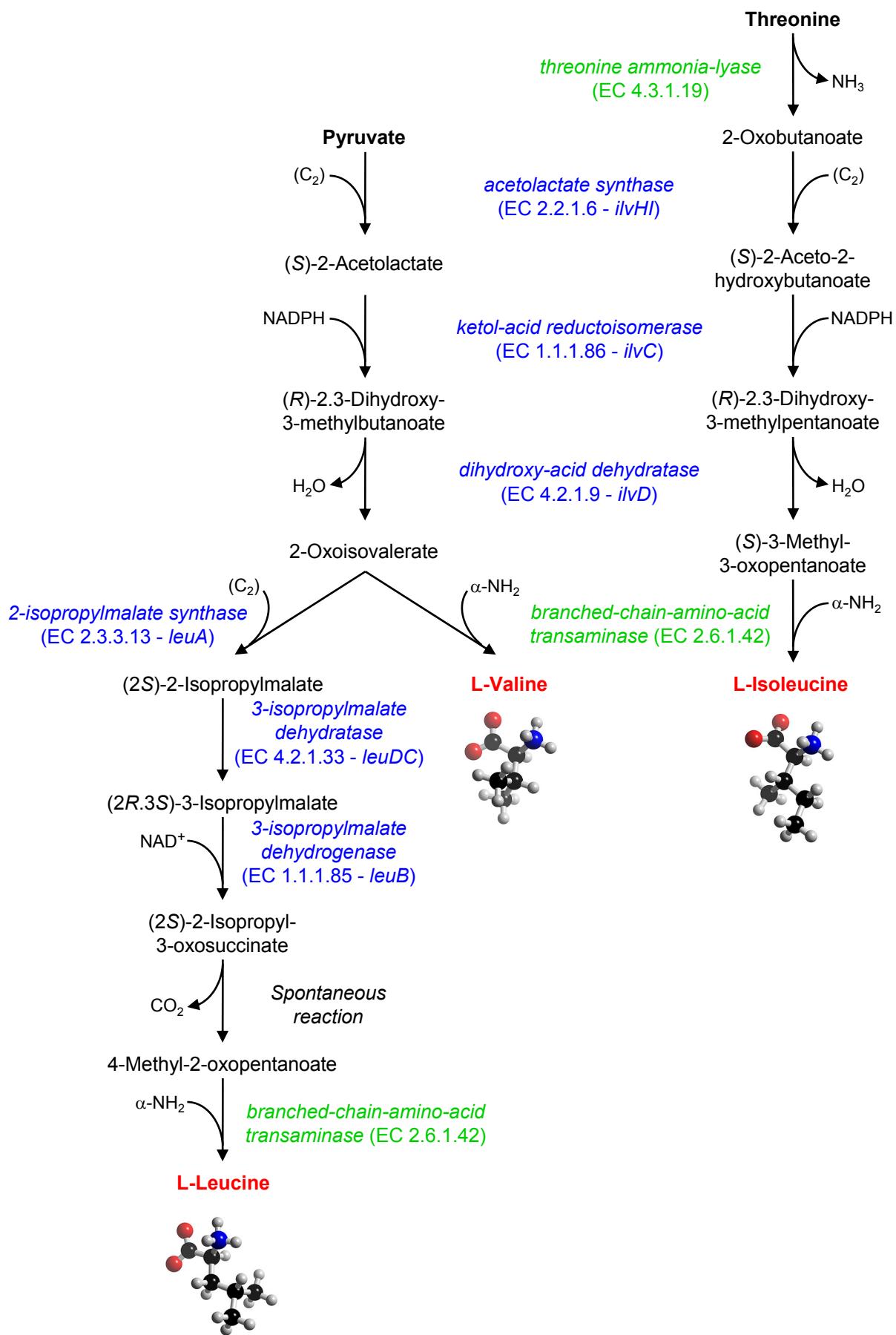
\*For correspondence.  
E-mail [federica.calevro@insa-lyon.fr](mailto:federica.calevro@insa-lyon.fr)  
Tel. (+33) 472 43 79 88  
Fax (+33) 472 43 85 34

† Present address:  
Université Lyon 1, FST, Lyon, F-69003, France ; CNRS, UMR 5534, CGPhiMC, F-69622  
Villeurbanne, France.

**Supporting Information**

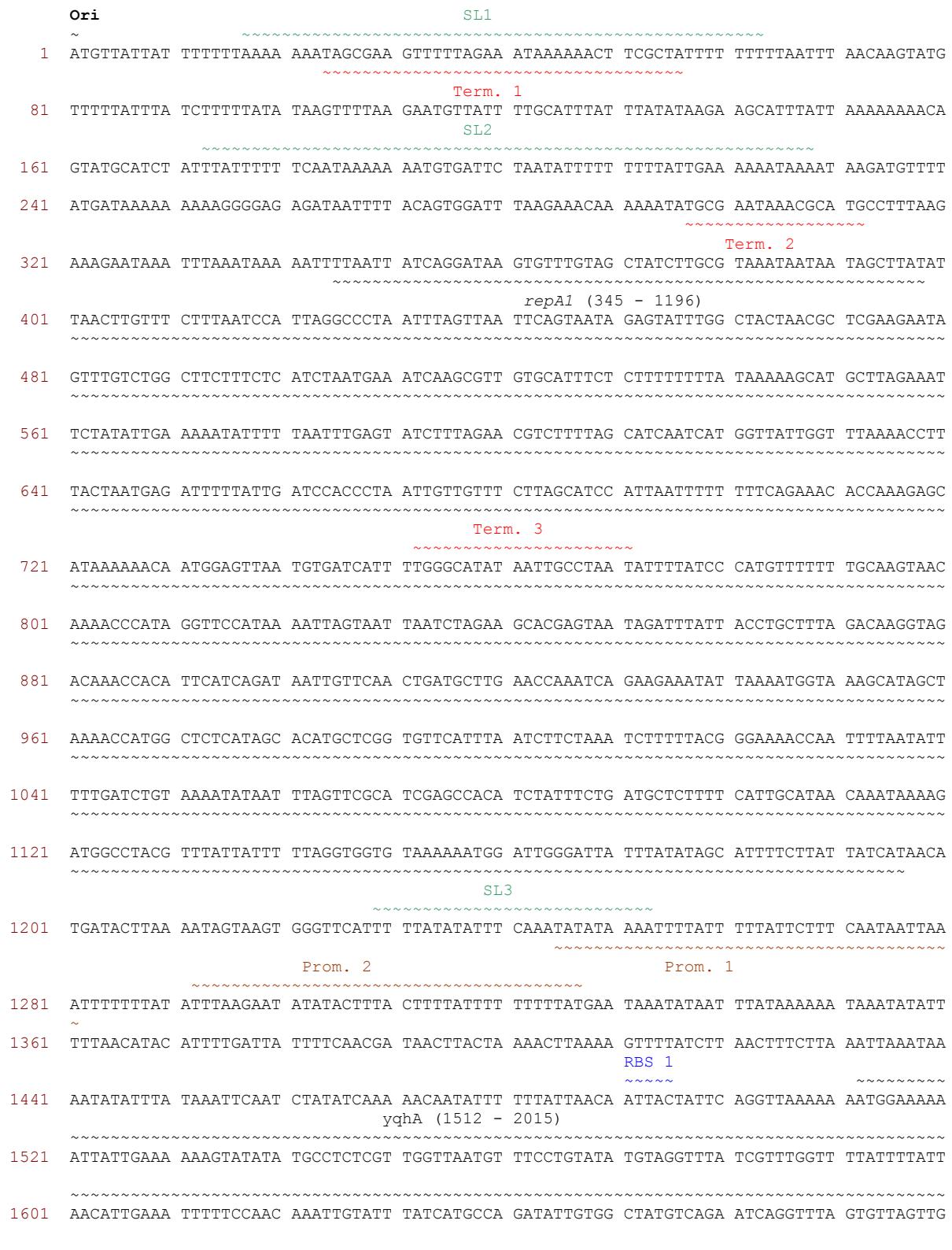
### Figure S1. Pathways for the biosynthesis of isoleucine, leucine and valine.

The pathways were modified from <http://acyclic.cycadsys.org/> and completed with data from Wilson and colleagues (Wilson *et al.*, 2010). The enzymes encoded by *B. aphidicola* but absent in the pea aphid, and vice versa, are specified in blue and in green, respectively.



**Figure S2. Features map of the reannotated pLeu plasmid in *B. aphidicola* from *A. pisum*.**

Stem-loop structures are shown in green (SL), promoter sequences in brown (Prom.), rho-independent terminators in red (Term.) and ribosomal binding sites in blue (RBS).



1681 TTTTGTCTATT AATTGATATT GCATTAGTAG GTGGTCTATT AGTTATGGTT ATGTTTCTG GATATGAAAA TTTTATTC  
~~~~~  
1761 AAAATGGATA TACAAGATAA TCAGAAAAGA TTAGGGTGGA TGGGAACCAT GGATGTTAAT TCAATAAAA ACAAAAGTAGC  
~~~~~  
1841 ATCTTCAATA GTGCCATAT CTTCTGTTCA TCTTTACGT CTTTTATGG AAGCAGAAA AATATTAGAT GATAAAATTA  
~~~~~  
1921 TGTTATGTGT TATTATTCAT TTAACTTTG TATTATCGC TTTGGAATG GCATATATTG ATAAAATGAG CAAAAAA  
Prom. 3  
~~~~~  
2001 CATATCCTTC ATTAGTTTT GTTTAAAAAA AATTATTTT TTTTATTAA TTTTTATAT TTTTTAAAAA AACTAATAT  
RBS 2  
~~~~~  
2081 AAAAGTATAT TGTTCAATAT ATGATTATC AAAAACCTT CTTTAAAAAA AATATTTAC TTTAATTAA GTGTATTTAG  
repA2 (2160 - 2912)  
~~~~~  
2161 TGCCTAGAAA AAATTATATA TATAATCTAA ACCATTTT TAATCCATCT AAAATGAAA GAAAAAAATC TACATTATT  
~~~~~  
2241 TGTTATGCAA TGAAAAAAGT ATCAGAAATA GACGTTGCTA GAAGTCATTT AAATCGTGCA TTATTACCAA TAGATCCTAA  
~~~~~  
2321 AACTGGTAAT GTTCTGCCTC GATTCAGACG ATTTAATAAA CATCGAGCAT GTGCTATGAG AGCTATAGTA CCTGCTATGT  
~~~~~  
2401 TGTATTATTT TAATATTGAC TCTAAATTAG TCGAAGCTTC TATTGAAAAA TTACAGATG AATGTGGATT ATCTACTTTA  
Term. 4  
~~~~~  
2481 TCAGATTCTG GTAATAAACAT CATCACTCGC GCTTCTCGTT TAATAAGTGA GTTTTGGAA CCAATGGGTT TTGTTAAATG  
~~~~~  
2561 TAAAAAAATA AATAGTAAAT CAATGAGTAA TTATATACCT AAAAAAATT TTTTAACTCC CATGTTTTT ATGTTATGTG  
~~~~~  
2641 GTATTCACC ATCAGAAATA AATCATTTT TATCAAAAAA AATAAACCCA TTAAAAAAAT TAAAAAAACA AGAAAAAAAGT  
~~~~~  
2721 GCATTTATTT CTTTACAGA TATGAAGATT ATATCGCAAT TAGATGAAAG ATCAGCTAGA ACAAAAATT TAAATGCTT  
~~~~~  
2801 AATTAATTAT TACACAGCTA GTGAATTAAAC AAAAATAGGT CGAAGGGTT TAAAAAAA GATAGATATT GAATATAGTA  
SL4  
~~~~~  
Prom. 4  
~~~~~  
2881 ATTTATGTAA TTTATATAAA AAAAATCAT AAAATAAATT TATACAATAA AAATTCATG AGACATCATA TACAAAATT  
RBS 3  
~~~~~  
2961 TTTGTATAT GATGCTTAT GAAATTTA TTGTATAAT TTATTTAT CCAATAAAC ATTCCATTAC CTTAATTGG  
leuA (3051- 4610)  
~~~~~  
3041 AAAAATTGTT GTGAATCTA AAGTTGTTAT TTTGATACC ACGCTACGCG ATGGAGAAC AGCATTACAA GCAAGCTTA  
~~~~~  
3121 GTGTTAAAGA AAAATTACAA ATTGCACTAT CTTAGAAAA ATGTGGATA GACATTTAG AAATAGGATT TCCTGTTCA  
~~~~~  
3201 TCACCCGGAG ATTTAAATC AGTCAAACT ATATCTAAA ATATTAAGAA TAGTCGAATA TGTAGTTAG CTCGTTGTAT  
~~~~~  
3281 AGAAAAAGAC ATCGATGCAG CCGGAGAAC TATGCTTCA TCTGATTCTT TTCGAATTCA TATTTTTA GCTACTTCAA  
~~~~~  
3361 CACTCATAT GGAATCTAAA TTAAAGAAAA ATTTAACGA AATAATAGAT ATGGCTGTT TTTCAGTAAA AAAAGCCTTA  
~~~~~  
3441 CGTTTAACTG ATGATATTGA ATTTCTTGT GAAGATGCTA CTAGAACTAC AATGGATAAT TTATGTCGTA TTGTAGAAC  
~~~~~  
3521 ATTAATTAAA TCAGGTGTGA AAACAATTAA TATCCCCGAT ACAGTAGGAT ATACTGTACC CAACGAATTAA TCTTGTATAA  
~~~~~

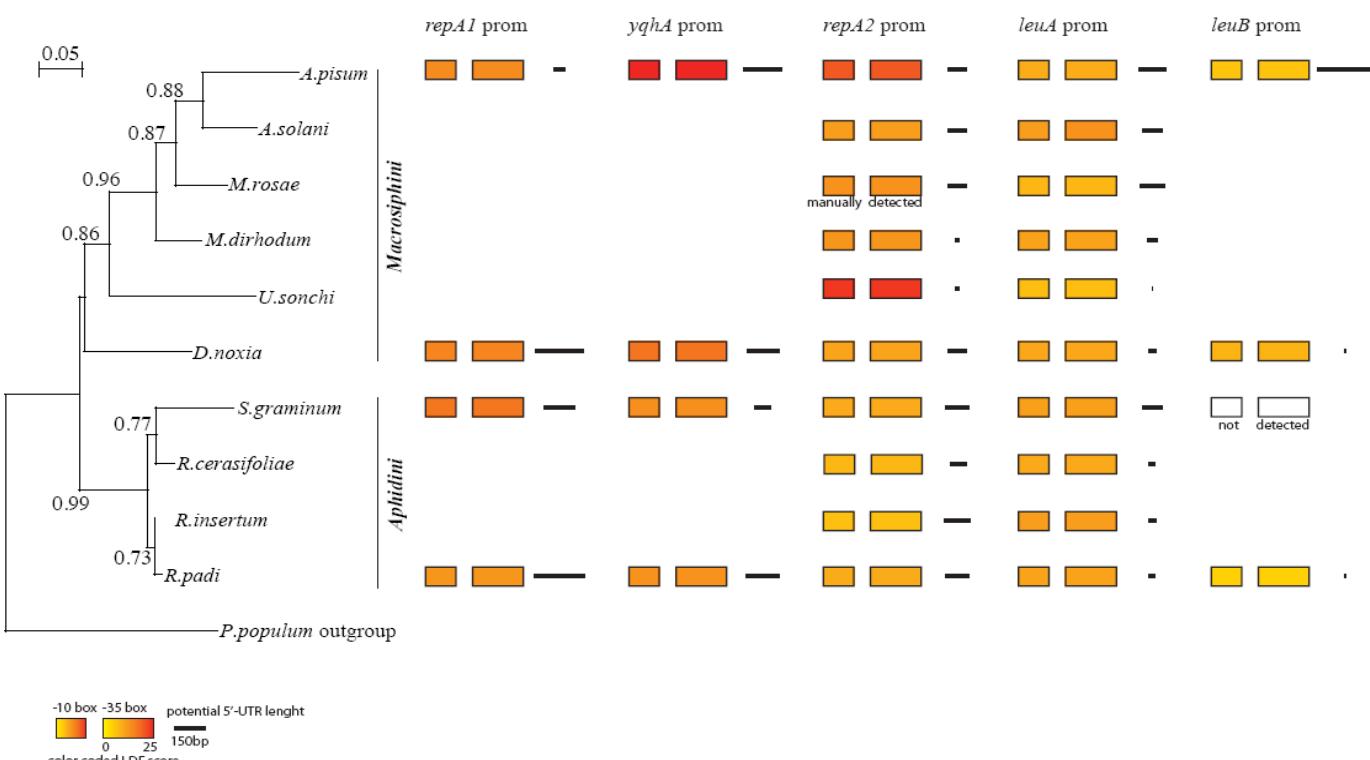
3601 TAAAAAATTT GCTTGAAAGA GTACCTAAC A TTCAAAATC TATAATTCA GTTCATTGTC ATGACGATT AGGCATGGCT  
 ~~~~~  
 3681 GTGGGAAATT CAATATCAGC TATACAGGCA GGTGCTAGAC AAATAGAAGG GACTATAAAC GGAATTGGCG AAAGAGCTGG  
 ~~~~~  
 3761 TAATACAGCA TTAGAAGAAA TAATTATGGC TATAAAAGTA AGAGAAGATA TCTTAAGTGT TTCCACTAAT ATTAATTATA  
 ~~~~~  
 3841 AAGAAAATTTA TCGAACTAGT AAAATTGTTA GTCAGATTG TAATATGCCA ATCCCATCTA ATAAAGCTAT AGTAGGTAGT  
 ~~~~~  
 3921 AATGCGTTG CACATTCTC AGGTATCCAC CAAGATGGTG TATTAAGAAA TAGAAAAAAT TATGAAATTA TGGAACCAAG  
 ~~~~~  
 4001 TAGTATTGGT TTAAAAGAAG TAAAACCTAA CTTAACCTCT CGTTCTGGAA GAGCAGCAGT AAAACATTAT ATGGATGAAA  
 ~~~~~  
 4081 TGGGTTATAA TAATAGTGAT TATAATATAG ATGAACCTTA TATTGTTTT TTAAAGTTAG CAGATAAAA AGGTAGGTT  
 ~~~~~  
 4161 TTTGACTATG ATTTAGAAGC ATTAGCTTTT ATTAATAAAC ACAAGATGA ATGGGAATAC TTTCTTAA AATTTTTAG  
 ~~~~~  
 4241 TGTGCAGTCT ATTTCTAATA GTTTATCTAC TGCATCAGTA AAATTATTAT GTGGCAAAA AACATACACA GAATCTTCTA  
 Prom. 5  
 ~~~~~  
 4321 CTACAAGCAA TGGACCAGTT GATGCTATT ACCAAGCATT AAATAGAATT GCATGTTTC CTATTATATT ACAAAAATTT  
 ~~~~~  
 4401 CAACTGTAG CTAAGGGGAA GGGTAAAGAT GCGTTAGGTC AAGTAGATAT CTTGGTTGAA CATAAAAAGGAAAGTTCA  
 ~~~~~  
 4481 TGGAGTGGGT TTGGCTACTG ATATTATTGA AGCATCAGCT CAAGCAATGA TCAATGTACT AAATAACATA TGGAAAGCAA  
 RBS 4  
 ~~~~~  
 4561 AACAAAGTTAA CAAAAAATTA AAAATTAA AAGATTAA AAAAAAATAA TACATCTAGA TATAGAAATA TAATATTTT  
 leuB (4671- 5762)  
 ~~~~~  
 4641 TGTTGATTT TATTAAGAG AAAAATTAA ATGAAAAAA ATTATCGTAT CGCTGTATTA TCTGGTATG GAATAGGTCC  
 SL5  
 ~~~~~  
 Term. 5  
 ~~~~~  
 4721 TGAAGTCATG CAAGAACAT GTAAAATTT AAATGTTTA AAAAATATT TTTTCTATC TTAGAAATA CAAAATTTA  
 ~~~~~  
 4801 ATATTGGTGG TATAGCTATT GAACGTGAAG GTGTTGCTTT GCCAAAAACT ACATTGCTAG GATGTGAAAA TTCTGATTCA  
 ~~~~~  
 4881 ATTTTATTAG GTTCTGTAGG TGGTAAGAAG TGGGATAACC TTCCAGTAGA ACAACGTCC GAAAGAGCAG CTTTATTGCC  
 ~~~~~  
 4961 TTTAAGAAAA TACTTAATC TTTTTCTAA TTTAAGACCA GCAAAATTAT ATCCAGAACT AAAATGTTA TCACCTCTC  
 ~~~~~  
 5041 GTTCAGATAT TGTAAGAAAT GGTTTGATA TATTATGTGT AAGAGAATTAA ACAGGTGGAA TTTATTGGA TGAACCTAA  
 ~~~~~  
 5121 GGCTTGAA ATAAAACAA TACTAAATAT GCTTTGATA CAGAAATTAA TCATGAGTAT GAAATTATCC GTATTGCTCA  
 ~~~~~  
 5201 TTTAGCTTT AAATTGGCAC GATCTAAGAA GAAGAAAGTC TGTCTATAG ATAAATCAA TGTCTTCGA AGTCTATT  
 ~~~~~  
 5281 TATGGGAAA AGTAGTTGAA AGTGTCTA AAAAATACCC AGATGTTCAT TTATCTCATT TATATATTGA TAATGCTGCT  
 ~~~~~  
 5361 ATGCAAATAA TTAAAGATCC TAATCAGTTT GATGTATTAT TATGCTCAA TCTTTTGGA GATATTATTT CTGATGAATG  
 ~~~~~  
 5441 TGCTACAATT ACAGGTTCAA TTGGGATGTT ACCATCAGCA AGTTTAATG AAAAATTT TGGATTATAT GAGCCGGCAG  
 ~~~~~

5521 GCGGATCAGC ACCTGATATT GAAGGTAAAA ATATTGCTAA TCCGATTGCT CAAATACTTT CACTTCTAT GTTAGTTAGA  
~~~~~  
5601 TATGGCATGA ATTTAACATCA GATAGCAGAT AAAATTGATA AAGCTGTTAA TAATGTATTA AAAAAAGGTT ATAGAACCTC  
RBS 5  
~~~~~  
5681 AGATATATCT CATGATAATA ATTTTTAAA AACAGATGAA ATGGGTGATC TTATTGTTGA TTCTTAAATT AATGGTAAAT  
*leuC* (5764 - 7179)  
~~~~~  
5761 GACATGAAGA AAACATTATA CGATAAAAATA TATGATTAC ATATTGTTA TGAAGAGAAA AATAATACAT CTCTTTATA  
~~~~~  
5841 TATAGATTTA CATTACTTC ATGAGGTTAC ATCACCTCAA GCCTTGATT CATTACGAGA TAAAATCGA AAGGTTAGAC  
~~~~~  
5921 AGCCTAAAAA AACTTTGCT ACAATGGATC ATAATGTTTC GACAACTAGT CAAGATATTA ATGCTTCAGG TTCTATGGCA  
~~~~~  
6001 AAGGTACAAA TGCAAGAATT AATTAAAAAT TGTCGAGT TTAATATATC ATTATATGAT ATAAAAAAATC CTAATCAAGG  
~~~~~  
6081 GATTGTTCAT GTTATTAGTC CTGAAAAAGG GATGACTTTG CCGGGTGTGA CTATTGTATG TGGTGAETCA CATAACATCGA  
~~~~~  
6161 CGCATGGCGC ATTTGGAGCA TTATCTTTG GTATTGATAC TTCAGAAGTT GAACATGTC TTGCAACTCA AACATTTAAA  
~~~~~  
6241 CAACAGCGTT TTAAAAATAT GAAAATAGAA ATTACAGGAG AAATTCAAAA ATTTGTCACT GCTAAAGATT TGATTTGTT  
~~~~~  
6321 TATTATTGGA AAATTAGGAT CATCTGGAGG TGCAGGATAT ATAATTGAAT TTTGTGGAA TGTGATTGAA AAAATGAGCA  
~~~~~  
6401 TGGAAGAAG GATGACAATT TGTAATATGG CAATTGAAAT AGGTGCAAAG TCAGGTTGA TAGCACCAGA TGAAGTGAC  
Term. 6  
~~~~~  
6481 TTTTCTTATC TGAAAATAA AATGTATGCA CCACGTGGTG TTTTTGGAA AAAAGCATTA AATTTTGGAA AGAATTGAA  
~~~~~  
6561 ATCTGATAAA AATGCTTTT TTGATAAAGT TGTGAATATA AATATTCTG ATCTCTCGCC ACAAATTACC TGGGGACAA  
~~~~~  
6641 ATCCTGATCA GGTGATCTCA ATCGATCAA AAATACCTGA TTTTAGTAGT TTTGATAATT TAATAAGAA AGATTAGCG  
~~~~~  
6721 AAATCTGCAT GTAAATATAT GGGTTAAAA ATAGGTACTT ATTTAACTAA TATTACTGTT GATGAAGTT TTATTGGATC  
~~~~~  
6801 TTGACTAAT GGAAGAATAG AAGATTAAG AGCTGCCTCA AAGATACTTA AAGATAAAA AATTGCTAAT AATGTTAAAG  
~~~~~  
6881 CTATTGTTGT TCCTGGATCA AGTCAGTTA AGAGAGAAGC TGAAAATGAA GGTTAGATA AAATATTAT TAATGCAGGA  
~~~~~  
6961 TTCGAATGGC GTTACCTGG TTGTTCTATG TGTTAGGTA TGAATAAAGA TAGATTAAT GATGGTGAAC GTTGTGCGTC  
~~~~~  
7041 TACTAGCAAT CGAAATTTG AGGGTCGCCA GGGGAGAGGG GGGCGAACAC ATTTAGTAAG TCCTATTATG GCGCCTGCAG  
RBS 6  
~~~~~ leuD (7182 - 7805)  
~~~~~  
7121 CTGCTGTATA CGGTAATTG GTTGATGTTA GAAAATTATA TAATGGTGAG AATAATTAAA TATGTTAAA TTTACTGGAC  
~~~~~  
7201 ATGCTGGTAT CGTTGTTCCCT TTAGATATAT CTAATATAGA TACAGATATT ATTATTCCGA AGCAATTGTTT GAAAAGAGTA  
~~~~~  
7281 AACAAAATTG GATTTGGTAA ATATTATTT CATGACTGGC GTTTTATTGA TGCAAATCAA TTAGTAAAAA ATGAGGATT  
~~~~~  
7361 TATACTCAAT AAAAAAATTG ATAAAAACGC AAGTATTTA TAACTAGAG AGAATTGGG TTGCGGCTCA TCGAGAGAAC  
~~~~~

7441 ATGCTGTTG GTCTCTAGTA GACTATGGAT TCAAAGTAAT AATTGCACCT AGTTTGCTG ATATTTTTA TAATAATAGT  
~~~~~  
7521 TTTAACATA AGCTTCTTT AATCACTTA AGTCGAGTG AAATTACTTT TTTATTTGAT ATTGTAAAAAA ATAATATAGG  
~~~~~  
7601 TATTACTTT GATGTAAGTT TAGTCGAAAA AACGGTTACT GTAAATAAGG AAGTATTTTC ATTTGAATTA GATGATTTC  
~~~~~  
7681 ATTACTTTG TTTGTTAAAT GATTTAGATA ATATTGATT GACTATGAAG CATTATCAG AAATAAAATC TTATGAAAGT  
~~~~~  
7761 CGTATATCTG ATTTTTTATT AGAAAGAAGA GATTCCAAT CTTAA

### Figure S3. Comparative evolutionary analysis of pLeu promoters in the Aphididae family.

The analysis was performed on ten aphid species belonging to two tribes of the Aphididae family which diverged more than 50 Myr ago: the Macrosiphini (*Acyrtosiphon pisum*, *Aulacorthum solani*, *Macrosiphum rosae*, *Metopolophium dirhodum*, *Uroleucon sonchi* and *Diuraphis noxia*) and the Aphidini (*Schizaphis graminum*, *Rhopalosiphum cerasifoliae*, *Rhopalosiphum insertum* and *Rhopalosiphum padi*). We were able to locate: (i) the *repA2* promoter in 9/10 species (mean LDF  $\pm$  SE =  $-12.0 \pm 1.6$ ); we identified however the *M. rosae* boxes by visual homology, the negative BPROM resulting from a one-base pair difference thresholding effect; (ii) the *leuA* promoter in 10/10 species (mean LDF  $\pm$  SE =  $-9.6 \pm 0.44$ ); (iii) the *yqhA* and *repA1* promoters in 4/4 species (mean LDF  $\pm$  SE being  $-16.2 \pm 3.0$  for *yqhA* and  $-14.2 \pm 0.9$  for *repA1*); and (iv) for *leuB*: the alternate promoter signal was located as a conserved internal signal to *leuA* cds for two *Macrosiphini* species, whilst only one potential signal was found for the *Aphidini* species, immediately upstream of the *leuB* cds in *R. padi*. All these *leuB* signals were the weakest found with BPROM, whether included, or not, in coding regions (LDF =  $-6.2 \pm 1.0$ ).



**Table S1. Neosynthesis of amino acids from the carbons of sucrose in the symbiotic pea aphid reared on diets with leucine concentrations ranging from 0 to 80 mM.**

Values (means  $\pm$  SE, n = 5) are expressed in sucrose equivalent (nmoles) per unit of aphid fresh weight (mg).

Amino acid	Dietary leucine concentration					P-value *
	0 mM	20 mM	40 mM	60 mM	80 mM	
Aspartic acid	9.1 $\pm$ 0.6	9.9 $\pm$ 0.5	10.1 $\pm$ 0.3	10.1 $\pm$ 0.4	10.2 $\pm$ 0.4	0.49
<b>Threonine</b>	<b>2.4 <math>\pm</math> 0.4<sup>a</sup></b>	<b>2.8 <math>\pm</math> 0.2<sup>a</sup></b>	<b>3.6 <math>\pm</math> 0.2<sup>b</sup></b>	<b>4.3 <math>\pm</math> 0.2<sup>b</sup></b>	<b>3.9 <math>\pm</math> 0.3<sup>b</sup></b>	<b>&lt; 0.001</b>
Serine	5.7 $\pm$ 0.3	5.9 $\pm$ 0.1	6.3 $\pm$ 0.3	6.8 $\pm$ 0.3	6.4 $\pm$ 0.5	0.22
Glutamic acid	18.8 $\pm$ 0.9	17.8 $\pm$ 0.7	18.3 $\pm$ 0.6	17.5 $\pm$ 1.0	18.5 $\pm$ 0.5	0.77
Proline	10.4 $\pm$ 0.2	12.0 $\pm$ 0.4	11.8 $\pm$ 0.5	11.6 $\pm$ 0.5	12.3 $\pm$ 0.6	0.14
Glycine	4.2 $\pm$ 0.2	3.9 $\pm$ 0.1	3.9 $\pm$ 0.3	4.1 $\pm$ 0.2	4.1 $\pm$ 0.1	0.77
Alanine	13.6 $\pm$ 0.9	13.9 $\pm$ 0.5	14.5 $\pm$ 0.5	14.8 $\pm$ 1.1	15.8 $\pm$ 0.2	0.21
<b>Valine</b>	<b>5.6 <math>\pm</math> 0.2<sup>a</sup></b>	<b>6.8 <math>\pm</math> 0.2<sup>b</sup></b>	<b>8.2 <math>\pm</math> 0.2<sup>c</sup></b>	<b>8.9 <math>\pm</math> 0.6<sup>c</sup></b>	<b>9.0 <math>\pm</math> 0.7<sup>c</sup></b>	<b>&lt; 0.001</b>
<b>Cystine</b>	<b>0.7 <math>\pm</math> 0.1<sup>a</sup></b>	<b>0.7 <math>\pm</math> 0.1<sup>a</sup></b>	<b>0.3 <math>\pm</math> 0.05<sup>b</sup></b>	<b>0.5 <math>\pm</math> 0.05<sup>ab</sup></b>	<b>0.5 <math>\pm</math> 0.1<sup>ab</sup></b>	<b>0.02</b>
Methionine	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2	0.8 $\pm$ 0.1	0.6 $\pm$ 0.1	0.25
Isoleucine	5.9 $\pm$ 0.4	4.4 $\pm$ 0.2	5.3 $\pm$ 0.3	5.4 $\pm$ 0.6	5.1 $\pm$ 0.3	0.14
<b>Leucine</b>	<b>19.7 <math>\pm</math> 1.4<sup>a</sup></b>	<b>6.7 <math>\pm</math> 0.6<sup>b</sup></b>	<b>4.8 <math>\pm</math> 0.3<sup>c</sup></b>	<b>3.3 <math>\pm</math> 0.4<sup>cd</sup></b>	<b>2.4 <math>\pm</math> 0.3<sup>d</sup></b>	<b>&lt; 0.001</b>
<b>Tyrosine</b>	<b>3.4 <math>\pm</math> 0.3<sup>a</sup></b>	<b>3.2 <math>\pm</math> 0.3<sup>a</sup></b>	<b>5.0 <math>\pm</math> 0.3<sup>b</sup></b>	<b>5.2 <math>\pm</math> 0.2<sup>b</sup></b>	<b>5.2 <math>\pm</math> 0.7<sup>b</sup></b>	<b>0.002</b>
Phenylalanine	6.4 $\pm$ 0.3	6.2 $\pm$ 0.2	7.0 $\pm$ 0.2	7.0 $\pm$ 0.5	7.4 $\pm$ 0.5	0.12
<b>Lysine</b>	<b>3.8 <math>\pm</math> 0.4<sup>a</sup></b>	<b>3.4 <math>\pm</math> 0.2<sup>a</sup></b>	<b>4.8 <math>\pm</math> 0.2<sup>b</sup></b>	<b>4.9 <math>\pm</math> 0.6<sup>b</sup></b>	<b>5.4 <math>\pm</math> 0.4<sup>b</sup></b>	<b>0.006</b>
Histidine	nd <sup>†</sup>	nd	nd	nd	nd	
Arginine	nd	nd	nd	nd	nd	

\* P-values of ANOVA (diet effect) performed for each amino acid. When the diet effect was significant (bold values, F-test P-value < 0.05), means were further compared with Student-Newman-Keuls test; columns with different letters are significantly different.

† nd = null value or below the detection threshold.

**Table S2. Sequence information on primers used for real-time quantitative PCR and operon validation experiments.**

For each couple of primers, sequences, lengths, annealing temperature and amplicon length are specified.

Gene	Primer	Sequence primer 5'- 3'	Primer length (in bp)	Primer Ta (in °C)	Amplicon length (in bp)
<i>atpA</i>	atpA-For	AAATGATTATTGCCTAA	19	47.1	99
	atpA-Rev	CAGAAAATATGAATATAGGGA	20		
<i>ilvH</i>	ilvH-For	GAATCAGGTGCACTATCAAG	20	46.8	90
	ilvH-Rev	AGGATCTCAGTAGGTGCTA	20		
<i>ilvI</i>	ilvI-For	TCTTTAATGGGATTAGGTAG	20	47.5	183
	ilvI-Rev	AACAATCGCATTG	15		
<i>leuA</i>	leuA-For	GATATACTGTACCCAACGAA	20	46.8	178
	leuA-Rev	ATTCCGTTTATAGTCCT	18		
<i>leuB</i>	leuB-For	TTGCCAAAAACTACATTGC	19	47.1	93
	leuB-Rev	TTCTACTGGAAGGTTATCCC	20		
<i>leuC</i>	leuC-For	AGCCTTGATTCATACGAG	20	47.4	127
	leuC-Rev	TACCTTGCCATAGAACCT	19		
<i>leuD</i>	leuD-For	ACATGCTGGTATCGTTGTC	20	44.6	125
	leuD-Rev	AACGCCAGTCATGAA	15		
<i>leuABCD</i>	ABCD-For	TAAACGGAATTGGCGAAAGA	20	50.8	3697
	ABCD-Rev	GATGAGCCGCAACCAAA	17		
<i>repA1</i>	repA1-For	ATGGGTTTGTTACTTGC	18	46.4	154
	repA1-Rev	TATTGATCCACCCTAATTGT	20		
<i>repA2</i>	repA2-For	ATTATATCGCAATTAGATGA	20	43.6	105
	repA2-Rev	TAAACCCCTCGGACTT	16		
<i>rplX</i>	rplX-For	AAAAAGAAGCACCTATTCAT	20	44.4	93
	rplX-Rev	TCCCTTCTCAAACCTAA	18		
<i>rpmC</i>	rpmC-For	GGCATTGGTAGAATTAGAA	20	46.8	184
	rpmC-Rev	CTCTCCTTGAATCTAGCA	20		
<i>yqhA</i>	yqhA-For	TTATCATGCCAGATATTGTG	20	47	174
	yqhA-Rev	CCATCCACCCCTAATCTT	18		

**Table S3. Descriptive statistics from the BestKeeper software of the three candidates for data normalization, the *atpA*, *rplX* and *rpmC* genes.**

On the three tested genes, only *atpA* and *rplX* met the criteria imposed by the Bestkeeper analysis: standard deviation  $\leq 1$  CP between the three tested conditions at each time point.

Abbreviations: n: number of samples; geo Mean [CP]: the geometric mean of CP; ar Mean [CP]: the arithmetic mean of CP; Min [CP] and Max [CP]: the extreme values of CP; std dev [ $\pm$  CP]: the standard deviation of the CP; CV [% CP]: the coefficient of variance expressed as a percentage on the CP level. Conditions showing significant gene variation ( $Cp >> 1$ ) are in red.

	12 hours			1 day			2 days			3 days			7 days		
	<i>atpA</i>	<i>rplX</i>	<i>rpmC</i>												
n	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
geo Mean [CP]	25.90	26.30	26.27	24.64	24.41	24.86	24.88	21.44	25.65	24.65	21.68	24.83	25.92	23.54	23.64
ar Mean [CP]	25.92	26.32	26.46	24.65	24.43	24.90	24.91	21.47	25.76	24.67	21.71	24.94	25.94	23.54	23.65
min [CP]	24.77	24.8	23.68	23.78	23.33	22.77	23.5	19.94	22.68	22.74	20.31	21.33	24.7	22.77	22.81
max [CP]	27.55	28.04	31.44	25.72	26.41	26.67	26.49	23.62	28.34	25.75	23.45	27.17	27.9	24.27	24.65
std dev [ $\pm$ CP]	1.01	0.98	3.02	0.67	1.07	1.22	0.99	0.89	2.07	1.05	0.98	2.13	0.92	0.49	0.58
CV [% CP]	3.89	3.73	11.42	2.70	4.37	4.90	3.97	4.15	8.03	4.25	4.53	8.54	3.56	2.09	2.43

