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ANALYZING VARIABILITY IN NECTAR AMINO ACIDS: COMPOSITION IS LESS VARIABLE THAN CONCENTRATION

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Abstract—Thirty species of flowering plants were analyzed for floral nectar amino acid composition. High-performance liquid chromatography (HPLC) was used in conjunction with AccQtag derivatization to produce accurate and precise data. For any one species, the total concentration of amino acids varies greatly (average coefficient of variation 0.65), but composition is much less variable (average correlation among samples from a single species 0.85). Absolute concentration of individual amino acids is much more variable than the relative abundance (coefficients of variation 0.98 and 0.77, respectively; $N = 544$, $t = 16.98$, $P < 0.001$). When amino acids that occur in only small relative abundance (<1%) are removed from the analysis, the difference is even more marked (0.78 and 0.51, respectively; $N = 344$, $t = 15.13$, $P < 0.001$). The results highlight the need for large sample sizes when making observations concerning the absolute amounts of amino acids in nectar and for sensitive analyses of the composition, as even small changes may be biologically significant.

Key Words—Nectar, amino acid, correlation, HPLC, flowering plant, composition, variation.

INTRODUCTION

Although early studies showed that the nectar of flowering plants contained substances other than sugars (Ziegler, 1956; Luttge, 1961), it was not until Baker and Baker (1973) showed that amino acids were largely ubiquitous in nectar that studies began to examine variation in composition both between and within species. Early studies used simple ninhydrin staining techniques to quantify total amino

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acid concentration (Baker and Baker, 1975). Despite limitations, the method is still used (Bernardello et al., 1991; Forcone et al., 1997). Other studies use thin-layer chromatography (TLC) to separate and identify component amino acids (Baker and Baker, 1976, 1977, 1979; Baker et al., 1978), with many studies electing to use a relative scale of concentration for the various component amino acids. More recent investigations employ high-performance liquid chromatography (Gottsberger et al., 1989; Lanza et al., 1995; Rusterholz and Erhardt, 1998), which provides a more precise and accurate determination of amino acid complement and concentration in a mixed sample (Cohen and Micheaud, 1993).

Based on TLC analysis, Baker and Baker (1977) stated that the amino acid complement of nectar was "remarkably constant" within a plant species. The relative concentrations of the amino acids present in each species were given, which, upon inspection, appeared to show impressive constancy of amino acid complement. However, the study by Lanza et al. (1995) determined that variation existed among individual plants within a single population of *Impatiens capensis* and between separate populations. Nectar from different flowers of a single individual showed no significant variability. More recently, Gardener and Gillman (2001) showed that the amino acid complement could be altered by soil fertilizer treatment, highlighting the fact that the use of HPLC allows greater sensitivity in detecting small changes that might remain undetected using previous techniques.

We present here the first quantitative assessment of the variability of nectar amino acid composition for a wide range of plant species. We also examine the variability of total amino acid concentration in those species. This is important, as previous studies have made assertions concerning the role of amino acids in nectar based upon measurements of the total concentration (e.g., Baker and Baker, 1973, 1977; Gottsberger et al., 1984). It is necessary for future studies to appreciate the variability in composition and concentration if we are to fully understand the ecological role of amino acids in nectar.

METHODS AND MATERIALS

Nectar Collection. Nectar was collected from plants growing wild in the vicinity of the Open University campus in Milton Keynes, Buckinghamshire, United Kingdom. In all cases, samples were taken from a number of individuals in the same neighborhood to minimize variability among populations or caused by soil conditions. Samples were taken at the same time of day (14:00–16:00 hr) and from flowers of approximately the same age (first day of dehiscence). This was to minimize effects of flower aging that have been shown to affect amino acid concentrations in nectar samples (Gottsberger et al., 1990; Petanidou et al., 1996). Predehiscent flowers were covered with a fine net (dress net, 1-mm mesh size) to prevent visitation by insects and so possible contamination or nectar removal. The following day the nectar of those flowers was withdrawn using 5-μl glass

graduated micropipet tubes, capillary action being enough to draw in the nectar. Precautions were taken to minimize possible contamination with pollen, which can release free amino acids in solution (Erhardt and Baker, 1990). In some species, the inflorescence was cut with sharp scissors, allowing the anthers to fall away, revealing the ovary. In other species (e.g., *Epilobium hirsutum*), this was not necessary, as the nectaries were easily accessible. In such species, care was taken to avoid touching the anthers with the pipet. The volume of each sample was determined by measuring the fluid column in the pipet. This measurement was a source of variability. Most samples were in the range of 1–2 μl. With 15 mm representing 1 μl and a measurement accuracy of 0.5 mm, this represents a random error in the range of 1–3.5%. Each sample was aspirated into a glass chromatography vial (Chromacol 02-CTVG) and frozen (at -40°C) shortly afterwards until analysis by HPLC. In total, samples from 30 plant species were collected.

Analysis of Nectar. Samples were thawed and amino acids derivatized using the AccQtag protocol (Waters Corp.) (Cohen and Micheaud, 1993) in a 0.02 M borate buffer (pH 8.6). HPLC was performed, with standards every four samples, using the following equipment: Waters 712 WISP autosampler, Waters 600 pump controller, Waters 600 HPLC pump with 510 pump-heads. Separation was achieved using a Novapak C_{18} (15 cm $\times$ 4.6 mm) cartridge with guard column. The binary solvent system was a 6:4 acetonitrile–water mix and a TEA–phosphate (pH 5.0) buffer. Detection was via a Waters 474 scanning fluorescent detector (excitation at 295 nm and detection at 350 nm). The system was monitored and data collected using the Waters Millenium^{32} software. Chromatograms were analyzed and compared to standards for identification of individual amino acids. Standard amino acids were made up to a concentration of 100 pmol/μl. In addition to all the protein-building amino acids, standards of hydroxyproline, ornithine, taurine, AABA, and GABA were used. Peak areas were compared to standards to determine the concentration of individual amino acids. From these data, the total concentration of all amino acids was determined and the proportion that each made to the total was calculated. A summary of the amino acid composition for each species is given in Appendix 1.

RESULTS

Total Concentration of Amino Acids. For each species the variability in total amino acid concentration among samples was determined by using the coefficient of variation, i.e., the standard deviation divided by the mean. Table 1 shows the coefficients for all the species analyzed. The mean coefficient was 0.67. Given this variability, the error arising from measurement of nectar in the collecting capillary is negligible.

Comparison of Composition. Each nectar sample produced a range of amino acids in varying proportions. In general, a sample contained a few, abundant amino

TABLE 1. SPECIES EXAMINED FOR NECTAR AMINO ACIDS BY HPLC[a]

Species	*N*	Corr	Vc
Agrostemma githago	42	0.91	0.6
Ajuga reptans	8	0.85	1.3
Calystegia sylvatica	7	0.83	1.2
Cardamine pratensis	7	0.94	0.9
Centaurea nigra	6	0.81	0.9
Centranthus ruber	18	0.92	0.2
Chamaenerion angustifolium	7	0.75	0.7
Cirsium vulgare	6	0.82	0.6
Convolvulus arvensis	6	0.93	0.3
Corydalis lutea	7	0.89	0.4
Epilobium hirsutum	7	0.74	1.0
Epilobium montanum	5	0.88	0.3
Lamium purpureum	6	0.99	1.0
Lamium album	7	0.93	0.5
Lavatera arborea	5	0.96	0.5
Lonicera hecrotii, Goldflame	4	0.83	0.3
Lotus corniculatus	10	0.75	1.0
Lunaria annua	5	0.99	0.8
Lychnis flos-cuculi	9	0.84	0.5
Lythrum salicaria	7	0.88	0.9
Primula veris	6	0.92	0.9
Primula vulgaris	6	0.82	0.8
Prunella vulgaris	7	0.96	0.3
Pulmonaria officinalis	3	0.91	0.4
Scrophularia scorodonia	5	0.91	0.6
Silene dioica	36	0.92	1.1
Stachys sylvatica	6	0.86	0.3
Trifolium pratense	7	0.94	0.4
Vicia sativa	6	0.84	1.0
Vinca major	6	0.96	0.4
Average		0.88	0.67

[a] Data columns are: number of samples analyzed (N), average intraspecific correlation (Corr), and coefficient of variation for total concentration of nectar amino acids (Vc). All correlations are statistically significant ($P < 0.001$).

acids that each contributed greater than 10% towards the total concentration, a number of smaller components with fractions in the range of 5–10%, and a larger number of amino acids each contributing <5% towards the total. These latter amino acids, many contributing <1% towards the total concentration, would undoubtedly have remained undetected if less sensitive techniques had been used.

The extent of the similarity of composition between two nectar samples can be determined by correlation of the amino acid concentrations (e.g., Figure 1). In this case, Pearson correlation coefficients were determined for all comparisons of compositions within each species. The mean correlation coefficient could be used

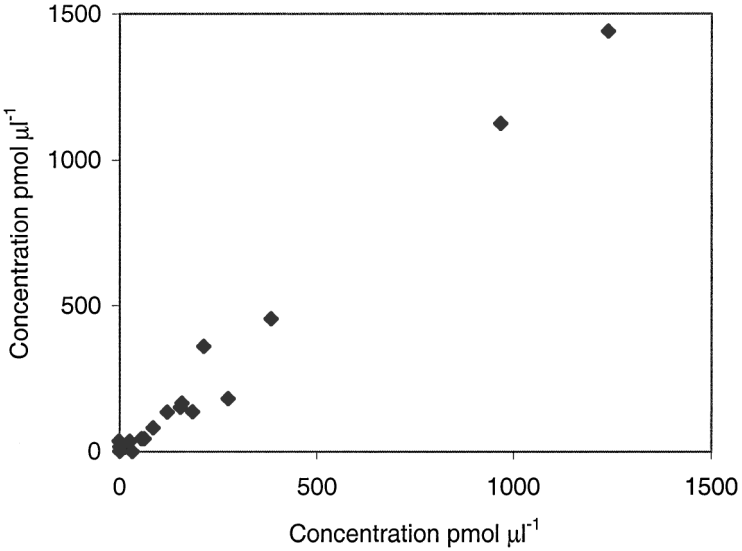

FIG. 1. Example of positive correlation of a pair of nectar samples (*Corydalis lutea*, $R = 0.993$, $N = 19$, $P < 0.001$).

to represent the variation in composition. This is a conservative (and potentially biased) measure, as one errant sample would have a greater effect upon the final mean by virtue of multiple paired comparisons. Table 1 shows the mean coefficient for each species analyzed. The mean of all the coefficients of variation was 0.88.

Correlation provides a useful tool to examine the similarity of samples. It would be expected that nectar samples from conspecifics would be more highly correlated to one another than to heterospecifics, and this could form the basis for an interesting analysis of nectar evolution, perhaps by comparing a cluster analysis of nectar samples to a molecular phylogeny.

Comparing Variability of Composition and Concentration. Although the correlation coefficient is useful as a guide to the constancy of composition, it is not directly comparable to the coefficient of variation of total concentration. In order to improve the comparison, we considered the set of samples from each plant species in turn. For each amino acid in the set of samples, a coefficient of variation for its absolute concentration was calculated. A similar coefficient was calculated for the fraction that amino acid contributed to the total (i.e., the relative abundance of the amino acid). The process was repeated for all 30 of the plant species, producing a database of 544 comparisons.

For each amino acid of each species, the coefficients of variation in absolute amount and relative amount were compared. The data were analyzed by using a

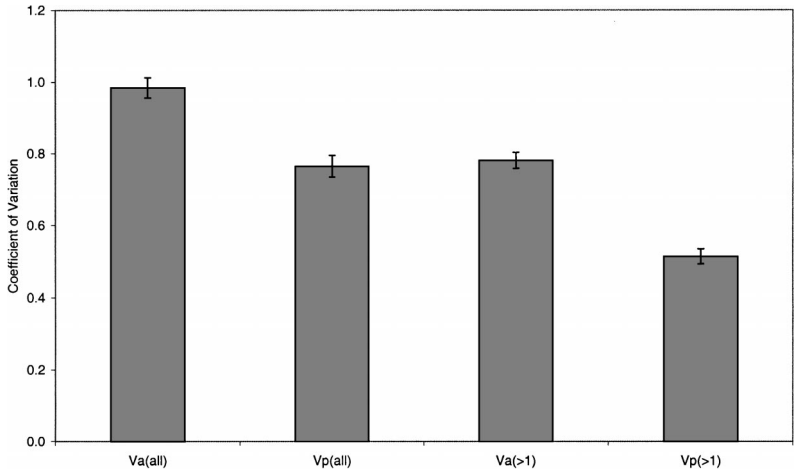

FIG. 2. Coefficients of variation for absolute concentration of amino acid and proportion (Va and Vp, respectively) for amino acids in nectar samples. First group represents all data while the second group is for amino acids contributing >1% to the sample. Error bars are standard error.

t test for paired samples, showing that the variability in absolute concentration was significantly higher than the variability in relative abundance (0.98 and 0.77, respectively, $N = 544$, $t = 16.98$, $P < 0.001$, Figure 2). It might be expected that the more abundant amino acids (>10%) would be less variable than the less abundant ones. To test this, the amino acids were categorized into abundance classes. Removing the last category (0–1%) of amino acids from the analysis, the difference in variability between absolute and relative concentration is more pronounced (0.78 and 0.51, respectively; $N = 344$, $t = 15.13$, $P < 0.001$, Figure 2). Variability of absolute concentration, in the range 0–1% was higher than all other frequency ranges (one-way ANOVA, $F_{6,537} = 18.205$, $P < 0.001$. post-hoc LSD test $P < 0.001$; Figure 3). Variability of relative abundance was also higher for this 0–1% group (one-way ANOVA, $F_{6,537} = 26.791$, $P < 0.001$, post-hoc LSD test $P < 0.001$; Figure 4).

The data in Figures 3 and 4 can be combined to produce a simple index of amino acid variability expressed as the ratio of variability in absolute concentration to variability in relative abundance (Va/V%). The ratio of variability of absolute to relative abundance changed as the component amino acids increase their share of the total (Figure 5). There appear to be three groups, the first being amino acids, contributing >20% to the total. Post-hoc LSD tests showed this group to have significantly higher Va/V% than all other groups ($P < 0.001$, one-way ANOVA, $F_{6,537} = 12.185$, $P < 0.001$). The second group was amino acids occurring in the range of 1–20%. Post-hoc tests showed no significant differences for any of

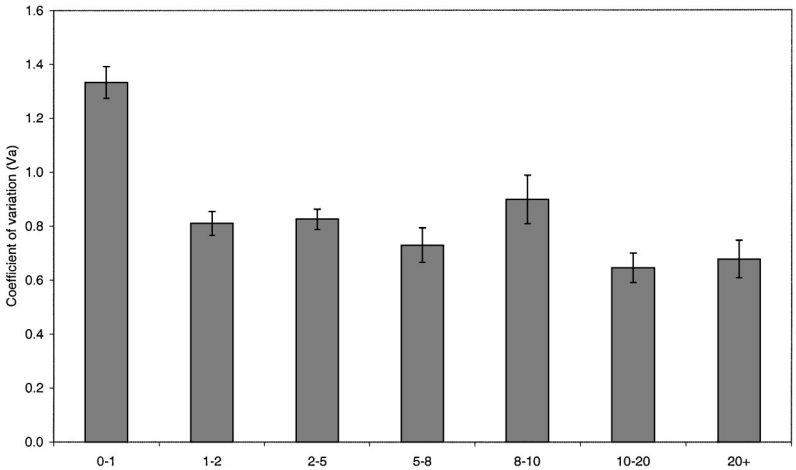

FIG. 3. Variability of absolute concentration of amino acids in nectar for various relative abundance ranges e.g., A0–1 = amino acids that contribute 0–1% to the total in the sample (one-way ANOVA, $F_{6,537} = 18.205$, $P < 0.001$).

the abundance classes within this range. A final category covers amino acids that contribute <1% to the total of the sample in which they occur. Post-hoc LSD tests show this group to have a lower Va/V% ratio than all the others ($P < 0.01$) except for the 8–10% group ($P = 0.08$) and the 10–20% group ($P = 0.13$).

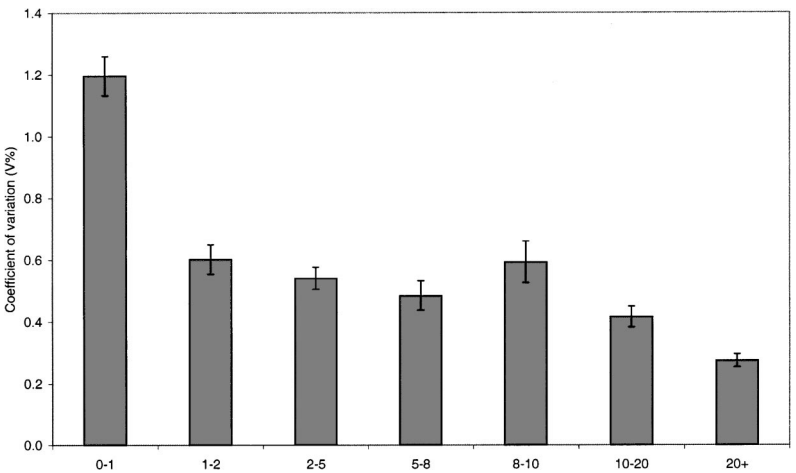

FIG. 4. Variability of relative abundance of amino acids in nectar for various relative abundance ranges (one-way ANOVA, $F_{6,537} = 26.791$, $P < 0.001$).

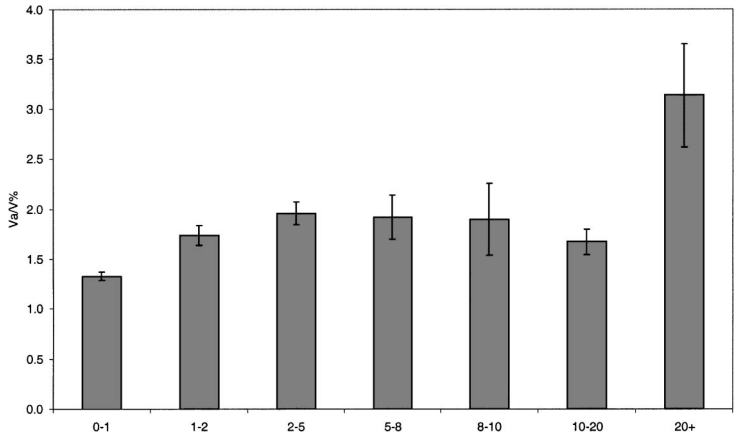

FIG. 5. The ratio of variability in absolute concentration (Va) to variability in relative abundance (V%) for amino acids occurring in nectar samples in various relative abundance ranges (one-way ANOVA, $F_{6,537} = 12.185$, $P < 0.001$).

DISCUSSION

These analyses show that the total concentration of amino acids in the nectar of any one species may vary widely. When making comparisons between species, therefore, it is important to ensure adequate replication and to use appropriately sensitive techniques. The composition, on the other hand, is much less variable. This is not entirely unexpected. The structure of the plant tissues that contribute to nectar production—the nectaries, phloem, and surrounding cells—are fixed by genetic processes and produce nectar of a certain species-specific, composition (Baker and Baker, 1977), although there can be variability among populations of a species (Lanza et al., 1995). The production of nectar is an active, energy-requiring process, which is curbed by respiratory inhibitors (Findlay and Mercer, 1971). Day-to-day environmental variations, in temperature and sunlight for example, are factors that will influence the metabolic processes of nectar production and may lead to changes in overall concentration of the nectar components. Physiological processes such as water relations may influence nectar concentration at the production stage, and evaporation may influence concentration afterwards. Longer-term environmental variables operating within a growing season, such as soil nutrients (Gardener and Gillman, 2001) and CO_2 (Rusterholz and Erhardt, 1998), are more likely to alter nectar composition by a variety of mechanisms e.g., altered metabolite availability and concentration, altered growth of plant tissues.

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APPENDIX 1. NECTAR AMINO ACID COMPOSITION DATA[a]

	Agrostemma githago ($N = 42$)		*Ajuga reptans* ($N = 8$)		*Calystegia sylvatica* ($N = 7$)		*Cardamine pratensis* ($N = 7$)		*Centaurea nigra* ($N = 6$)		*Centranthus ruber* ($N = 18$)	
N	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE
Hyp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Asp	510	64.0	115	37.6	303	158.5	138	54.3	422	269.4	216	11.9
Ser	766	106.3	240	67.4	0	0.0	251	101.8	734	471.8	497	44.3
Asn	33	16.3	49	25.3	4565	2301.6	17	18.5	2085	805.2	0	0.0
Glu	327	32.2	97	28.2	19	19.1	180	52.4	456	329.3	153	18.3
Gly	312	45.1	122	35.6	60	24.1	142	72.0	347	227.1	1118	61.5
Gln	6,193	700.4	1421	970.8	1321	718.9	1583	523.3	3955	1380.7	352	48.0
His	194	21.7	7	3.2	47	15.5	49	15.0	106	36.1	21	5.0
Tau	5	2.9	11	3.8	15	10.5	1	1.1	4	3.8	0	0.0
Arg	287	25.9	125	33.8	406	180.4	152	53.7	412	152.3	105	10.5
Thr	55	5.3	27	5.2	76	19.0	21	8.1	185	44.5	124	9.3
Ala	459	64.1	56	13.3	184	95.0	62	39.0	668	299.1	179	26.6
Pro	1,243	178.6	38	18.3	801	277.4	235	55.5	1697	743.4	31	14.3
Gaba	876	152.3	0	0.0	120	62.3	113	87.8	105	83.8	93	26.9
Aaba	2	1.9	0	0.0	0	0.0	0	0.0	3	3.1	0	0.0
Cys	13	5.3	0	0.0	220	100.3	8	8.3	0	0.0	0	0.0
Tyr	54	5.3	13	4.0	36	11.2	8	6.3	42	19.5	53	3.4
Val	305	26.8	32	8.1	368	185.3	84	28.2	243	80.0	497	18.4
Met	38	6.1	2	1.5	40	8.8	1	1.5	0	0.0	43	2.6
Orn	104	31.6	81	21.4	17	8.3	41	37.1	261	206.8	85	10.0
Lys	118	14.4	12	4.9	29	12.4	22	12.7	99	42.5	28	3.8
Ile	160	11.7	22	5.1	206	102.4	47	17.0	101	35.4	33	2.7
Leu	171	22.9	21	5.3	162	79.0	45	15.2	70	25.9	42	3.4
Phe	496	22.5	15	3.4	732	381.1	22	7.9	76	28.0	23	2.5
Trp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	12,730	1268.2	2507	1139.6	9726	4486.2	3226	1104.3	12070	4438.2	3692	195.4

APPENDIX 1. CONTINUED

	Chamerion angustifolium ($N = 7$)		*Cirsium vulgare* ($N = 6$)		*Convolvulus arvensis* ($N = 6$)		*Corydalis lutea* ($N = 7$)		*Epilobium hirsutum* ($N = 7$)		*Epilobium montanum* ($N = 5$)	
N	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE
Hyp	0	0.0	3	3.2	0	0.0	0	0.0	0	0.0	0	0.0
Asp	6	3.0	76	34.3	398	74.9	139	9.6	16	9.4	212	91.6
Ser	22	7.3	296	77.9	473	158.1	361	49.1	0	0.0	186	26.2
Asn	0	0.0	357	77.9	2332	373.8	109	41.3	39	7.4	0	0.0
Glu	14	3.1	140	37.7	266	74.9	137	14.7	3	2.3	95	23.5
Gly	2	1.7	141	36.6	264	74.9	140	25.6	5	3.6	81	12.5
Gln	66	18.9	976	293.3	1089	185.6	2231	474.6	22	8.7	200	43.5
His	12	6.4	49	9.0	60	23.8	37	3.1	19	5.5	63	21.1
Tau	0	0.0	4	2.3	92	24.2	1	1.5	0	0.3	0	0.0
Arg	30	2.8	209	51.2	447	97.4	297	24.6	17	3.4	46	4.8
Thr	10	4.4	29	9.3	39	11.0	12	7.8	1	0.6	4	3.9
Ala	3	2.1	176	53.3	266	62.3	85	7.6	47	38.4	0	0.0
Pro	12	11.7	465	57.3	64	42.1	53	27.5	212	93.3	1140	214.5
Gaba	0	0.0	34	15.2	68	36.4	7	4.8	1	1.5	131	56.4
Aaba	0	0.0	0	0.0	0	0.0	0	0.0	1	0.8	0	0.0
Cys	0	0.0	12	11.5	426	92.0	0	0.0	0	0.0	0	0.0
Tyr	1	0.8	38	7.1	55	15.5	21	3.0	1	0.7	0	0.0
Val	1	0.6	79	21.9	452	86.3	33	6.5	3	1.3	31	5.4
Met	0	0.5	0	0.0	29	16.9	56	9.2	0	0.0	25	4.1
Orn	0	0.0	77	23.1	124	37.3	105	20.8	0	0.0	33	13.4
Lys	1	0.7	19	7.5	47	9.4	43	5.5	1	0.7	6	6.4
Ile	2	0.7	49	11.5	320	63.2	14	1.5	2	0.8	18	2.9
Leu	3	0.5	33	11.5	194	45.9	1104	191.0	1	0.9	25	5.1
Phe	6	1.5	58	11.5	3074	305.9	228	33.9	5	2.0	14	2.0
Trp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	81	81.1
Total	190	51.1	3318	792.8	10580	1441.2	5198	695.3	397	157.2	2392	369.4

APPENDIX 1. CONTINUED

	Lamium album ($N = 7$)		*Lamium purpureum* ($N = 6$)		*Lavatera arborea* ($N = 5$)		*Lonicera hecrotii Goldflame* ($N = 4$)		*Lotus corniculatus* ($N = 10$)		*Lunaria annua* ($N = 5$)	
N	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE
Hyp	8	4.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Asp	10	5.6	124	35.4	178	38.8	53	8.4	551	156.6	264	75.4
Ser	55	17.5	155	31.0	0	0.0	130	45.9	385	136.1	200	68.2
Asn	32	8.7	138	50.1	1805	482.7	0	0.0	1876	952.0	288	97.2
Glu	0	0.0	89	24.4	0	0.0	48	5.0	347	115.5	181	83.8
Gly	14	6.1	83	16.7	9	4.3	55	20.2	182	43.5	17	14.7
Gln	142	61.2	3668	1686.4	246	86.3	257	106.4	1066	703.7	5606	2194.4
His	9	2.4	3	3.0	23	3.3	14	5.1	151	72.8	15	9.8
Tau	1	0.7	0	0.0	0	0.0	3	1.5	7	7.4	0	0.0
Arg	54	21.5	51	9.8	85	11.9	83	8.5	335	87.5	61	18.0
Thr	19	4.7	24	9.3	11	2.6	13	1.2	106	31.8	0	0.0
Ala	83	28.8	36	14.2	289	114.1	29	10.3	316	88.1	26	20.0
Pro	625	118.3	69	21.2	1005	153.5	418	30.1	1382	241.5	49	20.3
Gaba	0	0.0	46	16.9	0	0.0	0	0.0	52	34.9	0	0.0
Aaba	0	0.0	0	0.0	0	0.0	48	28.1	14	14.3	0	0.0
Cys	0	0.0	0	0.0	0	0.0	3	2.9	115	70.0	0	0.0
Tyr	243	42.3	3	3.0	7	0.7	8	3.3	1924	547.3	6	4.0
Val	14	3.8	46	9.3	19	6.5	28	6.5	167	51.0	87	29.4
Met	3	1.1	20	3.5	0	0.0	3	0.7	40	11.0	0	0.0
Orn	8	4.1	35	12.7	0	0.0	25	9.6	94	22.7	0	0.0
Lys	3	1.6	3	3.3	0	0.0	8	1.9	70	32.1	5	5.3
Ile	18	4.6	26	4.7	9	2.1	18	4.6	76	20.6	41	9.4
Leu	8	2.2	31	6.1	20	3.1	20	4.9	90	26.9	14	4.4
Phe	627	130.0	16	2.8	5	0.7	8	3.1	1879	519.3	16	4.3
Trp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	1976	386.9	4668	1922.3	3712	843.1	1271	202.2	11228	3472.4	6878	2580.9

APPENDIX 1. CONTINUED

	Lychnis flos-cuculi ($N = 9$)		*Lythrum salicaria* ($N = 7$)		*Primula veris* ($N = 6$)		*Primula vulgaris* ($N = 6$)		*Prunella vulgaris* ($N = 7$)		*Pulmonaria officinalis* ($N = 3$)	
N	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE
Hyp	0	0.0	4	2.9	0	0.0	0	0.0	0	0.0	0	0.0
Asp	195	47.7	230	93.3	86	37.1	81	12.2	67	12.7	150	61.6
Ser	402	91.5	266	130.4	0	0.0	112	14.6	356	30.4	264	118.3
Asn	150	24.4	244	91.3	278	122.0	210	102.4	0	0.0	16	16.0
Glu	248	49.9	201	119.6	169	54.3	164	31.7	49	15.5	162	61.1
Gly	44	9.6	95	36.3	61	26.3	63	9.3	182	14.6	125	64.8
Gln	3691	818.6	590	242.2	162	63.7	714	208.8	130	21.3	603	47.7
His	62	12.5	32	12.5	12	4.8	3	2.8	28	6.7	0	0.0
Tau	0	0.0	6	4.4	0	0.0	5	5.1	1	1.2	0	0.0
Arg	358	60.6	354	69.5	52	18.8	17	7.7	168	26.7	242	33.2
Thr	138	38.9	69	16.1	14	3.9	19	13.9	25	4.7	11	11.3
Ala	297	86.3	169	58.9	371	137.9	113	38.1	80	11.8	57	24.5
Pro	478	56.4	992	367.2	1022	372.7	726	317.9	955	118.7	141	30.1
Gaba	211	77.5	157	104.4	2	1.6	0	0.0	0	0.0	0	0.0
Aaba	0	0.0	0	0.0	0	0.0	0	0.0	40	40.1	0	0.0
Cys	0	0.0	8	7.9	0	0.0	0	0.0	2	2.4	0	0.0
Tyr	140	30.0	16	7.6	7	3.2	0	0.0	27	4.8	0	0.0
Val	277	60.3	143	43.1	30	10.7	32	6.1	38	5.3	38	11.3
Met	17	3.1	26	10.3	10	3.5	20	1.7	16	5.9	7	6.8
Orn	43	7.5	45	20.4	42	17.9	18	11.2	136	23.8	64	25.7
Lys	131	29.3	37	15.6	25	9.8	8	5.1	29	7.6	0	0.0
Ile	225	47.5	93	37.6	17	6.2	20	2.3	25	3.4	22	8.3
Leu	232	48.7	83	27.5	20	7.2	18	2.9	27	3.6	25	8.2
Phe	1437	244.4	2365	784.2	8	3.1	15	3.1	15	2.3	14	5.5
Trp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	8777	1561.2	6224	2049.8	2387	867.7	2358	744.6	2396	252.5	1941	484.4

APPENDIX 1. CONTINUED

	Scrophularia scorodonia ($N = 5$)		*Silene dioica* ($N = 36$)		*Stachys sylvatica* ($N = 6$)		*Trifolium pratense* ($N = 7$)		*Vicia sativa* ($N = 6$)		*Vinca major* ($N = 6$)	
N	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE	Av C	SE
Hyp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Asp	29	11.1	78	14.0	26	6.2	277	36.4	193	66.6	135	17.1
Ser	194	72.9	118	23.1	64	15.0	0	0.0	1057	504.8	0	0.0
Asn	0	0.0	5	4.7	0	0.0	1820	332.9	299	138.3	310	102.1
Glu	28	12.0	47	12.0	18	2.3	0	0.0	257	100.7	96	18.9
Gly	411	54.8	59	13.7	30	9.3	88	21.0	258	99.8	4	4.4
Gln	2190	779.2	1261	283.7	129	15.9	472	89.8	601	224.8	77	25.6
His	40	16.1	13	2.1	9	3.2	38	6.8	98	51.3	17	3.0
Tau	0	0.0	4	1.0	2	1.2	0	0.0	0	0.0	0	0.0
Arg	21	20.7	56	9.6	52	6.5	152	29.9	569	151.7	238	33.6
Thr	386	117.6	16	2.2	7	3.5	17	4.7	97	21.7	49	7.3
Ala	20	7.9	80	13.9	15	3.4	211	26.6	515	309.2	158	60.1
Pro	0	0.0	32	10.0	0	0.0	563	175.4	0	0.0	1281	217.6
Gaba	0	0.0	12	5.3	0	0.0	156	38.8	65	65.0	0	0.0
Aaba	0	0.0	0	0.0	0	0.0	49	30.2	0	0.0	0	0.0
Cys	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Tyr	19	4.9	8	1.4	6	2.3	18	4.0	40	22.2	0	0.0
Val	75	8.5	28	5.3	11	2.6	83	15.7	140	65.3	12	2.6
Met	10	3.5	2	1.2	4	1.3	19	4.0	8	5.6	9	1.4
Orn	37	8.7	31	8.8	15	4.4	55	15.8	82	28.6	2	2.3
Lys	814	132.2	6	1.8	6	4.1	16	4.6	28	13.3	3	1.8
Ile	9	4.4	13	2.5	8	2.2	49	9.8	89	46.8	7	1.5
Leu	11	5.1	24	5.6	10	4.1	40	8.7	104	49.3	20	2.6
Phe	10	7.4	11	1.4	22	7.1	28	5.3	80	37.4	3	1.6
Trp	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	4304	1124.9	1904	350.8	435	55.8	4151	626.7	4581	1928.1	2422	397.5

[a] Each amino acid and totals are given as picomoles of amino acid per microliter of nectar (equivalent to millimolar).

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