

SIMILARITY OF AMINO ACIDS IN NECTAR AND LARVAL SALIVA:  
THE NUTRITIONAL BASIS FOR TROPHALLAXIS  
IN SOCIAL WASPS

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Larvae of social wasps of the family Vespidae produce an oral exudate that is imbibed by attendant adults. Passage of this liquid from larvae to adults is the key feature of trophallaxis behavior (Wheeler, 1918), which has recently been reviewed by Wilson (1971), Spradbery (1973), and Jeanne (1980). Exchange of alimentary liquid among adults is also generally viewed as a component of trophallaxis, but this exchange is distinct from the larva-adult exchange (Hunt, 1982), and we will not consider it here.

Maschwitz (1966) showed that the larval exudate in *Vespula vulgaris* is chemically identical to the labial gland secretion. Morimoto (1960) fed larvae of *Polistes chinensis antennalis* a radioactively labeled solution, and he noted that no label appeared in the trophallactic liquid after 30 min, but at 3 h and thereafter label did appear. In another experiment Morimoto (1960) used radiotracers to confirm the uptake of larval liquid by the adults. Nutritive value of the liquid has been demonstrated by several studies (Table 1).

Attractiveness of the liquid for the attendant adult wasps has been widely reported. Roubaud (1916) proposed, in fact, that adult attraction to the larval secretion was the *raison d'être* of wasp sociality. This extreme position has been challenged by West-Eberhard (1978), who argues that polygynous social groupings of wasps antedate the monogynous social species, and so the attraction to larval secretions is not necessary to explain group living in wasps. While we feel that this may be true, it does not fully dismiss the potential importance of the larval secretions in the evolution of wasp sociality. Hunt (1982) has specified larva-adult trophallaxis as one of five varied feeding behaviors that may have affected social evolution in Hymenoptera. In this paper we will present new data on the nutritive value of the larval tro-

phallactic secretion, propose an hypothesis on the origin of larva-adult trophallaxis, and briefly discuss the significance of such trophallaxis in the social evolution of wasps.

#### MATERIALS AND METHODS

Nests of *Polistes* and *Vespula* species were collected near St. Louis, Missouri; a nest of *Vespa crabro* was collected near Hillsborough, Orange Co., North Carolina. Immediately following collection, samples of the larval trophallactic liquid were taken by teasing the mouthparts of medium to large larvae with a transfer pipette. The liquid exuded by the larvae in response was taken into the pipette and spotted onto Whatman #1 chromatography paper. Both single spots from single larvae and pooled spots from several larvae were taken. The dried samples were then analyzed for amino acid presence and concentration using techniques described for floral nectars by Baker and Baker (1976). Total protein was analyzed by the technique of Flores (1978); for the *Vespa crabro* samples, additional protein determinations were obtained using a fluorescent method that involves labeling with ANSA (8-anilino-naphthalene-1-sulphonic acid, ammonium salt, obtained from Turner Associates). Sugars were identified by single direction descending paper chromatography, followed by staining to detect glucose, sucrose, and fructose using oxalic acid dissolved in chloroform and acetic acid (Gal, 1968) and by staining to detect trehalose using anthrone (Weiner et al., 1970). Sugar concentrations in freshly collected saliva of two species were measured using a hand refractometer.

#### RESULTS

Table 2 presents the amount of liquid per droplet, total amino acid concentration, and total protein for the larval oral exudates of seven social wasp species.

TABLE 1. *Previously reported constituent analyses of social wasp larval salivas.*

Species	Sugars (%)	Protein (%)	Free amino acids (%)	Ammonia (%)	Uric acid (%)
<i>Vespula vulgaris</i>	9.0 <sup>2</sup>	—	—	.035 <sup>2</sup>	.004 <sup>2</sup>
<i>Vespula germanica</i>	8.3 <sup>2</sup>	1.8 <sup>1</sup>	1.4 <sup>1</sup>	—	—
<i>Vespa crabro</i>	8.0 <sup>2</sup>	1.8 <sup>1</sup>	1.4 <sup>1</sup>	—	—
<i>Vespa orientalis</i>	5.5 <sup>3</sup>	1.3 <sup>3</sup>	0.13 <sup>3</sup>	—	—

<sup>1</sup> Maschwitz, U., 1965, cited in Spradbery, 1973.

<sup>2</sup> Maschwitz, U., 1966.

<sup>3</sup> Ishay and Ikan, 1968.

TABLE 2. Free amino acid and protein concentrations in the larval saliva of seven social wasp species.

Species	Amount saliva/droplet ( $\mu$ l)	Amino acid concentration (mM)	Protein $\ddagger$ ( $\mu$ g/ $\mu$ l)
<i>Vespa crabro</i>	18.04 $\pm$ 16.76 (10)*	36.1 (10)*	0.174 (5)* (0.137–0.238) $\ddagger$
<i>Vespula maculata</i>	2.61 $\pm$ 1.49 (15)	32.1 (4)	1.568 (4) (0.563–2.280)
<i>Vespula maculifrons</i>	1.28 $\pm$ 0.49 (12)	32.4 (3)	1.049 (4) (0.422–1.604)
<i>Polistes annularis</i>	2.28 $\pm$ 0.96 (8)	27.2 (2)	1.781 (1)
<i>Polistes carolina</i>	7.46 $\pm$ 2.69 (15)	48.0 (3)	2.419 (4) (1.980–3.164)
<i>Polistes exclamans</i>	3.20 $\pm$ 1.34 (8)	95.1 (4)	1.138 (2) (0.563–1.712)
<i>Polistes fuscatus</i>			
nest 77-1a	1.60 $\pm$ 0.83 (8)	61.3 (2); 70.7 (2)	2.210 (3) (1.780–2.640)
nest 77-2	4.63 $\pm$ 0.40 (8)	48.1 (2)	3.164 (1)

\* Sample size

 $\ddagger$  Range. $\ddagger$  Values for *V. crabro* derived from fluorescent method using ANSA, all other values derived by the technique of Flores (1978).

Our analyses revealed that from 18 to 24 individual free amino acids are present per species. Table 3 presents the array of amino acids found and their concentrations in molar percentages. The assays for sugars revealed glucose to be present but not sucrose or fructose; no trehalose was detected. Table 4 presents sugar concentrations in the saliva of two species.

#### DISCUSSION

Adult wasps feed primarily on nectar and other plant juices, although some females do feed to a limited extent on the prey they capture (Evans and Eberhard, 1970 p. 13). The existence of a well developed, muscular proventriculus (the organ that regulates food flow from crop to midgut) gives evidence in its pump-like morphology of the strong adaptation of Hymenoptera to a liquid diet (Eisner, 1957). Bohart and Menke (1976) note that most members of the largest wasp family, Sphecidae, have short tongues and tend to frequent flowers with short corolla tubes such as in Compositae, Euphorbiaceae, Polygonaceae, and Umbelliferae. The early evolutionary history of both Angiospermae and higher Hymenoptera (i.e., Aculeata: true wasps, ants, bees) is obscure, but it seems clear that flower visitation by hymenopterans played a significant role in the radiation of both groups (Baker and Hurd, 1968). The visitation of flowers by wasps thus seems to be both well developed and of very long standing.

We suggest that a valid comparator for nutritive value of the larval secretion in social wasp species is floral nectar. Not only are floral nectars widely used as nourishment sources by adult aculeate hymenopterans, but the extensive studies of Baker and Baker (1973a, 1973b, 1975; Baker, 1977, 1978) have shown a convincing correlation between pollinator type (e.g., bee vs. bird vs. fly) and concentration of amino acids in nectar. Based on these correlations and other stud-

ies it now seems highly probable that many nectars have significant nutritive value to anthophilous animals in addition to the obvious carbohydrate component.

The larval salivas we examined are generally richer in concentration and variety of amino acids than are the vast majority of flower nectars. The amino acid concentrations of flower nectars have usually been scored on a "histidine scale," where the highest score (10) was equal to 25.0 mM (Baker and Baker, 1973b). The amino acid concentrations reported here for wasp larval secretions range from a low of 27.2 mM (*P. annularis*) to a high of 95.1 mM (*P. exclamans*) in histidine equivalents. The variety of free amino acids in wasp larval secretion is also greater than in the majority of floral nectars, with from 18 to 24 individual amino acids being found. By these comparisons, then, the social wasp larval secretions can be seen as significantly more nutritious, in terms of free amino acids, than are most floral nectars. However, "wasp-flower" nectars are unusually high in amino acid concentrations compared with, for example, bee flowers or those visited by bats or hummingbirds (Table 5).

The usefulness to adults of the protein and amino acids in the larval trophallactic secretion has been seen differently by various authors. Roubaud (1916) discussed trophallaxis behavior in the context of a reciprocal food exchange, with larval provisioning as the complementary behavior. Other functions ascribed to the larval saliva include a role in regulation of nest temperature and humidity (Weyrauch, 1936, in Wilson, 1971), larval excretion (Brian and Brian, 1952), and as an aid in the ingestion and digestion of solid foods (Spradbery, 1965). Maschwitz (1966, in Wilson, 1971 p. 283) indicated that the Brian and Brian excretory hypothesis is based on incorrect experimental evidence. Wilson (1971 p.

TABLE 3. Proportions of each acid\* in total (on molar basis).

Amino acid	<i>V. crabro</i>	<i>V. maculata</i>	<i>V. maculifrons</i>	<i>P. annularis</i>	<i>P. carolina</i>	<i>P. exclamans</i>	<i>P. fuscatus</i>
Alanine	.0523	.0123	.0152	.0144	.0453	.0515	.0818
Arginine	.0451	.0862	.0374	.0834	.0136	.0048	.0102
Aspartic acid	.0294	.0185	.0066	n.d.	.0109	.0129	n.d.
Asparagine	n.d.	.0172	.0094	.0417	n.d.	n.d.	.0934
Cysteine	.0425	.0074	.0073	.0628	.0952	.0145	.0076
Cystic acid		.0529	n.d.				
Glutamic acid	n.d.	.0123	.0230	n.d.	.1088	trace <sup>?</sup>	n.d.
Glutamine	.0621	n.d.	.0349	.1451	n.d.	.0781	.1863
Glycine	.1112	.0308	.0529	.0550	.0870	.0966	.1626
Histadine	.0392	.0049	.0047	.0056	.0122	trace	trace
Isoleucine	.0340	.0191	.0187	.0223	.0204	.0592	.0117
Leucine	.0366	.0271	.0132	.0295	.0245	.0555	.0165
Lysine	.0320	.0222	.0081	.0778	.0102	.0258	.0354
Methionine	.0327	.0382	.0062	n.d.	.0102	.0044	.0025
Phenylalanine	.0432	.0356	.0264	.0726	.0408	.0724	.0190
Proline	.1962	.2917	.5620	.1667	.2910	.2221	.1743
Serine	.0458	.0418	.0200	.0239	.0422	.0190	.0200
Threonine	.0589	.0209	.0111	.0339	.0354	.0185	.0203
Tryptophan	.0425	.0160	.0087	.0137	.0085	.0137	.0051
Tyrosine	.0582	.0283	.1058	.0893	.0925	.1288	.0759
Valine	.0379	.1046	.0028	.0257	.0340	.0668	.0379
$\gamma$ -NH <sub>2</sub> -butyric		.0357	n.d.	.0316	n.d.	n.d.	.0407
Unknown		.0640			.0109	.0097	
Unknown		.0117			.0122		
Unknown		.0006					

\* Free amino acids.  
n.d. = not detected.

283), citing the work of Maschwitz (1966) and Montagner (1963, 1964), dismisses the protein and amino acids as "present in the saliva but at only one-fifth the concentration in the hemolymph [of the larvae]." Wilson (1971) echoes the position of Roubaud (1916) in viewing larva-adult trophallaxis as an exchange, with the larvae trading sugar for protein (the solid provisions fed to them by adults). Spradbery (1973), by comparison, cites the studies of Ishay and Ikan (1968) on oogenesis in *Vespa orientalis*, noting that a queen *V. orientalis* produces about 4,000 eggs, weighing about 8,000 mg, over the course of a season. He notes that the queen drinks large quantities of larval saliva, containing amino acids, thus enabling her to sustain her high rate of oogenesis. The review of trophallaxis by Jeanne (1980) also stresses nutritional aspects of the behavior. The data we have

TABLE 4. Sugar concentration, in sucrose equivalents, of the larval saliva of two social wasp species.

Species	$\bar{x}$	% Sugar range	N
<i>Polistes fuscatus</i>	7.4	(6.7-7.7)	4
<i>Polistes metricus</i>			
nest 79-1	6.6	(5.4-7.3)	4
nest 79-2	7.8	(6.8-8.3)	4
nest 79-3	6.6	(5.8-7.7)	4

presented here confirm and clarify the nutritional value to adult wasps of the larval saliva.

All social wasps of the family Vespidae malaxate (macerate) prey before offering the prey to their larvae. (Malaxation is absent in the "social" sphecid *Microstigmus comes* [Matthews, 1968a, 1968b]) though the evidence for eusociality in this species is equivocal [West-Eberhard, 1978]). It is known that

TABLE 5. Means of total amino acid concentrations in wasp larval salivas and in floral nectars grouped according to type of predominant pollinator. Floral nectar data are from Table 3.6 in Baker (1978).

Source	No. of determinations	Amino acids (mM)
Wasp larval saliva	9	50.111
Floral nectars:		
Settling moths	51	1.150
Wasps	32	.875
Butterflies	84	.820
Short-tongued bees	76	.650
Flies	22	.585
Hawkmoths	34	.450
Long-tongued bees	49	.398
Bats	15	.275
Hummingbirds	55	.255

*Polistes* females extract hemolymph from prey items during malaxation (Yoshikawa, 1962; Hunt, unpubl. data). The ingested hemolymph is typically regurgitated to larvae, though a portion may be retained by the adult (Hunt, unpubl. data); the extent of adult nourishment by this means is unknown. The significant point of this behavior as regards the present discussion is that the food mass remaining after malaxation that is fed to larvae must certainly be at least sometimes rather dry or sticky. Spradbery's (1965) suggestion that exudation of saliva by a larva to facilitate ingestion of the provision seems reasonable. The close contact between a provisioning adult and the larva makes it likely that an adult would encounter this saliva. We suggest that the impetus for larva-adult trophallaxis was set in motion when provisioning adults first encountered saliva that bore at least a faint resemblance to the primary adult food, floral nectar. We feel that selective value would have accrued to the adult female feeding on such saliva via increased longevity and, hence, increased fecundity. In order for such selection to occur it is not necessary that the larvae themselves benefit directly from the trophallactic exchange.

West-Eberhard (1978) has argued that trophallaxis need not be invoked as a mechanism to explain the origin and maintenance of group life in wasps. We agree. We feel, however, that though group living among cooperating adults may have been antecedent to the origin of sterile castes as West-Eberhard (1978) proposes, trophallaxis may still be seen as a significant component of wasp social evolution. Malaxation of prey items is a significant antecedent to wasp sociality (Evans, 1958; Evans and Eberhard, 1970). Trophallaxis, as a behavior derived from malaxation, may have contributed to social evolution in any of three ways. (1) Adult wasps engaging in trophallaxis may be freed of the need to gather floral nectars for self-sustenance. These wasps therefore may restrict flights from the nest to searching only for larval provisions and nest construction materials, and so they may be more efficient in foraging and in larval care per unit time than are wasps that do not practice trophallaxis. (2) The reliably available and highly nourishing food made available to adults may be an important contributor to reproductive longevity, which is a critical component of wasp eusocial evolution (Evans, 1958; Evans and Eberhard, 1970). That is, the larval regurgitation might conceivably provide adult nourishment adequate to sustain the foundress through periods of inclement weather or dearth of flowers that might be fatal to a solitary wasp. (3) If larvae that engage in trophallaxis diminish their own nourishment, then such trophallaxis may contribute directly to the trophogenesis of a worker caste, as first proposed by Marchal (1897). Wilson (1971 p. 180) has said: "In two articles published in 1896 and 1897 Paul Marchal postulated that the physical divergence of queen and worker castes of the Vespinae is based on nutritional discrimination during larval growth. He considered the workers to be victims of 'nutritional castration' (*castration nutritivale*). Deprived of adequate nourishment in their

larval period, these individuals have ended up as stunted adults with underdeveloped ovaries. In spite of its simplistic sound, Marchal's idea may well be close to the truth in social wasps generally."

Detailed examination of these three propositions seems a fruitful area for further research. We do not propose, however, that trophallaxis is an essential component of hymenopteran eusocial evolution in general, for the behavior is entirely lacking in bees.

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## SEED DISPERSAL AND THE EVOLUTION OF DIOECISM IN FLOWERING PLANTS—A RESPONSE TO HERRERA

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Bawa (1980) and Givnish (1980) found that a disproportionately high number of dioecious species in angiosperms and gymnosperms have fleshy fruits. To explain this correlation, they proposed that the probability of a male-sterile mutant spreading in species dispersed by animals, especially birds, might be greater than in species with other modes of dispersal, if an increase in maternal reproductive expenditure in the form of greater number of fruits resulted in a disproportionate gain in fitness via increased seed dispersal. The two assumptions implicit in the model were that 1) the larger the size of the fruit crop, the greater the proportion of seeds dispersed by specialist

frugivores (Howe and Estabrook, 1977); and 2) male sterile mutants accrue gains in maternal reproductive investment by reallocating resources from paternal to maternal functions. Herrera (1982) has indirectly tested the seed dispersal hypothesis for the evolution of dioecism by comparing fruit crops of hermaphroditic (including monoecious species) and dioecious species which have their seeds dispersed by birds. Although Herrera concedes that a valid test of the hypothesis advanced by Bawa (1980) and Givnish (1980) must await *within-species* comparisons, he makes broad *between-species* comparisons of fruit quantity and fruit quality and concludes that his re-