

The presence of the digestive enzymes in the excreta of insects, appears at first, to be a wasteful process. In *S. inferens* and *C. infuscatellus* they may have the function of keeping the microenvironment congenial, since the larval and pupal life of these insects is passed in a tunnel in sugarcane in which the excreta is also discharged.

**Zusammenfassung.** In den Fäces von *S. inferens*-Larven wurde eine beträchtliche Aktivität von Aminotripeptidase, Leucin-Aminopeptidase und Glycyl-L-Leucin-Dipeptidase, sowie eine sehr schwache Aktivität von Trypsin, Prolinase und Glycyl-Glycin-Dipeptidase festgestellt. In den Fäces von *C. infuscatellus*-Larven wurde eine Aktivität von Aminotripeptidase, Leucin-Amino-

peptidase und Prolinase, jedoch nur eine geringe Aktivität von Trypsin und Glycyl-Glycin-Dipeptidase gefunden. Es konnten keine Carbohydrasen festgestellt werden.

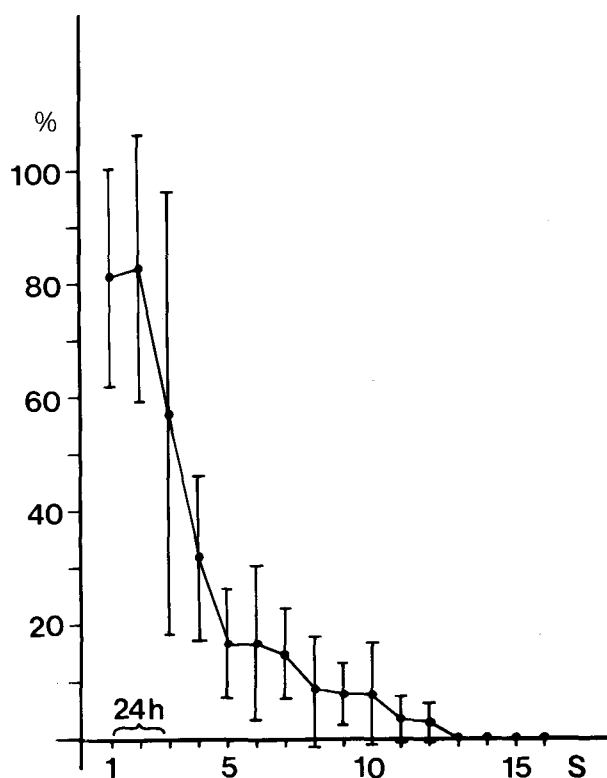
A. K. AGARWAL<sup>8</sup>

Department of Zoology, University of Lucknow,  
Lucknow (India), 9 October 1974.

<sup>8</sup> Acknowledgments. The author wishes to express his thanks to Prof. R. RAKSHPAL for his valuable guidance, to Dr. S. B. SRIVASTAVA for translating the summary into German and to University Grants commission, India for awarding a junior Research Fellowship to him.

### Long-Term Habituation to Species-Specific Alarm Calls in a Songbird (*Fringilla coelebs* L.)

When their species-specific alarm call, the 'pink' vocalization, is played back to chaffinches (*Fringilla coelebs* L.), they respond with a variety of behaviour patterns, one of which is 'freezing'<sup>1,2</sup>. Our study is concerned with how such a behaviour pattern changes, from a quantitative point of view, when captive birds are presented with a constant alarm call pattern stimulus twice daily. We also determined to what extent the changes could be considered as 'stimulus specific'. — This investigation was conducted in order to elucidate the effects of natural signal variation on the behaviour of conspecifics.



Long-term habituation to the species-specific alarm calls in the chaffinch (*Fringilla coelebs*): Changes in the response 'freezing' after repetitive stimulation twice daily with a 'pink' call pattern. Ordinate: Duration of 'freezing' (mean and standard deviation of the mean for  $n = 4$ , in % of the maximal value). Abscissa: sequence of stimulus presentations (S).

The birds were placed in a visually and acoustically isolated room 3 days before the experiments started. Locomotory reactions as well as freezing were registered by perch contact and transcribed directly on a multi-channel event recorder. Other responses were recorded on the same equipment over a keyboard by a hidden observer. The illumination was a 12:12 h on/off light cycle. The birds were fed every other day. The stimulus consisted of a series of 10 trisyllabic alarm calls at normal intensity with an interval of 1 sec between them. These were played at a constant volume through a loudspeaker placed near the cage. The length of 'freezing' brought about by the stimulus decreased with the number of stimulus exposures (Figure). Other behavioural parameters showed a similar decrease. This decrease was more marked at the beginning than at the end of the experimental series. A 'warming-up-phase' was only observable in a few of the experimental animals. After approximately 12 stimulations, the animals showed none of the responses observed at the start of the experiments. Only some of the orientation components, which are not included here, remained.

The question as to whether it was the one specific 'pink' alarm pattern which no longer acted as a releaser, or whether the response to other types of stimulus constellations was affected, was tested on conclusion of the above experiments. The animals were presented with stimuli varying both qualitatively and temporally (Table). Practically every change in the original stimulus resulted in a response from the birds (exception: decreased volume, Table, B). The response strength in the tests, however, never reached the maximum or original level. When the original stimulus pattern was presented daily throughout the above test series, the response to it was always nil (Table, K). The 'inhibition' of the releasing effect was thus shown to be stimulus specific and of long duration.

This 'inhibition' can be interpreted as long-term habituation. Operationally defined, this type of habituation is considered as a relatively long-term stimulus specific decrease in response to a repetitive uniform stimulus, not followed by a reinforcement<sup>3,4,1</sup>. Owing to its long-term effects, habituation is considered to be a form of learning. Here specific stimulus parameters, which act as releasers at first, are stored with a negative prefix and computed in the functional context of the releasing

<sup>1</sup> P. MARLER, *Ibis* 98, 231 (1956).

<sup>2</sup> W. FLEUSTER, *J. Ornith.* 114, 417 (1973).

<sup>3</sup> W. H. THORPE, *Learning and Instinct in Animals* (Methuen, London 1963).

<sup>4</sup> S. E. FILE, *Anim. Behav.* 21, 585 (1973).

Specificity tests: Response of habituated chaffinches (*Fringilla coelebs*) to modified forms of the species-specific alarm call pattern

Test	Stimulus	Duration of 'freezing' (mean and SD of the mean for $n = 4$ , in % of the maximal value)
	e.s.: 10 trisyllabic 'pink'-calls with 1 sec intervals	
A	same as above, from another individual, more noisy	$81.5 \pm 19.8$
B	e.s., half volume	$25.3 \pm 19.5$
C	e.s., intervals 2 sec	$0.0 \pm 0.0$
D	10 bisyllabic 'pink'-calls with 1 sec intervals	$4.3 \pm 4.9$
E	e.s., double volume	$25.0 \pm 21.6$
F	10 trisyllabic 'pink'-calls with 5 sec intervals	$9.0 \pm 7.0$
G	15 bisyllabic 'pink'-calls with 1.3 sec intervals	$2.5 \pm 1.5$
H	e.s., half speed	$12.3 \pm 4.5$
I	e.s., double speed	$2.3 \pm 2.3$
K	e.s. daily, alternating with the tests	$33.3 \pm 11.8$
		$0.0 \pm 0.0$

The first response to the experimental stimulus (e.s.) is given above for comparison.

<sup>5</sup> PH. GRAMET, *Annls. Epiphyt.* 13, 111 (1962).

<sup>6</sup> W. KEIL, *Jber. wetterau. Ges. ges. NaturKde.* 123/124, 15 (1973).

<sup>7</sup> R. A. HINDE, *Proc. R. Soc., Lond. B* 142, 331 (1954).

<sup>8</sup> M. SCHLEIDT, *Z. Tierpsych.* 11, 417 (1954).

<sup>9</sup> Thanks are due to Dr. O. ANNEF, RASA, Marburg/Lahn, who kindly translated the manuscript. This investigation was supported by the Deutsche Forschungsgemeinschaft.

mechanism. These criteria for a long-term habituation can be applied to our results: we could show that, under laboratory conditions, chaffinches habituate to a species-specific alarm call pattern. This type of behavioural adaption has also been observed in the acoustical control of bird flocks on airfields or in crops<sup>5,6</sup>, but apparently has not been studied from a quantitative point of view nor in terms of learning processes. Habituation to visual stimuli, such as owl models and even living owls, has already been shown<sup>7</sup>. Phenomena of this type are probably basically different from short-term habituation which is also known for birds<sup>8</sup>. The almost complete loss of response to an enemy, as well as habituation to alarm calls, appears to have little adaptive value. In the field it would not occur to this extent, owing to habituation preventive factors such as the animal's continuously varying external and internal situation. Whether a generalized habituation to variant acoustical signals occurs, is to be tested by further experiments.

**Zusammenfassung.** Isoliert gehaltene Buchfinken (*Fringilla coelebs* L.) reduzieren fast alle ihre Verhaltensreaktionen auf Tonaufnahmen arteigener Alarmrufe bei täglich zweimaliger Darbietung desselben Musters nach ca. 6 Tagen auf einen Nullwert. Die fehlende Auslöserwirkung ist an das spezifische Muster der Gewöhnungsattrappe gebunden. Geringfügige Änderungen in verschiedenen Parametern riefen wieder Reaktionen hervor.

H. ZUCCHI and H.-H. BERGMANN<sup>9</sup>

*Fachbereich Biologie der Philipps-Universität Marburg, Ketzerbach 63, D-355 Marburg/L. (German Federal Republic, BRD), 21 February 1975.*

## The Growth-Retarding Effect of Guanidino-Methylated Arginines on the Tobacco Tissue Cultures

Guanidino-methylated derivatives of L-arginine/ $N^G$ -mono-methyl-L-arginine (MMA),  $N^G, N^G$ -dimethyl-L-arginine (DMA) and  $N^G, N^G$ -dimethyl-L-arginine (DMA') have recently been detected in some animal<sup>1</sup> and plant proteins<sup>2</sup>, as well as in free state in various tissues and biological fluids<sup>3</sup> as catabolic compounds of the enzymic methylated proteins.

The role of these methylated basic amino acids in the living organisms has not been cleared up so far. On the basis of the well-known growth-promoting effect of arginine<sup>4</sup>, as well as of the tumour growth promoting<sup>5</sup> and growth promoting effect on the tobacco tissue cultures of  $N^G$ -methylated lysines<sup>6</sup>, we supposed the guanidino-methylated arginines to have an inhibitory effect on growth.

The present studies continued the comparative investigation of the effect of L-arginine and its 3 guanidino-methylated derivatives (MMA, DMA' and DMA) on the tobacco tissue cultures, and examined the stability of guanidino-methylated arginines in culture medium and in tissue cultures.

The test material used in our investigations was a secondary callus tissue isolated from tobacco (*Nicotiana tabacum* L.) stem. The tissue consisted of a yellowish-green cell population, was of intensive growth, and on standard culture medium did not show organ formation but only some tissue differentiation.

Of the amino acids used, L-arginine was a commercial product (Reanal Chemical Works, Budapest, Hungary),

while the guanidino-methylated L-arginine derivatives were prepared by synthesis<sup>7</sup>. The different amino acids were applied at concentrations of 10.0–100.0 mg/l agar-agar culture medium<sup>8</sup>. In the Erlenmeyer dishes, each piece of tissue was placed on 50 ml culture medium, with an initial weight of 200 mg. The tissues grew for 62 days in a thermostat of  $28 \pm 2^\circ\text{C}$  temperature with a natural alternation of day and night.

The added amino acids can be extracted with 80% alcohol from tobacco tissue and culture medium after 62 days. The identification of amino acids in alcoholic extracts can be pointed out by one- and two-dimensional thin-layer chromatography on Fixion 50  $\times$  8 chromato-

<sup>1</sup> W. K. PAIK and S. KIM, *Biochem. biophys. Res. Commun.* 40, 224 (1970). – P. R. CARNEGIE, *Biochem. J.* 123, 57 (1971). – G. S. BALDWIN and P. R. CARNEGIE, *Biochem. J.* 123, 69 (1971).

<sup>2</sup> E. TYIHÁK, B. SZENDE, K. LAPIS, A. PATTHY and K. SIKOS, *Abstr. Commun. 9th Meet. Fedn. Eur. Biochem. Soc. Budapest 1974*, p. 421.

<sup>3</sup> J. KAKIMOTO and S. AKAZAWA, *J. biol. Chem.* 245, 5751 (1970). – E. TYIHÁK and A. PATTHY, *Acta agronom. hung.* 22, 445 (1973).

<sup>4</sup> E. SCHWERTFEGER, *Qualitas Pl. Mater. veg.* 20, 347 (1971).

<sup>5</sup> B. SZENDE, E. TYIHÁK, L. KOPFER and K. LAPIS, *Neoplasma* 17, 433 (1970). – L. KOPFER, B. SZENDE, K. LAPIS and E. TYIHÁK, *Neoplasma* 18, 251 (1971).

<sup>6</sup> E. TYIHÁK, M. MARÓTI and D. VÁGUJFALVI, *Bot. Közlem.*, Budapest 58, 85 (1971).

<sup>7</sup> S. BAJUSZ and A. PATTHY, *Process for the Production of Guanidino-Methylated Arginines*, Hung. Pat. No. 165 543, Jul. 1974.

<sup>8</sup> M. MARÓTI, *Bot. Közlem.*, Budapest 56, 85 (1969).