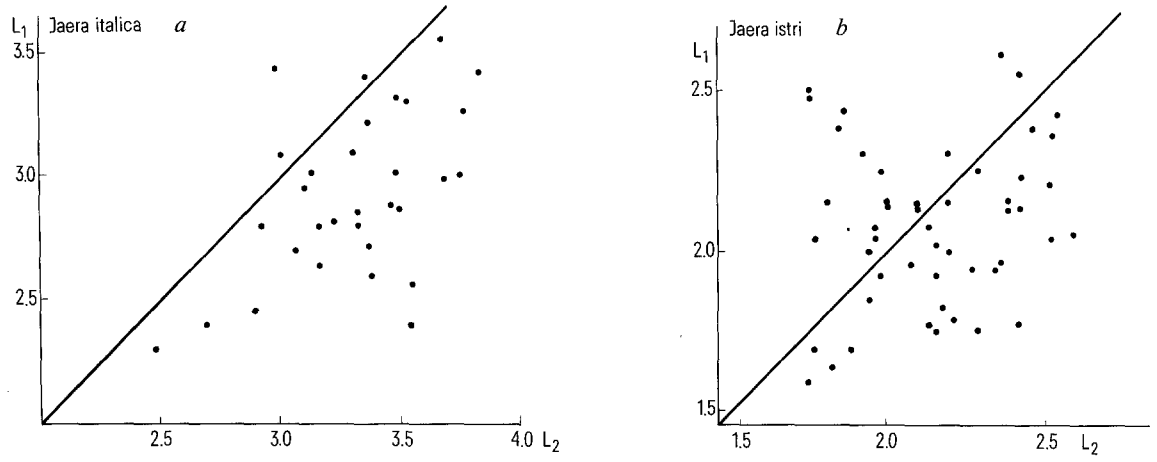

Length of the non-mated male (L_1) as a function of the length of the mated male (L_2) in competition experiments (mm): *a* in *Jaera italica; b* in *Jaera istri.* Values lying below the bisector indicate assays in which the mating male was the largest.

whenever both of them were simultaneously large or small. A positive correlation exists between the lengths of the competitors ($r=0.51$). This verifies the conclusion that, in each case, the outcome of the competition was determined by an interaction between their relative sizes. The length of the mating male was not determined by the size of the female, since no significant correlation was found between them.

We cannot, however, conclude that sexual selection was due to the males only, since the female's ability to discriminate between males according to their length is not excluded.

In *J. istri,* the largest male was paired with the female in only 32 cases out of 50 (figure, b), so that the panmictic hypothesis cannot be ruled out (probability level $p=0.05$). No significant difference in mean length was found between both series of males.

It was formerly assumed that sexual selection was responsible for the evolution of sexual dimorphism in body size in *Jaera.* The latter is apparent in our experiment when measuring the probability of the mean length being different in the 2 sexes (table). The difference is not significant in *J. istri,* while it is definite in *J. italica.* This is easily explained as a result of selection: since large males are more likely to reproduce, their greater relative contribution to the next generation will favour the expansion in the population of genetic factors for large size. This mechanism would increase the mean lengths of males in *J. italica.* The observations made in each species conform to this hypothesis, while the reason for which sexual competition evolved in 2 divergent ways remains still to be explained.

1 Acknowledgments. We are indebted to Prof. J. David for helpful criticism of the manuscript.
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The effect of two new peptide antibiotics, the hypelcins, on mitochondrial function

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Summary. The action on mitochondria of 3 peptide antibiotics, hypelcin-A, hypelcin-B, and alamethicin, was examined. The results showed that they are unique uncouplers of oxidative phosphorylation, with the same mechanism of action.

Recently, Fujita et al.[1] isolated 2 new peptide antibiotics, hypelcin-A and -B, from *Hypocrea peltata.* Both antibiotics consist of 19 amino acid residues and an amino alcohol, leucinol. Hypelcin-A is a neutral peptide, which does not contain any dissociable acidic residues, while hypelcin-B is an acidic peptide containing a glutamic acid residue instead of a glutamine residue in hypelcin-A. The chemical compositions of these peptide antibiotics are very similar to that of alamethicin, which consists of 18 neutral amino acid residues, an acidic amino acid residue, glutamic acid, and an amino alcohol, phenylalaninol[2]. Since alamethicin has effects on biomembrane systems by forming channels[3,4], or by activating Ca^{2+}- and Na^+-K^+ -ATPase[5,6] it is of interest to examine the effect of hypelcins on biomembrane functions and compare them with that of alamethicin.

This paper deals with the effects of hypelcin-A and -B on mitochondrial function, since these peptides were found to have an inhibitory action on the growth of the mushroom

Lentinus edodes, and this action can be considered to be directly related to the inhibition of energy transduction in the mycelia.

Materials and methods. Hypelcin-A and -B were isolated as reported previously[1]. Alamethicin was kindly supplied by C.B. Whitfield, Jr, The Upjohn Company, Kalamazoo (USA). Oligomycin was obtained from Sigma Chemical Co., St. Louis (USA), and ADP was from Kyowa Hakko Co., Tokyo (Japan). Rat liver mitochondria were isolated by the method of Hogeboom[7] as described by Myers and Slater[8]. The respiratory rate of mitochondria was measured with a Clark oxygen electrode (Yellow Spring Instruments) at 25 °C in medium consisting of 200 mM sucrose, 2 mM $MgCl_2$, 1 mM EDTA and 10 mM potassium phosphate, pH 7.4 (phosphate-medium). When the effect of inorganic phosphate was examined either Tris·Cl buffer or Tricine buffer was used instead of phosphate buffer (Tris-medium, Tricine-medium, respectively).

Results and discussion. Figure 1 shows the effect of hypelcin-A and -B on state 4 mitochondria in phosphate medium using succinate (with rotenone) as substrate. Both antibiotics stimulate the respiration of state 4 mitochondria, and the effects become greater as the concentration increases. The maximum effect was attained at about 1.0 µg/ml with hypelcin-A, and at about 1.5 µg/ml with hypelcin-B. At the concentration where the maximal activity was observed, hypelcin-A accelerated the respiratory rate of state 4 mitochondria about 9-fold, and hypelcin-B about 5-fold. When glutamate plus malate was used as substrate stimulation of respiration by these antibiotics was also observed. Hypelcins released the oligomycin-inhibited respiration of mitochondria almost completely at the concentration where the maximal stimulation of state 4 respiration was induced, as shown in figure 2. The effects of alamethicin on mitochondria were essentially the same as those of the hypelcins, and the maximum activity was exhibited at about 1 µg/ml. The effects of the antibiotics including alamethicin are characteristic of the effects of uncouplers of oxidative phosphorylation in mitochondria[9,10]. Thus we conclude that hypelcins and alamethicin act as uncouplers on mitochondria.

However, when the Tricine-medium was used as reaction medium, hypelcins and alamethicin failed to stimulate the respiratory rate to the level induced with the phosphate-medium, and the addition of inorganic phosphate (Pi) caused a stimulation of the respiration (table). It is noted that the Pi-requirement for releasing the state 4 respiration was typically observed with hypelcin-B. Other permeant anions, such as acetate and thiocyanate, had little effect on the respiration induced by these 3 antibiotics as shown in the table. Similar results were obtained with the Tris-medium.

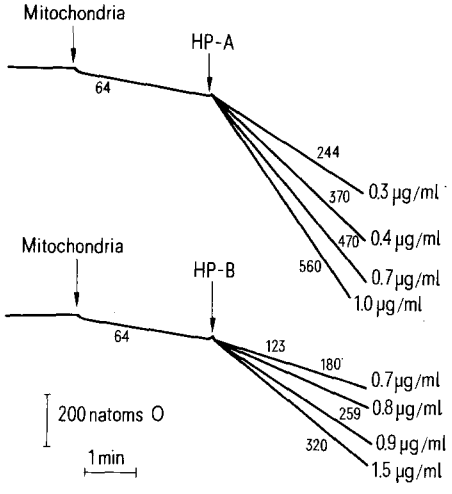

Fig. 1. Effect of hypelcin-A(HP-A) and hypelcin-B(HP-B) on the respiration of state 4 mitochondria. The phosphate-medium was used as reaction medium. Numbers adjacent to the traces are respiratory rates in natoms O/min. Substrate: 10 mM succinate with 3 µg rotenone. Mitochondria: 0.7 mg protein/ml. Total volume of the reaction mixture: 4.35 ml.

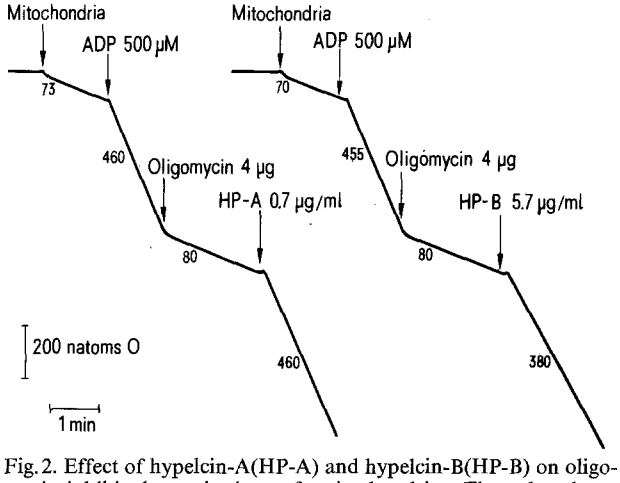

Fig. 2. Effect of hypelcin-A(HP-A) and hypelcin-B(HP-B) on oligomycin-inhibited respiration of mitochondria. The phosphate-medium was used as reaction medium. Numbers adjacent to the traces are respiratory rates in natoms O/ml. Substrate: 10 mM succinate with 3 µg rotenone. Mitochondria: 0.7 mg protein/ml. Total volume of the reaction mixture: 4.35 ml.

Effect of various anions on the stimulation of respiratory rate of state 4 mitochondria induced by peptide antibiotics*

Peptide antibiotic	Added anion**	Respiratory rate (natoms 0/min/mg protein) in the presence of anion			
		0 mM	1 mM	5 mM	10 mM
Hypelcin-A (0.8 µg/ml)	Phosphate	86	184	–	252***
	Acetate	86	89	96	110
	Thiocyanate	86	87	73	82
Hypelcin-B (1.5 µg/ml)	Phosphate	42	72	92	141***
	Acetate	41	41	44	46
	Thiocyanate	41	38	35	31
Alamethicin (0.8 µg/ml)	Phosphate	72	130	192	206***
	Acetate	73	79	86	123
	Thiocyanate	73	76	66	79

* TheTricine-medium was used as reaction medium with succinate (10 mM) plus rotenone (3 µg) as substrate. The respiratory rate of state 4 mitochondria in the absence of peptide antibiotics was 23 natoms O/min/mg protein with theTricine-medium, and was 33 natoms O/min/mg protein with the phosphate-medium. Total volume of the reaction mixture was 7.0 ml. Mitochondria, 0.7 mg/ml. ** Potassium salt was used. *** The value with the phosphate-medium.

When 70 µM N-ethylmalenimide (NEM), which completely inhibited the formation of ATP, due to the inhibition of Pi-translocation across the mitochondrial membrane, was present in the reaction medium, the respiratory rates induced by these 3 peptide antibiotics in the presence of Pi were the same as those induced in the absence of Pi. The results with hypelcin-A are shown in figure 3. Thus the translocation of Pi is concluded to be very important for exhibiting the uncoupling of hypelcins and alamethicin.

Furthermore, to know whether the effects of these antibiotics are directly related to the ionophoric action in mitochondria as observed with valinomycin[11], the effects of Na^+ and K^+ on the activity of the antibiotics were examined using Na^+ and K^+-depleted medium where all the acids were neutralized with Tris·Cl. It was found that these cations have no effect on the action of hypelcins and alamethicin.

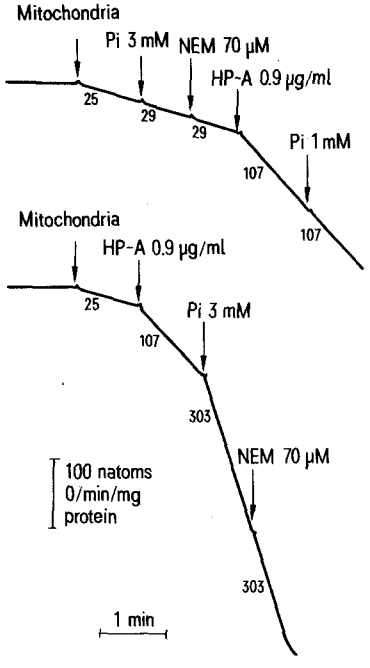

Fig. 3. Effect of N-ethylmalenimide (NEM) on the uncoupling of hypelcin-A(HP-A) in the presence of inorganic phosphate (Pi). The Tricine-medium containing 10 mM succinate with 3 µg rotenone was used as reaction medium in a total volume of 7.0 ml. Numbers adjacent to the traces are respiratory rate in natoms O/min/mg protein. Mitochondria: 0.7 mg/ml.

These results indicate that hypelcins and alamethicin are unique uncouplers of oxidative phosphorylation in mitochondria; they exhibit uncoupling action effectively in the presence of inorganic phosphate. The inorganic phosphate-requiring uncoupling action has sometimes been observed with cationic uncouplers, such as the cyanine dye NK-19[12], and peptide ionophors, such as valinomycin[11], though the role of inorganic phosphate is not clear. It is of interest that hydrophobic peptides, such as hypelcins, alamethicin, and valinomycin act on mitochondria with a common requirement of inorganic phosphate, like that of cationic uncouplers. It should be noted that a neutral antibiotic, hypelcin-A, exhibited about the same activity as the acidic antibiotic alamethicin did, while the peptide hypelcin-B, which is more alamethicin-like with regard to chemical composition, exhibited less activity than alamethicin. The presence of an acidic residue (glutamic acid) in the peptide chain seems not to be directly related to the uncoupling effect of these peptide antibiotics, although the presence of a dissociable proton is generally regarded as essential for exhibiting the action of weakly acidic uncouplers[13,14]. The activity of these antibiotics may be related to the membrane modifying action found with alamethicin[15].

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Lycorine – a resistance factor in the plants of subfamily Amaryllidoideae (Amaryllidaceae) against desert locust, *Schistocerca gregaria* F.

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Summary. Lycorine, an alkaloid was isolated from the bulbs of *Hymenocallis littoralis* Salisb. The percentage of lycorine was 0.00083. When sprayed on cabbage leaves this alkaloid inhibited the feeding of the desert locust, *Schistocerca gregaria* at 0.05% concentration.

The plants of the subfamily Amaryllidoideae, popularly known as lilies, are famous for their beautiful fragrant flowers. They are also of pharmacological interest[3,4]. A few plants are reported to contain a toxic principle[4,5]. *Crinum asiaticum* Linn. is reputed to be repellent to noxious insects[3]. Because of the above characteristics of the plants, it was thought desirable to screen the plants of this subfamily.

When pieces of leaves from 5 plant species, viz. *Crinum asiaticum* Linn., *C. bulbispermum* Milne-Redhead and Schweikerdt, *Hymenocallis littoralis* Salisb., *Zephyranthes grandiflora* Lindl. and *Hippeastrum hybridum* Herb.