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On the induction of metamorphosis of Lepidoptera by means of ecdysone and 20-hydroxyecdysone

Data on 268 hybrid and non-hybrid Sphingidae and 14 Bombyces

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Summary

Metamorphosis and adult eclosion were induced by the injection of appropriate amounts of 20-hydroxyecdysone or ecdysone into the pupae of female hybrids of Sphingidae (Lepidoptera) which otherwise would have remained in diapause until death. Non-hybrid pupae were also treated. Ecdysone produced better results than 20-hydroxyecdysone. For hybrids, the optimum dose of hormone was assessed at six mg per g live weight ; the dose might be considerably higher for non-hybrids. The duration of metamorphosis correlated weakly with the injected dose ; the correlation with temperature was pronounced. Interspecies differences were small. Ecdysone-induced imagines fed and mated normally, produced fertile sperm and eggs, and displayed normal longevity. Shipment of the injected pupae had a deleterious effect on metamorphosis in the majority of cases.

Résumé

La métamorphose et l'éclosion de femelles adultes hybrides de Sphingidae (Lepidoptera) ont été causées par l'injection de quantités appropriées de 20-hydroxyecdysone ou ecdysone dans leurs chrysalides, sans quoi celles-ci seraient restées en diapause jusqu'à leur mort. Des chrysalides non-hybrides furent également traitées. L'ecdysone produisit de meilleurs résultats que la 20-hydroxyecdysone. Pour les hybrides, la dose hormonale optimale était fixée à six µg par gramme de poids vivant ; la dose pourrait être considérablement supérieure pour les non-hybrides. Il y avait une corrélation faible entre la durée de la métamorphose et la dose injectée ; la corrélation avec la température était élevée. Les différences interspécifiques étaient faibles. Les imagos obtenus par l'injection d'ecdysone se nourissaient et s'accouplaient normalement, produisant du sperme et des oeufs fertils et montrant une longévité normale. L'envoi de chrysalides injectées avait un effet néfaste sur le déroulement de la métamorphose dans la majorité des cas.

Key words : Ecdysone, ecdysteroids, metamorphosis, Lepidoptera, Sphingidae, hybrids, shipment

Introduction

Female hybrids of certain species of the Sphingidae family enter metamorphosis spontaneously only in exceptional cases or not at all (Fischer, 1932). The first successful attempt to break this barrier was accomplished by means of transfusion of haemolymph taken from normal metamorphosing pupal instars (Meyer, 1953). The transfusion procedure was, however, hazardous ; therefore this procedure was never developed and applied. It was only after purified hormones became available that experimental work on the induction of metamorphosis gained momentum (Williams, 1968 ; Ohtaki & Williams, 1970). The hormones were also injected into female hybrid pupae predestined to die in Papilionidae (Clarke and Willig, 1977) and in Bombyces (Ades *et al.*, 1989), but obviously not yet in Sphingidae (Nijhout, 1994).

In 1983, our series of experiments was started. Initially, the results varied markedly. In 1989, by pure chance, we switched from 20-hydroxyecdysone to ecdysone ; immediately our results were more successful. We could now assess the dose/effect relationship, possible interspecies differences, and the relationship between the duration of metamorphosis and the effect of ambient temperature on pupae.

In non-hybrid pupae we tried to synchronize the eclosion of males and females which, under mid-European climatological conditions, only haphazardly enter metamorphosis.

Materials And Methods

Experimental animals (Table 1)

The majority of pupae referred to under Results (Tables 2 and 3) were derived from well-known hybrid and non-hybrid species (Ades *et al.*, 1989 ; De Freina, 1991 ; De Freina & Witt, 1987 ; Denso, 1913 ; Fischer, 1931 ; Gehlen, 1933 ; John, 1932 ; Jordan, 1913 ; Meerman, 1988 ; Meerman & Smid, 1988 ; Rothschild & Jordan, 1903). Novel hybrids are indicated under Results as hybr.nov.Loeliger (Table 1).

Most pupae were maintained on dry sand. Only pupae known to be susceptible to dessiccation (*elpenor, gschwandneri, harmuthi*, and *rydbergi*) were kept in an atmosphere of sufficient humidity. After the injection of ecdysteroid, the pupae were stored at room temperature unless otherwise stated.

Table 1

Sphingid hybrids

Combination of species			Eponym
Published (Denso, 1913 ; Jordan, 1913 ; John, 1932 ; Gehlen, 1933 ; De Freina, 1991)			
♂		♀	
H. euphorbiae	x	*H. vespertilio*	*epilobii*
H. euphorbiae	x	*P. elpenor*	*harmuthi*
H. gallii	x	*H. hippophaes*	*fromkei*
H. gallii	x	*H. vespertilio*	*carolae*
H. gallii	x	*P. elpenor*	*gschwandneri*
H. hippophaes	x	*H. euphorbia*	*hippophorbiae*
H. hippophaes	x	*P. elpenor*	*rydbergi*
Unpublished (Hybr. nov. Loeliger)			
♂		♀	
H. dahlii	x	*P. elpenor*	no eponym
H. gallii	x	*H. tithymali himyarensis*	*gallyarensis*
H. nicaea	x	*H. euphorbiae conspicua*	*paranicaea conspicua*
H. nicaea	x	*H. euphorbiae cretica*	*paranicaea cretica*
H. nicaea	x	*H. euphorbiae gecki*	*paranicaea gecki*
H. nicaea	x	*H. vespertilio*	*nicertilio*

Table 1 : Synopsis of eponyms for the experimental hybrid female pupae, which usually remain in permanent diapause until they die.

Other materials

Injection needles : Microlance™, Becton, Dickinson & Company ; 30 g½ 0.3 × 13 bl lb.
Syringes : Jecton-S™, 1 ml, subdivided into 0.02 ml.
Water and physiological saline : pyrogen-free and sterile.
Alcohol : aethylalcohol (ethanol) and isopropylalcohol (isopropanol), > 99%.
Collodium : 3%.
Iodine : 1%, in ethanol 65%.
Ecdysteroids : Ecdysone from SIGMA, St. Louis, USA ; FLUKA, Buchs, CH ;
ROHTO, Osaka, J. Abbreviations used : SIGMA = S, FLUKA = F, and ROHTO = R.
20-hydroxyecdysone from SIGMA and from ROHTO.

Injection fluid : not until 1992 was alcohol used in a concentration ⩾ 10%, sufficient to hold ecdysone in solution for the time needed for the injection (see legends to Tables 2 and 3).

Table 2

Experiments with 20-hydroxyecdysone

Species year of breeding/ injection	Injection fluid	Number of pupae	mg/g live weight	Handling	Result
Hybr. *rydbergi* 1982/1983	[1]	2	5.0		1 ++ ; 1 ±
		2	1.25		both –
		2	0.25		––
		2	0.05		––
Hybr. *rydbergi* 1982/1983	idem	4	2.5		2 – ; 2 ––
		4	3.75	shipped	1 ± ; 3 –
		4	5.0		1 ++ ; 3 –
		4	6.25		1 ++ ; 3 –
H. dahlii ♂ x *D. elpenor* ♀ 1982/1984	[2]	4	5.0	3 shipped	4 –
Hybr. *rydbergi* 1983/1984	idem	1	5.0	shipped	–
Hybr. *fromkei* 1983/1984	idem	3	3.0		1 ++ ; 2 ––
		3	5.0		3 ++
Hybr. *fromkei* 1983/1984	idem	22	5.0	4 non-shipped	–
				18 shipped	1 ++ ; 2+ ; 14 – ; 1 ––
Hybr. *hippophorbiae* 1983/1984	idem	8	5.0	4 non-shipped	1 ++ ; 2 – ; 1 ––
				4 shipped	1 – ; 3 ––
Hybr. *carolae* 1984/1985	[3]	10	5.0	2 non-shipped	2 ++
				8 shipped	3 ++ ; 3 + ; 2 –
Hybr. *fromkei* 1983/1985	idem	10	5.0	shipped	all –
Hybr. *hippophorbiae* 1983/1985	idem	8	5.0	shipped	all –
Hybr. *epilobii* 1983/1985	idem	1	5.0	non-shipped	all –
Hybr. *fromkei* 1983/1985	idem	5	5.0	1 non–shipped	all –
				4 shipped	
Hybr. *carolae* 1984/1985	[4]	3	8.0		all –
Hybr. *fromkei* 1986/1987	[5]	9	3.0	5 non-shipped	2 – ; 3 ––
				4 shipped	1 ++ ; 1 ± ; 2 –
H. centralasiae siehei 1986/1987	idem	7	3.0		3 – ; 4 ––
Hybr. *fromkei* 1988/1989	[6]	3	5.0		all –
	[7]	3	3.3		all –
	[8]	3	4.0		1 + ; 1 ± ; 1 –

Present procedure for preparation of the injection fluid

To obtain a 0.1% hormone solution in 12% alcohol, first 0.12 ml alcohol are mixed with 1 mg of the hormone crystals which rapidly dissolve. To this mixture 0.08 ml water is added in order to enhance the sterilizing capacity of alcohol (65% is optimal). Ten minutes later 0.8 ml water are added resulting in the final concentrations.

Injection technique

The apex of the head of the pupa is iodinized about 10 minutes before puncture. Anaesthesia is achieved by exposing the pupa for 20-40 minutes to CO_2 evaporating from dry ice. To protect the pupa against cold, it is placed on a 2-3 cm layer of linen covering the ice.

The anaesthetized pupa is held in the horizontal position while the needle is inserted through the iodinized apex of the head deep through the thorax into the upper abdomen, along the sagittal axis. The injection is slow. The needle remains *in situ* for about 10 seconds, to allow diffusion of the injected fluid. After removal of the needle, the puncture site is immediately covered with collodium. Twenty-four hours later, the pupa can be handled normally.

Results

Tables 2 and 3 represent the results in chronological order. The synopsis given in Table 4 is an attempt to identify preconditions for successful eclosion of adults. It appears that during the first six years (20-hydroxyecdysone experiments ; Table 2), a much smaller number of pupae was treated under optimum conditions than during the next three years (the ecdysone experiments ; Table 3). Therefore, the eclosion

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Table 2 : Chronological assessment of the results of 20-hydroxyecdysone injections, from 1983 to 1989. The injection fluid was consistently filtered. The composition varied considerably : 1) 0.1% beta-ecdysone"S" in 2.5% ethanol and 0.81% NaCl ; 2) 0.01%"R" further idem ; 3) 0.05%"S" further idem ; 4) same solution after storage at -20°C for 3 months ; 5) 0.04%"S" in 4% ethanol and 0.54% NaCl ; 6) same solution as under 3) after storage at -20°C for 4 years ; 7) same solution as under 5), after storage at -20°C for 26 months ; 8) 0.04%"S" in 4% ethanol.
Under "Results" the quality of metamorphosis is indicated by : ++ = perfect eclosion ; + = almost perfect eclosion ; ± = crippled imago ; - = signs of metamorphosis but no eclosion ; -- = no signs of metamorphosis. From hybr. *hippophorbiae* treated in 1985 through *H. centralasiae siehei* treated in 1987, the injection site was sealed with cyanoacryl glue.

Table 3

Experiments with ecdyson

Species year of breeding/ injection	Injection fluid	Number of pupae	µg/g live weight	Handling	Result
Hybr. *fromkei* 1988/1989	[1]	3	4.0		2 ++ ; 1 --, [a]
Hybr. *fromkei* 1988/1989	[2]	2	4.0		1 ++ ; 1 ±
Hybr. *paranicaea cretica*, 1988/89	[3]	6	5.0	3 non-shipped 3 shipped	3 ++ ; ovaria unripe 3 ++
Hybr. *paranicaea conspicua*, 1988/89	idem	2	3.0	1 non-shipped 1 shipped	--, reinj. 1990 --
idem, inj. 1990	[4]	1 1	10.0 18.0 [5]		++ ++
Hybr. *fromkei* 1988/1990	idem	2 2 2 2	2.0 4.0 8.0 12.0	26°C	1 ++ ; 1 ± 1 ++ ; 1 ± 2 + 2 ±
G. isabellae ♂ x *A. luna* ♀, 1989/90	[6]	2	6.0	shipped	1 ++ ; 1 –
Hybr. *fromkei* 1988/1990	[4]	1	3.0 à 4.0	shipped	+
Hybr. *fromkei* x *fromkei*, 1989/90		2	3.0 à 4.0	shipped	--
H. centralasiae siehei 1986/1991	[7]	1	3.0 à 4.0		--
Hybr. *fromkei* 1990/91	idem	4	3.0 à 4.0		all 4 ++, [b]
E. imperialis ♂ x *E. magnifica* ♀, 1989/91	idem	6	?		no eclosion remained alive
Hybr. *gschwandneri* 1991	idem	2	?		--, remained alive
Hybr. *gallyarensis* 1991/1992	[8]	4 4 4	8.0 4.0 2.0	2 non-shipped 2 shipped 2 non-shipped 2 shipped 2 non-shipped 2 shipped	2 ++ 1 ++ ; 1 + 2 ++ 2 – 2 ++ 1 – ; 1 --

Table 3 (continued)

Experiments with ecdyson

Species year of breeding/ injection	Injection fluid	Number of pupae	µg/g live weight	Handling	Result
Hybr. *nicertilio* 1991/1992	idem	2	8.0		2 ++
		1	4.0		++
		2	2.0		––
Hybr. *gschwandneri* 1991/92	idem	4	8.0	2 non-shipped	both –
				2 shipped	1 ± ; 1 –
		3	4.0	2 non-shipped	both –
				1 shipped	+
		4	2.0	2 non-shipped	1 ± ; 1 –
				2 shipped	1 – ; 1 ––
Hybr. *harmuthi* 1991/1992	idem	1	8.0		±
		1	4.0		–
		1	2.0		±
Hybr. *nicertilio* 1991/1992	[9]	17	6.0	7 non-shipped	6 ++ ; 1 +
				10 shipped	1 + ; 1 ± ; 8 –
		3	12.0		1 ++ ; 2 –
					(not optimal pupae)
Hybr. *gallyarensis* 1991/1992	idem	5	6.0	2 non-shipped	1 ++ ; 1 +
				3 shipped	1 ++ ; 1 + ; 1 –
		6	6.0	2 non-shipped	1 ± ; 1 –
				4 shipped	2 ++ ; 2 –
Hybr. *fromkei* 1991/1992	idem	4	6.0		1 ++ ; 1 ± ; 2 –
Hybr. *paranicaea Gecki*, 1991/1992	idem	4	6.0	3 non-shipped	3 ++
				1 shipped	–
B. europaea, 1990/1992	idem	6	6.0	shipped	no eclosions, survived
Hybr. *gschwandneri* 1991/92	idem	10	6.0	7 non-shipped	2 ++ ; 2 ± ; 3 –
				3 shipped	1 + ; 2 –
Hybr. *gallyarensis* 1991/1992	idem	2	8.0		1 + ; 1 –
		2	4.0		2 ++
		2	2.0		1 – ; 1 ––, [c]
Hybr. *rydbergi* 1992	idem	1	6.0	35° C	++, [d]
Hybr. *nicertilio* 1991/1992	idem	4	6.0	shipped	all 4 –
Hybr. *rydbergi* ♂ 1992	idem	1	6.0		++

Table 3 (continued)

Experiments with ecdyson

Species year of breeding/ injection	Injection fluid	Number of pupae	µg/g live weight	Handling	Result
H. euphorbiae mauretanica ♀, 1992	idem	1	6.0		++
H. euphorbiae conspicua ♂♂, 1992	idem	2	6.0		1 ++ ; 1 +, [e]
D. elpenor ♂, 1992	idem	1	6.0		++
D. elpenor 3 ♂♂ and 3 ♀♀, 1992	[10]	6	6.0		2++; 4––, surviving, eclosing 1993
P. proserpina 3 ♂♂ and 3 ♀♀, 1992	[10]	6	6.0		––, surviving and eclosing 1993

Table 3 : Chronological assessment of the results of the ecdysone injections, from 1989 through 1993. After treatment of hybr. *paranicaea cretica*, the injection fluid was no longer filtered ; the composition varied : 1) 0.04%"S" in 4% ethanol ; 2) same solution after storage at -20°C for 6 weeks ; overt turbidity ; 3) 0.05%"F" in 9% ethanol ; 4) same, after storage at -20°C for 1 year ; overt turbidity ; 5) addition through same needle of 4 mg/g live weight beta-ecdysone "S" which had been stored at -20°C for 1 year, displaying no turbidity ; 6) as under 3), but freshly prepared ; 7) 0,035%"R" in 9% ethanol ; 8) 0.05"F" in 5% ethanol, displaying slight turbidity, further diluted with water ; 9) 0.1"F" in 13% ethanol ; 10) same solution after storage at -20°C for 8 days ; overt turbidity.
Flawlessly eclosed imagines behaved normally : a) one specimen mated with a spontaneously eclosed male hybr. *fromkei*, began oviposition 5 days later (5 eggs), died 4 weeks after eclosion, with about 180 ripe eggs *in abdomine* ; b) one specimen mated with *H. gallii* and started oviposition on *E. angustifolium* 5 days later ; of the 82 eggs 76 were fertile ; c) one mated with *D. elpenor*, but died 8 days later without having laid eggs ; it contained about 50 ripe eggs ; the other specimen began oviposition 30 days after eclosion (49 sterile eggs) ; it lived for 67 days ; d) this pupa, injected 4 days after ecdysis and kept at 35°C, eclosed 15 days later, containing about 100 ripe eggs ; e) mated with a spontaneously eclosed female which produced 383 fertile eggs. Qualification of metamorphosis as for Table 2.

rates calculated for the two periods should be assessed carefully. However, the higher eclosion rates for ecdysone are overly clear. Irrefutable is the deleterious effect of shipment on injected pupae, particularly on those of hybrid *nicertilio* : only one of the 14 shipped pupae underwent eclosion and produced a more or less flawless imago.

Sealing the puncture with cyanoacryl glue turned out to be toxic; the puncture sites sometimes turned black, especially among *Hyles centralasiae siehei* pupae injected in 1987. This sealing procedure was abandoned after 33 pupae had produced only one acceptable imago.

Figure 1 illustrates the interrelationship between the duration of metamorphosis and the temperatures to which the injected pupae were exposed. At temperatures of 30°C to 35°C less than a fortnight was needed for procreation; in contrast, at temperatures $< 20°C$ metamorphosis took more than five weeks. The data presented in Figure 2 demonstrate the species-specificity of the interrelationship, hybrid *nicertilio* requiring about ten percent more time than hybrid *gallyarensis* to complete the process of metamorphosis. Moreover, the higher the dose, the faster the process.

Table 4

Eclosion rates after ecdysteroid injections

	Pupae injected number	Flawless eclosions	
		Number	percentage
20-HYDROXYECDYSONE			
optimal conditions (sphingidae hybrids only)	18	7	39
$\leqslant$ 3 mg/g live weight (suitable hybrid pupae only)	13	1	8
shipped pupae (suitable hybrid pupae only)	42	6	14
unsuitable conditions	54	1	2
TOTAL	127	15	12
ECDYSONE			
optimal conditions sphingidae-hybrids	42	28	66
non-hybrids	22	6	27
$\leqslant$ 3 mg/g live weight (suitable hybrid pupae only)	7 [1]	2	29
shipped pupae (suitable hybrid pupae only)	34	8	24
unsuitable conditions	51	6	12
TOTAL	156	50	32

[1] one pupa is counted a second time under "unsuitable conditions"

Table 4 : Grouping of the results according to the type and dosage of the ecdysteroid, the quality of the pupae and treatment procedure. "Optimal conditions" indicate a good pupa, a sufficient amount of hormone injected, a flawless injection technique, and no shipment. "Unsuitable conditions" mean a deep-frozen ecdysone solution, imperfect pupa, pupa older than one year, insufficient dosage, substantial loss of haemolymph after the injection, and shipment.

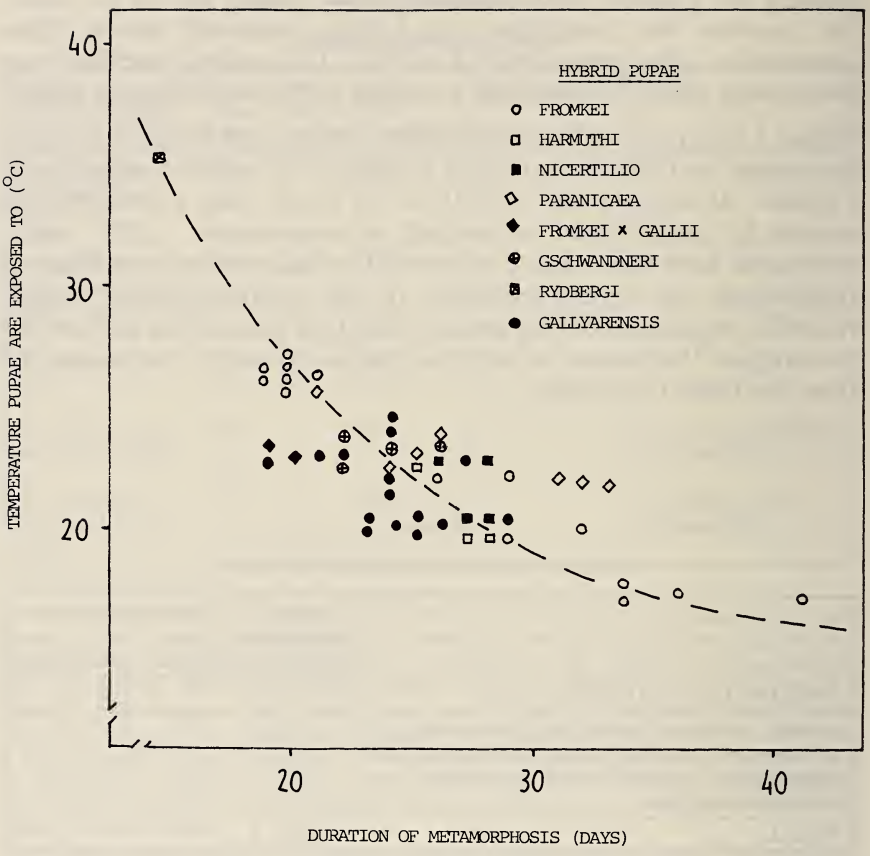

Fig. 1. Relationship between the duration of metamorphosis and the temperature of storage of the injected pupae. The season-dependent temperatures indicated are rough assessments. Temperature was not controlled except for eight of the hybrid *fromkei* specimens injected in 1990 (26° C), and the hybrid *rydbergi* specimen injected in 1992 (35° C).

Doubling the amount appears to shorten the period, also by about ten percent.

When we switched from 20-hydroxyecdysone to ecdysone (June 1, 1989) at a dose of four mg per gram live weight, the duration of the process of metamorphosis was found to differ between the two hormones : among hybrid *fromkei* pupae kept at room temperature, the appearance, on translucence, of the eyes occurred three days later in those treated with ecdysone than in those treated with 20-hydroxyecdysone under the same conditions. The delays in the appearance of the wing pattern

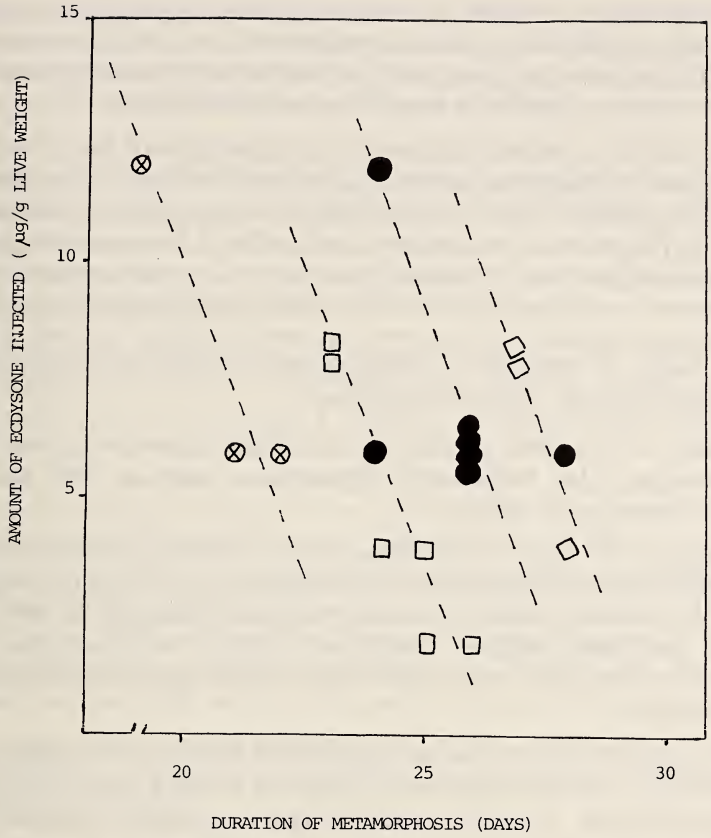

Fig. 2. Relationship between the amount of ecdysone injected and the duration of metamorphosis. The mean duration for hybrid *nicertilio* injected on July 4th, 1992 (◇) and July 22nd, 1992 (●), was 26.2 days, whereas that for hybrid *gallyarensis* injected on July 4th (□) and July 22nd (⊗) was 23.1 days, approximating a difference of ten per cent. The broken lines roughly indicate the relationship between the dose applied and the duration of metamorphosis.

and the eclosion of the imago were similar. In contrast, there was no difference in the duration of metamorphosis of the two hybrid *paranicaea conspicua* specimens, one of which was treated with a combination of the two ecdysteroids (see Table 3).

Discussion

When we started this series of experiments in 1983, nothing was known about the induction by the injection of ecdysteroids of the

metamorphosis of females of certain hybrid species of the family Sphingidae, which do not eclose spontaneously or only in exceptional cases. By trial and error, the goal of our endeavor to terminate diapause in these females as well as in normal pupae was achieved.

In the beginning, special attention was directed toward the prevention of infection of the pupae, isotonicity, and non-toxicity of the injection fluid. The injection site was iodinized about ten minutes before the injection. Filtration of the injection fluid to free it from possible pathogenic microorganisms, a routine procedure in the early stages of our experiments, was abandoned after the switch from 20-hydroxyecdysone to ecdysone. Instead, ecdysone was exposed to diluted alcohol, 65 per cent in water, to enhance its sterilizing effect. With these precautions, microbial infections were never observed.

Isotonicity of the fluid seemed to be of little importance, since discontinuation of the addition of physiological saline in 1985 did not cause a change in the results.

The apex of the head of the pupa as the preferred injection site in Sphingidae pupae was adopted from experienced investigators (Meyer, 1953). It obviously does not cause prohibitive damage of the brain nor the prothoracic region. Abdominal injection sites may give raise to untoward blood loss once pupae start to move again after awakening from narcosis.

Signs of alcohol toxicity were not apparent. The ethanol or isopropanol concentration was increased step by step, up to the 12 per cent needed to keep ecdysone in solution long enough to complete successful injection of a series of pupae. In storage, at low temperatures in particular, flocculation of ecdysone takes place if the solution contains one mg per ml ; after flocculation, ecdysone seems to loose much of its activity.

With this ecdysone solution — one per mille in 12 per cent alcohol/water — the volume injected remains below one per cent of the pupal volume and the intrapupal pressure barely increases, so that the procedure does not induce undesirable loss of haemolymph as long as anaesthesia is effective and the injection site is sealed.

Results with 20-hydroxyecdysone were unsatisfactory in spite of a meticulous injection procedure. For non-shipped pupae treated with sufficient amounts of hormone, the success rate remained below 50 per cent. Three to five mg per g live weight did not always induce complete metamorphosis. Six mg per g might be the optimum amount. Our results obtained with 20-hydroxyecdysone correspond closely to those

reported earlier for other hybrids : in 34 female pupae of a Bombyx species, the eclosion rate obtained at a dosage averaging 3.9 mg per g live weight was almost 50 per cent, the percentage of flawless imagines being approximately 15 per cent (Ades *et al.*, 1989).

It was by pure chance that ecdysone was introduced in our series of experiments. The first application, in female hybrid *fromkei* pupae, in June 1989, resulted in a complete success : one of the imagines mated normally, started oviposition soon afterwards, and displayed normal longevity. The vitality of this specimen was sufficient reason to continue our study with only ecdysone.

The minimum effective dose of ecdysone was assessed at three mg per g live weight, $\geqslant 4$ mg per g never failing to induce the process of metamorphosis. Six to 12 mg per g regularly produced flawless adults. In one instance (hybrid *paranicaea conspicua*, 1990) a dose as high as 18 mg per g was just as successful, indicating a wide range from the optimum dosage of ecdysone for hybrids, and confirming results reported for non-hybrids (Williams, 1968 ; Ohtaki & Williams, 1970 ; Sláma *et al.*, 1973). it is interesting that the addition of 20-hydroxyecdysone (four mg per g live weight) to ecdysone (18 mg per g) did not appreciably accelerate the process of metamorphosis : the two hybrid *paranicaea conspicua* specimens, one treated with the combination and the other with ten mg per g only, eclosed simultaneously.

We suggest that six mg ecdysone per g live weight will be sufficient for induction of the metamorphosis of female hybrids of Sphingidae remaining in diapause.

Using this dosage, we recently were successful in breeding an F_2 of the hybrid *vespertilioides* Boisduval (Loeliger & Karrer, *in press*).

In hybrids of Papilionidae, the optimum dose is probably much lower (Clarke & Willig, 1977). For non-hybrids, the optimum dosage has not yet been assessed. Depending on the stage of diapause, it might be considerably higher, although procedural failures and/or flocculation of the ecdysone solution might have been the reason for non-induction of metamorphosis in *D. elpenor* and *P. proserpina* in 1992.

From observations of hybrid *gallyarensis, nicertilio*, and *rydbergi*, we conclude that the process of metamorphosis is about halfway when the eyes become visible on translucency ; moreover, the period between the appearance of the wing pattern and eclosion equals 15 to 20 per cent of the total duration of metamorphosis. These assessments are in good agreement with observations of *Samia cynthia* (Williams, 1968).

It is quite clear that the process of metamorphosis is highly dependent on the ambient temperature for the injected pupae (Figure 1). Our results agree with those reported for spontaneously developing hybrids (Harbich, 1982) and non-hybrids (Harbich, 1976 and 1977). Figure 2 demonstrates that the process is also dose-dependent, though to a much lesser degree. Moreover, the overall values presented indicate a species specificity, the difference amounting to ten to 15 per cent of the total duration.

After the injection of ecdysone it takes about ten per cent more time to obtain the adult than after injection of 20-hydroxyecdysone. A similar difference was observed when hybrid *fromkei* pupae received the two hormones simultaneously in 1989. It reflects the time needed for the biotransformation of the inactive ecdysone into the active 20-hydroxyecdysone well-known in slow release reactions in endocrinology.

Our investigations of the feeding and mating behaviors of hormone procreated imagines is limited to only a few specimens receiving ecdysone (see Legend to Table 3). Feeding was not always easy because the two parts of the proboscis were insufficiently adjusted to each other or did not curl, mainly in hybrid nicertilio. But females of hybrid *gschwandneri, gallyarensis,* and *vespertilioides* F_2 (Loeliger & Karrer, *in press*) could be fed and remained in good health for up to two months. The ovaries of *gschwandneri* were atrophic, whereas *gallyarensis* produced eggs from the 30th day after eclosion on. Another *gallyarensis* mated within 24 hours after eclosion with *D. elpenor*, but died nine days later without having deposited any of the eggs it contained. Hybrid fromkei, which eclosed in June 1990, mated with *H. gallii* and remained alive for three weeks. The offspring of this couple were flawless adults, very much resembling *gallii*. Fertility of the F_2 of the hybrid *vespertilioides* was surprisingly high (Loeliger & Karrer, *in press*).

Among the injected non-hybrids, the *H. euphorbiae conspicua* male adult, eclosing in September 1992, mated with a spontaneously eclosed female of the same species that produced more than 350 eggs, the vast majority of which were fertile. Finally, four female *H. euphorbiae conspicua* specimens, eclosing in late December 1993, appeared on section to contain numerous ripe eggs.

The low eclosion rate observed for shipped pupae was disappointing. Only one of the 14 shipped hybrid *nicertilio* displayed satisfactory eclosion, compared to ten of the 13 non-shipped specimens. For hybrid *gallyarensis*, the deleterious effect of shipping was somewhat less

impressive, the respective figures amouting to 4/11 and 9/14. Considered together, successful eclosion of 70 per cent of the non-shipped pupae occurred, compared to only 20 per cent of those shipped. Bumping and rocking during shipment must account for the disturbance of metamorphosis.

We may conclude with the assumption that ecdysone, due to its continuous slow release biotransformation — the biological t½ as assessed in *Samia cynthia* was approximately six hours (Ohtaki & Williams, 1970) — produces sufficient amounts of physiologically active 20-hydroxyecdysone to induce metamorphosis if doses of six mg or more are injected per g live weight of a dormant pupa.

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The Checklist is intended to demonstrate our current state of knowledge of the Australian fauna of Lepidoptera. It therefore includes a large number of nomenclatural changes formally published for the first time ; new synonymies, new combinations, changes of status and reinstatements are clearly indicated as new in the Checklist. A complete index of all names is included together with a CD-rom containing all the actual Checklist files in ASCII format. The Checklist is multi-authored with one or more authors responsible for each family with contributions by A. Atkins, I. F. B. Common, E. D. Edwards, K. D. Fairey, M. Horak, F. Komai, T. Kumata, M. S. Moulds, P. B. McQuillan, E. S. Nielsen, G. S. Robinson, M. Shaffer and G. Tarmann. The family classification adopted in this work is that developed by I. F. B. Common and E. S. Nielsen for Moths of Australia (Common, 1990) and the treatment of Lepidoptera in The Insects of Australia (Nielsen & Common, 1991), with minor changes. The editors are to be congratulated for the high professionalism of the present achievement. This work presents a wealth of information in concise form and is absolutely indispensable to anyone working on Australian Lepidoptera.

Alain Olivier

Errata in and acknowledgement to *Nota lepidopterologica* Vol. 19, 1996

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In the articles of Loeliger & Karrer, published 21.XI. and 21.XII.1996, pages 113-128 and 243-260, respectively, quantities of ecdysone injected into pupae are erroneously presented a thousand times too high in most instances, i.e. in mg instead of in µg. Correct figures are given in the first publication under Material and Methods, Table 3 and Figure 2, and the French Summary, on pages 113, 117-120 and 123, resp. ; and in the second article under Material and Methods on page 247.

The first author's address is also incorrectly given. In The Netherlands two capitals separate the numbering and wording of the domicile (vide supra).

As the author responsible for reviewing the proofs I apologize for the flaws.

Delightful news is the approval, just before publication, of our request for financial support by the Uyttenboogaart-Eliasen foundation in Amsterdam. Its gift satisfactorily compensates the costs of the colour printing of the figures 1-7, illustrating the results of our cross breeding experiments published in volume 19 (3/4) of *Nota lepid.*