

# Sterols of the pine processionary caterpillar; effects of diflubenzuron (Dimilin®)<sup>1</sup>

J. Cl. Denneulin and M. Lamy

Université de Bordeaux I, Laboratoire de Neuroendocrinologie-ERA 850 CNRS, Avenue des Facultés, F-33405 Talence (France), 25 May 1981

**Summary.** Cholesterol in pine processionary caterpillars (*Thaumetopoea pityocampa* Schiff.; Lepidoptera) is produced mainly from  $\beta$ -sitosterol present in their food (pine needles); it can also be synthesized from acetic acid. This synthesis is stimulated by the insecticide Dimilin but inhibited by the antibiotic tetracycline.

In general, insects are unable to synthesize cholesterol because they cannot form the cyclic part of this molecule<sup>2</sup>. The alimentary sterols satisfy their needs. The silverfish is an exception to this rule<sup>3</sup>. It has also been demonstrated that traces of cholesterol are synthesized by symbionts or parasites<sup>4</sup>.

We have studied a) sterols present in the pine processionary caterpillar, b) sterols contained in their staple food, the maritime pine tree, and c) the biosynthesis of cholesterol in the larval and pupal stages. Sterols were analyzed after extraction, saponification and column purification. The procedures used were: thin-layer chromatography, gas-liquid-chromatography and mass-spectrometry.

Animals were injected with <sup>14</sup>C<sub>2</sub> acetate dissolved in Ephrussi and Beadle liquid (20  $\mu$ l containing 3  $\mu$ Ci per animal) in order to study sterol biosynthesis. Experiments were carried out during the indispensable feeding (middle of the larval stage) and fasting periods (spinning, prepupa and pupa). Sterols were extracted 24 h after injection and collected in digitonid complexes. One group of caterpillars was treated with a synthetic insecticide Dimilin (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea mixed with a solu-

tion of labeled acetate in the ratio of 300 ppm. Other caterpillars had an antibiotic, tetracycline (integrated dosis between 1/20<sup>3</sup> and 1/100, added to their food).

In the pine processionary caterpillar, TLC shows only 1 main type of sterol which has only 1 double bond in the B cycle (C5-C6). Detectable sterols polyunsaturated on the lateral chain are too low in concentration to be measurable. The GLC distinguished 3 dominant sterols: cholesterol, campesterol and  $\beta$ -sitosterol present in the caterpillars as well as in the prepupa and the pupa. Mass spectrometry corroborates these results. Spectra show the characteristic peaks of cholesterol (M=386, 371, 368, 353, 301, ...).  $\beta$ -sitosterol (M=414, 399, 396, 381, 329, ...) and campesterol (M=400, 385, 382, 367, 315, ...). In addition, sterols C<sub>27</sub> and C<sub>28</sub>, as stigmasterol and furcosterol (m/e 412), methylene-24-cholesterol, brassicasterol (m/e=398) and dehydro-22-cholesterol (m/e=384) are present in non-measurable traces. The presence of sterols with a double bond on the lateral chain is confirmed by the existence of peaks which correspond to the ions m/e=271, 273, 299, 300, 301, 314. In the pine processionary caterpillar's food, the same 3 sterols predominate but in different proportions (table).

The labeled acetate is incorporated in small amounts into the sterols of the pine processionary caterpillar and the intensity of incorporation depends on the age of the 5th larval instar caterpillar, with 2 apparent peaks: 5-7 days after the last larval molt and a few days before the pupal molt (fig. 1). In the animals treated with Dimilin we found

Sterol-percentage of the pine processionary caterpillar at different stages, and of the pine needles that serve as food. Results obtained by gas-liquid chromatography and mass-spectrometry

	Cholesterol		Sitosterol		Campesterol	
	GLC	MS	GLC	MS	GLC	MS
Alimentary phytosterols	3%	7.3%	90%	76.4%	7%	16.3%
Caterpillars	89.6%	86.3%	5.7%	9.4%	4.7%	4.2%
Preynympha	90.5%	90%	5.9%	7%	3.6%	3%
Nympha	92.1%	90.1%	4.3%	7%	3.5%	2.9%

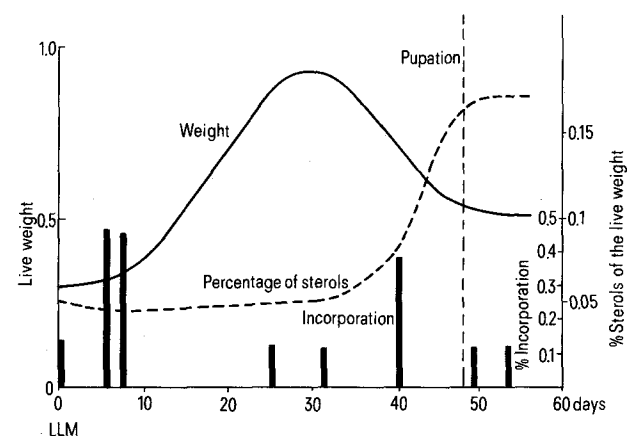


Figure 1. <sup>14</sup>C acetate incorporation in sterols (■) expressed in percent of the total radioactivity related to the weight variation (—) and the percentage of sterols related to the live weight (---); LLM = last larval molt.

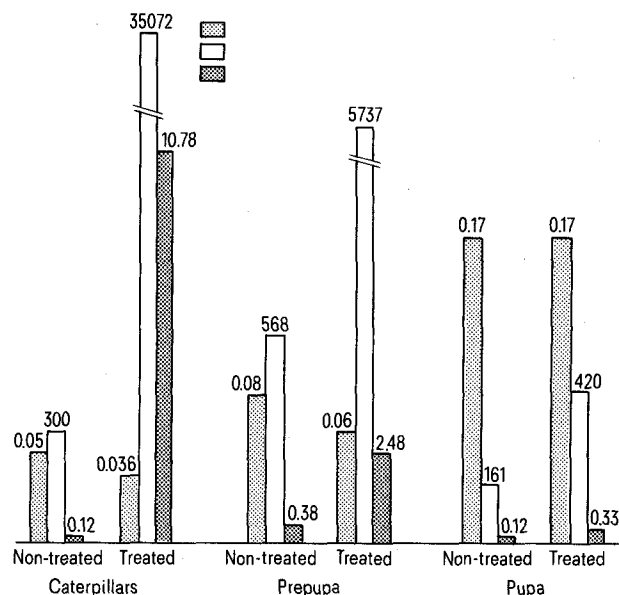


Figure 2. <sup>14</sup>C acetate incorporation in the sterols of the pine processionary caterpillar. Comparison between non-treated and Dimilin-treated animals. ■ Sterols for 100 g live weight. □ Radioactivity: cpm/mg of sterols. ■ Percent of the radioactivity incorporated in the sterols.

a large increase in the incorporation of labeled acetate in the sterols, especially in larvae (fig. 2). We confirmed by means of preparative GLC-analysis that labeled acetate is in fact incorporated in the cholesterol. With animals treated with tetracycline, cholesterol synthesis disappears but only with a high concentration of antibiotics (1%). This could mean that symbiotic microorganisms are involved in this synthesis.

For *Thaumetopoea pityocampa* caterpillars, the cholesterol originates therefore mainly from pine needle  $\beta$ -sitosterol but also from biosynthesis from small molecules (acetic acid) either through symbiotic micro-organisms or by the animal itself. Furthermore, biosynthesis is greatly stimulated by Dimilin. Its other effects in addition to the specific action on the cuticle of insects<sup>5</sup> include (a) a rise in the respiratory quotient<sup>6</sup>, (b) stimulation of the pentose cycle<sup>6</sup>, (c) accumulation of hemolymphatic glucose<sup>7</sup>, and (d) the disappearance of the polysaccharides in oenocytes<sup>8</sup>. Stimulation of cholesterol synthesis by Dimilin could help

to explain why ecdysteroids accumulate in some treated insects<sup>9</sup> and why the effects of this insecticide are far more varied than is generally supposed.

- 1 This work has been partly realized at the Institut de Chimie des Substances naturelles of CNRS. Thanks to the amiability of Mr Michel Barbier.
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## Genetic basis of moult, wing length, and body weight in a migratory bird species, *Sylvia atricapilla*

P. Berthold und U. Querner

Max-Planck-Institute for Behavioral Physiology, Vogelwarte Radolfzell, D-7760 Radolfzell-16 (Federal Republic of Germany), 3 December 1981

**Summary.** In hand-raised groups of 4 populations of Blackcaps, different patterns of juvenile moult as well as differences in wing length and body weight, adaptive to the various degrees of migratory performance, could be demonstrated. 33  $F_1$ -hybrids which were produced experimentally with 2 of these groups (1 African  $\times$  1 European) showed an intermediate expression of these adaptations relevant to migration, indicating that these physiological and morphological features are under strong direct genetic control.

Patterns of juvenile and postnuptial (premigratory) moult, as well as morphological features such as wing length and body weight, are known to be adaptive to migratory performance in birds. As a rule, long-distance migrant birds are characterized by early, short and intensive moult whereas less marked, shorter-distance migrants moult later, over a longer time and with a lower intensity. Strongly migratory birds also often have longer and more pointed wings and greater premigratory body weight than shorter-distance migrants. Such differences have been demonstrated in different species as well as in different populations of the same species<sup>1,2</sup>. The interactions of genetic and environmental influences upon these migratory adaptations are largely unclear<sup>1-3</sup>. It is, however, likely – on the basis of studies in other animals – that these morphological patterns are strongly determined genetically, and there is also some empirical evidence of this among birds<sup>1,3-5</sup>. On the other hand it is to be expected that environmental conditions (e.g. differences in photoperiod, temperature, or food supply under which individuals grow up) can essentially influence the expression of these migratory adaptations<sup>1,6,7</sup>. The 'genetic hypothesis' would be supported if  $F_1$ -hybrids of birds from 2 populations that migrate over different distances and are characterized by different physiological and morphological adaptations, showed intermediate characteristics relevant to migration<sup>8,9</sup>.

The blackcap, *Sylvia atricapilla*, which has a wide distribution from about 15°–65° N. Lat. (from the Cape Verde Islands and Africa to northern Europe) is a suitable species for such a cross-breeding experiment. First, we hand-raised nestlings from each of 3 European populations and 1 African population in the spring of 1976 and 1977 in identical light conditions in Germany, and their juvenile

moult, wing length and body weight were measured. Birds of these different populations differ extremely in their migratory performance<sup>10</sup>, in the time course of their juvenile moult (fig. 1A) and in their morphology (fig. 2). In accordance with the decreasing distances of north-to-south travel in the free-living populations, and in agreement with the decreasing magnitude of migratory activity and a progressively later onset of the first migratory period<sup>10,11</sup> there was also a progressively later onset of the juvenile moult from Finnish through German and French to Afri-

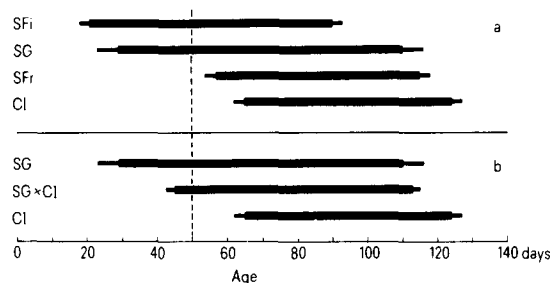


Figure 1. Time course of juvenile moult of blackcaps (with SE for the duration of the moult). A Data from birds of different populations, SFi, southern Finnish birds (n=26), 60° N. Lat.; SG, southern German (n=25), 47° N. Lat.; SFr, southern French (n=25), 43° N. Lat.; CI, birds from the Canary Islands, Tenerife, Africa (n=26), 28° N. Lat. B Data from hybrids (SG  $\times$  CI, n=33) and their parental birds (SG, CI, as above). Vertical broken line: transfer to a constant light/dark ratio of 12.5/11.5 h, in order to be able to measure the migratory performance<sup>10</sup> in all groups in identical conditions.