60–67 (1960); Stevens, J. C., and S. S. Stevens, "Warmth and cold: dynamics of sensory intensity," *J. Exptl. Psychol.*, **60**, 183–192 (1960); Stevens, S. S., and J. R. Harris, "The scaling of subjective roughness and smoothness," *J. Exptl. Psychol.*, **64**, 489–494 (1962); Stevens, S. S., and M. Guirao, "Subjective scaling of length and area and the matching of length to loudness and brightness," *J. Exptl. Psychol.*, **66**, 177–186 (1963).

[2] Luce, R. D., and E. Galanter, "Psychophysical scaling," in *Handbook of Mathematical Psychology*, ed. R. D. Luce, R. R. Bush, and E. Galanter (New York: John Wiley and Sons, 1963), vol. 1, pp. 245–307.

[3] For a description see Stevens, J. C., and M. Guirao, "Individual loudness functions," *J. Acoust. Soc. Am.*, **36**, 2210–2213 (1964).

[4] Stevens, S. S., "Calculation of the loudness of a complex noise," *J. Acoust. Soc. Am.*, **28**, 807–832 (1956); Stevens, S. S., "Problems and methods of psychophysics," *Psychol. Bull.*, **55**, 177–196 (1958).

[5] Stevens, S. S., "On the brightness of lights and the loudness of sounds," *Science* (Abstracts), **118**, 576 (1953); Onley, J. W., "Light adaptation and the brightness of brief foveal stimuli," *J. Opt. Soc. Am.*, **51**, 667–673 (1961); Stevens, J. C., and S. S. Stevens, "Brightness function: effects of adaptation," *J. Opt. Soc. Am.*, **53**, 375–385 (1963).

[6] Stevens, S. S., "Procedure for calculating loudness, Mark VI," *J. Acoust. Soc. Am.*, **33**, 1577–1585 (1961); Harper, R., and S. S. Stevens, "Subjective hardness of compliant materials," *Quart. J. Exptl. Psychol.*, **16**, 204–215 (1964).

[7] Lane, H. L., A. C. Catania, and S. S. Stevens, "Voice level: autophonic scale, perceived loudness, and effects of sidetone," *J. Acoust. Soc. Am.*, **33**, 160–167 (1961).

[8] Stevens, J. C., and S. S. Stevens, "Brightness function: effects of adaptation," *J. Opt. Soc. Am.*, **53**, 375–385 (1963).

---

*JUVENILE HORMONE ACTIVITY FOR THE BUG PYRRHOCORIS APTERUS**

BY KAREL SLÁMA† AND CARROLL M. WILLIAMS

BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

Communicated June 23, 1965

When transported from Prague to Boston and reared in the Biological Laboratories at Harvard University, the bug *Pyrrhocoris apterus* failed to undergo normal metamorphosis. Approximately 1500 individuals were reared from eggs. Instead of metamorphosing into normal adults, at the end of the 5th larval instar all molted into 6th instar larvae or into adultoid forms preserving many larval characters. Indeed, as illustrated in Figure 1, some continued to grow and molted into still-larger 7th instar larvae. Without exception, all individuals died without completing metamorphosis or attaining sexual maturity.

During 10 years of culturing *Pyrrhocoris* in Prague, not a single instance of this sort had been observed. Additional larval instars, in *Pyrrhocoris* as in other species, had been induced only by the implantation of active corpora allata (the endocrine source of juvenile hormone), or by the injection or topical application of substances showing juvenile hormone activity.[1] Evidently, when reared at Harvard University, the bugs had access to some unknown source of juvenile hormone.

An audit of the culture conditions at Harvard versus Prague suggested 15 differences. By systematic study, 14 were eliminated. The source of juvenile hormone activity was finally tracked down to exposure of the bugs to a certain paper towel

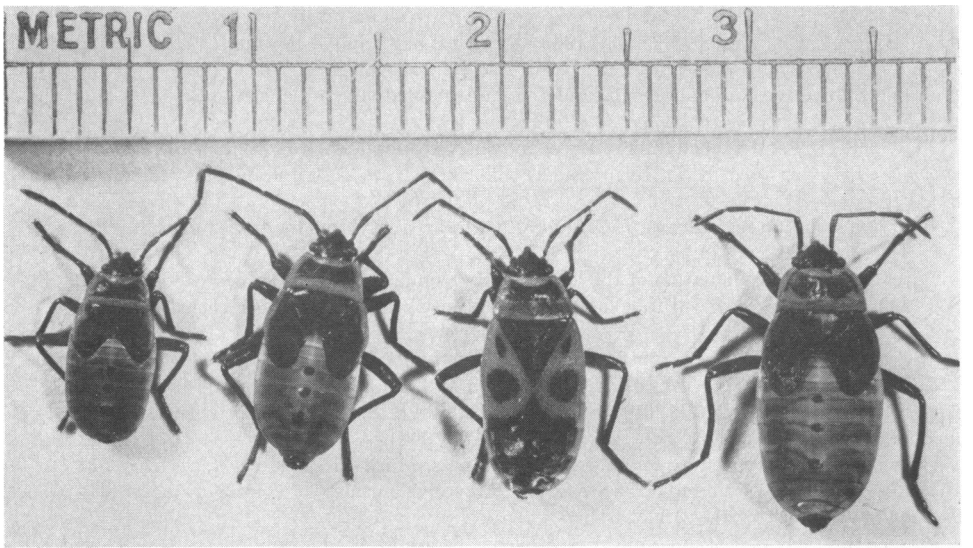

FIG. 1.—The effects of contact with the active principle extracted from paper material is illustrated by these four specimens. On the left is a 5th instar *Pyrrhocoris* larva which normally transforms into the winged adult (*third from left*). When exposed to the paper extract, the larva continues to grow without metamorphosis to form a 6th instar larva (*second from left*) which, in turn, may form a still-larger 7th instar larva (*fourth from left*). (*This print was prepared by Frank White from a color transparency by Muriel V. Williams.*)

("Scott, brand 150") which had been placed in the rearing jars. When this toweling was replaced by Whatman's filter paper, the entire phenomenon disappeared and all individuals developed normally.

At first we thought that the effect of the toweling was due to some chemical added during its manufacture. However, of 20 other brands of American towels and tissues, 18 provoked the same extraordinary juvenile hormone effects when tested on *Pyrrhocoris*. Indeed, pieces of American newspapers and journals (*New York Times*, *Wall Street Journal*, *Boston Globe*, *Science*, and *Scientific American*) showed extremely high juvenile hormone activity when placed in contact with *Pyrrhocoris*. The *London Times* and *Nature* were inactive, and so were other paper materials of European or Japanese manufacture.

Extracts prepared from the Scott toweling were subjected to biological assay. The latter was accomplished merely by soaking a disk of filter paper in the extract, evaporating the solvent, and allowing young 5th instar *Pyrrhocoris* to walk upon it. After 5 days at 25°C, the presence of juvenile hormone activity was signaled by their molting to 6th instar larvae, or, in the case of weaker reactions, by molting to creatures which showed a mixture of larval and adult characters. The absence of activity was recognized by molting to normal adults after 7 days at 25°C.

Like all previous materials with juvenile hormone properties,[2–5] the active principle in the toweling was found to be insoluble in water, but freely soluble in methanol, acetone, ether, and petroleum ether. The surprisingly large amount of material obtained by methanol extraction of toweling was purified by evaporation of solvent and extraction of the residue into petroleum ether. The active principle was found to be heat-stable (100°C) and destroyed by vigorous saponification.

Further purification was achieved by chromatography on a column of silicic acid ("Unisil"). The active fraction was retained after perfusion with benzene but was eluted with an equal-part mixture of benzene and diethyl ether.

This partially purified extract showed extraordinary juvenile hormone activity when assayed on *Pyrrhocoris*. Contact with a 10-cm disk of filter paper impregnated with 100 μg of extract caused all of scores of larvae to transform into 6th instar larvae or adultoid forms. As little as 0.01 μg of extract provoked the formation of 6th instar larvae when topically applied to individual bugs. Even when the extract was placed on the tip of an antenna or leg, or at any other site, the full effect was realized. This demonstrated that the active principle readily penetrates the cuticle and is distributed throughout the insect. The extract was fully effective when tested on *Pyrrhocoris* larvae from which the corpora allata had been removed at the outset of the fifth instar—a finding which shows that the corpora allata are not involved in the reaction to the extract.

We would emphasize that the active material was without any detectable effects on the growth, molting, or viability of *Pyrrhocoris* larvae prior to the stage when metamorphosis ordinarily supervenes. This result is intelligible since larval growth normally proceeds in the presence of endogenous juvenile hormone secreted by the corpora allata.[4] Metamorphosis occurs only when the corpora allata stop secreting juvenile hormone; consequently, it is at this specific stage that the insect becomes sensitive to the materials possessing hormonal activity.

Through the kindness of Professor Irving W. Bailey, authentic samples of seven species of gymnosperms were obtained from the venerable wood collection of the Harvard Herbarium. Each specimen was first rinsed in acetone and then cut up on a drill press with an acetone-washed drill. The pulverata were shaken with acetone, filtered, and the solvent evaporated to obtain the acetone-soluble materials. The latter were dissolved in small volumes of methanol, impregnated onto filter paper, and assayed as described above.

Extracts of balsam fir (*Abies balsamea*), hemlock (*Tsuga canadensis*), and yew (*Taxus brevifolia*) showed high juvenile hormone activity. Extracts of American larch (*Larix laricina*) showed intermediate activity, while red spruce (*Picea rubra*), European larch (*Larix decidua*), and the southern pine (*Pinus echinata*) showed barely detectable activity. Evidently the substantial juvenile hormone activity in American paper products is mainly derived from the indigenous American pulp tree, the balsam fir.

We were astonished to find that our most active extracts were without any detectable effects when injected or topically applied to previously chilled pupae of the *cecropia* or *polyphemus* silkworms—even when they were tested in the ultrasensitive wax-wound assay for juvenile hormone.[3, 5, 6] Equally surprising was the additional finding that purified extracts of *cecropia* oil, of extremely high juvenile hormone activity when assayed on *cecropia* or *polyphemus* pupae, showed only a trace of activity when tested on *Pyrrhocoris apterus*.

What this implies is that molecules with juvenile hormone activity for one species of insect are not necessarily active on other species. Evidently during the millions of years of insect evolution, the detailed chemistry of the hormone has evolved and diversified. The scant sensitivity of *Pyrrhocoris* to *cecropia* hormone documents this fact and shows, moreover, that the evolution of the hormone has been accom-

panied by the biochemical "retuning" of the receptor mechanism at the cellular level.

At the present time additional studies are under way to clarify the chemistry of the active material and to define the types of insects sensitive to this particular variant of juvenile hormone. It is already clear that the towel extract is without any detectable effects on most laboratory insects, including two other species of heteroptera, *Oncopeltus fasciatus* and *Rhodnius prolixus*. In point of fact, the only sensitive species which we have thus far encountered is *Pyrrhocoris apterus* itself.

The family Pyrrhocoridae includes a substantial number of insect pests, such as the notorious "red cotton bug" (*Dysdercus cingulatus*) of eastern Asia, and various species of "cotton stainers" endemic to Australia, the West Indies, South America, and southern United States. It seems not unlikely that the hormonally active material may be effective in the selective destruction of at least certain of these pests, as well as any other insects which show the same hormonal sensitivities as *Pyrrhocoris apterus*.[2] This possibility is worthy of attention because the active material is available on an unlimited scale in American newspapers and journals.

Summary.—Materials composed of American paper pulp contain an extractable, heat-stable lipid which exhibits extremely high juvenile hormone activity when injected or topically applied to the European bug *Pyrrhocoris apterus*. The active principle in the paper materials is derived from certain species of pulp trees, more particularly the balsam fir, *Abies balsamea*. Larvae exposed to the active material ultimately die without completing metamorphosis or attaining sexual maturity.

Despite its extremely high activity for *Pyrrhocoris apterus*, the extract is without any detectable effects on silkworm pupae; conversely, juvenile hormone extracts prepared from *cecropia* silkmoths show only a trace of activity when tested on *Pyrrhocoris*. These findings point to a diversification of the detailed chemistry of juvenile hormone during insect evolution.

The factor extracted from paper materials promises to be an effective agent for the selective control of insect pests which show the same endocrine sensitivities as *Pyrrhocoris apterus*.

* This investigation was supported, in part, by grant GB-3232 from the National Science Foundation.

† Permanent address: Department of Insect Physiology, Entomological Institute of Czechoslovak Academy of Sciences, Prague.

[1] Sláma, K., *Zool. Jb., Physiol.*, **70**, 427 (1964).

[2] Williams, C. M., *Nature*, **178**, 212 (1956).

[3] Schneiderman, H. A., and L. I. Gilbert, *Science*, **143**, 325 (1964).

[4] Wigglesworth, V. B., *Advan. Insect Physiol.*, **2**, 247 (1964).

[5] Williams, C. M., and J. H. Law, *J. Insect Physiol.*, **11**, 569 (1965).

[6] Schneiderman, H. A., and L. I. Gilbert, *Biol. Bull.*, **115**, 530 (1958).