References and Notes

1. M. Cohn, J. Monod, M. R. Pollock, S. Spiegelman, R. Y. Stanier, *Nature* **172**, 1096 (1953).
2. R. Liberman, *Science* **135**, 372 (1962).
3. D. Krech, M. R. Rosenzweig, E. L. Bennett, *J. Comp. Physiol. Psychol.* **53**, 509 (1960).
4. D. Nachmansohn, *Science* **134**, 1962 (1961).
5. H. Hyden and A. Pigon, *J. Neurochem.* **6**, 57 (1960).
6. J. A. Deutsch, *Ann. Rev. Physiol.* **24**, 270 (1962).
7. H. Hyden and E. Egyhazi, *Proc. Natl. Acad. Sci. U.S.* **48**, 1366 (1962).
8. F. M. Burnet, *Perspectives Biol. Med.* **3**, 447 (1960).
9. C. W. Thomson, J. C. McGaugh, C. E. Smith, W. J. Hudspeth, W. H. Westbrook, *Can. J. Psychol.* **15**, 67 (1961).
10. J. L. McGaugh and L. Petrinovitch, *Am. J. Psychol.* **72**, 99 (1959).
11. M. Moroz, *J. Comp. Physiol. Psychol.* **52**, 172 (1959).
12. W. C. Corning and E. R. John, *Science* **134**, 1363 (1961).
13. S. M. Beiser and S. W. Tanenbaum, *Nature* **189**, 659 (1961).

14. I am indebted for critical analysis and useful suggestions to several colleagues, particularly Drs. Harold J. DeBey and Calvin W. Thomson.

27 July 1962

Fatty Acids in Pollen of Some Coniferous Species

Abstract. Fatty acids in pollen of five coniferous species were isolated and analyzed by gas-liquid chromatography. It was found that 0.76 to 0.89 percent of the dry weight of pollen was fatty acid in three species of *Pseudotsuga* and 1.25 to 1.33 percent in two species of *Pinus*. Major components in *Pseudotsuga* were oleic, palmitic, and linoleic acids, whereas in *Pinus* they were linolenic, oleic, palmitic, and stearic acids.

In the course of developing methods for preserving pollen for plant hybridization, the chemical composition of pollen from various conifers was determined. Fatty substances in pollen have received little attention because of the small amounts available for analyses (*1*). This paper, for the first time, reports the quantitative and qualitative determinations of fatty acids in three species of *Pseudotsuga* and two species of *Pinus*. There may be a correlation between the chemical findings and the phylogenetic relationships of the two genera studied.

Pollen of Formosan Douglas fir, *Pseudotsuga wilsoniana* Hay, was collected in Ta Chia Chi, Taiwan, in the middle of February, 1962. Branches of big-cone Douglas fir, *Pseudotsuga macrocarpa* (Vasey) Mayr, bearing mature male flowers, were shipped from southern California to Corvallis in the early part of April, in cartons which contained moist paper, and the pollen sac was forced to dehisce in the laboratory. Mature pollen of Douglas fir, *Pseudotsuga menziesii* (Mirb) Franco, was gathered in Corvallis from a single tree by a vacuum pollen collector in early April. Pollen of ponderosa pine, *Pinus ponderosa* Dougl., and lodgepole pine, *Pinus contorta* Dougl., was obtained from trees on the Oregon State University campus in the early part of May. That all pollen samples were highly viable at the time of extraction was indicated by germination tests.

Duplicate samples of 1 gram of fresh pollen were inactivated in 10 ml of boiling isopropanol; after cooling, 20 ml of peroxide-free diethyl ether was added. Extraction was conducted at room temperature (21°C), for 16 hours with occasional stirring, followed by two successive extractions of ether for 4 hours. Extracts were filtered with the aid of vacuum, combined, and washed; the solvent was removed in a rotary vacuum evaporator. The extracted fatty substances were saponified by 2 percent ethanolic sodium hydroxide for 2 hours; the ethanol was removed by a stream of nitrogen gas. The soap was dissolved in water, and the nonsaponifiable material was removed by hexane. The soap solution was acidified, and the fatty acids were recovered in ether and methylated with diazomethane.

The mixtures of the methyl esters of the fatty acids were separated, in a temperature-programmed gas chromatograph by two columns which contained 10 percent and 20 percent diethyleneglycol succinate on acid-washed Chromosorb w (90 to 100 mesh). The identification of components was conducted by cochromatographic technique with pure methyl esters or by matching retention time of known mixtures. The quantitative analysis of the components was obtained by weighing the material from the peaks after each column was calibrated against the standard mixture.

The total fatty acid content, expressed as percentage of dry weight, of the pollen of each species was as follows: Douglas fir, 0.79; Formosan Douglas fir, 0.89; big-cone Douglas fir, 0.76; ponderosa pine, 1.33; and lodgepole pine, 1.25. The quantitative analysis of fatty acid in each species is shown in Table 1. Oleic, palmitic and linoleic acids are the major components in the Douglas firs, and linolenic, oleic, palmitic, and stearic acids are the major components in the pines.

It is interesting to note the similarity between the fatty acid composition of Douglas fir and Formosan Douglas fir and between the two pines, while the composition of big-cone Douglas fir stands as an intermediate between the other two species of Douglas fir and pines.

The unknown component in big-cone Douglas fir pollen is not margaric acid, a saturated C_{17} fatty acid, since the unknown formed a shoulder when margaric acid was cochromatographed. The unknown could be an unsaturated C_{16} fatty acid, for the peak disappeared when the mixture was hydrogenated and an increase of palmitic acid was observed.

Taxonomic relationships of plant waxes which were analyzed by gas chromatography were shown by Eglinton (*2*), and characterization of plant families by major fatty acids has been indicated in the literature (*3*, *4*). Ivanov considers oil content to be an inherited characteristic of plants whereas iodine value or composition of unsaturated fatty acids changes with climate (*3*). The data presented are

Table 1. Distribution of fatty acids (as percentage, by weight, of methyl esters) in the pollen of five species of Pinaceae.

Fatty acid	Douglas fir	Formosan Douglas fir	Big-cone Douglas fir	Ponderosa pine	Lodgepole pine
Caproic, C_6			0.2		
Caprylic, C_8			0.3	0.5	0.8
Capric, C_{10}			0.6	2.5	1.8
Lauric, C_{12}			0.5	4.9	6.1
Myristic, C_{14}	0.2	0.1	0.8	2.0	1.8
Palmitic, C_{16}	20.9	26.5	26.4	17.6	13.4
Palmitoleic, C_{16}*	0.2	0.2	0.2		
Unknown			1.7		
Stearic, C_{18}	2.7	2.5	15.6	10.9	12.2
Oleic, C_{18}*	62.2	52.9	39.0	23.1	16.5
Linoleic, C_{18}†	11.9	16.4	8.0	5.4	4.4
Arachidic, C_{20}	0.2				
Linolenic, C_{18}‡	0.9	0.9	4.5	24.1	31.5
Eicosenoic, C_{20}*	0.4	0.3	1.3	2.5	2.9
Behenic, C_{22}	0.3	0.2	0.9	3.1	3.1
Erucic, C_{22}*				3.6	3.5

* One carbon-to-carbon double bond. †Two carbon-to-carbon double bonds. ‡Three carbon-to-carbon double bonds.

consistent with this proposal, including the apparent anomaly of *Pseudotsuga macrocarpa*, which may be an environmental effect. Any taxonomic value of this report, however, awaits more general survey of the species in the family and between families. Furthermore, genetic make up of varieties (*5*), climate (*3, 4, 6*), and maturity (*7*), have altered the composition of fatty acids in seed. A parallelism which might exist in pollen material might be revealed by further systematic study (*8*).

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References and Notes

1. B. M. Johri and J. K. Vasil, *Botan. Review* **27**, 326 (1961).
2. G. R. Eglinton *et al.*, *Nature* **193**, 739 (1962).
3. J. B. McNair, *Botan. Rev.* **11**, 1 (1945).
4. E. W. Eckey, *Vegetable Fats and Oils* (American Chemical Society Monograph, Reinhold, New York, 1954); M. L. Meara, *Handbuch der Pflanzenphysiologie* (Springer Verlag, Berlin, 1957), vol. 7 p. 11.
5. D. M. Yermanos and P. F. Knowles, *Crop Sci.* **2**, 109 (1962).
6. H. B. White *et al.*, *J. Am. Oil Chemists Soc.* **38**, 113 (1961).
7. W. Crocker and L. V. Barton, *Seed Physiology* (Chronica Botanica, Waltham, Mass., 1953).
8. The pure methyl esters were procured from the Hormel Institute, University of Minnesota, Minneapolis. The known mixtures were provided by the Metabolism Study Section, Division of Research Grants, National Institutes of Health. The margaric acid was given by Dr. P. K. Stumpf, University of California, Davis. Support from the National Science Foundation (G 16032) and the Louis W. and Maud Hill Family Foundation.

10 September 1962

Temperature-Independent Morning Emergence in Lizards of the Genus Phrynosoma

Abstract. An investigation of the relationship of morning emergence and body temperature in *Phrynosoma* demonstrated a rhythmic anticipation of conditions favorable for normal activity. Such a rhythm offers a mechanism by which ectothermic reptiles can use safe nocturnal shelters without loss of activity time in the morning.

The daily existence of terrestrial "cold-blooded" animals, especially reptiles, depends upon their ability to utilize available external heat. Their activity is restricted when environmental conditions prove too hot or cold. In many diurnal reptiles there appears to have been selection for high body temperatures with resulting enhanced neuromotor control and celerity (*1*).

Along with the advantages accruing to high body temperatures, these animals must also suffer from a decrease in mobility at low temperature levels. Many of them use underground nocturnal retreats and might not be expected to begin daily activity until sufficient heat had penetrated into these shelters to warm them. Such a delay of emergence would result in the loss of valuable activity time on the surface during the morning. As an alternative, ectothermic animals could remain on the surface exposed throughout the night, but in the resulting cold-comatose condition they would be subject to predation and possibly freezing. However, I have found that horned lizards, *Phrynosoma*, in captivity regularly emerge before sunrise at body temperatures of 19°C, almost 15°C below temperatures of normal activity (*1*). These observations prompted an investigation of the relationship of morning emergence and body temperature.

The nature of morning emergence behavior in horned lizards is particularly advantageous for the study of this problem. In these animals daily activity is initiated by two distinct behavioral patterns. First, the animals may move upward in the sand until their heads are exposed and remain in this position until warmed to their activity levels. Alternatively, they emerge completely and begin basking in a fully exposed position. In either case inactive animals stay buried in the sand and are not visible. With the above criteria for activity, a simple experiment was devised to demonstrate whether there is any direct relationship between body temperature and morning emergence.

Two mixed groups of 15 *Phrynosoma coronatum* and *P. cornutum* were separated and kept in the laboratory at constant ambient temperatures of 18° and 27°C. The animals had access to radiant heat from infrared lights for 8 hours daily. Light was provided during this time and for four additional hours after the heat lamps were turned off; 12 hours were passed in relative darkness. The energy from the heat lamps was sufficient to allow the lizards to achieve normal activity temperatures (34° to 38°C). Although both groups had been adjusted to this daily schedule for several weeks, they were maintained in the new situation for 3 days before measurements were taken.

The lizards were active during the entire period of infrared radiation. All burrowed within 2 hours after the cessation of heating. The horned lizards spent the night buried from 2 to 8 cm in the sand. Many animals in both groups emerged in the darkness shortly before initiation of heat and light. Records on the time of emergence of individual animals were made on four consecutive days. These are shown plotted as a percentage of the total number of animals in Fig. 1. The initiation of daily activity prior to the availability of heat to warm them occurred in both groups.

The first animal came out of the sand about 40 minutes before experimental "sunrise." By 15 minutes before the initiation of heat 70 percent of the animals were active. Body temperatures at this time were the same as environmental temperatures, 18° and 27°C, respectively.

I believe these data show that the initiation of activity in the morning is independent of temperature, at least when the animals are warm enough to move. The similarity of emergence time in the two groups suggests the operation of an endogenous or circadian rhythm.

The possibility of a circadian rhythm integrated with temperature regulation in reptiles is of considerable significance with regard to their biology. By this mechanism emergence in the morning may be timed to place the animal in a situation where it can warm rapidly to normal activity levels, although exposing only a part of the body may mediate this to some extent. This is corroborated by observations of lizards in the field. Norris (*2*) has found that simultaneous morning

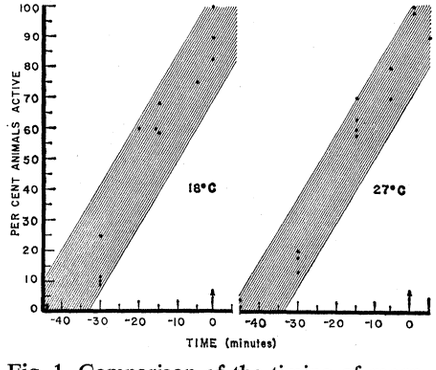

Fig. 1. Comparison of the timing of morning emergence for four consecutive days of two groups of horned lizards maintained at different temperatures. Activity begins prior to experimental "sunrise" indicated by the arrow at time 0.