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Melanophore-Stimulating Substances in Amphibia

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Workers visit between nests. Extensive marking of thousands of individual ants at each of 7 nests in a group of nests, together with the results of earlier years permit the following statements.

Workers visit between nests up to a distance of 82 feet. Visiting rate is low, of the order of 1½ to 3½, with a greater visiting rate between bud nests and presumed parental nests, than between adjacent established nests. Workers from one nest visit at several nests, and individual nests are, in turn, visited by workers from several different nests. Attempts to influence the visiting rate by heavy feeding at one nest and partial mound destruction at another have been unsuccessful.

The operation of a pattern of visiting and the low rate of production of sexuals suggest that colonies in the population do not function independently of one another, but are integrated to form a social organization at the level of the population of societies.

It is planned that future work will continue the population census, attempt to relate microclimate changes to behavioral events at the mating flight, investigate the pattern of feeding and attempt to trace the course of individual ants while visiting at nests. The suspected existence of male polymorphism in this species will be investigated during the coming year using a sample of 500 males collected during the summer.

Assisted by Daniel C. Smith, Glendale College.
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Melanophore-Stimulating Substances in Amphibia
William Thurmond
California State Polytechnic College
Project Number 122

A study of the Northwestern tiger salamander, Ambystoma tigrinum melanostictum (Baird), has confirmed a study of the Pacific tree frog, Hyla regilla, that a melanophore-stimulating substance is produced in the infundibulum of the embryo and young larva. In addition, this study has confirmed the observation that this melanophore-stimulating substance can be detected at an earlier stage in the developing embryo than can the melanophore stimulating substance, intermedin, from the adenohypophysis.

Thirty-five infundibula from embryos of modified Harrison stages 32 to 37 were homoplastically transplanted to subcutaneous tissue of 14-16 mm. albino larvae previously hypophysectomized. Thirty-four grafts evoked a pigment response in the hosts melanophores and one was negative. All seven infundibular grafts from hypophysectomized larvae evoked pigment responses.

Sixty one of sixty-six grafts of anterior forebrain, mandibular mesenchyme, and stomodeal and flank ectoderm did not evoke pigment responses. All seven control grafts of larval tissue from the telencephalon and medulla gave negative responses.
Determination of the first evidence of melanophore-stimulating activity of the infundibular and adenohypophysial grafts required that each graft be observed at least once and sometimes twice a day and that the stage of the donor be recorded at each observation. The results are shown in the following table.

<table>
<thead>
<tr>
<th>Age of Graft (in stage of donor)</th>
<th>Infundibular Grafts (34)</th>
<th>Adenohypophysial Grafts (16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>3% (1)</td>
<td>0</td>
</tr>
<tr>
<td>37</td>
<td>29% (10)</td>
<td>0</td>
</tr>
<tr>
<td>38</td>
<td>85% (29)</td>
<td>6% (1)</td>
</tr>
<tr>
<td>39</td>
<td>97% (33)</td>
<td>56% (9)</td>
</tr>
<tr>
<td>40</td>
<td>97% (33)</td>
<td>77% (13)</td>
</tr>
<tr>
<td>41</td>
<td>100% (34)</td>
<td>94% (16)</td>
</tr>
<tr>
<td>42</td>
<td>100% (34)</td>
<td>100% (17)</td>
</tr>
</tbody>
</table>

By stage 38, 85% of the infundibular grafts had evoked melanin dispersion in the host's melanophores adjacent to them whereas only one of the adenohypophysial grafts had initiated a response by this stage. Not until stage 40 or 41 do most of the adenohypophysial grafts become active.

In normal and hypophysectomized larvae of *Ambystoma tigrinum melanostictum* pigment cells become visible at stage 37 and then stellate during stages 38 and 39. During stage 40 the melanin granules in pigment cells of hypophysectomized animals become aggregated and give the cells a punctate appearance whereas the melanin granules remain dispersed in the cells of the normal larvae. This same sequence of events as occurred in the cells of hypophysectomized larvae occurred in the melanophores in the control graft of flank ectoderm. Preliminary observations indicate that the pigment cells in embryos of stages 37 to 40 exhibit the "Babak response". This is a response in which the pigment cells remain punctate in the dark and stellate in bright light. This response appears to be lost at stage 40 to 41. At stage 40 the larvae are just emerging from their jelly membranes. Ninety-three percent of 116 larvae hatched at this stage.

Thus it appears that there are coincident but independent developmental phenomena occurring about stage 40. Four events observed are: (1) the release of intermedin from the adenohypophysis; (2) melanophore dependence upon intermedin for melanin dispersion; (3) loss of the "Babak response"; and (4) hatching.

The report of Drager and Blount, (141) that the adenohypophysis begins to release intermedin at stage 38, or earlier, in *Ambystoma maculatum* embryos should now be questioned. The work this summer on *Ambystoma tigrinum* would suggest that they may have been detecting a melanophore-stimulating substance produced by the infundibulum since they tested extracts of the heads of embryos rather than the adenohypophysis itself.
Identification of the hypothalamic melanophore-stimulating substance is still to be determined. Localization of the source of this substance in the brain of the adult salamander may cast some light on this subject. Certainly the extensive work of Judson Herrick on, "The Brain of the Tiger Salamander", as well as Paul G. Roofe's work on the circulation of the brain in this animal will aid significantly in localizing the area of activity of this melanophore-stimulating substance in the brain.

Supported by the New York Zoological Society.

A Taxonomic Study by Chemical Differentiation of the Genus Artemisia
Alvin Young
University of Wyoming
Project Number 128

Work this past summer has involved a study of the chemical composition of sagebrush and how this composition varies within species. The particular area in and around Teton County was selected because of the availability of eight distinct types of Sagebrush.

1. A. arbuscula subsp. arbuscula - (Low Sagebrush)
2. A. arbuscula subsp. thermopola - (Hotsprings Sagebrush)
3. A. longiloba - (Alkali Sagebrush)
4. A. cana subsp. cana - (Silver Sagebrush)
5. A. cana subsp. viscidula - (Mountain Silver Sagebrush)
6. A. tridentata subsp. vaseyana - (Big Sagebrush)
7. A. tridentata subsp. vaseyana f. spiciformis - (Subalpine Sagebrush)
8. A. tripartita subsp. tripartita - (Threetip Sagebrush)

Leaf and flower extracts were prepared in ethanol. Two drops of the extracts were then applied to thin layers (Silicon oxide) chromatographic plates. The prepared plates were then run in a 65:30:5 solvent of n-propanol, water, and ammonium hydroxide. While the plates were still wet they were examined under ultra violet light and all fluorescent spots were marked off and Rf values determined.

Results showed that each of eight species varied distinctly both in color and number of fluorescent spots. Variations in complexes and forms were obvious. It was found also that these spots varied only slightly when examining a given population, and where variation occurred, it was found that these plants were different morphologically as well as chemically. In some cases these abnormal plants could be shown to be hybrids between two known species, i.e. A. tridentata subsp. vaseyana X A. cana subsp. cana, in this particular case the resultant proved to give the same chromatographic patterns as those obtained for A. tridentata subsp. vaseyana f. spiciformis.