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References and Notes

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Territorialism in Two Species of Salamanders

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Among the vertebrates it is well known that certain activities of individuals of a species may be limited to more or less roughly defined areas or home ranges. Territories may be established in the defense of such areas or portions thereof. Relatively little information is available on the home ranges and territories of the poikilothermal tetrapods. Examples of investigations that have yielded meaningful results are those of Nichols (*1*) on the home range of the box turtle, *Terrapene carolina* and Evans (*2*) on territorial defense in *Anolis sagrei,* the Cuban lizard. The display of aggressive territoriality in the South American frog, *Phyllobates trinitatis* has recently been described by Test (*3*). Aggressiveness was most pronounced in adult females in the defense of their home sites and feeding grounds.

In the urodeles, females of several species are customarily found on or near the developing egg masses. Such a situation has been thoroughly described by Wood (*4*) in the four-toed salamander, *Hemidactylium scutatum.* His investigations seem to indicate that this is not a case of territorialism per se, for the majority of the females present at nesting sites are gravid, having congregated for the purpose of laying eggs rather than maintaining a vigil over them.

This paper reports preliminary studies of territorialism in *Hemidactylium scutatum* and the two-lined salamander *Eurycea bislineata.* Studies were conducted in aquariums provided with a terrain of moss and forest debris in order to simulate the natural habitat of the animals. Each habitat thus established occupied an area 18 by 9 in. In the first experiment four specimens of *E. bislineata* were introduced into this environment. Two individuals immediately selected stations under bark fragments at opposite ends of the area. After a period of several weeks it became apparent that these were permanent stations and it was possible to plot a home range for each individual. This extended for a radius of 5 to 6 in. from the centrally located shelter site except where limited by the walls of the chamber. Repeated experiments, in which as many as six individuals were used, showed that the laboratory habitats described above would support no more than two resident individuals at a time. In almost every case there was a slight overlapping of the home ranges. Although food was supplied throughout the environment, established individuals were observed eating outside their home area on only two occasions. It is reasonable to assume that in this species the home range corresponds roughly to the general feeding grounds.

Animals that did not occupy home ranges wandered at random. When an errant individual entered the home range of another member of its species, it was allowed to approch within 2 in. of the shelter site. At this point the resident advanced and placed its snout in direct contact with that of the intruder. After the antagonists had remained in this posiiton for several minutes, the intruder always backed away from the area and continued its course in a different direction. Such intimidation of the intruder was the usual method of defense displayed by *E. bislineata.* On occasion, however, this technique was abandoned in favor of direct assault, in which the intruder was bitten about the snout or tail, not infrequently losing large portions of the latter member during the foray. These investigations indicate that the actual territory of *E. bislineata* is limited to a region in the immediate vicinity of the shelter site. The undefended portions of the home range cover a considerably larger area.

The number and composition of the home ranges established by *H. scutatum* corresponded closely to those of *E. bislineata.* However, territorialism was not as aggressively maintained as in the two-lined salamander. Intimidation was the only method of defense observed. An intruder usually halted as a resident advanced toward it from the shelter site. Although there was seldom any direct contact between the two animals, the mere presence of the resident seemed to be sufficient intimidation to cause the intruder to withdraw. Intruders were occasionally allowed to pass unmolested over shelter sites. However, since two animals never occupied the same shelter and since no resident was ever evicted from his established domain, it is reasonable to conclude that in *H. scutatum* the integrity of the territory is maintained, although aggressive territorialism is but weakly developed. It is interesting to note that specimens of *H. scutatum* and *E. bislineata* that did not establish a home range consistently refused to accept food under any conditions and eventually starved to death.

Territorialism has been observed in *E. bislineata* and *H. scutatum* kept in the laboratory. In both species the integrity of the shelter site is maintained and a territory thus defined. However, it should be noted that in nature the home ranges and territories of these species can be expected to vary greatly in size and shape according to the available food supply, population density, terrain, and so forth. The selective advantage afforded individuals possessing a sheltered territory is obvious, for animals deprived of such a retreat must sustain heavy predation. Furthermore there is some evidence that the proper feeding reactions of an individual are dependent on its possession of a home range and associated shelter site. Much additional work is called for in the study of territorialism in the amphibians. It would be particularly interesting to determine what specific reactions are involved in defense by intimidation when there is little or no display apparent.

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On the Conversion of Anthranilic Acid to Indole

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Isotope studies on the utilization of anthranilic acid as a precursor of tryptophan in *Neurospora* have indicated that during the conversion of anthranilic acid to indole the carboxyl group is lost (*1*) and the amino group is retained (*2*). Somewhat different conclusions concerning indole synthesis in *Escherichia coli* have been arrived at by Bergmann *et al.* (*3*) on the basis of inhibition analyses. These workers suggest that in *E. coli* the carboxyl carbon of anthranilic acid is retained during conversion to indole.

The question of whether or not the carboxyl carbon of anthranilic acid is lost in *E. coli* has recently been examined in tracer experiments performed with this organism. The results obtained (*4*) showed conclusively that in *E. coli,* as in *Neurospora,* the carboxyl group of anthranilic acid is removed during the formation of indole. In the synthesis of indole from anthranilic acid, therefore, two carbon atoms must be added to form the pyrrole ring of indole. Since the carboxyl group of anthranilic acid is removed during this process, the pyrrole ring of indole could be formed through either the 1- or the 3-position of the benzene ring of anthranilic acid. The results of an experiment designed to distinguish between these possibilities are presented in this paper (*5*).

Washed cell suspensions of certain tryptophan auxotrophs of *E. coli* readily convert anthranilic acid to indole, and cell-free extracts of such cells also carry out this conversion (*4*). 4-Methylanthranilic acid can be substituted for anthranilic acid, in which case a methylindole is formed. If ring formation in the production of methylindole from 4-methylanthranilic acid occurred through the 1-position of the benzene ring, the product of the reaction should be 6-methylindole (Fig. 1). If, however, the pyrrole ring is formed through the 3-position, 4-methylindole should result. If the ring could be formed through either position, as, for example, if aniline were the product of anthranilic acid decarboxylation, then a mixture of 4- and 6-methylindole might be produced. Thus identification of the methylindole formed from 4-methylanthranilic acid would permit distinguishing between the various possibilities.

Washed cell suspensions of a tryptophan auxotroph of *E. coli* were incubated (with shaking) in the presence of 4-methylanthranilic acid (40 μg/ml), glucose (2mg/ml) and hydroxylamine hydrochloride (200 μg/ml) (*6*). After a 30-min incubation period at 37°C an additional portion of hydroxylamine hydrochloride (200 μg/ml) was added. After 60 min, incubation was stopped and the cells were removed by centrifugation. A few milliliters of alkali were added to the supernatant solution (enough to make it slightly alkaline), which was then extracted with several portions of ether. The ether extracts were combined and concentrated to a few milliliters. Approximately 5 ml of 0.1*N* NaOH were then added to the ether extract and the mixture distilled in vacuum until less than 1 ml of solution remained. At this point the ether-free distillate contained all the methylindole.

The quantity present was determined colorimetrically with Ehrlich's reagent, using 6-methylindole as standard. The distillate assayed 10.2 mg methylindole (assuming that it was 6-methylindole). Picric acid (100 mg) was added to the distillate and the picrate that formed was collected by filtration; 83 percent of the methylindole was recovered as the picrate. The picrate melted (melting points are uncorrected) at 156 to 160°C. The melting point of the picrate of an authentic sample of 6-methylindole was 159 to 161°C. The melting point of a mixture of the two picrates was 158 to 160°C. Reported melting points for the 4- and 6-methylindole picrates are 194 to 195° (*7*) and 157°C (*8*), respectively. The infrared spectra of the isolated material and authentic 6-methylindole picrate were determined by H. Wasserman of Yale University. Comparison of the spectra indicated that the two samples were identical. Since the isolated material is 6-methylindole, ring formation must occur through the 1-position of the benzene ring of 4-methylanthranilic acid (*9*).

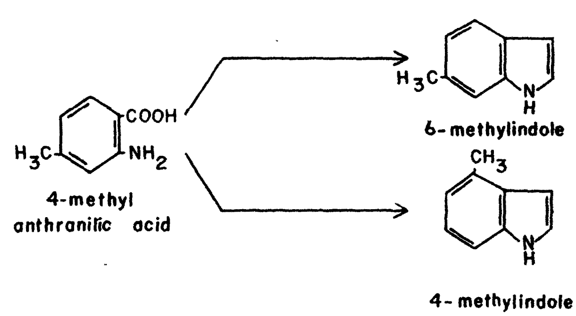

Fig. 1. The two methylindoles that would be produced depending upon whether the pyrrole ring is formed through the 1- or the 3-position of the benzene ring of 4-methylanthranilic acid.