Reports and Letters

Behavior Pattern in Migratory Fishes

I wish to call attention to a behavior pattern in certain migratory fishes which may be a very common phenomenon. So far the evidence for its universality is relatively slight, yet there are indications that it may be both widespread and of some importance to the economy of the affected species. The hypothesis may be stated as follows: In anadromous or potamodromous (*1*) fishes the earliest arrivals in a particular spawning tributary will travel to the farthest reaches of the acceptable breeding area, the later arrivals occupying territories closer to the mouth of the stream.

My first awareness of this type of behavior came when I was working on a small coastal stream in California (*2*) and I referred to it, for want of a more concise terminology, as a "phenomenon of differential distribution." Since that time attempts have been made to collect further information, both from the literature and through correspondence with persons engaged in research on the life-cycles of such fishes.

Stone (*3*) and Hobbs (*4*) both noted that the earlier runs of king salmon (*Oncorhynchus tshawytscha*) go farther upstream and that the later fish take places below them. In 1939, Pritchard and Cameron (*5*) observed the distribution of tagged sockeye salmon (*O. nerka*) in a small creek but found no significant correlation between the location on the spawning areas and the time of entry into the stream. In this case it may be that the presence of a weir, which would somewhat delay the spawning run, and the handling of adults during the tagging process had some effect on their subsequent distribution. It is interesting to note that a report by Schaefer (*6*), also describing work with the sockeye salmon, does refer to a differential distribution on the spawning grounds.

During his observations on the life-history of the sea lamprey (*Petromyzon marinus*), Applegate (*7*) found a well-marked differential distribution. The most recent observations (*2*) of this phenomenon were made on the Pacific lamprey (*Entosphenus tridentatus*), the silver salmon (*O. kisutch*), and the steelhead trout (*Salmo gairdneri*). Briefly, the results of communication with other biologists have been encouraging because many have mentioned casual observations which tend to show that this distributional pattern is repeated, at least occasionally, in other areas and with other migratory species such as the eastern brook trout (*Salvelinus fontinalis*) and the Atlantic salmon (*Salmo salar*).

As is stated in a foregoing paragraph, the hypothesis is given as a possibly typical behavior, but I recognize that a number of situations might exist which would so modify the effect of this behavior that its expression may be more or less inhibited. These are some factors that must be taken into consideration when observations are made for the purpose of testing this hypothesis: (i) The breeding area of a particular stream may be cut off or otherwise affected by dams, log jams, or waterfalls. (ii) An unsuitable spawning substrate might be present over a portion of the area. (iii) Local pollution or unfavorable temperatures may have noticeable effects. (iv) The behavior may not be shown by species that typically spawn very close to salt water or in brackish situations. (v) There might be a great range in size in a particular species, so that the larger individuals would be inclined to seek deeper water for spawning purposes.

The most logical explanation of why such a phenomenon should exist is that it is genetic in nature and such early arrivals are offspring of parents that showed a similar behavior. The principal objection to this theory is that there are certain selective disadvantages operating against such a state of affairs. The fishes that spawn earliest (at least in some species) travel upstream under conditions of relatively low water, with the result that passage is more difficult, and are, presumably, under greater threat from predatory animals. However, this disadvantage may be offset by the favorable conditions that are found by the young upon their emergence. They would have a headstart over the progeny of the later arrivals, an especially important factor in such fishes as salmon or trout, which are quite cannibalistic.

In some Pacific salmon another cause may lie in the territorial behavior of the female. Once the territory is selected and the spawning activities are begun the female becomes a very zealous guardian and remains so for a considerable period after the eggs are deposited. This may discourage later females from penetrating farther into the spawning area, especially if the stream is small. On the other hand, territorial behavior is only poorly developed in the steelhead trout and the sea lamprey, and, as was mentioned previously, these two species have been observed to follow the differential distribution pattern.

This "rule" of behavior is presented here as a hypothesis, one that does seem likely in view of the information obtained so far. It is hoped that its publication will stimulate the curiosity of those engaged in research on the life-histories of migratory fishes.

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References and Notes

1. The terms *anadromous* and *potamodromous* are used according to the restricted definition of G. S. Myers [*Copeia* 2, 94 (1949)]: *anadromous* refers to marine fishes that migrate to fresh water to breed, and *potamodromous* means those fishes that migrate wholly within fresh water.
2. J. C. Briggs, *Calif. Div. Fish and Game, Fish Bull.* 94, 26 (1953).
3. L. Stone, in *The Fisheries and Fish Industries of the United States* by G. B. Goode, Sect. 1, 483 (Govt. Print. Off., Washington, 1884).
4. D. F. Hobbs, *New Zealand Marine Dept., Fish Bull.* 6 (1937).
5. A. T. Pritchard and W. M. Cameron, *Progr. Repts. Pacific Biol. Sta., Fisheries Research Board Canada* 43, 16 (1940).
6. M. B. Schaefer, *Bull. Intern. Pacific Salmon Fish Comm.* 4 160 (1951).
7. V. C. Applegate, *U.S. Fish Wildlife Service, Spec. Sci. Rept. Fisheries* 55, 93 (1950).

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Concentration and Purification of Polio Viruses

Methods have been developed recently that employ heavy metals in the separation of various components of plasma (*1*). These techniques have been utilized in the concentration and purification of influenza and Rous sarcoma viruses (*2*). We have applied modifications of these methods to the concentration and purification of polio viruses. Highly significant results have been obtained, and since vaccines made from these agents currently occupy much attention, a brief mention of our results appears to be timely.

Polio viruses types I, II, and III grown in monkey kidney cells in 199 medium have been quantitatively precipitated by the addition of zinc lactate to a concentration of 2.5 to 5 mmoles/lit at *p*H 7.5. All manipulations are carried out at 2°C. The precipitate (which contains greater than 99 percent of the virus) is separated at about 3000 rev/min in the refrigerated angle centrifuge. The insoluble zinc-virus compound is washed at various *p*H levels with reagents such as 0.85-per cent NaCl or H_2O. The zinc may be removed by adding a small amount (1 to 100 ml) of $0.1M$ sodium citrate to the zinc-virus compound followed by dialysis against H_2O or 0.85-percent NaCl. An alternate method is the removal of zinc utilizing ion-exchange resins (*3*). Analyses show that either dialysis or resin exchange will remove zinc to a residual concentration of less than $2 \times 10^{-5}M$.

Titration of the virus concentrate in monkey kidney tissue culture demonstrates that no loss of virus occurs. The titers expressed in infective units for dose $\log_{10}$ of type II MEF_1 virus before zinc precipitation, and of the supernatant after zinc precipitation were $10^{7.7}$ and $10^{4.2}$, respectively, and of type III Saukett were $10^{5.5}$ and $10^{1.5}$, respectively, or approximately 99.9 percent of the virus was removed. The stability of the zinc precipitate has been followed for 10 wk at 4°C without perceptible loss of infectivity for monkey kidney tissue culture. (It is interesting to note that the titer of the virus is unaltered even when zinc is combined with it.)

The method described here has many obvious applications. It provides a technique for safety testing of the finished vaccine, since any desired concentration may be effected. The process provides a simple way of removing various components of the medium, such as antibiotics, amino acids, and metabolites. It should simplify the production of the vaccine, since large volumes of virus may be conveniently and rapidly concentrated for processing and killing. This should make possible better standardization of virus inactivation with formaldehyde on other agents. With the removal of the growth medium, selection of a preservative and antigenic stability of the vaccine may no longer be a problem.

The method would appear to have wide application. Highly purified, stable herpes simplex virus has been obtained (*4*).

A complete report concerning work with poliomyelitis viruses is in preparation.

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References

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3. J. L. Oncley, and H. M. Dintzis, report available from the department of biophysical chemistry, Harvard University, Cambridge, Mass.
4. J. Smolens, A. Gray, T. F. McNair Scott, in preparation.

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Some Analogs and Derivatives of the Insecticide DDT

A casual inspection of recent chemical literature reveals that no fewer than 102 organic compounds, either isomeric or analogous DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], have been prepared. An inclusion of related condensation products would provide even a far more imposing list. We have recently prepared five additional analogs of DDT that have not been previously reported (*1*). In connection with the preparation of these five compounds, a more exhaustive study has been made of several other variations.

The general method of preparation consisted in the condensation of chloral hydrate, bromal, or butyl chloral with several mono- or disubstituted benzenes in the presence of fuming sulfuric acid. In several instances, the resulting compounds were subsequently treated with alcoholic potassium hydroxide in order to eliminate hydrogen halide. These ethylenic products were then further halogenated or oxidized to related derivatives. Various nitro derivatives were also prepared by the direct nitration of the original condensation products. The results of 33 types of attempted condensations under a variety of conditions that involved several hundred syntheses seemed to indicate that chloral hydrate and monohalogenated benzenes condense readily in the presence of fuming sulfuric acid. Bromal and monohalogenated aromatic compounds formed condensation products that were, in general, relatively unstable, as was previously reported (*2*). Butyl chloral and chlorobenzene formed condensation products of unknown and variable structure. Ethyl benzoate and chloral hydrate formed an apparent condensation product that was unstable to treatment with alkali. The condensation of chloral hydrate with *p*-dichlorobenzene, *p*-dibromobenzene, and *p*-chlorobromobenzene formed products, the structure of which has not been fully established. *m*-Dichlorobenzene, *o*-dichlorobenzene, and *o*-chlorotoluene did not condense readily with chloral or its hydrate under comparable conditions using fuming sulfuric acid.

The specific experimental data in the preparation of these analogs is as follows.

1) 1,1-Dichloro-2,2-bis(*p*-iodophenyl)-ethylene: A mixture of 10 g of 1,1,1-trichloro-2,2-bis(*p*-iodophenyl)ethane (*3*) and 6 g of potassium hydroxide in 400 ml of 95-percent ethanol was refluxed for 4 hr. The reaction mixture was poured into ice water, filtered, and recrystallized from absolute ethanol, yielding 4.8 g (51-percent theory) of product; melting point, 146.4 to 147.0°C (corrected). As proof of structure, this compound was oxidized with chromium trioxide in glacial acetic acid to *p-p'*-diiodo benzophenone; melting point, 235° to 236°C (*4*).

2) 1,1,1-Trichloro-2,2-bis(3-nitro-4-iodophenyl)ethane: A solution of 10 g 1,1,1-trichloro-2,2-bis(*p*-iodophenyl)-ethane in 160 ml of glacial acetic acid and 160 ml of fuming nitric acid (specific gravity, 1.60) was heated on a steam bath for 5 hr. The resulting nitrated product was poured into ice water, filtered, and recrystallized from absolute ethanol, yielding 5 g (45-percent theory) of product; melting point, 173.7° to 174.3°C (corrected). It was assumed that one nitro group entered the 3-position *o*- to the iodine. Analytic calculated for $C_{14}H_7Cl_3I_2N_2O_4$: N, 4.46 percent; found: 4.52 percent.

3) 1,1,1,2-Tetrachloro-2,2-bis(*p*-bromophenyl)ethane: A warm solution of 20 g of 1,1,1-trichloro-2,2-bis(*p*-bromophenyl)ethane (*5*) in carbon tetrachloride was chlorinated with strong illumination during 4 hr. The carbon tetrachloride was removed by vacuum distillation, and the solid was recrystallized from absolute ethanol, yielding 12 g (57-percent theory) of product; melting point, 95° to 96°C. Theoretical silver halide for $C_{14}H_8Cl_4Br_2$: 1.980 g/g of compound; found: 2.018 g/g of compound.

4) 1,1,1-tribromo-2,2-bis[2(5)-chloro-5(2)-bromophenyl]ethane: A 1.0- to 2.0-mole ratio of bromal and *p*-chlorobromobenzene was heated at 85° to 90°C while 400 ml of 15-percent fuming sulfuric acid was slowly added with vigorous stirring. The reaction mixture was heated for an additional 2 hr at the same temperature, resulting in the formation of a yellow precipitate. The product was filtered and then washed, first with 10-percent sodium bicarbonate solution and then with water. After recrystallization from a 50–50 mixture of ethanol and trichloroethylene, followed by absolute ethanol, 50 g (50-percent theory) of a yellow product was obtained, melting point, 163.0° to 163.7°C (corrected). Theoretical silver halide for $C_{14}H_7Cl_2Br_5$: 1.898 g/g of compound; found: 1.895 g/g of compound. We are inclined, in the light of orientating tendencies and steric hindrance, to assume that the coupling in the phenyl group is *o*- to the chlorine and *m*- to the bromine, although the converse might be true. Final confirmation on this structure is still lacking.

5) 1,1,1-Tribromo-2,2-bis(2,5-dibromophenyl)ethane: A similar condensation was made with a 1.0- to 2.0-mole ratio of bromal and *p*-dibromobenzene that was heated with 720 ml of 15-percent fuming sulfuric acid and stirred at 75° to 80°C for 2 hr. The brown prod-