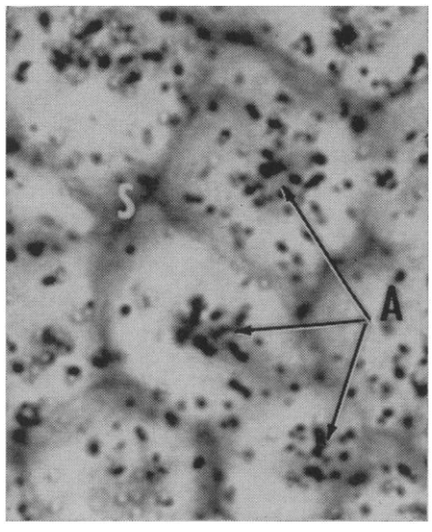

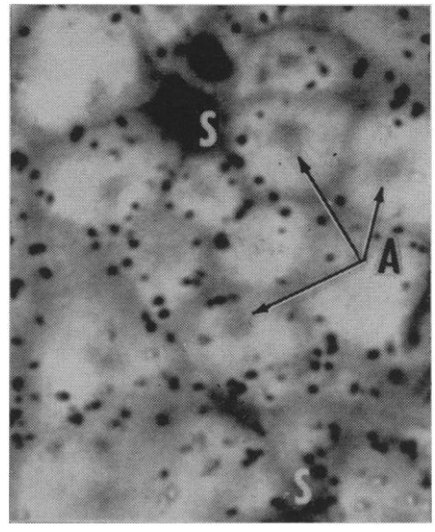

Fig. 2 (left). At 27 hours, that is, relatively early after injections of leucine-H^3, no significant radioautographic reaction was observed over the axons of the sciatic nerve (*A*), but silver grains were located over Schwann cell nuclei (*S*) and cytoplasm. Fig. 3 (right). After 16 days, silver grains were concentrated over the axons (*A*) in a distal region of the sciatic nerve, while the reaction over Schwann cell cytoplasm (*S*) is lessened.

cell bodies and of the cells located along the nerves. By this time, radioactivity was back to a low level over the axons in the proximal region (Fig. 1) but had reached a high level over those in the distal region (Fig. 3). It may be emphasized that the presence of silver grains over every single axon indicates that all nerve fibers, whether sensory or motor, were involved.

It is known that the radioactivity detected by radioautography in histological sections soon after leucine-H^3 injection consists of newly synthesized proteins (*8*). Therefore, the presence of radioactivity at the early time interval in the cells of spinal cord and ganglia indicates synthesis of protein [in confirmation of data showing continuous protein synthesis in nerve cell bodies (*9*)]; but there is no demonstrable synthesis in the axons of the sciatic nerve. Nevertheless, labeled proteins were detected in the axoplasm of the proximal region of the sciatic nerve 4 days, and in that of the distal region 16 days, after the first injection. Hence, the proteins synthesized in the nerve cell bodies must have migrated along the axons at a speed such that the proximal region was reached by 4 days and the distal region by 16 days (Fig. 1). The data permit only a very rough estimate of the migration rate of axoplasmic proteins—about 1.5 mm per day.

The present results offer the first direct evidence in support of the axonal flow hypothesis. Further direct evidence may be available in the near future, since a recent abstract by Weiss *et al.* (*10*) indicated that the migration of axoplasmic material may be seen in vitro in mouse peripheral nerves explanted with their ganglia. It is concluded from the present data that proteins migrate along the motor and sensory axons of the sciatic nerve of the rat (*11*).

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24 May 1962

Chromatographic Validation of Two Morphologically Similar Hybrids of Different Origins

Abstract. The origin of natural hybrids of the plant *Baptisia* was determined by chromatographic analyses of leaf extracts of both hybrid and parent plants. This method demonstrated a pattern of inheritance in the hybrid of certain species-specific components of both parents.

After the first demonstration that chromatographic techniques are useful in validating the hybrid nature of suspected interspecific hybrids of *Baptisia* (*1*), the method was applied to the study of a trihybrid population to determine its composition (*2*) and also to validate a number of other combinations of suspected interspecific hybrids in the genus (*3*). One important generalization derived from these investigations is that hybrids of *Baptisia* tend to accumulate the sum of the species-specific components of both parents. Other investigators have reported similar results with different genera (*4*). This pattern of inheritance of species-specific components makes possible the determination of the origin of a particular hybrid, in some instances through chromatographic analyses alone and in other less favorable combinations through combined chromatographic and morphological analyses. Until now, it has not been possible to state unequivocally that a certain hybrid type could *only* be identified by means of a chromatographic analysis although some combinations bear a rather close morphological resemblance to each other. We can now report an example of hybridization involving three species, two of which are so similar in gross characters that it is only with great difficulty, if at all, that one can determine from external characters which of the two species is involved in hybridization with the third species in a particular instance. Chromatographic patterns can be used to establish the origin of these plants immediately, beyond doubt. Both of the nearly indistinguishable hybrid types have now been identified chromatographically from the same location.

In 1961 a putative hybrid between *Baptisia lanceolata* and *B. pendula* (*5*) was collected along state highway 56, 0.5 mile south of the Ogeechee River, Emanuel County, Georgia. Both *B. lanceolata* and *B. pendula* were observed in the area, and the former was seen in the immediate vicinity of the hybrid. A chromatographic analysis of

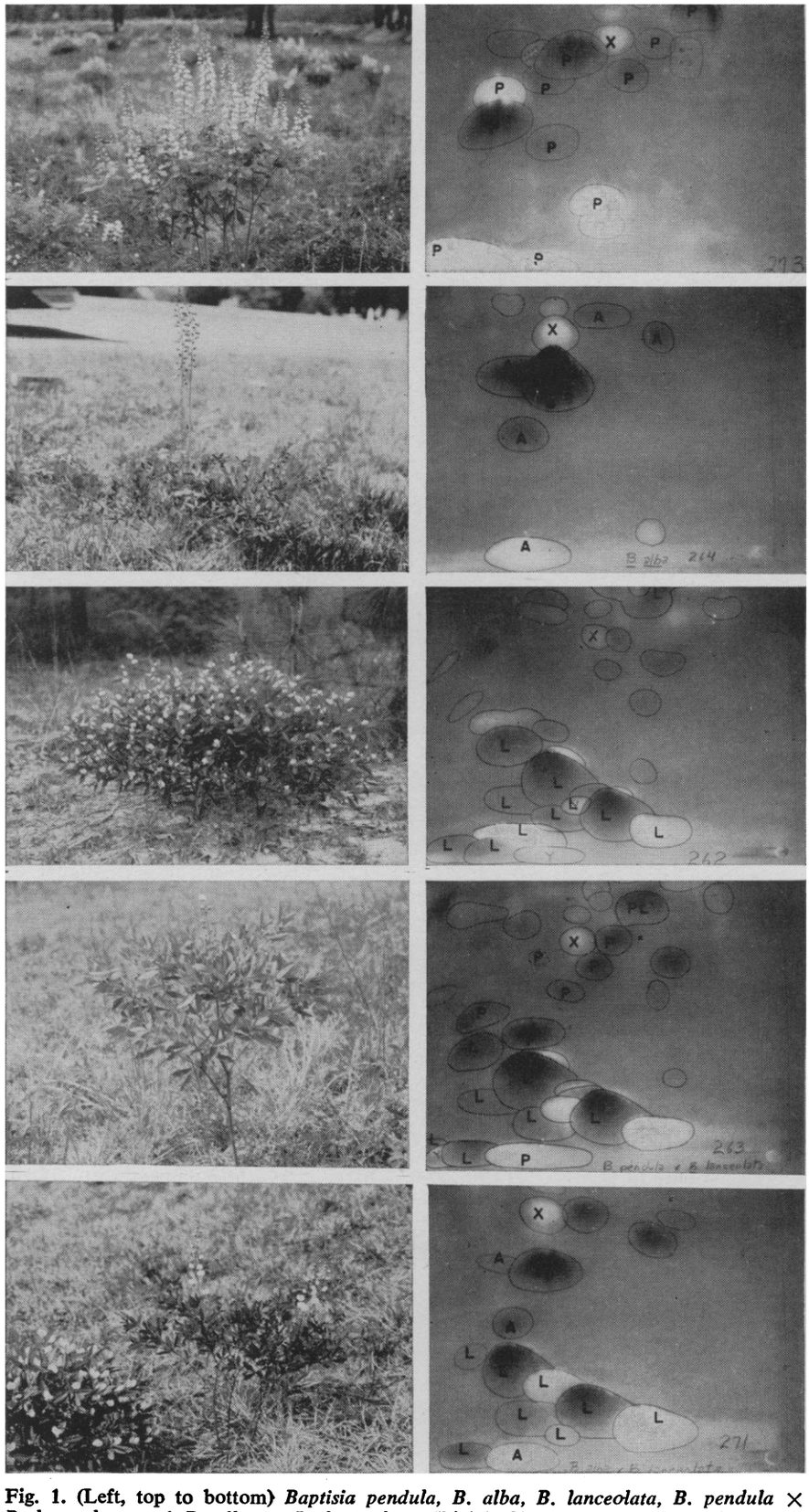

Fig. 1. (Left, top to bottom) *Baptisia pendula, B. alba, B. lanceolata, B. pendula* × *B. lanceolata,* and *B. alba* × *B. lanceolata.* (Right) Chromatograms of leaf extracts of parent plants and hybrids. *A*, spot characteristic of *B. alba; P*, spot characteristic of *B. pendula; L*, spot characteristic of *B. lanceolata; X*, common reference spot.

the hybrid indicated that it was a hybrid between *B. lanceolata* and *B. alba*, but *B. alba* was not detected in the area. It is pertinent that *B. pendula* and *B. alba* are both in the white-flowered group of *Baptisia* species and are morphologically rather similar, although it is possible to distinguish between them, especially through their fruit characters.

In 1962 another group visited the same location and discovered *B. alba* in the immediate vicinity of the original hybrid. Moreover, another definite hybrid type was found 100 yards south of the Ogeechee River together with *B. lanceolata* and *B. alba*, and close to *B. pendula.* This hybrid closely resembled the previously collected hybrid. Chromatographically, however, this plant was established as a hybrid between *B. lanceolata* and *B. pendula.*

Figure 1 illustrates the three species of *Baptisia* together with the two hybrids. To the right of each plant is its chromatographic pattern. The latter photographs were originally taken in color in ultraviolet light. Although the basic pattern of the species may be recognizable from these photographs alone, further evidence was based on the response of these substances to ammonia vapor and to a general phenol detecting reagent, *p*-dinitroaniline. With the latter reagent additional important components were disclosed. It is not possible in this discussion to treat the individual points of evidence in detail, and the photographs are included primarily to demonstrate the distribution and types of separation from which the evidence is derived. In our judgment the evidence is conclusive. Methods of extraction and chromatography have been discussed elsewhere (*3*).

It is evident that chromatographic techniques provide a new and valuable method for the study of natural hybridization. While in general the chromatographic approach may be expected merely to supplement existing methods of analysis, in *Baptisia* this approach has yielded data on population structure and, now, data on the origin of unresolved hybrids which could not have been obtained by any other means known to us (*6*).

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28 June 1962

Metabolic Requirements for the Swimming Activity of Three Antarctic Fishes

Abstract. The logarithm of the amount of oxygen consumed per unit swimming velocity in meters per minute for steadily swimming antarctic fishes at freezing temperatures is of the same order as the rate for temperate species at their lower temperature ranges. In contrast to temperate fishes, the antarctic fishes have their greatest swimming activity around −1.8°C and a level of no excess activity at about +2°C.

Field and laboratory observations of the swimming behavior of several fishes in McMurdo Sound have indicated that species of the genus *Trematomus* (family Nototheniidae) are generally sluggish. Demersal *Trematomus bernacchii* (*1*) and *T. centronotus* could not be induced to swim in a rotating, annular metabolism chamber which had been successfully utilized for arctic fish (*2*) and temperate fish (*3*). Demersal *T. hansoni* and *T. loennbergi* occasionally could be induced to swim slowly; pelagic, plankton-feeding *T. borchgrevinki* would swim steadily in the chamber. These species live in waters that are continuously at the freezing temperatures of −1.9° to −1.8°C. However, none of them would swim consistently at temperatures much higher than the natural temperatures, and at the highest laboratory temperatures to which they would become acclimated—about +2°C—they tended to cease swimming. Because this type of behavior contrasts sharply with temperate fishes for which similar data are available, metabolic requirements per unit swimming activity of the three swimming species were investigated.

The multiple regression technique (*2–4*) of relating oxygen consumption rates (Y = log milligrams of O_2 consumed per hour) as a measure of metabolism to the body weight (X_w = log grams), to the temperature (X_t = °C) at which the fish have been acclimated, and to the swimming velocity (X_s = m/min) has proved useful in the form:

$$Y_e = a + b_w X_w + b_t X_t + b_s X_s$$

In this form Y_e is the estimated logarithm of the number of milligrams of O_2 consumed per hour, b_w is the partial regression coefficient of increase in Y_e (usually about 0.8) for constant X_t and X_s, b_t is the increase in Y_e per degree increase in temperature (usually about 0.02 to 0.06) for constant X_w and X_s, and b_s (the respiration-swimming coefficient) is the increase in Y_e per unit swimming velocity in meters per minute for a constant X_w and X_t, and a is a constant whose value depends in part upon the degree of "cold adaptation" (*1, 2, 4, 5*).

In Table 1 are b_s values calculated over various experimental temperature and swimming velocity ranges as indicated for the three antarctic species. The usefulness of the b_s log milligrams of O_2 consumed per hour per unit swimming velocity in meters per minute is that energy expenditures are expressed in units independent of weight and absolute swimming velocity. The 1960–61 data are for fish acclimated at −1.8°C for a period of 48 hours before O_2 consumption rates were measured; 1961–62 data are for 5-day acclimation periods. Temperatures were raised at the rate of no more than 1.0°C per 24-hour interval, with 2- or 5-day acclimation periods at each of the successively higher temperatures before the respective O_2 consumption rate determinations. Among the fish that would swim in the chamber, pelagic *Trematomus borchgrevinki* would swim consistently for determinations over at least 10 minutes while the more sedentary *T. hansoni* and *T. loennbergi* would swim fairly steadily for 45-minute determinations.

From published data as indicated in Table 1, respiration-swimming coefficients can be calculated for a few diverse temperate and arctic fishes by taking the difference between the logs of the O_2 consumption rates at resting and at active conditions and dividing the difference by the swimming rate in meters per minute.

The maximum swimming activity for temperate species appears to be at temperatures somewhat below the seasonal maxima to which the species would normally be subjected (*6*). For the antarctic species the maximum tends to be at freezing temperatures.

For the 1960–61 *T. borchgrevinki*: 44 of 46 fish (−1.8° to −1.7°C) swam at an average velocity of 6.1 m/min; 17 of 22 (−0.8°C), at 6.1 m/min; 19 of 24 (+0.8° to +0.9°C), at 5.9 m/min; and 1 of 20 (+1.8°C), at

Table 1. Respiration-swimming coefficients with swimming velocity and temperature ranges for three antarctic species and for various temperate and arctic species.

Species	Temperature (°C)	Swimming rate (m/min)	Respiration-swimming coefficient	Source of data
Antarctic *Trematomus* spp:				
T. borchgrevinki; N = 112; all fish	−1.8 to +1.8	0–13.5	0.0307	Dec. 1960–Jan. 1961
T. borchgrevinki; N = 92; active swimming range	−1.8 to +0.8	0–13.5	.0345	Dec. 1960–Jan. 1961
T. borchgrevinki; N = 46; "natural" temp. range	−1.8 to −1.7	0–13.5	.0243	Dec. 1960–Jan. 1961
T. borchgrevinki; N = 63; "natural" temp. range	−1.8 to −1.7	0–11.4	.0406	Nov. 1961
T. loennbergi; N = 30	−1.8 to +2.4	0– 4.4	.1124	Jan. 1962
T. hansoni; N = 35	−1.8 to +2.2	0– 2.8	.0660	Jan.–Feb. 1962
Other species:				
Coregonus sardinella	8.3 to 10.8	1.0–10.0	.0243	Arctic Summer (*2*)
Oncorhyncus keta; males; Amur R. migration	12	80	.0111	(*9*)
O. keta; females; Amur R. migration	12	80	.0113	(*9*)
Salvelinus naymacush; 1-yr stock	16	31.4	.0227	(*10*)
S. naymacush; 2-yr stock	16	38.7	.0202	(*10*)
S. naymacush; 1-yr stock	9	21.3	.0402	(*10*)
S. naymacush; 2-yr stock	9	28.3	.0368	(*10*)
S. fontinalis	15	44.2	.0143	(*11*)
S. fontinalis	5	23.2	.0320	(*11*)
Lepomis macrochirus	22.0 to 26.5	1.0–14.4	.0138	Summer (*3*)
L. macrochirus	11.0 to 22.4	0–14.3	.0142	Autumn (*3*)
L. macochirus	8.2 to 12.0	0– 9.0	.0427	Winter (*3*)
L. macrochirus	14.0 to 23.0	0–12.5	.0250	Spring (*3*)
Trachurus trachurus	20	0–60	.0125	(*9*)
Carassius auratus	25	30.5	.0088	(*7*)
C. auratus	5	14.6	.0291	(*7*)