The bearing of *Phoxinus* (Cyprinidae) hybridity on the classification of its North American species[1]

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Three populations of *Phoxinus eos* × *P. neogaeus* were found to form single Mendelian populations, by comparison with the parental species through a discriminant function. The hybrids of one of these populations were found to be fertile. A fourth hybrid collection, studied by three discriminant functions, was found to contain *Semotilus margarita*, *P. eos* × *P. neogaeus*, and hybrids of *S. margarita* with at least *P. eos*, but possibly *P. eos* × *P. neogaeus*. The presence of this hybrid, when related to the chromosome number of the species concerned, suggests the transfer of *S. margarita* to the genus *Phoxinus*.

L'étude de trois populations de *Phoxinus eos* × *P. neogaeus* a mené à constater que chacune de ces populations est mendélienne. La comparaison avec des populations des espèces parentes qui a permis de tirer cette conclusion a été faite en utilisant une fonction discriminante. La fertilité des individus de l'une de ces populations a été vérifiée. Une quatrième collection fut étudiée à l'aide de trois fonctions discriminantes. Les spécimens furent identifiés comme appartenant à trois groupes: *Semotilus margarita*, *P. eos* × *P. neogaeus*, et des hybrides de *S. margarita* avec *P. eos* au moins, mais possiblement avec *P. eos* × *P. neogaeus*. La présence de ces hybrides et les nombres chromosomiques des espèces en question suggèrent que *S. margarita* serait mieux classifié dans le genre *Phoxinus*.

Introduction

The fishes under study hereafter pertain to the Eurasian genus *Phoxinus* that includes the former North American genus *Chrosomus* (Bănărescu 1964).

The existence of hybrids *Phoxinus eos* × *P. neogaeus* has been known for 40 years. When Hubbs and Brown (1929, p. 28) first mentioned them, they suspected them to be fertile: but this has never been demonstrated and the fish have usually not been considered as forming Mendelian populations, because hybrids *Phoxinus eos* × *P. neogaeus* (New 1962) and all other hybrid cyprinids (Nyman 1965) were thought to be sterile.

Collections of minnows from many localities in Québec, made by Prof. V. C. Wynne-Edwards of McGill University between 1931 and 1948, and unpublished distribution maps of these in the archives of the Québec Wildlife Service, Department of Tourism, Fish and Game, include some specimens identified as hybrids. For example, in 1932 he identified hybrids of *P. neogaeus* × *P. eos* from two localities in the Eastern Townships. In 18 of 20 other localities where he found *P. neogaeus*, *P. eos* was also present. Among these collections, a set of specimens from a lake north of the Ottawa River was later recognized by Mr. Vianney Legendre, of the Department of Game and Fisheries of the Province of Québec, as entirely consisting of hybrids *P. eos* × *P. neogaeus*. In 1966 the present author collected adult specimens of *Phoxinus* from three lakes near Saint-Hippolyte, Terrebonne Co. These were recognized as all being hybrids of these same species by the above-mentioned authority.

There is strong presumptive evidence, therefore, that *Phoxinus* populations in some lakes in Québec, and elsewhere perhaps, are entirely hybrid and must constitute self-sustaining populations. If this is so it follows that the hybrid fish must be interfertile at least to some degree.

Three populations of minnows considered to be hybrids have been studied in relation to representative populations of their parental species. Each parental sample was collected from areas where the other parent species and the hybrids have not been found: in this way we can be sure that specimens of only one of the species would be obtained from each area. The main part of this paper consists of a taxonomic study based upon morphometric data, making use of discriminant functions to determine the position of the assumed hybrid populations between the parental reference collections.

[1]Part of a thesis (Legendre 1969*a*).
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Another collection, containing *Semotilus margarita* as well as *P. eos* × *P. neogaeus*, together with supposed intergrading specimens, was studied by the same methods. No previous study has mentioned hybridization between *S. margarita* and *Phoxinus*. Finally, the present study examines boundaries of the genus *Phoxinus*.

Material and Methods

Two natural hybrids were studied: *Phoxinus eos* × *P. neogaeus* and *Phoxinus eos* × *Semotilus margarita*. Collections of specimens of the three parent species were also examined for comparison. When the name *S. margarita* is used, it is understood as being the subspecies *S. m. nachtriebi* (Cox).

Table I shows the localities where the author (unless otherwise specified) collected the specimens. Fishing was done by minnow traps in Caché, Triton, and Eos Lakes. A 35-ft long seine net, with ¼-in. mesh, was used in lakes Caché and Amikeus. Sampling sufficiently extensive and random, to assure that the sample was representative of all the *Phoxinus* present, was possible because of the small size of these four lakes (10 000 to 35 000 m² in area). A random sample of the fish of each lake has been used in the present study.

The collections were deposited at the Research Division of the Québec Wildlife Service, in Montréal.

The characters used were the ones that were known to vary in the hybrids, and were suitable for measurement. The meristic and metric characters (see Table II) were used mainly as defined by Hubbs and Lagler (1964). However, others are based on a new definition, more adapted to the present work. They are as follows.

Length from pectoral to pelvic fin—The longest straight distance between the points where the uppermost, outermost, or anteriormost ray of these fins is inserted into the body. This measurement, as the other paired measurements, was made on the left side of the body.

Length from the tip of the snout to the last pore of the lateral line—The anteriormost point of the premaxillary in the resting position is used as the anterior point of the straight line if the tip is the most anterior part of the snout. The last pore of the lateral line may not overpass the hypural plate: in this case, the length is equal to the standard length.

Length of orbit—The greatest longitudinal distance between the inside parts of the melanophore circle located on the rim orbital skin. (See Fig. 1).

Length of tail—From the most anterior point of the base of the anal fin to the midpoint of the depth of the fish, at the level of the end of the hypural plate.

Mandibulo-maxillary angle—The angle between the line touching the most anterior points of the mandible and the maxillary, and the line starting on the most anterior point of the mandible and going to the rear of the fish, parallel to the longitudinal axis of it (see Fig. 1).

Mouth angle—The mouth being closed but relaxed, this angle is the one between the straight line passing by the most anterior and the most posterior points where the upper lip touches the lower lip, and the line, from the most anterior point of junction of the lips, to the rear of the fish, parallel to the longitudinal axis of it (see Fig. 1).

Predorsal length—Length of the region where the so-called predorsal scales are. This region extends from the groove separating the head and the back, to the point where the first ray of dorsal fin is inserted into the body of the fish.

Snout length—The straight line from the most anterior point on the snout (on the upper lip relaxed when the lip is the most anterior point of the snout) to the interior margin of the most anterior part of the melanophore circle around the eye (see Fig. 1).

A dial caliper, with an accuracy of reading of 0.05 mm, was used to take the measurements in millimeters. The angles were measured in degrees with an arm protractor as described by Hubbs (1946).

From these measurements and counts, discriminant functions were computed by the IBM 360/75 computer of McGill University. The program used to calculate the functions is called "Discriminant analysis BMDO4M" (Dixon 1967, p. 185).

TABLE I

Origin of the specimens

Taxon	Date	Place	Coordinates	Source
Phoxinus eos	June 13, 1966	Caché (or: Parond) Lake, Qué.	74°29′25″ W, 46°05′42″ N	
Phoxinus neogaeus	June 3, 1954	Lichen Lake, Ont.	92°01′ W, 50°04′ N	Royal Ont. Mus.[a]
	June 15, 1942	Bog lake near Fort Albany, Ont.	81°41′ W, 52°14′ N	Royal Ont. Mus.[a]
P. eos × *P. neogaeus*	May 18, 1966	Triton Lake, Qué.	74°00′21″ W, 45°59′18″ N	
	May 28, 1968	Eos Lake, Ont.	78°21′35″ W, 45°35′35″ N	
	May 28, 1968	Amikeus Lake, Ont.	78°30′55″ W, 45°35′20″ N	
P. eos × *P. neogaeus*, *Semotilus margarita*, *P. eos* × *S. margarita*	May 15, 1968	Stream from Holly (Hawley) Lake, Ont.	76°06′23″ W, 45°36′20″ N	Nat. Mus. of Can.[b]
Semotilus margarita	Sept. 4, 1941	Belle-Rivière Lake, Qué.	71°42′ W, 48°13′ N	Qué. Wil. Serv.[c]

[a]Royal Ontario Museum of Zoology, Toronto, Ontario.
[b]National Museum of Canada, Ottawa, Ontario.
[c]Research Division, Québec Wildlife Service, Tourism Fish and Game Department of Québec, Montréal, Québec.

Other characters will be referred to in the text, such as the intermaxillary barbel, that is the "maxillary barbel in groove" of Scott and Crossman (1969, p. 5), the pharyngeal teeth, and the shape of the digestive tract. Figure 2 shows some examples of abnormal digestive tract patterns that we found in *Phoxinus eos* × *P. neogaeus*, in comparison with the parent species.

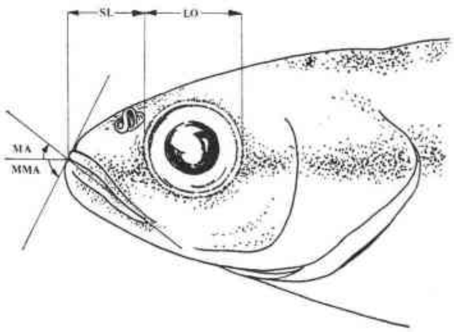

FIG. 1. Four of the characters used in the study are shown on the picture of the head of a specimen *Phoxinus eos*. LO: length of orbit; MA: mouth angle; MMA: mandibulo-maxillary angle; SL: snout length.

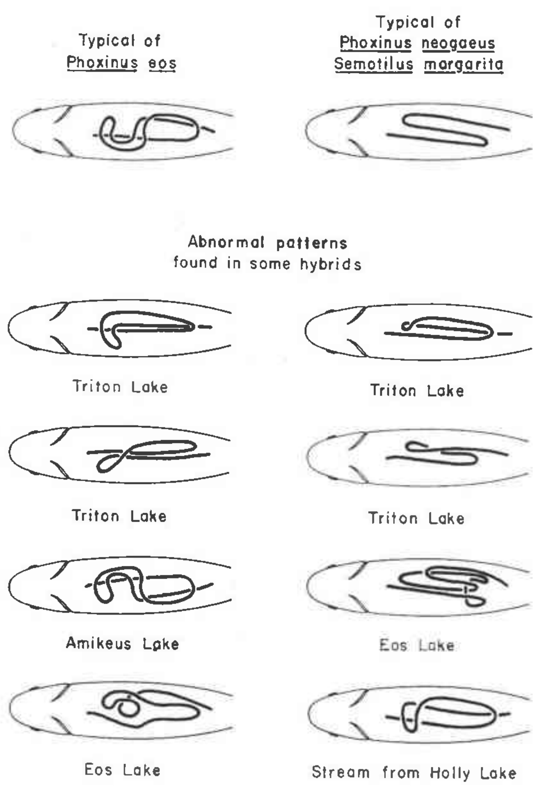

FIG. 2. Typical and abnormal digestive tract patterns (see text).

Results

Using four reference collections of minnows, representing the species *P. eos*, *P. neogaeus*, and *S. margarita*, four discriminant functions (Table II) were calculated to determine the ancestors of two other types of fishes suspected to be hybrids *P. eos* × *P. neogaeus* and *P. eos* × *P. neogaeus* × *S. margarita*. A discriminant function is a linear combination of characters that is optimum to discriminate between two groups of data. It is used as follows: given a specimen, each metric datum, or quotient of two data, is multiplied by the factor of the discriminant function that corresponds to this datum, and all the products concerning this specimen are summated, giving the position of the specimen on the discriminant axis.

Function A was calculated using 60 specimens of *P. eos* from Caché Lake and 28 specimens of *P. neogaeus* from Lichen Lake (5 spp.) and from the bog lake near Fort Albany (23 spp.). Fourteen characters were used, including standard length. Function B was calculated using 20 of the 60 specimens of *P. eos* from Caché Lake, and 30 specimens of *S. margarita* from Belle-Rivière Lake. Ten characters were used. Function C was calculated using the same 30 specimens of *S. margarita* from Belle-Rivière Lake and the 28 specimens of *P. neogaeus* used in function A. Nine characters were used. Function D was calculated using the 20 specimens of *P. eos* used in function B, and the 28 specimens of *P. neogaeus* used in functions A and C. Nine characters were used.

Considering the value, on the discriminant axis, of all the specimens studied in a given population, the mean value ($\bar{x}$) for this population, the standard error of the mean ($S\bar{x}$), and the standard deviation (s) were calculated on the populations presumed to be *P. eos* × *P. neogaeus*, as well as on the reference collections of *P. eos* and *P. neogaeus*, using discriminant function A. Similarly, the specimens presumed to be *P. eos* × *P. neogaeus* × *S. margarita* were computed in functions B, C, and D, and also the reference collections in each case. The results are presented in Figs. 4 and 6.

The two collections of *P. neogaeus* were pooled in these computations, even if Lichen Lake is part of the Winnipeg Lake basin, and the bog lake near Fort Albany is in the Albany River basin. The increase in the total range of variation on the discriminant axes, as well as in the standard deviation, is small, thus showing that the difference between the two populations is not large. This makes it possible, however, to get a better representation of the species *P. neogaeus.*

Analysis of the Results

(a) *The Major Discrimination Component Factors*

The mean value of each character was calculated in each of the reference populations, the populations used to calculate the discriminant functions A to D. All these values together form a sort of mean specimen representing the population. By multiplying the metric value of each character of the mean specimen by the corresponding factor of the discriminant function (Table II), and by comparing these data with the same set of data obtained with the mean specimen of the other species on which the discriminant function is built, it has been possible to classify the metric characters according to their capacity to discriminate between the two reference populations. This measure of the discriminating ability is given by the formula $\Delta = |f\bar{x}_1 - f\bar{x}_2|$ where f is the factor of the discriminant function, $\bar{x}_1$ is the numerical value of the corresponding character of the mean specimen of the species 1, and $\bar{x}_2$ is the numerical value of the

TABLE II

Multiplier factors of the discriminant functions A to D

Characters	Discriminant functions A	B	C	D
Length of orbit / Standard length	8.49491	13.47828	44.14528	19.48763
Depth of caudal peduncle / Standard length	−4.47233	−0.21994	−9.98850	−7.84565
Snout length / Standard length	7.77393	−25.01697	17.11438	6.85890
Predorsal length / Standard length	−1.17707	6.30220	−7.06106	−0.68605
Length of base of dorsal fin / Standard length	0.07654	—	—	—
Length of pectoral fin / Standard length	1.63912	—	—	—
Length of pelvic fin / Standard length	−0.83008	—	—	—
Length from pectoral to pelvic f. / Standard length	2.04742	—	—	—
Head length / Standard length	−6.34537	15.41182	−23.55312	−7.72419
Length of tail / Standard length	2.72241	9.29090	−3.36999	3.64850
Snout to last pore of lat. line / Standard length	0.32593	−9.15768	3.78914	0.81610
Rows of scales above lat. line	—	0.15371	—	—
Mouth angle	0.00235	0.01006	−0.01160	0.00647
Mandibulo-maxillary angle	0.00083	—	—	—

corresponding character of the mean specimen of the species 2. The characters that should be used in taxonomic keys are those which discriminate best. The Δ values of the major discrimination component factors are given in Table III, where A and D are the functions that discriminate between *P. eos* and *P. neogaeus*, B discriminates between *P. eos* and *S. margarita*, and C discriminates between *P. neogaeus* and *S. margarita*.

(b) Polymorphic Populations

Some of the populations are composed of more than one morph. This is the case of the population from Caché Lake, as shown in Figure 3*a*. A χ^2 test showed that this curve cannot be fitted to a normal curve. The hypothesis of a bimodal partition related to sex or age was tested and found to be untrue. However, this population will be considered as representative of the species *P. eos*, even if we have to keep in mind that this gene pool can hold genes of other origins, different lots of minnows having perhaps been brought by fishermen after poisoning of the lake in the early 50's. The validity of this procedure is supported by the small amplitude of the standard deviation and of the total variation range of the sample (Fig. 4).

The sample caught in the stream from Holly Lake shows three peaks when analyzed with functions B and C, and at least two peaks when analyzed with function D (Fig. 3*b*, *c*, and *d*). As these specimens are the ones suspected to be trihybrids, and because the non-metric characters separate the specimens into three groups (see paragraph *d* below), the shape of the frequency histograms has to be considered as a proof of the fact that the discriminant functions served to identify real differences in this case. This problem will be discussed in section *d*.

The specimens taken in Amikeus Lake are of two types: 5 specimens have the exterior colors of *P. neogaeus*, and show also the digestive tract pattern and the pharyngeal teeth formula of this species; the other 14 specimens in this collection have the colors, the teeth, and digestive tract pattern (except for one abnormal intestine) of *P. eos*. These two groups of specimens were caught in different portions of this small lake. Furthermore, the five neogaeus-type specimens form the extreme left of the distribution of this collection on discriminant axis A. The collection has then been split into two components, called respectively neogaeus-type and eos-type. The difference between these two components is statistically significant.

(c) Hybrid Populations

The samples from Eos Lake (60 specimens) and Triton Lake (60 specimens), as well as the 5 neogaeus-type specimens from Amikeus Lake, are significantly separated from the reference collections of *P. eos* (Caché Lake) and *P. neogaeus* (Lichen and bog lakes). They are intermediate between these reference collections, and they exhibit a large standard deviation and a

TABLE III

The discriminating power, Δ, of the major discriminating component factors in the discriminant functions A to D

Characters	Discriminant functions			
	A	D	B	C
Length of tail / Standard length	0.209	0.280	—	—
Snout to last pore of lat. line / Standard length	—	—	4.354	1.837
Head length / Standard length	0.112	0.136	3.844	0.249
Depth of caudal peduncle / Standard length	0.048	0.084	—	—
Mouth angle	—	—	0.854	—
Rows of scales above lat. line	—	—	0.815	—

large total variation, showing that they are part of a diversified gene pool. The 14 eos-type specimens form a distribution that is more difficult to explain: the mean is located outside the range of intermediateness between the reference collections of *P. eos* and *P. neogaeus*, and the distribution is statistically different even of the sample of *P. eos*. However, the total variation and the standard deviation are very large, showing the presence of a highly diversified gene pool. On the other hand, our remarks of section *b* above on the collection from Caché Lake may account for the relative position of these two distributions.

(d) Tri-hybrids

At least some of the 29 specimens from the stream from Holly Lake are suspected to be tri-hybrids (*P. eos* × *P. neogaeus* × *S. margarita*). The three discriminant functions calculated (B, C, and D) represent the three possible combinations of the suspected parent species.

Using several characters from identification keys, we divided these specimens into three groups: *the margarita-type*, which has the following characters: high number of perforated scales on the lateral line; intermaxillary barbel present, at least on one side; teeth (1 or 2), 5–4, (2 or 1 or 0); digestive tract forming a single S; this group is represented by specimens 1 to 5; *the eos-type*, with the following characters: low number of perforated scales on the lateral line; intermaxillary barbel absent; teeth 0, (4 or 5)–(5 or 4), 0; intestine longer; specimens 12 to 29 are in this group; *the hybrid-type*, combining the characters of the two above-mentioned types. Specimens 6 to 11 form this group.

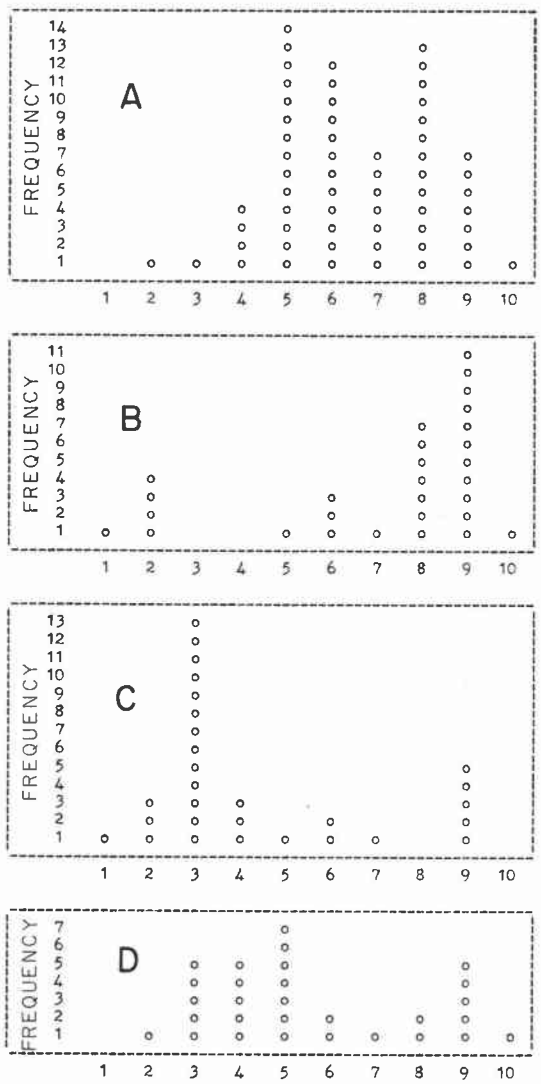

FIG. 3. Frequency histograms showing the distribution of the specimens of a collection on a discriminant axis. Each dot refers to a specimen. The range of variation of each population is divided into 10 classes. *a*: population from Caché Lake in discriminant function A; *b*, *c*, and *d*: population from the stream from Holly Lake in discriminant functions B, C, and D.

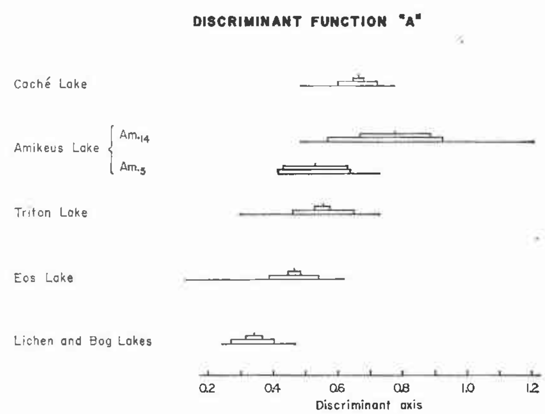

FIG. 4. Position of hybrid collections from Amikeus, Triton, and Eos Lakes on the discriminant axis A, compared with collections of *Phoxinus eos* (Caché Lake) and *P. neogaeus* (Lichen and Bog Lakes). The range of variation is shown by a horizontal line; the mean by a vertical line. The small rectangle represents two standard errors of the mean on each side of the mean. The longer rectangle indicates one standard deviation on either side of the mean.

Figure 5 shows the position of each specimen on the metric spaces formed by the functions B, C, and D, taken two by two. Three young eos-type specimens (nos. 27 to 29) are not considered here and in the following discussion, because they form a cluster on their own, even if the age is the only visible difference between them and the other eos-type specimens. The graphs (Fig. 5) show clearly that the so-called eos-type and margarita-type specimens form discrete clusters of points, while the other six specimens, with their abnormal combination of characters (Table

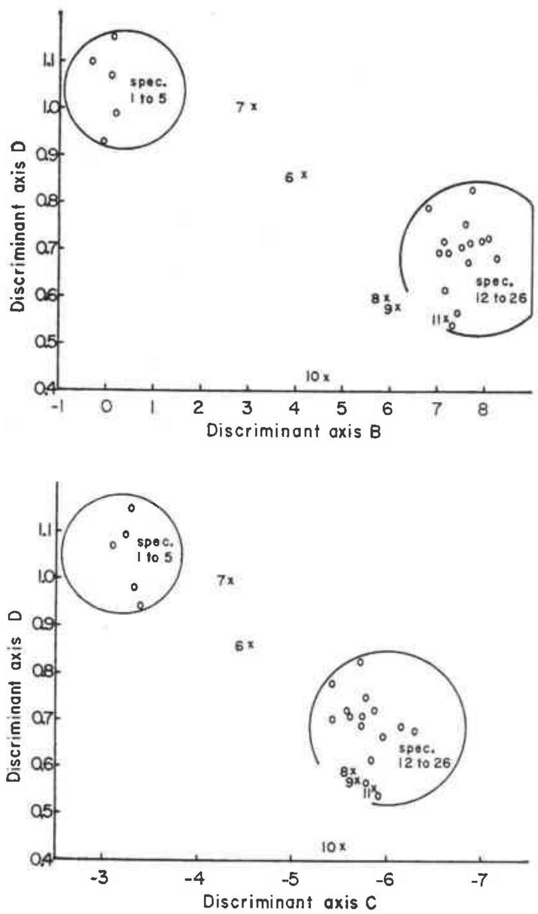

FIG. 5. Dispersion diagrams showing the position of each specimen of the collection from the stream from Holly Lake on the coordinates formed by the discriminant axes B and D (above) and C and D (below). Specimens 1 to 5 and 12 to 26, indicated by circles, form in each case two clusters of points. Specimens 6 to 11, indicated by an X, are more or less intermediate between these two clusters.

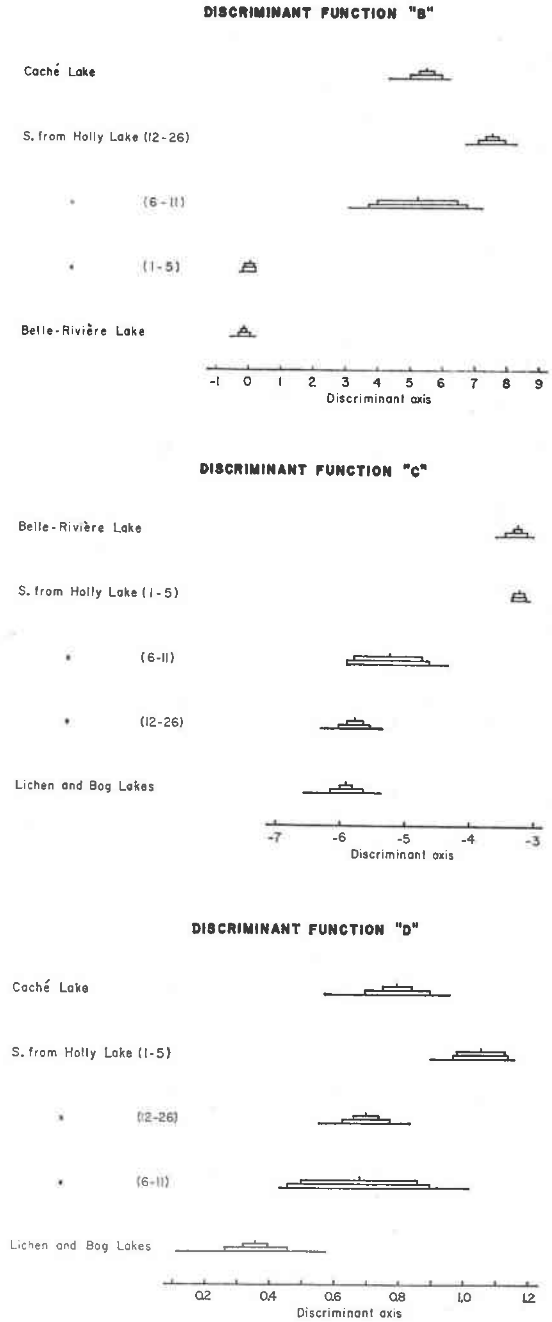

FIG. 6. Position of the three types of fishes found in the collection from the stream from Holly Lake on the discriminant axes B, C, and D, compared with collections of *Phoxinus eos* (Caché Lake) in B and D, *P. neogaeus* (Lichen and Bog Lakes) in C and D, *Semotilus margarita* (Belle-Rivière Lake) in B and C. (For meaning of the bars and rectangles, see Fig. 4.)

IV), occupy generally an intermediate position between them. The statistics of these groups of specimens are plotted in Fig. 6.

In functions B and C (Fig. 6) there is no significant difference between the distribution of the margarita-type specimens (nos. 1–5) and the distribution of the reference specimens of *S. margarita* from Belle-Rivière Lake. As the characters of these margarita-type specimens, enumerated above, are those of *S. margarita*, these five specimens are identified as belonging to this species. The two other groups of specimens are clearly different from *S. margarita*. The position of the margarita-type specimens in the D function has to be noted; they are out of the range on the intermediates between *P. eos* and *P. neogaeus*. This statement will be used later.

The position of the eos-type specimens (nos. 12–26) in function C (Fig. 6) has no significance, since the reference collection of *P. eos* is not used in this function. The interpretation is that these eos-type specimens are not intermediate between the species *S. margarita* and *P. neogaeus* on which function C is based. However, with respect to function D, the position of the eos-type specimens is intermediate between *P. eos* and *P. neogaeus*, and significantly different from both. This means that the specimens are hybrids *P. eos* × *P. neogaeus*. Almost all the hybrids from Triton and Eos Lakes studied above were, as here, eos-type in all their main characters. These specimens 12–26 are, with respect to function B, out of the range of intermediateness between *P. eos* and *S. margarita*, on the *P. eos* side. This suggests that the gene pool in which they share did not receive a contribution from *S. margarita*.

The hybrid-type specimens (nos. 6–11), in function C, are clearly intermediates between *P. neogaeus* and *S. margarita*. With respect to functions B and D, their distribution does not differ significantly from that of *P. eos*. But this does not mean that the specimens pertain to that species since, as shown in Table IV, none of them pertain to a pure species.

Considering Table IV, it might be possible to say that specimens 6, 7, and 10 are hybrids *P. eos* × *S. margarita*, instead of tri-hybrids, or that specimens 8, 9, and 11 are simply hybrids *P. eos* × *P. neogaeus*, as indicated also by Fig. 5. It is simply not possible to find the exact position of each specimen between the three pure species, because of the problems of dominance and of association of traits: not every combination of characters is viable. But the heterogeneity of these data is an adequate reason to consider these specimens as a distinct subsample.

Hereafter is an attempt to determine the relative position of the three groups of specimens by a graphical representation. The apexes of the triangle of Fig. 7 represent the pure species and the scale of each side is one of the discriminant functions B, C, and D. But there is no relation

TABLE IV

Analysis of the main characters of the hybrid-type specimens from the stream from Holly Lake. *Above*: the observations made. *Below*: classification of these observations into four categories: N for neogaeus-like; M for margarita-like; E for eos-like; h (hybrid) when a character state cannot be classified in the three above categories

Spec. no.	Digestive tract	Pharyngeal teeth	Inter-maxillary barbel	Number of perforated scales of lat. line	Rows of scales above lat. line
6	Short	1, 5–4, 1	Present	29	12
7	Short	1, 5–4, 1	Present	32	13
8	Intermediate	2, 4–4, 1	Absent	11	15
9	Long	1, 5–4, 2	Absent	6	15
10	Intermediate	1, 5–4, 1	Absent	20	14
11	Intermediate	1, 4–4, 1	Absent	3	16
6	N or M	N or M	M	E	M
7	N or M	N or M	M	E	M
8	h	N	E or N	N	h
9	E	N or M	E or N	N	h
10	h	N or M	E or N	E	M
11	h	N	E or N	N	E

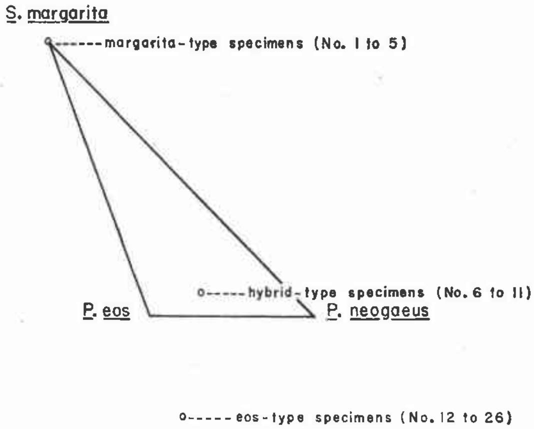

FIG. 7. Triangle showing the approximate relative phyletic position of the three species *Phoxinus eos*, *P. neogaeus*, and *Semotilus margarita* (apexes). The position of the mean of each of the three types of specimens found in the collection from the stream from Holly Lake, on this metric space, is shown by circles.

between the values given by these functions, which is necessary to determine the shape of the triangle.

We know that the margarita-type specimens are of the species *S. margarita*. The triangle is calculated by elementary analytic geometry, using the projection of the summit *S. margarita* on the side *P. eos* × *P. neogaeus*, as given by function D (Fig. 6), and in such a way that the mean value of the eos-type distribution in each of the three functions, represented by a corresponding perpendicular on each side of the triangle, are joined in a single point. Then the point representing the hybrid-type specimens is plotted by the same method. The three perpendiculars giving this point meet in a single point.

Discussion

(a) Natural Hybrids

The present study has been made in terms of Mendelian populations. After recognizing the parental species by the identification characters given in the keys (Legendre 1960; Hubbs and Lagler 1964; Scott 1967), the fish of the various collections were considered to be hybrids if some of their metric characters showed a significant variation from one parent species to the other, *and* if at least some specimens deviated in important non-metric characteristics from the most closely related species. For *P. eos* × *P. neogaeus*, almost all the specimens of each population tend to look like one of the parental species. For instance, most of the specimens that have a long digestive tract, have also the inclined mouth, the pharyngeal teeth, the lateral bands and the color of *P. eos*. The hybrids with an abnormal digestive tract, for example, represent only about 5% of the specimens investigated in this study. This may depend on linkage of characters or on the elimination of certain non-viable combinations of characters. Furthermore, the total variability of a population for a set of characters, such as obtained by the use of discriminant functions, is a good indication of its hybrid or non-hybrid origin.

According to these criteria, the populations from Triton and Eos Lakes, unimodally distributed on discriminant axis A, consist of only one type of fish, namely the hybrid *P. eos* × *P. neogaeus*, to the exclusion of both parents. Consequently these populations must consist of interfertile individuals that form Mendelian populations. This is supported by an experiment in which fertilized eggs from Triton Lake hatched successfully. Both parents were hybrids *P. eos* × *P. neogaeus*.

This conclusion applies also to the specimens from Amikeus Lake, the amplitude of the standard deviation of the distributions and the non-metric characters showing that the two groups of specimens are highly variable.

Even if part of the variation is determined by the environment, the genetic equilibrium attained in each of these populations is probably different, as shown by the position of the populations on the discriminant axis, and also by the difference in their number of vertebrae (Legendre 1970).

We showed the presence of three types of fishes in the collection from the stream from Holly Lake: *S. margarita*, *P. eos* × *P. neogaeus*, and hybrids between at least *P. eos* and *S. margarita*, if they are not tri-hybrids. However, the presence in the collection of *S. margarita* on one hand, and of *P. eos* × *P. neogaeus* on the other, is a strong presumptive evidence in favor of the argument that these two groups of fishes were probably the parents of the so-called hybrid-type specimens. Then these fish would be the result of a tri-hybrid combination. However, the small number of hybrids shows that even if *S. margarita* is crossable with *P. eos* × *P. neogaeus*, they are probably not miscible.

We can now use the point representing the mean of these specimens in the triangular graph (Fig. 7) to evaluate the genetic contribution of each parental species to the genetic constitution of the tri-hybrids: it is in the order of 55% *P. eos*, 35% *P. neogaeus*, and 10% *S. margarita*. We can also use the triangle to determine the relative phylogenetic differentiation between the three parental species, assuming that it is proportional to the relative length of the sides of the triangle: we would then obtain that $\overline{P.\ eos\ \ P.\ neogaeus} < \overline{P.\ eos\ \ S.\ margarita} < \overline{P.\ neogaeus\ \ S.\ margarita}$ where $\overline{A\ B}$ is the distance between species A and B.

(b) Implications on the Genus

McPhail (1963) explained how *P. neogaeus* migrated north, at the end of the last glaciation, from the Missouri refugium only, and then eastward from the Saskatchewan system to Lake Agassiz II, to Lake Duluth, and finally to the Great Lakes. It joined there the populations of *P. eos* that dispersed from the Mississippi refugium and hybridized with them. The western population of *P. neogaeus* that migrated eastward was allopatric with respect to the eastern populations of *P. eos*: hybridization between them was easier. This theory is emphasized by two facts: firstly, the unpublished maps of Prof. Wynne-Edwards mentioned in the introduction show that *P. eos* occurs alone in many lakes of Québec, while pure *P. neogaeus* is rare if not absent. So, *P. neogaeus* probably came later in these waters. Secondly, we examined carefully a collection of *P. eos* and *P. neogaeus* from Alberta (Institute of Fisheries, University of British Columbia, Vancouver, no. BC65-653) in which we failed to identify any hybrid specimen. It seems then that these two species do not cross in the West. Consequently, as proposed by Mayr (1969, p. 195) in the case of two species well isolated in a portion of their range but forming complete hybrid populations in another part, and because of the special circumstances that seem to have led to the formation of these hybrid populations, we recommend that *P. eos* and *P. neogaeus* be treated as full species. This judgment may of course be modified by future data on the western populations of these species.

The nomenclatural history of *Semotilus margarita nachtriebi* is very complex. It has been placed in many different genera, but one should note that it was placed three times in the same genus as *P. neogaeus* [1. *Leuciscus* (*Clinostomus*) *margarita* Cope *in* Günther, 1868, p. 246; *L. nachtriebi* U. O. Cox, 1896, p. 615; *L.* (*Phoxinus*) *neogaeus* Jordan and Evermann, 1896, p. 241; 2. *Phoxinus* (*Tigoma*) *margaritus* Jordan, 1887, p. 819; *P. neogaeus* Cope *in* Günther, 1868, p. 247; 3. *Richardsonius* (*Margariscus*) *margarita* Cockerell, 1909, p. 217; *R.* (*Margariscus*) *neogaeus* Cockerell, 1909, p. 217], the similarity of appearance of these two species being recognized by Jordan and Evermann (1896, p. 241) and by Hubbs (1926, p. 34).

The collection from the stream from Holly Lake indicates some close phyletic relationship existing between *S. margarita* and *P. eos* at least. This is emphasized by the fact that *S. margarita*, as well as *P. eos* and *P. eos* × *P. neogaeus*, has a diploid number of 50 chromosomes, contrarily to *S. atromaculatus* and *S. corporalis* that have a diploid number of 52 chromosomes (Legendre 1969*b*). Consequently it seems reasonable to transfer the species *Clinostomus margarita* Cope *in* Günther, 1868, to the genus *Phoxinus*. *Leuciscus nachtriebi* U. O. Cox, 1896, then receives the name *Phoxinus margarita nachtriebi*.

Conclusions and Summary

Morphological characters were used to calculate four discriminant functions, each one using 8 to 13 factors.

Fish populations from lakes Triton and Eos were each found to form single Mendelian populations identified as *Phoxinus eos* × *P. neogaeus*. Two distinct groups of hybrids were found in Amikeus Lake. The fishes of Caché Lake were identified as *P. eos*, but there were indications of two different gene combinations. A collection from the stream from Holly Lake contained three recognizable types: a group of *S. margarita*, a group of *P. eos* × *P. neogaeus*, and a third group consisting of hybrids of *S. margarita* with at least *P. eos*, but possibly with *P. eos* × *P. neogaeus*.

The metric characters found to discriminate more between each pair of species studied are as follows: for the pair *P. eos* and *P. neogaeus*, the length of the tail, the length of the head, and the depth of the caudal peduncle; for *P. neogaeus* and *S. margarita*, the length from the tip of the snout to the last pore of the lateral line and the head length. For *P. eos* and *S. margarita*, the two above characters, plus the mouth angle and the number of rows of scales above lateral line, can be used.

The hybrid *P. eos* × *P. neogaeus* was shown to be fertile.

S. margarita was found to be phyletically more distant from *P. neogaeus* than from *P. eos*.

The formation of *P. eos* × *P. neogaeus* hybrids in eastern North America seems to be the result of introgression. However, both parental feeding systems and digestive tract patterns seem to be better adapted than any hybrid combination.

The transfer of *Clinostomus margarita* Cope to the genus *Phoxinus* is proposed.

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