AN ANALYSIS OF MERISTIC, MORPHOMETRIC AND BIOCHEMICAL VARIATION IN THE JOHNNY DARTER, *Etheostoma nigrum* Rafinesque, IN THE NORTHERN UNITED STATES AND CANADA WITH BIOGEOGRAPHIC AND SYSTEMATIC CONSIDERATIONS

by

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A Thesis
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Fulfilment of the Requirements for the Degree of

Doctor of Philosophy

Department of Zoology
University of Manitoba
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SAM A. STEPHENSON

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ABSTRACT

An analysis of meristic, morphometric and biochemical variation in the johnny darter, *Etheostoma nigrum*, within the northern and central portion of its range was performed. Much of the variation exhibited in samples was correlated with geography, habitat and/or the dispersal history of the various phenotypes. A quantitative definition of the scaled phenotype was made and an evolutionary model developed to explain the variation, possible origin and probable distribution of the scaled and unscaled phenotypes from pre-Wisconsinan time to the present.

A total of 2111 specimens from 240 geographic locations were analyzed. Little morphometric difference existed between samples. The geographic distribution of the number of preoperculomandibular pores suggested an eastern and western unscaled form. Cluster analyses also supported an eastern and western form. Biochemical analyses of 20 allozyme loci suggested few distinct genetic differences between the scaled and unscaled forms. The upper and middle Ohio Basin unscaled form requires further study and may qualify for subspecific status due to several distinctive meristic traits. The scaled form is considered a valid subspecies.

The distribution of scaled specimens suggests a former continuous range with secondary dispersal. Squamation and several plesiomorphic traits vary little among specimens classified as scaled. The scaled form likely predates the Wisconsinan glaciations, surviving in an ancestral Hudson Bay drainage prior to that time.

Four forms are recognized; the scaled form (*E. nigrum eulepis*), an unscaled Ohio Basin and widespread unscaled eastern and western forms (*E. n. nigrum*). The scaled form is redescribed and a key given to separate *E. n. nigrum* and *E. n. eulepis*.

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INTRODUCTION

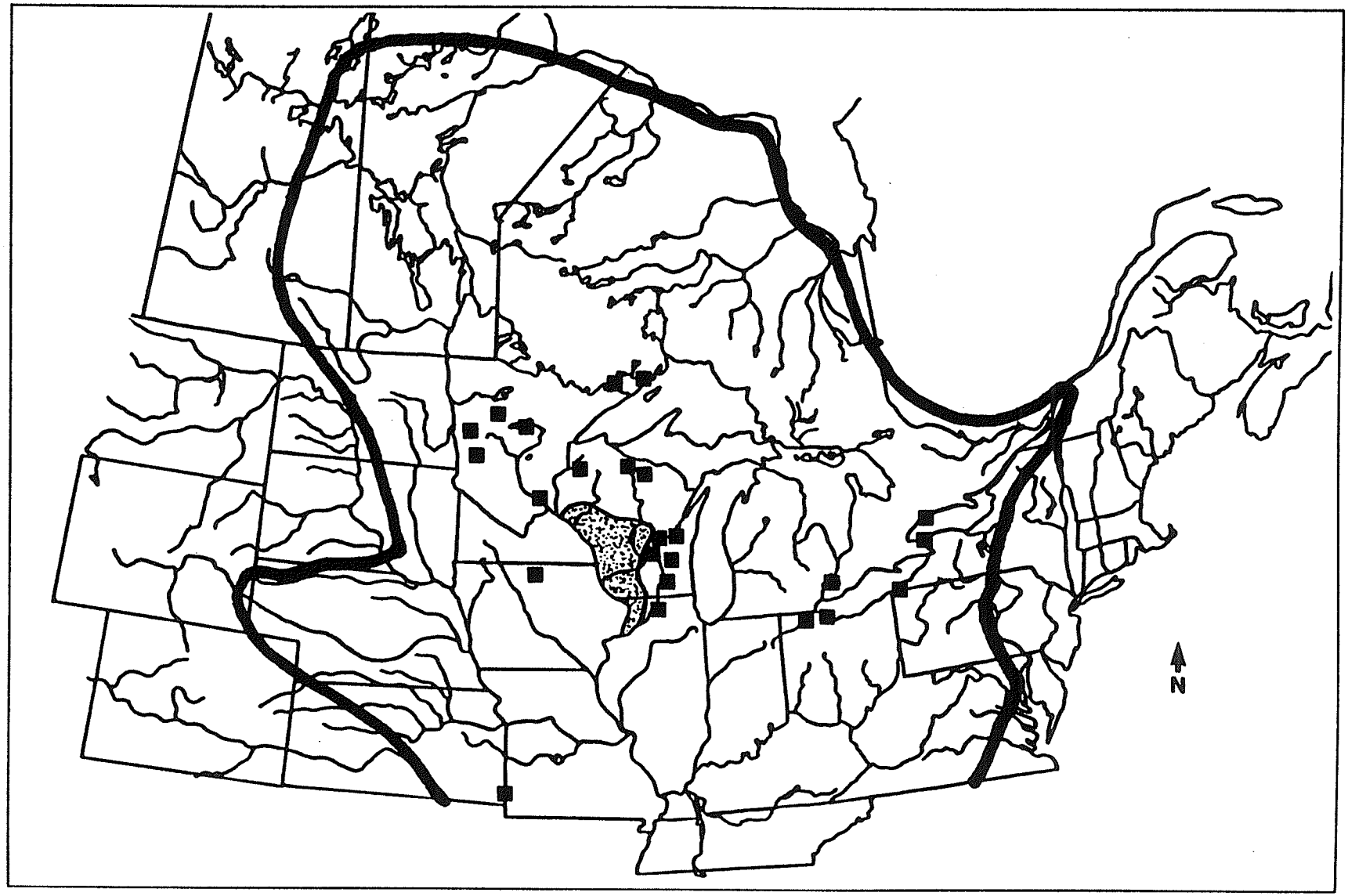
The johnny darter, *Etheostoma nigrum* (Percidae), was first described by Rafinesque as *Etheostoma nigra* in 1820 from specimens collected in the Green River, Kentucky. *Etheostoma nigrum* is a member of the subgenus *Boleosoma* which contains the tessellated (*Etheostoma olmstedi*), riverweed (*E. podostemone*), Waccamaw (*E. perlongum*) and longfin (*E. longimanum*) darters (Page 1983; Simons 1992). The riverweed, Waccamaw and longfin darters are restricted to only a few Atlantic Slope drainages in North Carolina or Virginia (Hubbs and Raney 1946; Jenkins 1980a,b; Shute 1980). The range of the tessellated darter extends from Florida to New Hampshire on the Atlantic Slope with additional populations in the Upper St. Lawrence, southern Lake Ontario and the Ottawa River (Lee and McAllister 1980). All darters are endemic to North America.

I/ Biology and Ecology of *Etheostoma nigrum*

Etheostoma nigrum has the largest geographic range of any darter in the genus *Etheostoma* (Page 1983) (Fig. 1). It is considered a generalist species due both to its wide distribution and the varying conditions under which it lives (Phillips *et al.* 1982; Becker 1983). Its range extends from the Hudson and James Bay drainages of Canada in the north to Alabama in the south and from Colorado in the west to Virginia and North Carolina, including Atlantic Slope populations in the James, Tar, Roanoke and Neuse rivers, in the east (Bruner 1980).

Etheostoma nigrum inhabits lakes, rivers and streams of all sizes and occurs sympatrically with a variety of warm and cool water fish species. In most areas of its

Figure 1: Distributional limits of *Etheostoma nigrum* within the study area (wide line), the Wisconsin Driftless Area (stippled), and known distribution of the scaled form prior to this study (locations marked by a square (■)). Distributions based on Greene (1935), Harlan and Speaker (1951), Trautman (1957), Hubbs and Lagler (1983), Underhill (1963), Page (1983) and Stephenson (1991).


N

range, however, it is confined to warmer, shallower waters such as those of bays and littoral areas (Becker 1983). Small streams with areas of moderate or no current with sand or small gravel seem to be the preferred habitats (Becker 1983). Sand-silt areas, those with aquatic vegetation and small riffles of streams are not as extensively utilized (Paine *et al.* 1982; Englert and Seghers 1983; Page 1983).

Few *E. nigrum* live beyond two years (Karr 1963; Becker 1983) and the maximum age reached is four (Page 1983). The maximum total length attained is approximately 77.0 mm (Becker 1983; Etnier and Starnes 1993). Males grow to a larger size than females (Page 1983). Individuals mature at age one and spawning occurs in early to mid-spring (Becker 1983; Page 1983). Males establish a territory around an elevated rock, log or other object on which eggs can be deposited (Winn 1958). Eggs are deposited on the underside of the object after the surface has been cleaned by the male (Winn 1958). Females may spawn with several males or spawn repeatedly with the same male (Karr 1963). The male guards the nest and defends the eggs against fish and invertebrate predators until the eggs hatch (Becker 1983; Rahel 1989). Hatching occurs in five to ten days, depending on water temperature (Page 1983).

Johnny darters play an important role as a converter of small, benthic materials to larger forms within the aquatic community (Scott and Crossman 1973). Chironomids (midge larvae) are the predominant food of young darters (Page 1983) while larger darters consume larger insects and chironomids (Karr 1963). When abundant, *E. nigrum* are an important forage item for walleye and other bottom

feeding fishes (Becker 1983; Stephenson and Momot 1991).

II/ Systematics of the *Boleosoma* Group

All darters were placed into the three currently recognized genera (*Percina*, *Ammocrypta* and *Etheostoma*) based on the concept of Bailey and Gosline (1955). The genus *Boleosoma* was relegated to subgeneric rank at that time. However, the systematic history of species assigned to this subgenus is marked by controversy. The long debate over the specific status of the tessellated and johnny darters (*e.g.*, Cole 1965, 1967, 1971; Scott and Crossman 1973; Hubbs and Lagler 1983) shows the great difficulty in resolving or accepting the specific status of members of this subgenus. Most recent works (*e.g.*, Page 1981, 1983; Robins *et al.* 1991; Simons 1992) have, however, recognized *Etheostoma olmstedi* as a distinct species. It possesses a greater number of rays in the dorsal fin and pectoral fin and a greater number of infraorbital pores than does *E. nigrum*. *Etheostoma olmstedi* is often considered the more scaled of the two forms, especially within the area where the two species are sympatric (Cole 1965; McAllister *et al.* 1972). Scott and Crossman (1973), despite earlier work by Cole (1965, 1967, 1972), felt that *E. nigrum* and *E. olmstedi* might be the same species and treated them as such, suggesting that further work had to be performed to determine if they were separate species. McAllister *et al.* (1972) examined morphological and electrophoretic differences between *E. nigrum* and *E. olmstedi* from the Ottawa region of Ontario and concluded that, although some interbreeding occurred, they were distinct species.

Most descriptions of the variation in *E. nigrum*, whether based on meristics or

morphometrics, have been limited either in geographic scope (Trautman 1957; Underhill 1963; Smith 1979) or in the number of specimens examined (Page 1981). Those studies which have examined variation over a larger area (*e.g.*, Kott and Humphreys 1978; Chapleau and Pageau 1985) have not done so in a geographically systematic manner and have treated only Canadian populations. The occurrence of both *E. nigrum* and *E. olmstedi* in the Lake Ontario and the Ottawa River area also suggests the possibility that the true distribution of the scaled form of *E. nigrum*, or perhaps the true distribution of *E. olmstedi*, has been masked. Both forms must be distinguished prior to any statistical analysis of either. Unless samples are correctly identified and possible areas of intergradation determined, parsimonious biogeographical hypotheses explaining the present distribution of either species cannot be constructed.

III/ The Scaled Phenotype of *Etheostoma nigrum*

Hubbs and Greene (1935) first noted the occurrence of *E. nigrum* from areas in Wisconsin with a high degree of cheek, nape and breast squamation, which they named as the subspecies *Boleosoma nigrum eulepis*. They also noted that Forbes and Richardson (1909) had earlier reported the presence of increased squamation on some Illinois specimens. Greene (1935) commented further on the occurrence of the scaled form within Wisconsin and suggested that it may have survived in, among other areas, the Driftless Area of Wisconsin during the Wisconsinan glaciation (Fig. **1**), perhaps inhabiting glacial Lake Wisconsin. Lagler and Bailey (1947) crossbred scaled and unscaled darters and showed that the offspring possessed intermediate squamation

thereby demonstrating a genetic basis for squamation. Underhill (1963) examined the squamation patterns of *E. nigrum* in Minnesota and, based on geographic distribution and a lack of ecological differences between the two forms, concluded that environmental factors did not cause the increased squamation. Based on the absence of scaled specimens from Lake Superior samples, he agreed with Greene (1935) that the scaled form had not entered Lake Superior. Although not reported at the time, Underhill (1963) stated that meristic characters (mainly the number of fin rays and spines) varied little between the scaled and unscaled forms. Kott and Humphreys (1978), examining johnny darters from eastern Lake Superior and the Nith River in southern Ontario, noted an absence of squamation and the presence of mainly subconical snouts in the Superior specimens in contrast with fully scaled specimens with blunt snouts in the south. Chapleau and Pageau (1985) suggested (based on a mixed sample of 767 specimens of *E. olmstedi* and *E. nigrum*) that Canadian *E. nigrum* are scaleless on the nape and cheek except in the Niagara River.

While examining *E. nigrum* from north shore tributaries of western Lake Superior, I (Stephenson 1991) noted the presence of heavily scaled *E. nigrum* in one Minnesota and four Thunder Bay area watersheds. I suggested that the scaled form entered the basin in advance of the unscaled form and survives in some areas due to lack of immigration by the unscaled form due both to barrier falls and the lack of suitable johnny darter habitat in the littoral zone of Lake Superior between tributaries.

The initial description of *Boleosoma nigrum eulepis* (Hubbs and Greene 1935) described the degree of squamation on the nape, cheek opercle and breast as slight,

moderate or complete. However, the number of specimens with a completely scaled nape, cheek, opercle or breast varied greatly in their tables. Greene (1935) described the form as possessing a fully scaled nape, cheek and breast but did not include data showing variation in the characters. Carlander (1941) described *Boleosoma nigrum eulepis* as having nape, cheeks and breast "well-scaled".

Although the opercle area, and to a lesser extent, the cheek area are naturally defined, the area of the breast and especially the nape, are not. For example, Kuehne and Barbour (1983) define the nape as the area "along the back from the head to the origin of the spiny dorsal fin". While this definition is correct for measurements, it does not have any lateral limitations. Without lateral limitations, some may call a nape partially scaled while others will not. Thus, descriptions of the nape and other body areas are required to assure consistency for all future studies.

No definition of "scaled" *E. nigrum* existed when I started this study although there have been several papers describing the variation between the "scaled" and "unscaled" form (*e.g.*, Hubbs and Greene 1935; Greene 1935; Lagler and Bailey 1947; Underhill 1963; Kott and Humphreys 1978). The lack of a precise definition was, perhaps, due to the noticeable differences between a darter which possesses squamation and one which does not. However, without a fixed, quantitative definition, *E. nigrum* which possess only limited squamation on a normally naked body area may be assigned inconsistently to either the scaled or scaleless form by different workers.

Analysis and comparison of possible meristic, morphometric or biochemical

variation exhibited by *E. nigrum* over a wide geographic area was incomplete. Although the scaled form of *E. nigrum* is known mainly south of the Great Lakes (Fig. 1), no study has attempted to determine the distribution of this phenotype. Additionally, no previous study has determined if other meristic or morphological trends exist among samples from discrete geographic areas.

Except for the possibility of adaptation to habitat, no one has offered an explanation as to why variation in the amount of squamation exists among specimens of *E. nigrum*. Without a thorough examination of samples from a major portion of its range, it is impossible to formulate a hypothesis which offers a suitable explanation for the appearance of this squamation. If, after examination of a large number of samples, habitat differences do not offer a suitable explanation for increases or maintenance of squamation, then a new hypothesis explaining the presence of the scaled form should be tendered.

IV/ Objectives

The objectives of this research were to analyze the meristic, morphometric and biochemical variation within *E. nigrum* within the northern and central portion of its range, and to determine whether the variation showed correlations with geography, habitat and/or presumed dispersal history of the various phenotypes. I also wanted to determine if the scaled form of *E. nigrum* did qualify for subspecific status based on some existing or revised definition of a subspecies. Finally, I developed an evolutionary model to explain the variation, possible origin and distribution of the scaled and unscaled phenotypes from pre-Wisconsinan time to the present.

To accomplish these objectives, I **1**) developed a means of separating *E. nigrum* from *E. olmstedi* using meristic characters, **2**) developed a quantitative morphologically-based definition of the scaled phenotype of *E. nigrum*, **3**) examined samples of *E. nigrum* representing a major portion of its range, and, **4**) examined the accepted definitions of subspecies and the basis these definitions had in declaring subspecific status among other members of *Etheostoma*.

METHODS

MERISTICS AND MORPHOMETRY

I/ Material Examined

In total, 2344 specimens of *Etheostoma* (*Boleosoma*) from 258 geographically separated locations north of 35°51'N latitude (Fig. **2**) were examined for morphometric and meristic analysis. The subgeneric name is used as some of these samples were known or thought to include either pure or mixed specimens of *Etheostoma olmstedi* or *E. nigrum* x *E. olmstedi*. Specimens examined for this study were borrowed from collections stored at the University of Michigan, Museum of Zoology (UMMZ), University of Manitoba (MZF), Lakehead University, Thunder Bay, Ontario (LU), the Royal Ontario Museum (ROM), the James Ford Bell Museum of Natural History, University of Minnesota (JFBM) and the Canadian Museum of Nature (NMC) (abbreviations follow Leviton *et al.* (1985) except for LU). Some donated or collected specimens remain amongst my personal collections (denoted as PC). Additional specimens were collected in Ontario and Wisconsin or donated by J.C. Underhill (JFBM) (Minnesota specimens) or W. Franzin (Dept. of Fisheries and Oceans (DFO), Freshwater Institute, Winnipeg, Manitoba) (Manitoba specimens). A list of the number of specimens examined is divided by major drainages and presented in Appendix **1**.

Samples (a collection of specimens from a single geographic location) were selected for analyses based on geographic location, major drainage, proximity to known areas of scaled populations and sample size. Samples located near the known

Figure 2a: Locations of examined samples of *Boleosoma* including specimens of *Etheostoma nigrum* and *E. olmstedi*. Numbers corresponding to sample locations are given in Appendix **2**. States in which only a single sample was examined are not denoted by number.

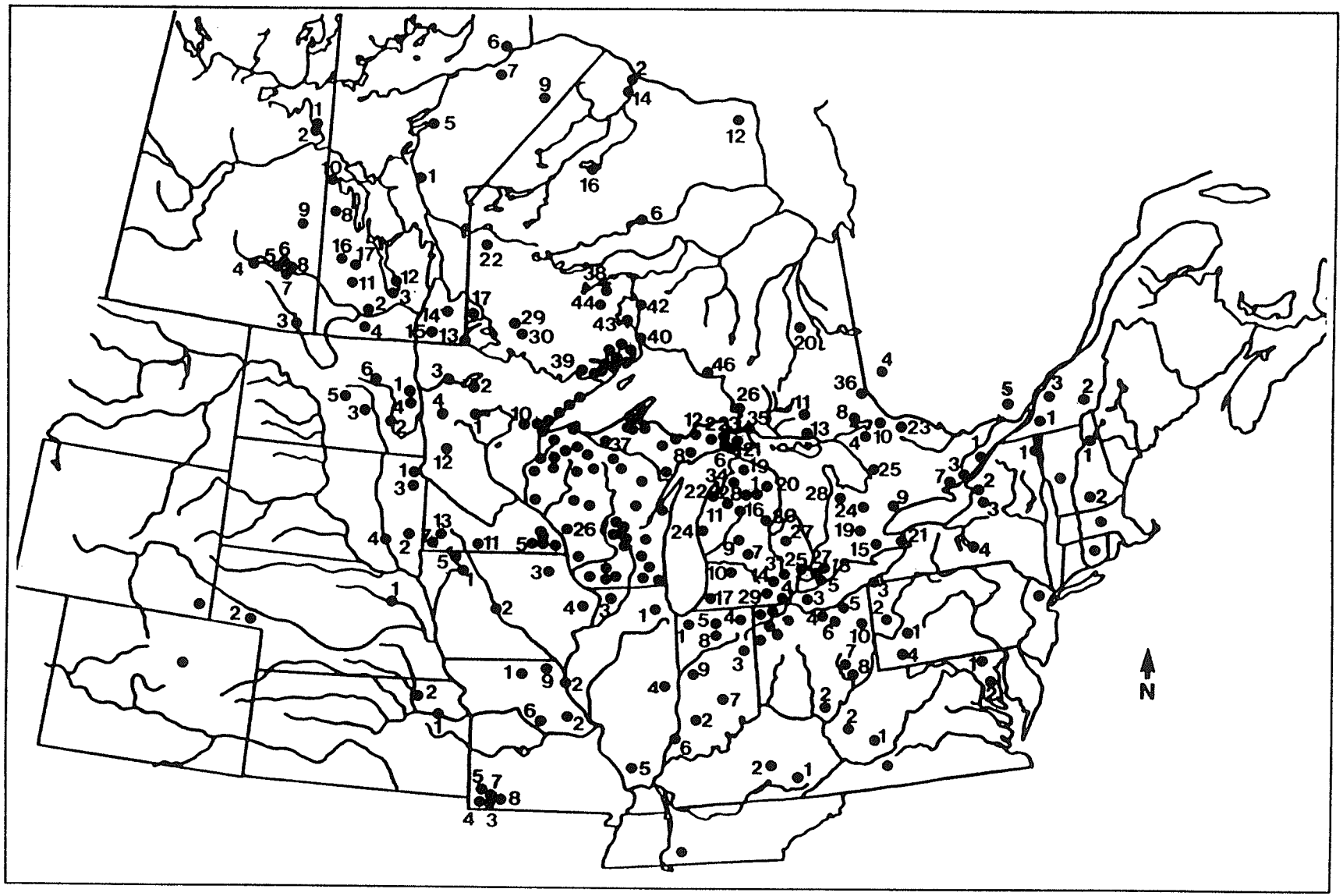

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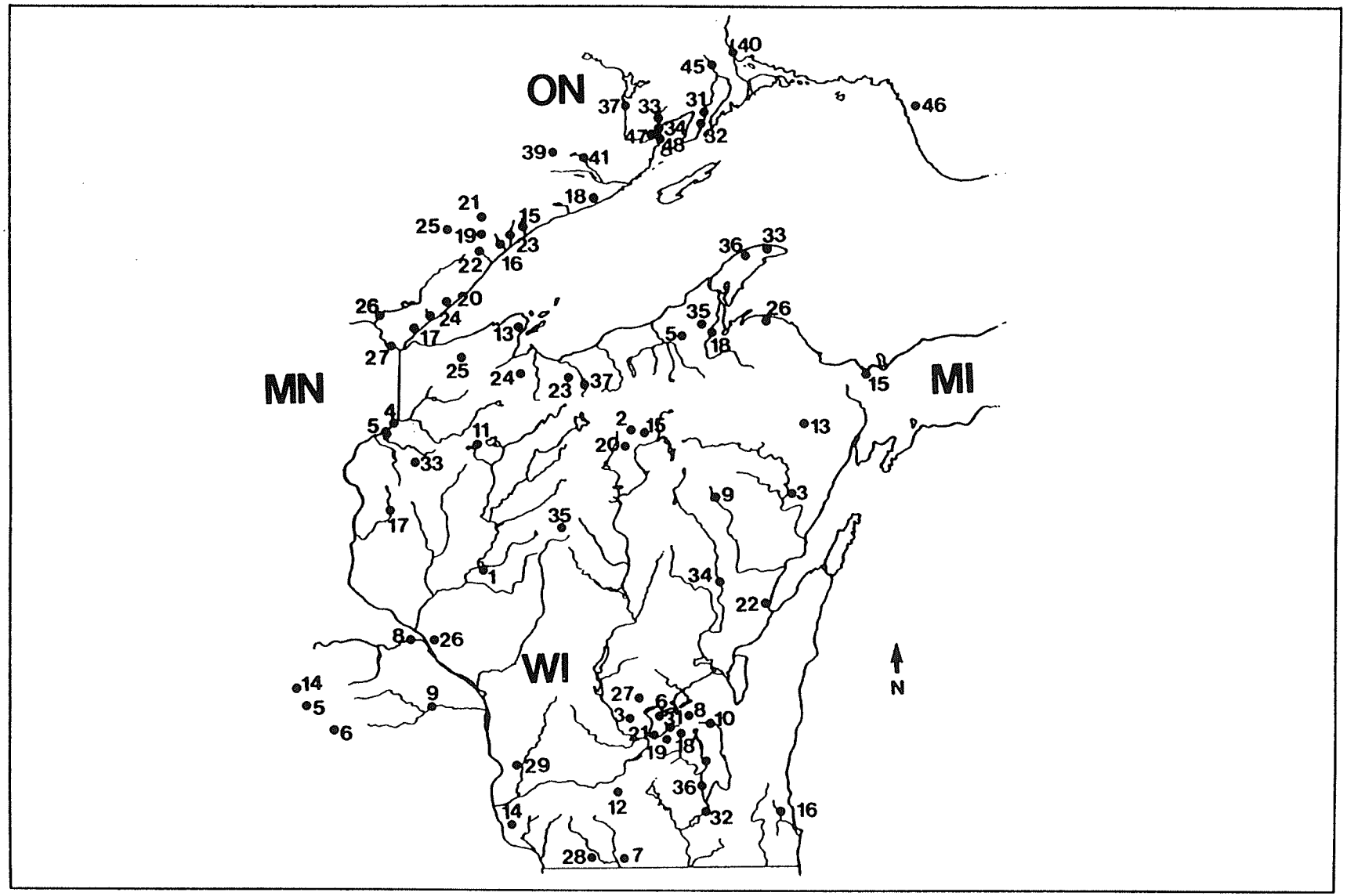
Figure 2b: Locations of examined samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario. Numbers corresponding to sample locations are given in Appendix **2**.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edge of the species' range were examined whenever possible. Suspected "scaled" samples and samples from locations near these areas were examined for evidence of intergradation. All available samples of *E. nigrum* stored at LU (60+) and MZF (50+) were examined for the presence of "scaled" specimens. Any collections which possessed specimens exhibiting increased squamation were included in further analyses. No attempt was made to examine an equal amount of samples from rivers or lakes as geographic location was considered *a priori* to be more informative than habitat.

When collection size permitted, ten specimens were selected randomly for examination without consideration for size or sex. All specimens from a collection were first placed in a 500 ml plastic container with a sufficient quantity of alcohol and water to prevent desiccation. Specimens were selected blindly as they were encountered by forceps. The only *a priori* requirement made for examination of specimens was that they be 26.0 mm or more in standard length (SL) (SL = the distance from the most anterior portion of the lip or snout to the end of the hypural plate on the last vertebrae). Specimens shorter than this length introduced a greater probability of error when performing counts or measurements and the possibility that all serial elements or scales had not yet formed. Miller (1968) reported that scale and fin ray development in *Etheostoma blennioides* was not complete until approximately 30.0 mm SL. Therefore, if a specimen less than 26.0 mm SL was selected, it was replaced with a specimen over 26.0 mm SL selected as described above. After the ten specimens were examined, the formula of Eckblad (1991),

$$\text{Sample size} \cong (\text{t-value}^2) \times (s^2) \div (0.10 \times \bar{x})^2$$

(where **t-value** equals the sample size (n-1) degrees of freedom from a t-table, s^2 equals the sample variance and $\bar{x}$ equals the sample mean) was used on all meristic variables to determine if the sample was large enough to be within $\pm$ 10% of the true mean for that variable at a 0.05 level of significance. If the sample was found to be too small, the required additional specimens were selected from the sample using the procedure described above and examined, after which the formula was again applied. All specimens greater than 26.0 mm SL from all samples were examined for the presence or absence of nape, cheek and breast scales. An estimate of the percent of the belly covered by scales was also made.

II/ Character Set

Meristic counts and morphometric measurements were made following Hubbs and Lagler (1983) except for squamation characters, which are defined below. Although an estimate of an area covered by scales is not considered a meristic character, the method of scoring the character (estimate of percent covered) is considered consistent with a quantitative (*i.e.*, meristic) character (Wiley 1981). Estimates of the area covered by scales are therefore listed under meristic characters. Measurements of morphometric characters were made to the nearest 0.5 mm using dial callipers which were recalibrated daily. All measurements were converted to percentage of standard length (variable length ÷ standard length) prior to analysis. All counts and measurements were made under magnification using a dissecting microscope.

Morphometric Characters

Prior to examining specimens for this study, a preliminary stepwise discriminant analysis of ten morphometric characters was performed on 75 randomly selected specimens of *E. nigrum* to determine which measurements might be most useful. These specimens, stored at Lakehead University, Thunder Bay, Ontario, represented 11 geographically separated samples collected from the Thunder Bay area. They included samples from lakes and rivers in two major drainages (Lake Superior-Atlantic and Hudson Bay-Arctic) and included specimens which ranged from heavily scaled to naked. Specimens were subjectively classed as either scaled or naked based on the presence or absence of squamation. These two classes were created solely to provide a means to carry out the discriminant analysis to select morphometric characters for further examination and were not used further in the study. Initial morphometric characters used for discrimination were selected based on either the success of those used in previous studies of *E. nigrum* (*e.g.*, Kott and Humphreys 1978; Starnes and Starnes 1979; Page 1981) or those thought to be the most informative. No attempt was made to separate sexes at this time. The measurements included standard length, pectoral length, head length, snout length, predorsal length, caudal peduncle depth, caudal peduncle length, distance between dorsal fins, length of spiny dorsal base and length of soft dorsal base. All measurements were made as described in Hubbs and Lagler (1983) with the exception of the distance between dorsal fins. The distance between the dorsal fins was measured as the distance from the base of the most posterior dorsal spine to the base of the most anterior soft dorsal

ray. Based on the results of this discriminant analysis, three morphometric characters were identified as being potentially valuable for this study: snout length (SNL), pectoral length (PECL) and predorsal length (PREDL).

Squamation Characters

The nape was defined as the area enclosed by a triangle with its apex at the insertion of the spiny dorsal fin with its base crossing the top of the head at the level of the nape-parietal juncture (Fig. 3). The number of scales within the nape was counted by placing a straight edge from the posterior end of the spiny dorsal to the middle of the eye (Fig. 3). Scales which fell on the line were counted if more than half of any such scale lay in the defined area. The scales on partially scaled napes were noted as being positioned predominantly at the edge or in the posterior or anterior portions of the defined area. Darters possessing nape scales which contacted the edge of the parietal bones (nape-parietal juncture) were enumerated and entered separately from the total nape scale count (Fig. 3). The belly was defined as the area within a rectangle delimited by the pelvic fin insertions anteriorly, extending posteriorly to the anterior margin of the genital aperture (Fig. 4). The percent of the belly covered by scales was estimated. The breast was defined as the area delimited anteriorly by the gill membranes and posterolaterally by the origin of the pelvic and pectoral fins (Fig. 4). The number of breast scales within this area was counted. The opercle was defined as that area underlain by the opercular and subopercular bones (Fig 5). Scales covering this area were counted. The cheek was defined as that area lying within the area beneath the infraorbital and lateral canals and above

Figure 3: Graphic representation of the definition of the nape (stippled area) used for this study.

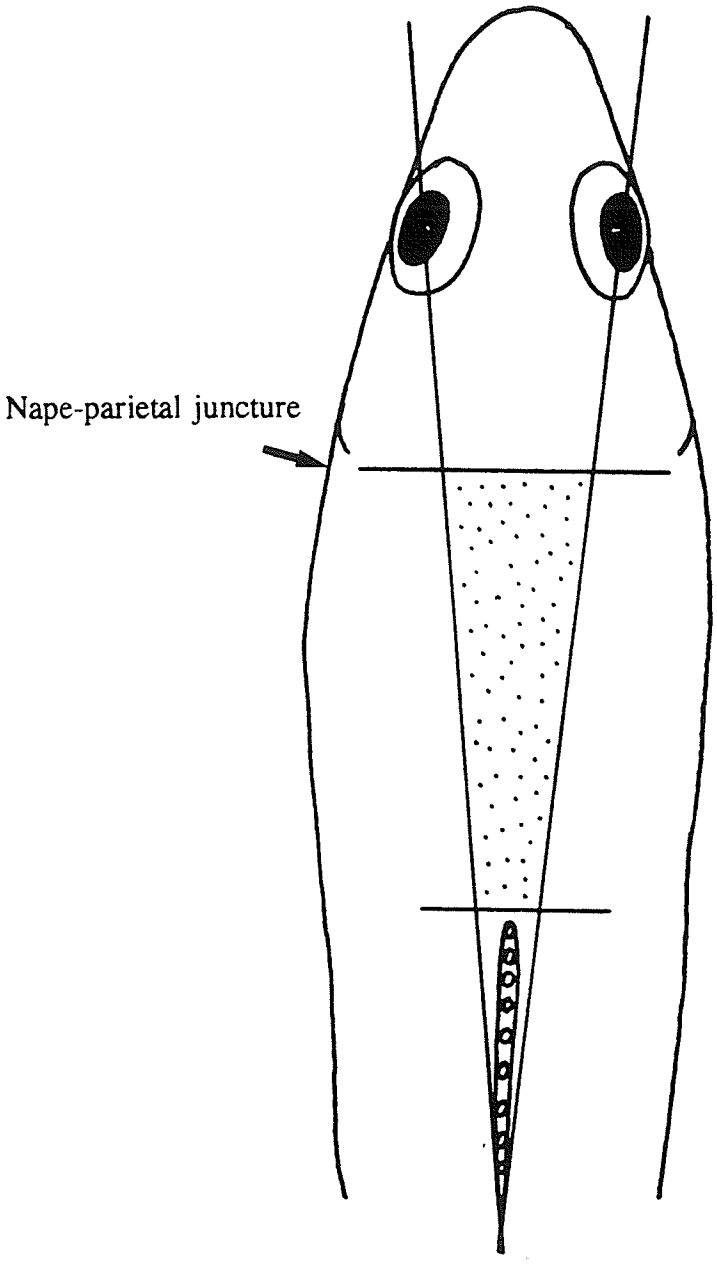
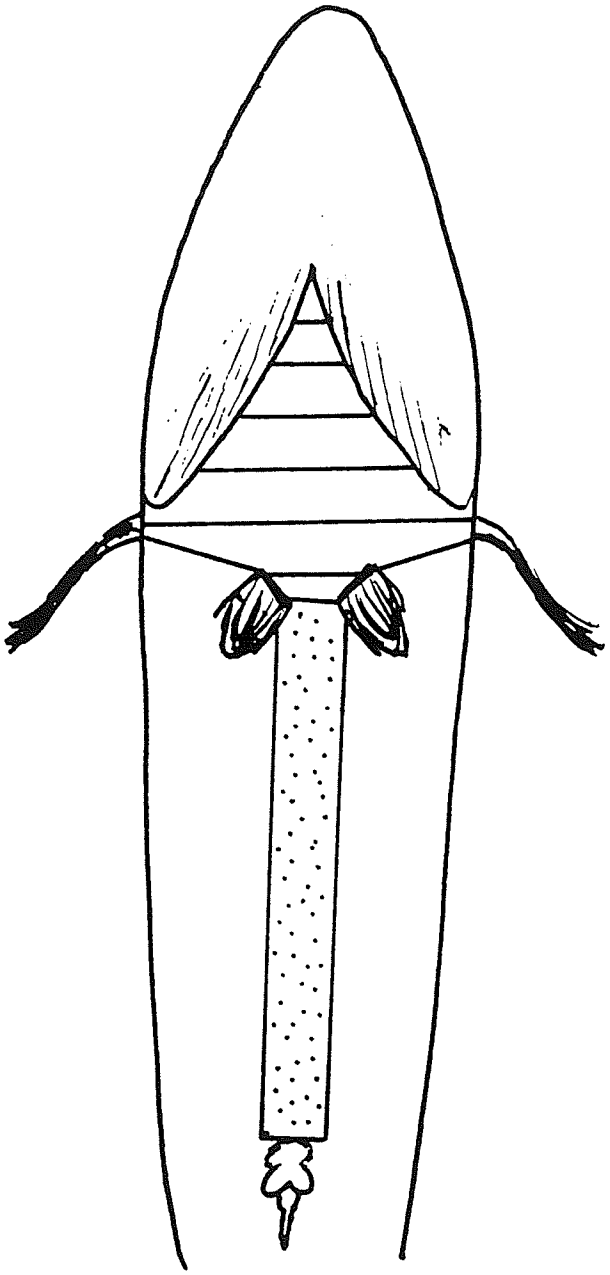

Nape-parietal juncture

Figure 4: Graphic representation of the definition of the belly (stippled area) and breast (hatched area) used for this study.



and anterior to the preoperculomandibular (POM) canal (Fig. 5).

Meristic Characters

Meristic characters used in this study were the following.

1: The number of scales covering (C) and an estimate of the percentage (P) of the area scaled on the opercle (OC and OP, respectively), cheek (CC and CP), nape (NC and NP), and breast (BC and BP) was made. Bilateral areas were counted on the left side only,

2: The number of scales at the nape-parietal juncture (NCT) was counted,

3: An estimate of the percentage of scaled area on the belly (BELLYP) was made,

4: The number of dorsal spines (DS) was counted,

5: The number of dorsal rays (counted as (DR1), the total number of dorsal rays counting the last two rays as one, even if they are cleft at the base as suggested by Hubbs and Lagler (1983) and (DR2), a count which was one ray higher than DR1 when it appeared that the posterior-most dorsal ray was not actually joined to the second last ray),

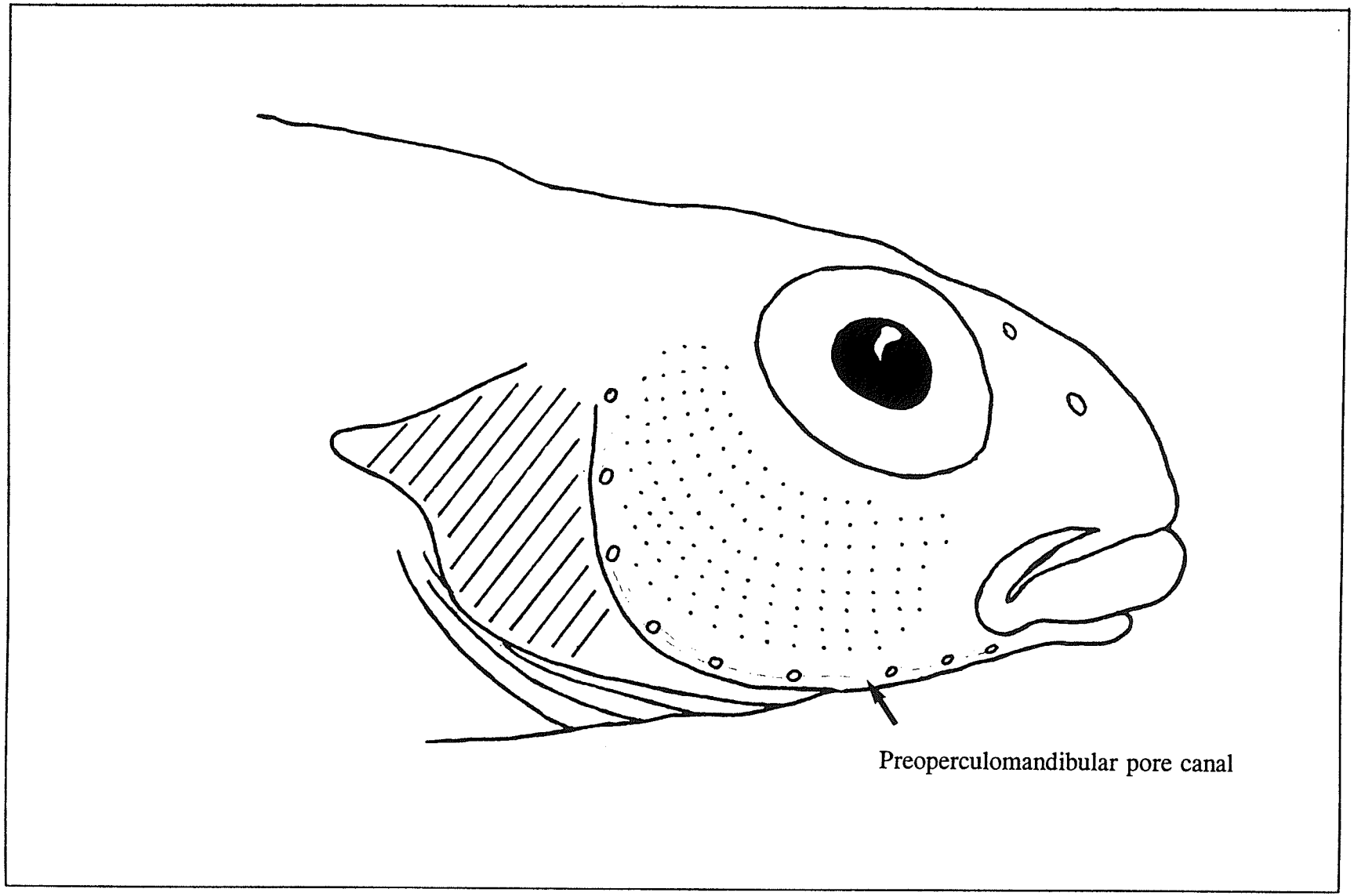
6: The number of anal rays (anal rays counted as (AR1) (AR2) as described above for the dorsal rays),

7: The number of left pectoral rays (PECR) was counted,

8: The total number of scales in the lateral line (LL) was counted,

9: The number of scales between the lateral line and the base of the anterior dorsal spine (LL1), and, the base of the posterior-most dorsal spine

Figure 5: Graphic representation of the cheek (stippled area) and opercle (hatched area) used for this study.


Preoperculomandibular pore canal

(LL2), counted diagonally from the dorsal spine down to and including the scale above the lateral line,

10: The number of scales below the lateral line (BLL) counted from and including the first scale above the origin of the anal fin upwards to and including the scale below the lateral line,

11: The number of branchiostegal rays (left (LBR) and right (RBR)) was counted,

12: The number of pores in the preoperculomandibular (POM) canal (left and right, counted as the total number (LPOP and RPOP) and the number confined to the opercle (LOPOP and ROPOP) as well as whether this canal system was interrupted, uninterrupted or confined to the opercle only (coded as 1, 2 or 3 respectively) (IN/UN)),

13: The number of anterior infraorbital pores (left (LANT) and right (RANT)) was counted,

14: The number of posterior infraorbital pores (left (LPOS) and right RPOS)) was counted,

15. The number of anal spines was determined. This character was not included in statistical analyses although it is discussed below.

The distribution of scales on the nape (including the scales at the nape-parietal juncture region), cheek, breast and opercle was noted as specimens were examined. It was then possible to draw the main patterns illustrating the distribution of the scales on these body areas. The resulting figures were based on examination of all

specimens. Contingency tables were constructed to determine if these patterns of squamation supported the possibility of several loci controlling squamation.

A small amount of methylene-blue was used to stain the cephalic lateral line canals on most specimens to better enumerate the infraorbital and POM pore systems and also to determine if they were interrupted or uninterrupted. Methylene-blue stain is non-permanent and may thus be used on museum specimens.

Other Characters

16: The sex of specimens was determined (SEX) as male (coded as 1), female (2) or unknown (0),

17: Snout-type (SNOUT) was assigned a number from 1-5 with "1" signifying a blunt snout and "5" signifying a subconical snout. A "3" was assigned for intermediate type snouts. An assignment of "2" or "4" was used to denote those specimens which did not appear to be intermediate in snout-type and leaned more towards one of the extreme types. Snout assignment was based on descriptions and photographs in Kott and Humphreys (1978),

18: The lateral line (LLSTAT) was scored as complete (1), incomplete with 1-3 unpored scales (2), or very incomplete (more than 4 unpored scales) and/or interrupted (3),

19: The presence or absence of a strong suborbital pigment bar was noted. The geographic occurrence of this character among samples is discussed below. This character was not included in statistical analyses largely due

to its subjective nature,

20: The presence or absence of a double bifurcation on the distal most portion of the individual soft dorsal rays was noted. This character was not included in statistical analyses although its geographic distribution is discussed below,

21: An *a priori* code number based on the estimated amount of squamation and divided into one of four "type" categories was assigned to each specimen. Those exhibiting scales estimated to cover 90% or more of the nape, cheek and breast were coded "1" (scaled darters); those without scales on the nape, cheek or breast were coded "2" (unscaled darters); those with more than 75% scaling on the nape, cheek and breast were coded "3" (intergrades); those with less than 75% scaling on any of the nape, cheek and/or breast were coded "4" (intermediates). "Type" codes were not included in any statistical analyses. The "type" categorizations allowed for comparisons with previously published results of classification of scaled *E. nigrum* based on subjective methods.

During data entry, specimens were entered with a state/province code number, a collection code number, a river or lake code number and an individual number.

All collections were assigned a drainage code based on major drainages; these were: Lake Ontario-St. Lawrence River=1, Mississippi River=2, Hudson Bay-James Bay (Ontario)=3, Ohio River=4, Missouri River=5, Atlantic Slope (excluding the St. Lawrence)=6, Hudson Bay (Manitoba-Saskatchewan, including the English-

Winnipeg rivers of Ontario)=7, Lake Erie=8, Lake Superior=9, Lake Huron=10 and Lake Michigan=11. Great Lakes drainages were coded individually to permit examination of regional trends in characters. The Hudson Bay-James Bay drainages of Ontario and the Hudson Bay drainages of Manitoba-Saskatchewan were considered separately due to climatic and habitat differences within each area. Additionally, the Hudson Bay Manitoba-Saskatchewan drainages have more recently been connected to the Mississippi drainage, possibly allowing movement of darters between drainages. Similar drainage divisions have been used by Underhill (1986) and Crossman and McAllister (1986) in their zoogeographic studies of North American freshwater fishes.

For each sample, the location was recorded by latitude and longitude. Locations were given the latitude and longitude of a one-degree latitude and longitude grid read from the southeastern corner of the quadrat. No samples fell on lines of latitude or longitude and therefore divisive methods were not required. The one-degree grids were further divided by major drainages such that one grid could be represented by two sample sets with the same latitude and longitude (*e.g.*, Ohio and Mississippi drainage). The grid method has been used by McAllister *et al.* (1986) to plot species density but can be utilized for cluster analyses or to examine data for clinal variation.

DATA ANALYSIS

Throughout this study, characters exhibiting variation which can be explained or interpreted in a biogeographical context are considered to exhibit meaningful variation. If, however, the variation in the character exhibits a random distribution,

does not exhibit agreement with variation in another related character or varies as much within samples as between samples, then the variation in that character will not be considered to exhibit meaningful variation.

Meristics and Morphometrics

After data entry, the entire set of meristic and morphometric data was examined to determine means, modes and ranges for each sample. Specimens known or thought to be *E. olmstedi* or *E. nigrum* x *E. olmstedi* hybrids (see below) were removed prior to analyses. Principal component analysis (PCA) using a correlation matrix was used to reduce the amount of total variation in the data to a more usable level. Principal component analysis reduces the amount of total variation within the data set by assigning the original variables (*e.g.*, number of dorsal spines) new component loadings or weights. These new variables are orthogonal to the original variables and are constructed so that the first principal component explains the largest amount of variation within the original data set. Characters which are less variable, and perhaps less informative in explaining a large amount of variation within the entire data set, are assigned lower component weights on the first few components but are gradually assigned higher weights as the last few components eventually explain the remainder of the variation within the data set. Therefore, examination of characters assigned the highest loadings on the first few components should explain the maximum amount of variation among all samples. These characters can then be used to cluster samples based on similarities and differences. Principal component analysis was run both with and without squamation variables, as it was known *a*

priori that PCA would identify the squamation variables with a high weight on the first or second component due to the great variation within the sample. Snout-type, which I considered to be subjective, was excluded from all PCA runs. Possible problems with codings for the condition of the lateral line and the completeness or incompleteness of the POM canal necessitated excluding them from PCA. Percentage of nape, cheek, breast and opercle covered by scales was also excluded from PCA for the reasons noted above. The estimated percentage of belly squamation was included only on runs which included other squamation variables as it was considered a potentially informative character in explaining variation between samples.

Principal component analysis was used to extract characters which had high loadings on the first two factors. The characters identified with high loadings were plotted on distribution maps using the previously determined means and modes of samples. The distributions of these characters were compared to abiotic variables including the mean number of growing degree-days above 5.6°C, average annual precipitation and length of frost-free period.

The means of meristic variables from individual samples (mean value for variable within a sample) were analyzed with PCA (correlation matrix) to examine how samples varied in relation to the component scores. Using the means of variables from samples for PCA is a method which should help eliminate some of the "noise" which is expected to be produced when using all specimens. Principal component analysis using sample means also provides a way to check the precision of the PCA performed using all specimens. Samples were plotted against the variable

scores in a biplot. Samples occurring in the same ordinate space as the examined variables were assumed to be most influenced by those variables (Podani 1994).

Morphometric data were analyzed with PCA using a correlation matrix. Four variables (standard length, pectoral length, predorsal length and snout length) were utilized.

The Pearson product moment correlation coefficient was calculated for all variables using the individual specimen matrix and the matrix of the sample means to determine the correlation between drainage, riverine/lacustrine habitat, latitude and longitude, and meristic variation among samples. The Pearson correlation coefficient was also calculated using the one-degree latitude and longitude grids to determine the correlation between variables within these grids with latitude and longitude. Using these three methods allowed for checking the precision of each method.

Univariate "*t*" tests were carried out to determine if males and females differed significantly in morphometric or meristic variables. A contingency table was used to determine if blunt or subconical snout-type was drainage dependent.

For this study, unscaled darters were defined as those that did not exhibit any squamation on the nape, cheek or breast. Intergrading specimens were those that did not meet the developed definition of scaled but possessed some squamation on the nape, cheek and/or breast.

A definition of a scaled darter was determined by first constructing frequency histograms of the number of scales on the nape, cheek, opercle and breast using all specimens identified as *E. nigrum*. Because the number of belly scales was not

counted, the estimated percent of belly scaled on a "scaled" darter was set at 100%. A 100% scaled belly is often the condition reported for *E. nigrum* and I reasoned a scaled darter should possess a completely scaled belly. The final frequency "peak" in each histogram was visually identified. Because scaled darters should be the ones possessing the greatest amount of squamation, they should be clustered on the right hand portion of the histograms. One sample standard deviation was therefore subtracted from these frequency peaks to arrive at the lowest number of scales that a specimen had to possess to be included as scaled for this study. I hypothesized that if there was any large, geographic trend in squamation variables for *E. nigrum*, using a similar method for all squamation variables would result in an approximately similar number of specimens being identified within each squamation histogram. Having set the lower limit of squamation characters required to meet the definition of scaled for this study, I defined scaled darters as those which met or exceeded these limits.

Once a definition of "scaled" had been developed, all specimens were examined with step-wise discriminant analysis to determine if additional characters might help separate the two forms and to classify intergrading specimens. Two analyses were performed; one included squamation characters (NC, NCT, CC, OC and BC) while the second excluded them. Estimated percent of area covered by scales, except for the belly, condition of the POM pore canal, snout-type, and those characters noted above, were not included in any analysis. All other meristic characters were included.

Meristic characters of all scaled and unscaled *E. nigrum* were compared.

Samples of the scaled and unscaled form from the Fox River system in Wisconsin were examined for statistical differences using a "*t*" test. Character index tables were created to determine if scaled and unscaled specimens could be separated using characters other than the obvious differences in squamation.

The Mann-Whitney *U*-test was used to examine the possible effects of habitat (lentic or lotic) on the total number of left POM pores. As a non-parametric test, the Mann-Whitney test does not require assumptions about the shape of the underlying distribution of the samples (SPSS 1992). This test was performed on the entire sample as well as on Ontario, Wisconsin, Manitoba and Michigan samples as the majority of lakes were located in these areas. Samples covering several states and/or provinces from the Mississippi River, Hudson Bay (Ontario and Manitoba) and Lake Superior drainages were also analyzed separately.

Principal component analysis and discriminant analysis was performed with SPSS (SPSS 1988) on a VAX mainframe computer. Summary statistics, Pearson correlation, "*t*" tests, contingency tables and the Mann-Whitney tests were performed via SPSS-PC+ (Ver. 5) (1992). Principal component analysis of sample means was performed using the SYN-TAX (Ver. 5) computer program (Podani 1993).

SEPARATION OF *Etheostoma nigrum* FROM *E. olmstedi*

All specimens borrowed from the UMMZ, including those from the Atlantic Slope, are labelled *E. nigrum*. Similarly, all specimens borrowed from the ROM are labelled *E. nigrum*, but the possibility that some of these collections may be *E. olmstedi*, or contain intergrade specimens, must be examined.

Museum collections from locations harbouring only *E. olmstedi* (based on previous work (Cole 1965; Smith 1979; Lee and McAllister 1980)) were examined to provide a basis for comparison with darters from areas where both *E. nigrum* and *E. olmstedi* might occur. Meristic and morphometric characters as given above were enumerated on these specimens. The eight samples examined are detailed in Table 1. Eight samples of *E. nigrum* were randomly selected from the north-central range of the species to provide baseline data for that species (Table 1). The north-central portion of the range was chosen due to its similar latitude and also to avoid any possible influence from *E. olmstedi* which may have occurred in more eastern samples. These samples were selected using the completed data sheets of *E. nigrum* samples examined for this study. Samples were chosen by randomly opening the binder which held these sheets until an approximately similar number of *E. olmstedi* and *E. nigrum* had been selected. All meristic and morphometric characters listed above were determined for these samples.

Frequency distribution tables were constructed for the eight samples of both species utilizing the most variable characters (excluding scaling and squamation characters). The frequency distributions of 17 samples of unclassified (*Boleosoma*) from Lake Ontario-St. Lawrence River tributaries and New York state were also compiled. These additional samples were selected based on their occurrence within a geographic area in which migration by either parental species might have occurred. The possibility of hybridized populations was thus high within this area. These 17 samples were compared to the sample means of the previously identified *E. olmstedi*

Table 1: Collection identification, location, number (N) and drainage of samples utilized in character indices and discriminant analysis to separate *Etheostoma nigrum* from *E. olmstedi*. Numbers following state or provincial abbreviations refer to identification numbers used in this study.

Waterbody	Drainage	State/Province	N	Collection Identification	
				Museum	This study
Etheostoma olmstedi					
Rock Creek	Atlantic	Maryland	9	ROM 49148	MD-01
Rock Creek	Atlantic	Maryland	20	ROM 49145	MD-02
Lake Dunmore	Atlantic	Vermont	15	ROM 28440	VT-01
Golden Gate Brook	Atlantic	Massachusetts	6	UMMZ 113392	MA-01
Gale River	Atlantic	New Hampshire	6	UMMZ 126712	NH-01
Otter Brook	Atlantic	New Hampshire	10	UMMZ 126705	NH-02
Scantic River	Atlantic	Connecticut	7	UMMZ 89271	CT-01
Raritan River	Atlantic	New Jersey	11	UMMZ 109832	NJ-01
Etheostoma nigrum					
Spirit Lake	Missouri	Iowa	8	UMMZ 101528	IA-05
Red Lake River	Hudson Bay	Minnesota	11	Private Col	MN-03
Sucker River	Lake Superior	Minnesota	6	JFBM 24293	MN-24
Roche Perch Creek	Mississippi	Missouri	7	UMMZ 148036	MO-06
Kawaguesaga Lake	Mississippi	Wisconsin	9	UMMZ 72447	WI-20
Pecatonica River	Mississippi	Wisconsin	11	UMMZ 76950	WI-28
Whetstone Creek	Mississippi	South Dakota	23	UMMZ 166905	SD-03
Clear Creek	Mississippi	Indiana	11	UMMZ 61966	IN-02
Unclassified samples					
Yamaska River trib.	St. Lawrence	Quebec	13	ROM 21566	QU-01
Lac Aylmer	St. Lawrence	Quebec	36	ROM 32993	QU-02
Prouxl Creek	St. Lawrence	Quebec	17	ROM 21582	QU-03
Lac Caron	St.Lawrence	Quebec	13	ROM 28472	QU-04
River du Nord	St. Lawrence	Quebec	9	ROM 24036	QU-05
Lake Champlain	Atlantic*	New York	11	UMMZ 140504	NY-01
Unnamed stream	Lake Ontario	New York	26	UMMZ 99153	NY-02
Oneida Lake	Atlantic*	New York	10	UMMZ 81282	NY-03
Cayuga Lake	Lake Ontario*	New York	8	UMMZ 84484	NY-04
Rideau Creek	St.Lawrence	Ontario	11	ROM 11138	ON-01
Prinyer's Cove	St. Lawrence	Ontario	8	ROM 4935	ON-03
Bateau Point	St. Lawrence	Ontario	6	ROM 9197	ON-07
Don River	Lake Ontario	Ontario	11	ROM 11693	ON-09
(no waterbody given)	Lake Ontario	Ontario	14	ROM 30611	ON-19
Niagara River	Lake Ontario	Ontario	5	ROM 40132	ON-21
Aumond Creek	Lake Ontario	Ontario	7	ROM 24563	ON-23
Credit River	Lake Ontario	Ontario	10	ROM 22765	ON-24

* variable due to construction of canals

and *E. nigrum*. Location and origin of all examined collections are presented in Table 1.

A character index (CI) was compiled using the results of the meristic data obtained from the two species. A CI is constructed by summing the variables for each specimen of each known species and arriving at a species mean (Cole 1965). At some point on a CI table, both species will be more completely separated than at any other point. The CI will show the best line of separation for the two species by way of a distinct break in the summed values of the characters presented in the table. The CI thus provides a simple means to sum the same characters from unclassified samples or specimens, and allows them to be assigned to the most likely species of actual membership. Unclassified samples were compared to the CI calculated for *E. nigrum* and *E. olmstedi* samples.

Although the CI was designed to evaluate the hybrid nature of intermediate specimens (Mayr and Ashlock 1991), it can be used as a simple, quick discriminant function for classifying specimens of uncertain lineage. The main difference between a CI and a discriminant function is the final multiplier of each character (Cole 1965). However, additional considerations such as the value range of the characters examined, whether or not the characters are commensurable, and the associated weighting that each character contributes to the CI, should be considered. Thus, the information conveyed by a CI has statistical limits and their results should be compared to those produced with multivariate methods. The main benefit of utilizing the CI is that it allows comparisons with earlier studies that utilized this method.

Two discriminant functions were prepared utilizing only known specimens of each species and using the most dissimilar characters between the two species. These functions were prepared via SPSS (SPSS 1988) using the stepwise method, forcing all variables to be utilized when creating the function.

Squamation and scaling characters for each species (mean number of scales on the nape, cheek, breast and opercle and number of scales in and below the lateral line) were compared to determine if any of these were suitable for separating the two species or for identifying samples for which the species membership was unknown.

TEMPORAL VARIATION ANALYSIS

Several studies in the literature have dealt with meristic variation in fishes at the same sample site over time (Lindsey 1953; Underhill and Merrell 1959; Underhill 1960, 1961; Nelson and Atton 1971). Most of these, however, have included only very short term data sets of three years or less. No studies exist on temporal variation of *E. nigrum*. Characters used in systematic analyses should have a genetic basis if the differences discovered between populations are to be considered valid for systematic purposes (Lagler and Bailey 1947; Reist and Crossman 1987). Any study which attempts to interpret geographic patterns of meristic variation should include a temporal component to determine if the patterns observed are consistent over time (Chernoff 1982).

Several Wisconsin areas, previously sampled during the early part of the century (Hubbs and Greene 1935; Greene 1935), were visited during 1992 for collection of specimens for meristic and morphometric or electrophoretic study. The

museum collections from these locations were borrowed from the UMMZ and meristic counts and morphometric data (same as above) were compiled for them. These museum samples are: Big Arbor Vitae Lake (72414), Flambeau River (78613), Whitefish Lake (96047), Rock River (77232) and Crawfish River (77165) (Table 2). These samples were compared to those collected in 1992 to see if characters had varied over time. These collections provide a 64-67 year gap between captures and, considering possible maturity at age one (Becker 1983), could represent the passage of between 32-64 generations. All specimens collected for this study have been deposited in the University of Manitoba collection of fishes. High water levels encountered in 1992 prevented the capture of the desired number of specimens from each location.

Meristic characters enumerated on all specimens from recent and historic samples were examined with stepwise discriminant analysis and compared by means of MANOVA and "*t*" tests. When either of the samples possessed a zero variance for the sample mean of that character, the character was excluded from analysis due to violations of the tests.

Discriminant analysis was used as a way to determine if recent and historic samples could be readily distinguished. The creation of a discriminant function which successfully separates a majority of the two samples indicates that significant changes have taken place over time and shows which characters have varied the most. Discriminant analysis was performed using SPSS (1988). A stepwise analysis in which variables were selected which limited the overall Wilks' lambda was used. The

Table 2: Collection site, origin of collection, number of specimens examined (N), date of collection, number of years difference between collections, and size range (total length) of specimens of *Etheostoma nigrum* from Wisconsin examined during temporal variation analyses. Specimens collected for this study have been deposited in the University of Manitoba collection of fishes.

Location	N	Collection Date	Difference in years	Size Range (mm)
Whitefish Lake				
UMMZ 96047	15	September 5, 1928	64	26-54
	7	August 13, 1992		31-38
Big Arbor Vitae Lake				
UMMZ 72414	30	September 19, 1925	67	26-57
	59	August 12, 1992		34-58
Flambeau River				
UMMZ 78613	10	August 28, 1928	64	49-60
	16	August 13, 1992		31-52
Crawfish River				
UMMZ 77165	11	August 26, 1927	65	32-45
	7	August 11, 1992		41-46
Rock River				
UMMZ 77232	14	August 27, 1927	65	35-49
	15	August 11, 1992		39-52

probability of "F" to enter or remove variables was set to 0.05.

Multiple analysis of variance was used to search for significant differences between the means of all characters which exhibited differences. It was not possible to enumerate the number of breast scales from four of the recent samples (Big Arbor Vitae Lake, Rock River, Flambeau River, Whitefish Lake) as electrophoretic preparation had destroyed portions of the breast. However, an additional sample from the Crawfish River, not used for electrophoresis, allowed for enumeration of the number of breast scales for that sample. Multiple analysis of variance was performed on this sample using both the four and five available squamation counts. Significance of the test was judged to occur at the $P < 0.05$ level.

Significance of the "*t*" test ($P < 0.05$) was judged based on the results of the Levene test ("F") for homogeneity of variances (SPSS 1992). If the observed significance of the Levene test was small ($P < 0.05$), the hypothesis that the population variances were equal was rejected and the unequal variance "*t*" value was used to determine the significance of the differences between sample means.

Comparisons were made between the recent and historic collections to determine if the percentage of scaled specimens making up the samples had changed. Because breast scale counts were not available for all recent collections, scaled specimens were identified two ways. In all historic collections, scaled specimens were first identified using the definition developed for the study. The number identified was then compared to the number identified when the number of breast scales was dropped from the qualifying definition. In the recent Crawfish River

sample, it was possible to identify scaled darters using the developed definition. Other recent collections were categorized by the number of nape and cheek scales only as the breast was partially destroyed during biochemical preparation.

Individual "*t*" tests were performed via SPSS/PC+ (SPSS 1992) while MANOVA was calculated using SPSS (1988) on a VAX mainframe computer.

CONSISTENCY OF SQUAMATION AND MERISTIC CHARACTERS

Squamation and meristic variables were examined to determine if more variation occurred within or between samples of *E. nigrum* which possessed a 100% nape or breast. Wisconsin specimens which possessed a 100% scaled nape or breast were compared with specimens in other samples, first within the state and then from distant geographic areas, to determine if there was any consistency in the number of scales needed to cover that body area to 100%. The Pearson product moment correlation coefficient was determined for all specimens from Wisconsin with a 100% scaled nape or breast to determine correlation with other squamation, meristic or morphometric characters. The estimated percent of the nape covered with scales for all Wisconsin specimens was plotted against the number of nape scales and frequency distribution tables produced.

Frequency distribution tables were produced showing the distribution of meristic variables of scaled specimens within major drainages. A Chi-square test was performed to determine whether scaled darters were equally distributed in both lacustrine and lotic environments. Univariate "*t*" tests were performed to test for significant differences in meristic variables of 100% scaled *E. nigrum* from lakes and

rivers in Wisconsin. Significance was judged based on the pooled or separate sample variances for that variable. If the Levene's test "F" value for equal sample variances had a probability of $P \leq 0.05$, the sample variances were considered unequal and the separate variance "*t*" test was used to determine the level of significance.

The Pearson product moment correlation coefficient between the number of scales on the nape and the predorsal length was determined for all Wisconsin darters which possessed a 100% scaled nape. The nape length was measured on 23 fully scaled specimens (nine from Minnesota, six from Wisconsin and eight from Ontario) with a 100% scaled nape to determine the correlation between nape length and the number of scales on this area. Nape and predorsal length were measured according to Hubbs and Lagler (1983). Scales were removed from the centre of the nape on these specimens to determine if nape scales were all the same size.

CLUSTER ANALYSES

Cluster analyses were carried out to determine if geographic assemblages of *E. nigrum* would be created, possibly suggesting areas with a unique history, and to determine if those samples identified as scaled remained clustered when squamation variables were removed from the algorithm. Cluster analyses were performed using minimum variance as a clustering algorithm on all samples identified as *E. nigrum*. Minimum variance clustering is a suitable resemblance measure for quantitative data (Podani 1994). Variable means were standardized by their range bringing all variables into the same scale (Podani 1994). Percentage difference, also called the Marczewski-Steinhaus difference (ratio of the sum of shared contributions of all

variables to the total scores of variables) was used as a resemblance coefficient (Podani 1994). Clusters were amalgamated using the reciprocal neighbour method which clusters pairs of samples which are closest to each other (Podani 1994). The sample means of characters identified by heavy weightings on the first and/or second PC axes based on PCA of all specimens or the means of samples were used as clustering characters. Using characters selected by PCA was deemed likely to provide higher information content than characters selected subjectively.

Cluster analysis was performed eight times using the different sets of heavily weighted characters identified by the different methods of PCA (PCA of individuals or PCA of sample means). The initial dendrograms were created utilizing those characters identified by heavy weighting on the first PC axis. The second set of analyses utilized characters identified on the first and second PC axes. Both of these analyses were performed using weights identified when squamation characters were included in the PC analysis. A third and fourth pair of dendrograms was created using those characters identified by heavy weighting on the first PC axes when squamation characters (estimated percent of belly scaled and number of scales on the nape, cheek, opercle, breast and the nape-parietal juncture area) were excluded.

An asterisk was added to the sample labels of samples identified as possessing scaled specimens so that dendrograms would show the position of these samples. This was done to aid in interpretation of dendrograms, such as when squamation was excluded. All clustered samples are identified by labels used in Appendix 2.

The two clustering methods (PCA on individuals and PCA on sample means)

were utilized to determine which method would be more informative. Characters identified through sample means was thought to remove some of the "noise" from the data set caused by specimens with extreme variation and thus provide a more informative dendrogram.

An additional dendrogram clustered groups of *E. nigrum* divided into 152 geographic areas based on one-degree latitude and longitude grids divided by major drainages. For each one-degree grid, sample means were calculated from all specimens within each of the 11 major drainages described above with the exception that the St.Lawrence River and Lake Ontario samples were grouped. In many instances, however, this method resulted in a single sample representing the entire grid. This method also resulted in some one-degree grids including samples of both heavily scaled and unscaled specimens. This clustering was performed using the methods outlined above. This analysis was performed using characters identified on the first and second PC axis with squamation variables removed and was based on variables identified using the results of PCA using sample means.

LATERAL ASYMMETRY

Studies in lateral asymmetry have suggested that populations under "stress" (environmental (Valentine *et al.* 1973) or genetic (Van Valen 1962)) may exhibit a large degree of asymmetry in bilateral characters as a result of the phenotype being unable to buffer itself against these disturbances. Levels of lateral asymmetry have been included in an increasing number of evolutionary studies (Merilä and Björklund 1995). The degree of asymmetry can be examined in samples of *E. nigrum* to

determine whether such stress has occurred in populations possessing intergrade, scaled and the unscaled phenotypes. Thus, if the scaled and unscaled forms of *E. nigrum* diverged during a period of separation, there may be a large amount of asymmetry present, especially in intergrading specimens.

Several meristic characters were counted on both the left and right sides of specimens. These characters included the number of branchiostegal rays, the number of anterior and posterior infraorbital pores, the total number of pores in the POM canal, and the number of these pores confined to the opercle. All *E. nigrum* were examined to identify samples or specimens within samples which exhibited differences in bilateral characters. The number of scaled specimens (as defined above), as well as the number of specimens possessing scaleless napes, breasts and cheeks, were also examined individually from the total sample to determine if one phenotype possessed greater asymmetry than the other.

Fluctuating asymmetry was assessed by summing the absolute differences between the counts on each side and dividing it by the sum of the sample size (Van Valen 1962; Moodie 1977). Thus any sample exhibiting fluctuating asymmetry will possess a value greater than 0 (*e.g.*, 7 - 6, 7 - 7, 6 - 6, 8 - 8, 7 - 7, 6 - 6, 6 - 6, 7 - 7, 8 - 8 = 1 ÷ 9 = 0.111), and those samples with a great degree of asymmetry will possess higher values than those with less variation. Most studies arrive at fluctuating asymmetry values of less than 1.5 (*e.g.*, Moodie 1977).

Only those samples with five or more specimens and possessing a minimum of 20% sample asymmetry for at least one character were investigated further using the

methods described above. Asymmetry levels less than 20% within samples were judged *a priori* to be "noise" (Van Valen 1962).

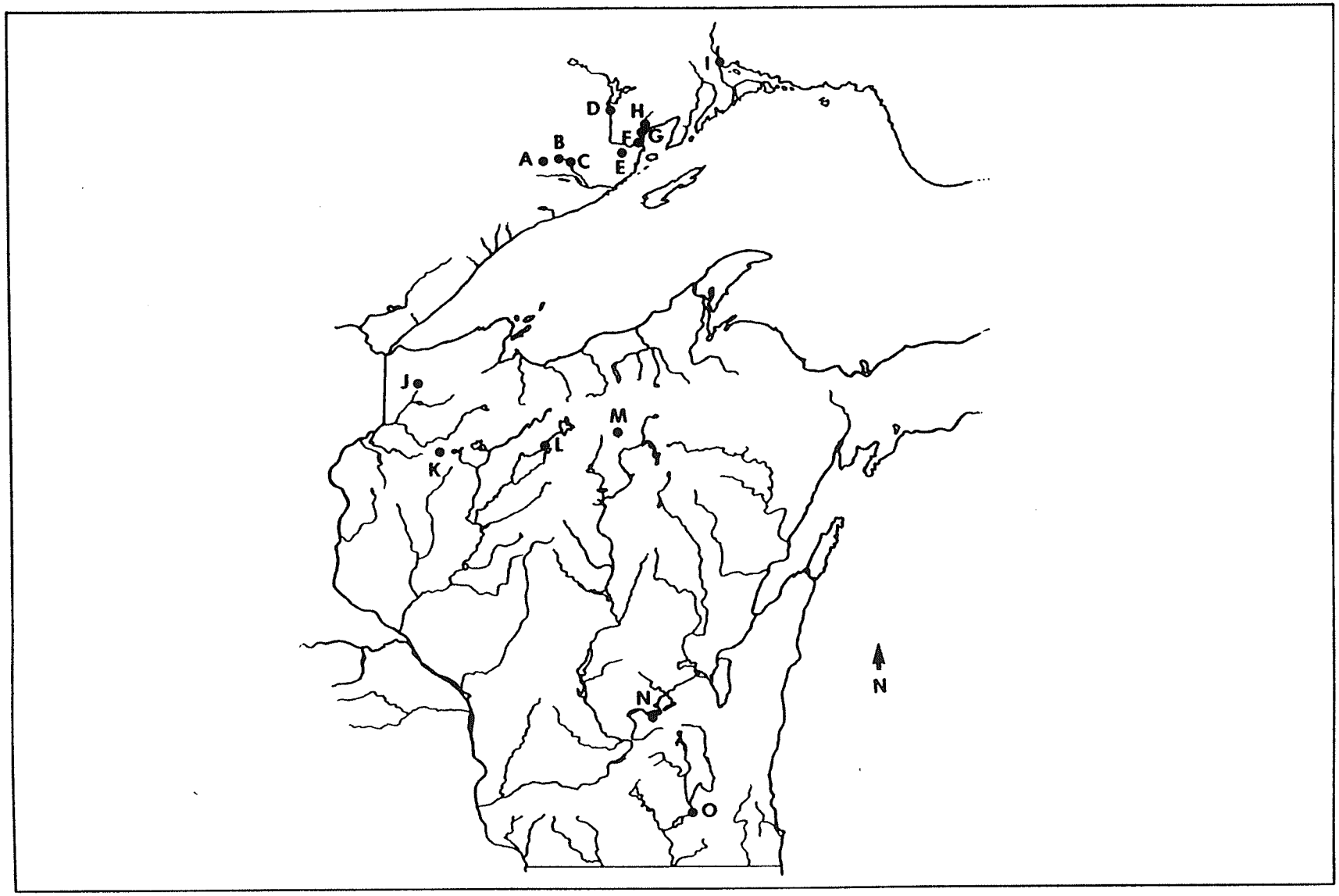
Direction of asymmetry was judged by noting the number of specimens in which either the right or left value was the greater. The magnitude of the differences between sides in the value of a character was ignored, so that if the left side had a higher pore count in more specimens than the right side, even if the total number of right pores exceeded those on the left (*e.g.*, 7-6, 7-6, 7-6, 6-9, 6-10), the direction of asymmetry was recorded as left.

The Pearson product moment correlation coefficient was used to examine the strength of the relationship between the amount of asymmetry and its occurrence on body areas both within all examined samples and within individual states and provinces. Correlations between the occurrence of asymmetry within lakes and rivers was also examined.

BIOCHEMISTRY

Horizontal starch gel electrophoresis was performed on 644 *E. nigrum* collected from 15 geographic locations in Wisconsin and northwestern Ontario (Fig. **6**). Mean sample size was 43 (range 7-97) with many samples being smaller than desired due to difficulty of capture because of high water conditions in 1992. Several smaller samples of darters collected in southern Manitoba (Lake Winnipeg (at Grand Beach; N = 4), Rat River (near St. Malo Provincial Park; N = 4), Roseau River (near Stuartburn; N = 5)) were also analyzed but were not included in analyses of biochemical variation (although they are occasionally referred to below). Trial runs

Figure 6: Locations of samples of *Etheostoma nigrum* from Ontario and Wisconsin used for biochemical analysis. A=Prelate Lake, B=Sandstone Lake, C=Roundtable Lake, D=Little Dog Lake, E=Silver Falls Creek, F=Neebing-McIntyre River, G=McVicars Creek, H=Boulevard Lake, I=Nipigon River, J=Upper St. Croix Lake, K=Whitefish Lake, L=Flambeau River, M=Big Arbor Vitae Lake, N=Puckaway Lake, O=Rock River.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performed with Boulevard Lake, Ontario specimens (not included in final analyses) allowed for determining the conditions under which each enzyme was best resolved. After performing trial runs with 15 stains, ten were chosen which allowed for both relatively simple and consistent scoring. Enzymes examined (abbreviation and Enzyme Commission number (in parentheses) from Shaklee *et al.* (1990)) were Lactate dehydrogenase (*LDH**; 1.1.1.27), Isocitrate dehydrogenase (*IDH**; 1.1.1.42), Phosphoglucomutase (*PGM-1**, *PGM-2**; 5.4.2.2), Xanthine dehydrogenase (*XDH**; 1.2.1.37), Glycerol-3-phosphate dehydrogenase (*G3PDH**; 1.1.1.8), Glucose-6-phosphate isomerase (*GPI**; 5.3.1.9), Phosphogluconate dehydrogenase (*PGDH**; 1.1.1.44), Malic enzyme (*sMEP**, *mMEP**; 1.1.1.40) (referred to as *ME-1** and *ME-2** in this study), Aspartate aminotransferase (*sAAT**, *mAAT**; 2.6.1.1.) and Esterase (*EST**; 3.1.1.-). Stain and buffer formulae are given in Appendix **3**.

Darters used for electrophoretic study were collected by seine and immediately placed on dry ice. Transfer was made to freezers kept at -20°C until the electrophoretic runs could be performed. Fish collected in 1991 were run within six months of collection while those collected in 1992 were run within three months. Fish were collected from two areas (McVicars Creek and Boulevard Lake, Ontario) in both 1991 and 1992 to determine if length of storage influenced results. Table **3** gives the location, number and date of collection of specimens of *E. nigrum* used for electrophoretic study. One specimen of *Percina maculata* was run to provide a comparison and help identify the *LDH-B** locus. Several specimens of *Etheostoma exile*, collected with samples of *E. nigrum*, were also run to provide comparisons

Table 3: Location, date of collection and number of *Etheostoma nigrum* examined using starch gel electrophoresis.

Location	Date Collected	Number Analyzed
McVicars Creek, ON	August 16, 1991	36
(48°26'4"N 89°12'8"W)	August 28, 1992	15
Boulevard Lake, ON	August 16, 1991	94
(48°27'9"N 89°12'9"W)	August 28, 1992	3
Neebing-McIntyre River, ON	August 1, 1991	56
(48°24'0"N 89°14'3"W)		
Silver Falls Cr, ON	August 28, 1992	9
(48°15'5"N 89°48'5"W)		
Roundtable Lake, ON	July 31, 1991	10
(48°13'0"N 90°10'0"W)		
Sandstone Lake, ON	July 31, 1991	92
(48°14'5"N 90°12'0"W)		
Prelate Lake, ON	July 31, 1991	52
(48°11'9"N 90°24'0"W)		
Little Dog Lake, ON	August 14, 1991	68
(48°27'9"N 89°36'0"W)		
Nipigon River, ON	August 13, 1991	66
(49°00'0"N 88°15'6"W)		
Rock River, WI	August 11, 1992	15
(42°59'0"N 88°55'0"W)		
Puckaway Lake, WI	August 11, 1992	15
(43°44'0"N 89°10'0"W)		
Upper St. Croix Lake, WI	August 13, 1992	34
(46°25'0"N 91°51'0"W)		
Big Arbor Vitae Lake, WI	August 12, 1992	59
(45°55'0"N 89°34'0"W)		
Whitefish Lake, WI	August 13, 1992	7
(45°50'0"N 91°28'0"W)		
Flambeau River, WI	August 13, 1992	16
(45°48'0"N 90°30'0"W)		

with previous work on darter *LDH** mobility (*e.g.*, Page and Whitt 1973a,b).

Specimens were removed from the freezer four or five at a time to prevent thawing and possible denaturation of enzymes. A "fillet" was removed from the right side of the fish and white muscle was separated from the skin and used as the sample. Care was taken that no viscera were included with this sample. All, or a portion, of the liver was also removed. These tissues were placed in the wells of sample trays which were kept on ice to prevent rapid thawing of tissues. After 30 samples were prepared, the tissues were crudely homogenized using glass rods. Sample buffer (Tris-HCl: pH 7.1), when required, was added to the wells to aid absorption of the homogenate onto wicks which were then placed on the pre-sliced gels. Gels with wicks in place were run under power for approximately ten minutes to allow the proteins to move into the gels. Power was turned off, the wicks removed, and the gels again run under power for approximately 4-5 hours (Appendix **4**). Gels were then removed, sliced and promptly stained to reveal the activity of proteins. Appendix **5** gives protocol for preparing gels. Scoring was performed immediately after the gels were stained darkly enough to read. The amount of movement was measured from the point of origin and recorded to the nearest 0.5 mm. Samples of many gels were preserved and/or photographed for future reference.

The terminology of Shaklee *et al.* (1990) was adapted for describing the movements of enzyme products and identifying loci. Thus, the most common allele from all samples was designated "100" and served as a reference point. Other alleles were designated by their relative mobility with reference to this "100" allele.

Genotype was denoted as "aa" when both loci coded for the most common allele (*e.g.*, "100"). The second most common genotype, whether anodal or cathodal relative to the "100" allele, was given the designation of "bb". Letters were used to identify loci when orthology with isozymes in the same or similar taxa had been demonstrated (*e.g.*, *LDH-B**). Numbers were assigned to loci which have not been demonstrated to be orthologous (*e.g.*, *PGM-1**). In these instances, numbers were assigned from the most anodal to most cathodal product. This method is comparable to other darter studies (*e.g.*, Martin and Richmond 1973; McKeown *et al.* 1984).

Measures of genetic variability calculated included mean number of alleles per locus, mean heterozygosity (unbiased) and percentage of polymorphic loci for each sample. Mean heterozygosity per locus was calculated as biased (based on Hardy-Weinberg expectations), unbiased (conditional expectations) and direct-count (the proportion of individuals actually heterozygous). The percentage of loci polymorphic using the 99%, 95% and "no criterion" methods were also calculated for each sample. Deviation from Hardy-Weinberg proportions was calculated by hand and also using the BIOSYS-1 computer program (Swofford and Selander 1989). Genetic divergence among samples was quantified through the heterogeneity chi-square statistic and calculation of F-statistics (Wright 1965). Calculation of F-statistics determines the partitioning and nature of variation within samples (F_{IS}), between samples (F_{IT}) and as a measure of genetic divergence between samples (F_{ST}). All F-statistics have a theoretical minimum value of zero (no genetic divergence) and a theoretical maximum of one (indicating fixation of alternate alleles in the samples). Estimates of

heterozygosity between and within samples were performed. Pairwise chi-square values were determined between samples to test for statistical differences in allele frequencies at individual loci. Nei's (1978) unbiased genetic distance and Nei's (1972) genetic distance were calculated and used in cluster analyses using the unweighted pair-group method with arithmetic averaging (UPGMA). Some calculations of allelic variation and the cluster analyses were carried out using the BIOSYS-1 computer program (Swofford and Selander 1989).

Canonical Correlation Analysis

As darters were being prepared for electrophoresis, specimens were identified with paper tags so that the biochemical results could be compared with meristic characters and geographic location. The characters used were the same as those described above for museum specimens. Morphometric measurements were not made due to damage to many specimens. These darters were statistically analyzed separately from museum specimens. Some difficulty arose with making meristic counts on specimens since some darters less than 26.0 mm SL were used for electrophoresis. These specimens were not utilized for canonical correlation analysis. The breast scale count was abandoned due to removal of portions of this area on most specimens during electrophoretic preparation. Therefore, only an estimate of the percentage area covered by scales was made for the breast. Some specimens also had damaged cheeks and opercles. This resulted in some counts being made on the right hand side of the specimen as opposed to the preferred left side. Additionally, two specimens had dorsal or anal rays either cut off during electrophoretic preparation or

being torn off while separating partially frozen specimens. In these instances, the specimens were assigned a count based on the mode of other, undamaged, specimens collected at the same location.

Canonical correlation analysis determines the correlation between linear combinations of two sets of variables; in this case, combinations of the meristic, biochemical, or environmental variables, and measures the strength of the association between these variable sets. Interset correlations may be a poor indicator of the relationship between the two variable sets if a single variable from each set is highly correlated. Intraset correlations express the contribution of variables to the canonical variates extracted from the same set of variables and thus have a higher interpretative value. Redundancy, or explained variance, acts as a direct expression of the interrelatedness of both variable sets (Podani 1994). A plot of the first axis score from each set of variables (*e.g.*, meristic vs. biochemical) should result in the objects lying close to the diagonal if the canonical correlation is high. Canonical correlation analysis was performed using the SYN-TAX computer program (Podani 1993).

Twenty-three meristic characters (number of scales in, above (LL1 and LL2) and below the lateral line, number of dorsal rays (DR1), anal rays (AR1), dorsal spines, pectoral rays, total left and right POM pores and the number confined to the opercle, number of left and right anterior and posterior infraorbital pores, condition of the POM pore canal, counts of the number of cheek, nape and opercle scales, an estimate of the percent scale coverage of the belly and breast and the number of scales at the nape-parietal juncture) and those enzymes exhibiting the greatest

polymorphisms were used as variables. Rare alleles which occurred in less than 5.0% of only one sample were not included in analyses. Environmental variables included latitude, longitude, the number of frost-free and growing degree-days above 5.6°C, stream order (1 = headwater lake or first order stream, 2 = second order stream, 3 = third order or higher stream, large rivers and lakes between streams or rivers), elevation (metres above sea level), and whether the specimen came from a lake or river. Elevations were determined from those shown on 1:250,000 topographic maps or using contour lines to determine the approximate elevation of the waterbody. Latitude, longitude, and stream order were determined from these same maps. Number of frost-free and growing degree-days were as reported by Phillips and McCulloch (1972). Biochemical results, habitat and the condition of the POM pore canal were entered as binary variables. Thus, specimens homozygous "aa", heterozygous "ab" and homozygous "bb" were entered as 1:0, 1:1 and 0:1. When three alleles were present, the presence of any allele was entered as a "1" while its absence was denoted with a "0".

Canonical correlation analysis was performed with 300 of the specimens used for biochemical analysis. All 146 Wisconsin but only 154 Ontario specimens were utilized in this analysis. Ontario specimens were selected based on the number of available specimens in each collection greater than 26.0 mm SL, biochemical variation and geographic location. Although all Ontario samples were represented by at least some specimens, those samples which exhibited limited or no biochemical variation were not included as frequently as those that exhibited greater variation.

RESULTS

SEPARATION OF *Etheostoma nigrum* FROM *E. olmstedi*

Means of the characters examined for each species are presented in Table 4. Based on the largest "*t*" values between means, four variables were selected for further analyses: the number of dorsal rays, number of left pectoral rays and number of pores in, and the condition of, the infraorbital canal. These characters have been utilized by previous researchers (*e.g.*, Cole 1965). Although different, POM pores were not included as recent work (Chapleau and Cooper 1992) suggests that possible eastern and western forms of *E. nigrum* have different POM pore counts.

Tables 5-7 present the frequency distributions for the mean number of dorsal rays, pectoral rays and infraorbital pores, as well as the condition of the infraorbital canal, for the eight samples of *E. olmstedi* and *E. nigrum*. The additional 17 samples from Lake Ontario-St. Lawrence River tributaries and New York state, which could be either species or represent samples with intergrade specimens, are also given.

A line of separation of between 12 and 13 pectoral rays separates 83.37% of the two species (Table 5).. A line of separation between 12 and 13 dorsal rays separates 82.34% of these same samples (Table 6). A line of separation between seven and eight pores in the infraorbital canal separates 94.10% of the two species (Table 7). If the condition of the infraorbital canal, either interrupted or uninterrupted, is considered, then an almost 100% separation can be made between the two species. Four specimens of the 84 *E. olmstedi* examined possess an interrupted infraorbital canal while none of the 86 *E. nigrum* examined possess a

Table 4: Means of 19 variables (SD in parentheses) enumerated from eight samples each of *Etheostoma olmstedi* and *E. nigrum*. Variables in bold denote those with the greatest differences between species. Results of "*t*" tests and statistical significance are given.

Character	*E. olmstedi*	*E. nigrum*	*t*	P
Dorsal rays	**13.06** (.68)	**11.88** (.66)	-11.43	0.001
Dorsal spines	8.79 (.56)	8.74 (.63)	-1.25	0.214
Pectoral rays	**12.74** (.52)	**11.81** (.47)	-12.14	0.001
Anal rays	8.37 (.66)	8.47 (.70)	.93	0.356
Left branchiostegals	6.00 (0.00)	5.98 (.24)	.45	0.657
Total Infraorbital pores	**8.01** (.63)	**6.43** (.70)	-15.55	0.001
Infraorbital pore condition$_1$	**0.96** (.19)	**0.00** (0.00)	-	-
Total POM pores	**10.07** (.83)	**7.29** (1.52)	-14.99	0.001
POM pores on opercle	**6.69** (.51)	**6.08** (.27)	-9.6	0.001
Lateral line scales	**45.75** (3.23)	**48.09** (3.05)	4.87	0.001
Scales below lateral line	**8.18** (.87)	**7.30** (.93)	-6.34	0.001
Scales above lateral line1	**4.42** (.52)	**4.17** (.51)	-3.06	0.003
Scales above lateral line2	**5.81** (.53)	**5.48** (.57)	-3.96	0.001
Lateral line condition$_2$	**1.0** (0.00)	**1.79** (.83)	-	-
Nape scales	**10.08** (13.27)	**19.10** (20.31)	3.44	0.001
Cheek scales	13.41 (14.94)	11.34 (16.03)	-1.22	0.224
Opercle scales	**18.32** (7.92)	**14.60** (6.60)	-3.33	0.001
Breast scales	**6.82** (13.15)	**42.19** (44.00)	7.13	0.001
Percent of belly scaled	**66.67** (27.98)	**89.07** (23.67)	5.63	0.001

$_1$ Infraorbital pore condition: 1 = complete, 0 = incomplete or interrupted

$_2$ Lateral line condition: 1 = complete, 2 = 3 or fewer unpored scales, 3 = more than 3 unpored scales and\or interrupted

Table 5: Frequency distribution of the number of left pectoral rays from samples of *Etheostoma olmstedi*, *E. nigrum* and unclassified samples from Quebec, Ontario and New York.

Etheostoma olmstedi	11	12	13	14	N	$\bar{x}$	SD
Maryland 1	-	4	5	-	9	12.56	0.53
Maryland 2	-	9	11	-	20	12.55	0.51
Vermont	-	3	12	-	15	12.80	0.41
Massachusetts	-	-	4	2	6	13.33	0.52
New Hampshire 1	-	-	6	-	6	13.00	0.00
New Hampshire 2	-	1	8	1	10	13.00	0.47
Connecticut	-	4	3	-	7	12.43	0.54
New Jersey	-	4	7	-	11	12.63	0.51
Species mean	-	25	56	3	84	12.74	

Etheostoma nigrum	11	12	13	14	N	$\bar{x}$	SD
Iowa 5	1	6	1	-	8	12.00	0.54
Minnesota 3	1	9	1	-	11	12.00	0.45
Minnesota 24	2	4	-	-	6	11.66	0.52
Missouri 6	3	4	-	-	7	11.57	0.54
Wisconsin 20	-	8	1	-	9	12.11	0.33
Wisconsin 28	3	8	-	-	11	11.73	0.47
South Dakota 3	4	19	-	-	23	11.83	0.39
Indiana 2	5	6	-	-	11	11.55	0.52
Species mean	19	64	3	-	86	11.81	

Unclassified samples	11	12	13	14	N	$\bar{x}$	SD
Quebec 1	-	5	8	-	13	12.62	0.51
Quebec 2	-	23	13	-	36	12.36	0.49
Quebec 3	-	11	6	-	17	12.35	0.49
Quebec 4	-	12	1	-	13	12.08	0.27
Quebec 5	-	9	-	-	9	12.00	0.00
New York 1	2	9	-	-	11	11.82	0.41
New York 2	1	12	12	1	26	12.50	0.65
New York 3	-	5	5	-	10	12.50	0.53
New York 4	-	3	5	-	8	12.63	0.52
Ontario 1	-	4	7	-	11	12.64	0.51
Ontario 3	-	4	4	-	8	12.50	0.54
Ontario 7	-	5	1	-	6	12.17	0.41
Ontario 9	4	7	-	-	11	11.64	0.51
Ontario 19	2	8	4	-	14	12.14	0.66
Ontario 21	-	4	1	-	5	12.20	0.45
Ontario 23	7	-	-	-	7	11.00	0.00
Ontario 24	3	7	-	-	10	11.70	0.48

Table 6: Frequency distribution of the number of dorsal rays from samples of *Etheostoma olmstedi*, *E. nigrum* and unclassified samples from Quebec, Ontario and New York.

Etheostoma olmstedi	11	12	13	14	15	N	$\bar{x}$	SD
Maryland 1	-	-	8	1	-	9	13.11	0.33
Maryland 2	-	3	11	6	-	20	13.15	0.67
Vermont	-	3	8	4	-	15	13.07	0.70
Massachusetts	-	-	4	2	-	6	13.33	0.52
New Hampshire 1	-	-	3	3	-	6	13.50	0.55
New Hampshire 2	-	2	4	3	1	10	13.30	0.95
Connecticut	-	3	4	-	-	7	12.57	0.54
New Jersey	-	5	6	-	-	11	12.54	0.52
Species mean	-	16	48	19	1	84	13.06	

Etheostoma nigrum	11	12	13	14	15	N	$\bar{x}$	SD
Iowa 5	1	5	2	-	-	8	12.13	0.64
Minnesota 3	-	10	1	-	-	11	12.09	0.30
Minnesota 24	4	2	-	-	-	6	11.33	0.52
Missouri 6	-	6	1	-	-	7	12.14	0.38
Wisconsin 20	-	4	5	-	-	9	12.56	0.53
Wisconsin 28	7	4	-	-	-	11	11.36	0.51
South Dakota 3	4	14	5	-	-	23	12.04	0.64
Indiana 2	8	3	-	-	-	11	11.27	0.47
Species mean	24	48	14	-	-	86	11.88	

Unclassified samples	11	12	13	14	15	N	$\bar{x}$	SD
Quebec 1	-	4	4	5	-	13	13.08	0.86
Quebec 2	-	4	17	15	-	36	13.31	0.67
Quebec 3	-	-	10	6	1	17	13.47	0.62
Quebec 4	-	3	10	-	-	13	12.77	0.44
Quebec 5	-	5	3	1	-	9	12.56	0.73
New York 1	-	2	3	5	1	11	13.46	0.93
New York 2	-	1	10	13	2	26	13.62	0.70
New York 3	-	-	2	8	-	10	13.80	0.42
New York 4	-	-	2	4	2	8	14.00	0.76
Ontario 1	-	6	5	-	-	11	12.46	0.52
Ontario 3	-	1	1	5	1	8	13.75	0.89
Ontario 7	-	1	5	-	-	6	12.83	0.41
Ontario 9	2	8	1	-	-	11	11.91	0.54
Ontario 19	-	2	7	4	1	14	13.29	0.83
Ontario 21	2	2	1	-	-	5	11.80	0.84
Ontario 23	2	5	-	-	-	7	11.71	0.49
Ontario 24	5	5	-	-	-	10	11.50	0.53

Table 7: Frequency distribution of the number of pores in the left infraorbital canal and percent of sample with an interrupted infraorbital canal in samples of *Etheostoma olmstedi*, *E. nigrum* and unclassified samples from Quebec, Ontario and New York.

Etheostoma olmstedi	4	5	6	7	8	9	10	$\bar{x}$	SD	N	%
Maryland 1	-	-	-	-	7	2	-	8.22	0.44	9	0
Maryland 2	-	-	-	1	15	4	-	8.15	0.49	20	5
Vermont	1	-	-	1	11	2	-	7.80	1.15	15	7
Massachusetts	-	-	-	-	6	-	-	8.00	0.00	6	0
New Hampshire 1	-	-	-	4	2	-	-	7.33	0.52	6	0
New Hampshire 2	-	-	-	-	9	1	-	8.10	0.32	10	20
Connecticut	-	-	-	-	6	1	-	8.14	0.38	7	0
New Jersey	-	-	-	-	10	1	-	8.09	0.30	11	0
Species mean	1	-	-	6	66	11	-	8.01		84	5

Etheostoma nigrum	4	5	6	7	8	9	10	$\bar{x}$	SD	N	%
Iowa 5	1	1	1	4	-	1	-	6.50	1.51	8	100
Minnesota 3	-	-	4	7	-	-	-	6.64	0.50	11	100
Minnesota 24	-	-	2	4	-	-	-	6.67	0.52	6	100
Missouri 6	-	-	3	4	-	-	-	6.57	0.53	7	100
Wisconsin 20	-	-	8	1	-	-	-	6.11	0.33	9	100
Wisconsin 28	-	-	9	1	1	-	-	6.27	0.65	11	100
South Dakota 3	-	-	14	8	1	-	-	6.43	0.59	23	100
Indiana 2	-	-	8	2	1	-	-	6.36	0.67	11	100
Species mean	1	1	49	31	3	1	-	6.43		86	100

Unclassified samples	4	5	6	7	8	9	10	$\bar{x}$	SD	N	%
Quebec 1	-	-	-	7	5	1	-	7.54	0.66	13	85
Quebec 2	-	-	1	5	9	19	2	8.44	0.91	36	44
Quebec 3	-	-	-	1	12	4	-	8.18	0.53	17	18
Quebec 4	-	1	11	1	-	-	-	6.00	0.41	13	100
Quebec 5	-	-	4	4	1	-	-	6.67	0.71	9	100
New York 1	-	1	5	3	2	-	-	6.54	0.93	11	100
New York 2	-	-	1	8	15	2	-	7.69	0.68	26	46
New York 3	-	-	4	5	1	-	-	6.70	0.67	10	100
New York 4	-	-	-	2	5	1	-	7.88	0.64	8	25
Ontario 1	-	-	5	5	1	-	-	6.64	0.67	11	100
Ontario 3	-	-	1	2	4	1	-	7.63	0.92	8	63
Ontario 7	-	-	1	-	2	3	-	8.16	1.17	6	33
Ontario 9	-	-	11	-	-	-	-	6.00	0.00	11	100
Ontario 19	-	-	-	3	10	1	-	7.85	0.53	14	43
Ontario 21	-	-	4	1	-	-	-	6.20	0.45	5	100
Ontario 23	-	-	2	5	-	-	-	6.71	0.49	7	100
Ontario 24	-	-	8	2	-	-	-	6.20	0.42	10	100

complete infraorbital canal. Therefore, typical *E. olmstedi* possess 13 dorsal rays, 13 pectoral rays and 8 pores in an uninterrupted infraorbital canal. *Etheostoma nigrum* possess 12 or fewer dorsal and pectoral rays and an interrupted infraorbital canal, usually with six or seven pores (2 posterior and 4 or 5 anterior).

Several samples, notably Ontario samples 1, 9, 21, 23 and 24 and Quebec samples 4 and 5 are likely *E. nigrum* due to their lower number of dorsal rays, generally lower number of pectoral rays and an always incomplete infraorbital canal system with fewer than 7 pores. Similarly, Ontario samples 3, 7 and 19 and Quebec samples 2 and 3 are *E. olmstedi* based on their greater number of dorsal and pectoral rays and, in most cases, a lesser number of specimens with an interrupted infraorbital canal with more pores than typical *E. nigrum*. The remaining samples are not as easy to classify due to the mixture of characters from both species on some specimens.

Quebec sample 1 possesses a pectoral ray count similar to and a dorsal ray count higher than typical *E. olmstedi*. The infraorbital canal has an intermediate number of pores (7.54) and is interrupted in 85% of the specimens.

New York sample 3 possesses higher dorsal ray counts than typical *E. olmstedi*. This sample also possesses a pectoral ray count much closer to that of typical *E. olmstedi*. However, the sample has an infraorbital pore count similar to the mean for *E. nigrum* and the infraorbital canal is always interrupted.

New York sample 1 possesses higher dorsal ray counts than typical *E. olmstedi*. This sample possesses pectoral ray counts and an infraorbital pore count similar to the mean for *E. nigrum*. However, all specimens in the sample possess an

interrupted infraorbital canal suggesting *E. nigrum*.

New York samples 2 and 4 are *E. olmstedi* based on their high pectoral ray count and a generally uninterrupted infraorbital canal with a mean of almost 8 pores. The dorsal ray counts for these two samples are higher than examined *E. olmstedi*.

Table **8** shows the frequency distribution of the CI for all *E. nigrum* and *E. olmstedi* examined. The mean CI for *E. olmstedi* is 34.76. The mean CI for *E. nigrum* is 30.09. However, a line between 32 and 33 (mean *E. nigrum* CI + mean *E. olmstedi* CI ÷ 2) separates the two species most completely and identifies 98.82% of the specimens correctly. The mean CI for each unclassified sample shows the possibility of their being either *E. nigrum* or *E. olmstedi*. Ontario samples 1, 9, 21, 23 and 24 and Quebec samples 4 and 5 are *E. nigrum* based on their low CI. Conversely, Ontario samples 3, 7 and 19 and Quebec samples 2 and 3 still appear to be *E. olmstedi* based on their high CI. New York samples 2 and 4 are *E. olmstedi* based on their very high CI score. Quebec sample 1 possesses a CI near the mean for *E. olmstedi* but, based on the interrupted infraorbital canal, may be *E. nigrum*. While New York sample 1 possesses a CI which strongly suggests *E. nigrum* as noted above, New York sample 3 has a CI which suggests *E. olmstedi*, which is the opposite of that previously noted.

A discriminant function prepared using the number of dorsal rays (DR), pectoral rays (PECR), infraorbital pores (INFP) and the condition of the infraorbital pore canal (IN/UN) resulted in the following function:

$$\mathbf{Y = .4032383(DR) + .6645918(PECR) + .6326663(INFP) + 6.152177(IN/UN).}$$

Table 8: Character indices for *Etheostoma nigrum*, *E. olmstedi* and unclassified samples from Quebec, Ontario and New York. Characters include the number of dorsal rays, pectoral rays, infraorbital pores and the completeness of the infraorbital canal. The best line of separation between *E. nigrum* and *E. olmstedi* is between 32 and 33.

	Character Index												
	27	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	$\bar{x}$	**N**
E. olmstedi	-	-	-	-	1	-	10	19	33	19	2	34.76	84
E. nigrum	1	5	19	30	23	7	1	-	-	-	-	30.09	86
Quebec 1	-	-	-	-	-	1	8	3	1	-	-	33.31	13
Quebec 2	-	-	-	-	1	3	2	11	7	7	5	34.69	36
Quebec 3	-	-	-	-	-	1	-	6	5	3	2	34.88	17
Quebec 4	-	-	-	4	7	2	-	-	-	-	-	30.85	13
Quebec 5	-	-	-	2	4	2	1	-	-	-	-	31.22	9
New York 1	-	-	-	3	2	2	2	2	-	-	-	31.81	11
New York 2	-	-	-	-	-	3	3	9	6	4	1	34.31	26
New York 3	-	-	-	-	-	2	6	2	-	-	-	33.00	10
New York 4	-	-	-	-	-	-	1	2	1	2	2	35.25	8
Ontario 1	-	-	-	1	3	5	2	-	-	-	-	31.73	11
Ontario 3	-	-	-	-	1	-	1	2	2	2	-	34.25	8
Ontario 7	-	-	-	-	1	-	1	1	3	-	-	33.83	6
Ontario 9	-	1	3	7	-	-	-	-	-	-	-	29.55	11
Ontario 19	-	-	-	2	-	-	1	7	1	3	-	33.86	14
Ontario 21	-	-	2	1	-	2	-	-	-	-	-	30.40	5
Ontario 23	-	1	2	4	-	-	-	-	-	-	-	29.43	7
Ontario 24	-	1	5	3	1	-	-	-	-	-	-	29.40	10

SD for *E. olmstedi* = 1.08
SD for *E. nigrum* = 1.12

This function gives the heaviest weight to the condition of the infraorbital canal. The frequency histogram for this function is shown in Figure 7. Substituting the average values for the characters examined from Tables 2-4, the average values for both species are: *E. olmstedi*, 24.65980491, and *E. nigrum*, 16.71161517. Therefore, unclassified specimens which possess Y values near 24.65 are *E. olmstedi* while those with a Y value near 16.71 are *E. nigrum*. Misclassifications may occur if the computed Y value for an unclassified specimen exceeds the average Y value for that species by more than half the difference between the two species (Fisher 1936). That is, a specimen of *E. nigrum* would be misclassified if its computed Y value exceeded 20.68571004.

The proportion of Y values for the species with the lower value (*E. nigrum*) which lie under the normal curve to the right of the point of misclassification (20.68571004) is equal to the probability of a normal deviate (Hill 1959). Thus the probability of misclassifications can be calculated by:

$$\mathbf{20.68571004 - 16.71161517 \div \sqrt{7.94818974} = 1.409626701}$$

Based on probits (Fisher and Yates 1963), the probability of this normal deviate is 0.921 and the error of misclassification becomes 7.9% for *E. nigrum*. Because of the assumed normal distribution of characters of *E. nigrum* and *E. olmstedi*, a similar error in misclassification is likely for both species. Therefore, the discriminant function should separate 92.1% of the specimens correctly. This is slightly less powerful in separating the two species than the four point CI but provides further predictive information.

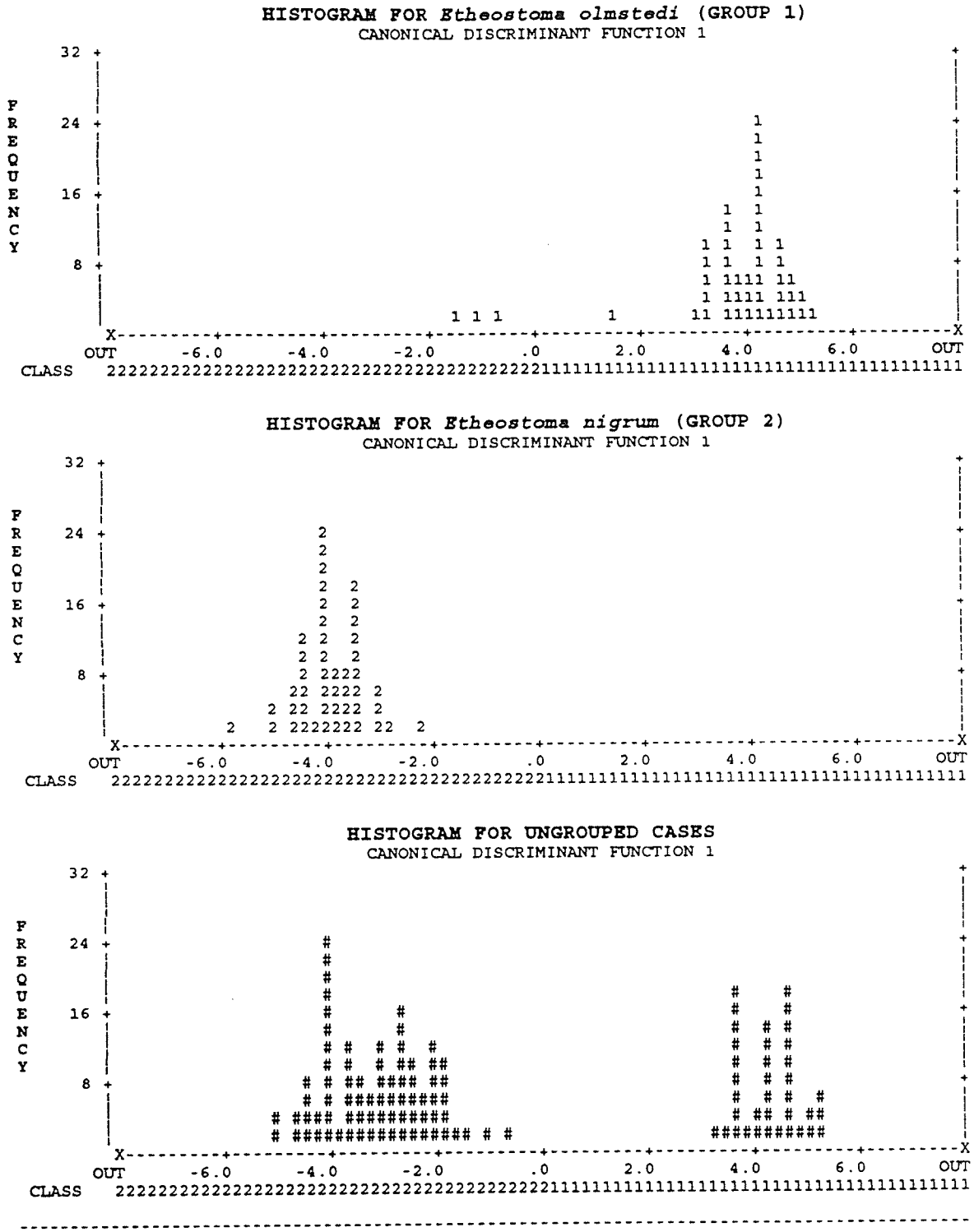


Figure 7: Frequency histograms from discriminant functions for *Etheostoma olmstedi*, *E. nigrum* and unclassified samples from Quebec, Ontario and New York. The discriminant function is based on four variables.

The discriminant function is most useful for those unclassified specimens, especially from New York (samples 1 and 3) and Quebec (sample 1), which may be either species and produced the conflicting results obtained by using the two methods. A sampling of several of the specimens possessing both the highest and lowest scores for these three samples results in the following (function multipliers have been rounded off in text only);

NY-01-1	12	x .403	+	12	x .664	+	6	x .632	+ 0 x 6.15 = 16.609959
NY-01-2	13	x .403	+	12	x .664	+	8	x .632	+ 0 x 6.15 = 18.2785299
NY-03-1	14	x .403	+	12	x .664	+	8	x .632	+ 0 x 6.15 = 18.6817682
NY-03-3	13	x .403	+	13	x .664	+	7	x .632	+ 0 x 6.15 = 18.3104554
QU-01-1	13	x .403	+	13	x .664	+	7	x .632	+ 0 x 6.15 = 18.3104554
QU-01-2	14	x .403	+	13	x .664	+	8	x .632	+ 1 x 6.15 = 25.498537

The Y values for specimens from New York samples 1 and 3 never approach the 20 mark, because none of the specimens from these two samples possesses a complete infraorbital canal. Therefore all specimens in both of these collections remain tentatively identified as *E. nigrum*. Quebec sample 1 possesses specimens with both a complete and incomplete infraorbital canal. However, only two specimens from this sample, both with a complete infraorbital canal, possess a Y value greater than 20 which suggests *E. olmstedi*.

A second discriminant analysis, prepared using only dorsal rays, pectoral rays and the number of infraorbital pores resulted in the function:

$$\mathbf{Y = .6879416(DR) + .9168853(PECR) + 1.092938(INFP).}$$

This function gives the heaviest weight to the number of pores in the infraorbital canal but the weight is considerably less than that previously given to the condition of the infraorbital canal. This function, based on average values for *E. nigrum* and *E. olmstedi* from Tables **2-4**, produces Y values of 29.4200694 for *E. olmstedi* and 26.02875294 for *E. nigrum*. Using this function, 82.2% of the specimens should be correctly classified. The range difference between species is less than half of that of the first function and specimens may be misclassified within a very narrow range (3.39131646). The frequency histogram for this function is shown in Figure **8**.

Examination of the same specimens reviewed above shows that (function multipliers rounded off in text only);

NY-01-1	12 x .687	+ 12 x .916	+	6 x 1.09 = 25.8155508
NY-01-2	13 x .687	+ 12 x .916	+	8 x 1.09 = 28.6893684
NY-03-1	14 x .687	+ 12 x .916	+	8 x 1.09 = 29.37731
NY-03-3	13 x .687	+ 13 x .916	+	7 x 1.09 = 28.5133157
QU-01-1	13 x .687	+ 13 x .916	+	7 x 1.09 = 28.5133157
QU-01-2	14 x .687	+ 13 x .916	+	8 x 1.09 = 30.2941953

The calculated Y values now show that several of the specimens classified as *E. nigrum* with the first function are now classified as *E. olmstedi*. Therefore, the two functions sometimes produce conflicting results which must be interpreted with care.

Specimens possessing a strongly pigmented suborbital bar are mainly those already identified as *E. olmstedi* (7 of 8 Atlantic Slope samples as well as Quebec

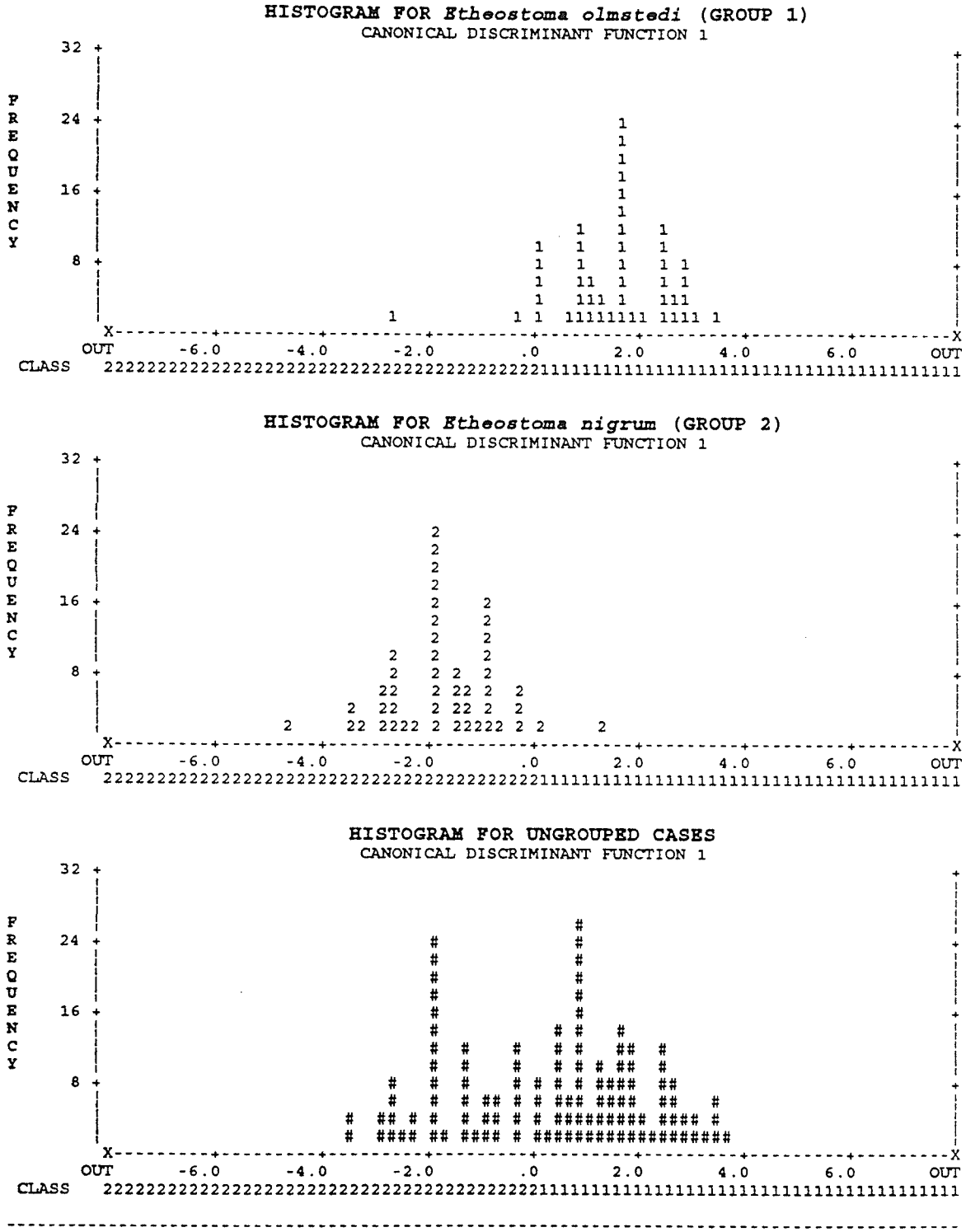


Figure 8: Frequency histograms from discriminant functions for *Etheostoma olmstedi*, *E. nigrum* and unclassified samples from Quebec, Ontario and New York. The discriminant function is based on three variables.

samples 1 and 3, New York samples 2, 3 and 4, and Ontario samples 3 and 7). All specimens in these samples possess the suborbital bar except for the Quebec samples, where roughly half of the specimens possess this trait.

Table **9** summarizes squamation and scaling characters enumerated on both the known and unclassified samples. Although most unclassified samples identified above as *E. nigrum* possess fewer scales on the nape, cheek, breast or opercle than those identified as *E. olmstedi*, this is not always true. Many *E. nigrum* are more heavily scaled than the baseline samples of *E. olmstedi* and squamation values for *E. olmstedi* are variable.

Based on these analyses, I removed the following samples known or suspected to contain specimens of *E. olmstedi* (QU-01, QU-02, QU-03, NY-01, NY-02, NY-03, NY-04, ON-03, ON-07, ON-19). Therefore, my samples were reduced from 258 to 240 with a total of 2111 specimens. These samples and specimens are used for all further analyses.

MERISTICS AND MORPHOMETRICS OF *Etheostoma nigrum*

The total number of *E. nigrum* examined from each state or province is given in Table **10**. The locations of those samples suspected of possessing *E. olmstedi* and removed prior to analyses are identified in Figure **9**. Summary statistics for variables examined are included in Appendix **6** and listed by sample (state and location). These give modes, means and ranges for discontinuous data, and means, standard deviations, and, in some cases, ranges for continuous variables. Males and females did not exhibit statistically significant differences in meristic characters. Anal spines

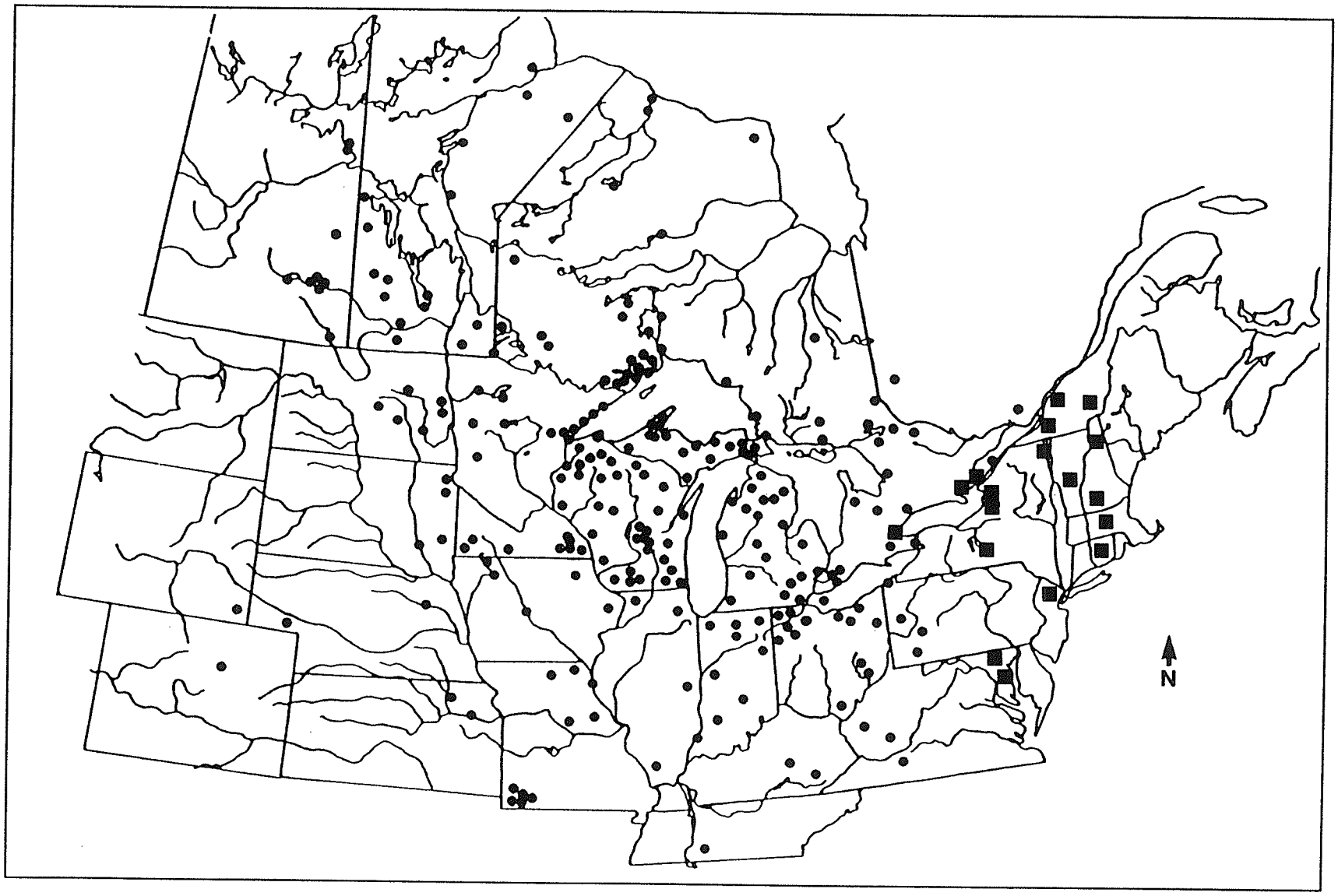
Table 9: Mean number of scales on the nape, cheek, breast and opercle, and number of scales in and below the lateral line for *Etheostoma nigrum*, *E. olmstedi* and unclassified samples from Ontario, Quebec and New York. Unclassified samples marked with an asterisk (*) are considered *E. olmstedi*.

Etheostoma olmstedi	Nape	Cheek	Breast	Opercle	Latline	BLL
Maryland 1	7.44	4.44	0.00	18.00	44.44	6.78
Maryland 2	6.30	3.55	0.00	22.00	42.25	8.10
Vermont	34.40	41.40	29.67	27.13	49.13	8.67
Massachusetts	10.67	16.00	13.50	15.67	46.00	8.33
New Hampshire 1	0.17	5.67	0.00	7.33	47.83	8.17
New Hampshire 2	1.5	7.80	1.70	7.10	47.50	8.30
Connecticut	2.14	6.14	0.43	12.43	47.14	8.71
New Jersey	3.91	18.91	0.82	21.46	44.82	8.27
Etheostoma nigrum	Nape	Cheek	Breast	Opercle	Latline	BLL
Iowa 5	47.75	43.75	108.25	26.50	48.50	6.75
Minnesota 3	19.46	8.18	54.46	13.64	50.91	6.91
Minnesota 24	11.33	2.00	28.50	11.17	45.67	6.17
Missouri 6	2.86	0.71	0.57	10.14	47.14	7.86
Wisconsin 20	50.33	27.67	109.67	21.33	51.89	7.89
Wisconsin 28	3.64	0.00	0.91	13.64	46.00	7.64
South Dakota 3	20.17	11.70	43.09	12.44	48.30	7.09
Indiana 2	0.18	0.00	0.00	12.18	45.46	8.00
Unclassified samples	Nape	Cheek	Breast	Opercle	Latline	BLL
Quebec 1*	21.69	21.00	25.69	15.69	46.46	7.38
Quebec 2*	42.61	41.00	72.72	25.92	50.64	8.00
Quebec 3*	9.53	7.94	1.94	16.35	45.41	7.41
Quebec 4	0.07	0.00	0.54	11.92	48.08	6.31
Quebec 5	0.44	0.00	0.00	13.11	42.33	8.00
New York 1*	15.09	7.46	10.73	9.46	51.64	7.64
New York 2*	31.62	34.35	66.85	23.54	50.73	8.00
New York 3*	15.50	31.40	69.10	19.90	48.20	7.00
New York 4*	28.13	23.13	66.38	13.38	51.38	8.13
Ontario 1	11.00	5.91	15.64	12.91	48.27	7.09
Ontario 3*	25.75	19.75	53.13	14.75	50.50	6.38
Ontario 7*	19.83	24.17	63.67	19.00	49.83	6.67
Ontario 9	0.55	0.00	0.00	9.09	42.73	6.73
Ontario 19*	12.71	24.71	24.50	22.43	46.64	8.21
Ontario 21	37.40	7.80	53.60	18.60	46.60	7.20
Ontario 23	6.29	0.00	0.29	13.86	45.29	7.29
Ontario 24	4.30	0.00	0.00	17.50	44.90	7.10

Table 10: Number of specimens and samples of *Etheostoma nigrum* examined from each state and province. Percent contribution of specimens from each state and province to the total sample is also given.

	Location	N	Percent of sample	Number of samples
United States				
	Colorado	33	1.6	1
	Illinois	31	1.5	5
	Indiana	94	4.5	9
	Iowa	39	1.8	5
	Kansas	9	0.4	2
	Kentucky	19	0.9	2
	Michigan	353	16.7	37
	Minnesota	229	10.8	27
	Missouri	42	2.0	9
	Nebraska	6	0.3	2
	North Dakota	48	2.3	6
	Ohio	92	4.4	14
	Pennsylvannia	31	1.5	4
	South Dakota	40	1.9	4
	Tennessee	10	0.5	1
	Virginia	19	0.9	1
	Wisconsin	407	19.3	36
	West Virginia	4	0.2	1
	Wyoming	7	0.3	1
Canada				
	Quebec	22	1.0	2
	Ontario	400	18.9	45
	Manitoba	145	6.9	17
	Saskatchewan	31	1.5	9
	TOTAL	**2111**	**100%**	**240**

Figure 9: Location of samples of *Etheostoma nigrum* examined for this study. Samples of *Etheostoma nigrum* are identified with a circle (●). Samples of known or suspected *Etheostoma olmstedi* are identified with a square (■).


N

are not included in these appendices as less than 1.0% of the specimens examined possessed two anal spines and no geographic trend was evident. The presence of a pigmented suborbital bar and the presence of a double bifurcation of rays on the dorsal fin are also not given as these characters were very sporadic in appearance. The geographic distribution of these two characters is reported below.

Means of variables of known or suspected samples of *E. olmstedi* are largely reported above although means for all samples appear in the appendices. The location and character states of samples of known or suspected *E. olmstedi*, or *E. olmstedi* x *E. nigrum* hybrids, are therefore excluded from maps showing variation in *E. nigrum*.

Principal Component Analysis

The inclusion or exclusion of the number of scales on the nape, cheek, breast, opercle and at the nape-parietal juncture has a significant effect on the PCA scores. When squamation characters are included in the analyses of all specimens, the first factor is interpreted as a "squamation factor" as these five variables, and to a lesser extent, the scales above the lateral line (LL1) and the estimated percent of the belly scaled, are the most heavily weighted (Table **11**). All of these variables are signed positive. The second factor gives high weight to the dorsal rays (DR1 and DR2), anal rays (AR1 and AR2), the number of left and right POM pores, the number of left and right branchiostegal rays and the number of pectoral rays. Left and right POM pore variables are negative while other variables are positive. Dorsal spines receive their highest weight on the second factor but contribute little overall to the total variation explained. None of these variables were given higher weights on any

Table 11: Principal component scores from the first two factors for 2111 *Etheostoma nigrum* performed with squamation characters both included and excluded (heavily weighted variables in bold).

	With Scaling		Without Scaling	
VARIABLE	**PC 1**	**PC 2**	**PC 1**	**PC 2**
LL	-.01256	.37473	**.36598**	.00083
LL1	**.63464**	-.08678	-.23907	.38539
LL2	.37261	-.13790	-.22632	.29077
BLL	.28052	-.12813	-.19560	.27695
DS	.03363	.15339	.14181	-.01686
DR1	-.06232	**.66411**	**.65693**	.21638
DR2	-.05889	**.66905**	**.66146**	.21907
AR1	-.06706	**.73840**	**.73385**	.16409
AR2	-.06349	**.73897**	**.73398**	.15915
LBR	.15855	**.51387**	.45382	.36028
RBR	.18036	**.50534**	.44151	.36515
LPOP	.29510	**-.54778**	**-.60889**	.34763
LOPOP	.14012	-.01319	-.05413	.20335
RPOP	.28073	**-.54263**	**-.60116**	.32992
ROPOP	.12268	.00796	-.03048	.16905
LPOS	.37652	-.06062	-.16239	**.73168**
LANT	-.05039	-.02798	-.00672	-.36507
RPOS	.39414	-.05314	-.15835	**.74402**
RANT	-.04550	.00478	.02396	-.35627
PECR	.03501	**.31560**	**.29434**	.11573
NC	**.86511**	.11194		
NCT	**.73637**	.03284		
OC	**.82443**	-.02243		
CC	**.78544**	.16763		
BC	**.83228**	.17041		
BELLYP	**.50881**	.22979		
% of Variation	**18.2**	**13.6**	**17.6**	**11.9**
Cumulative % of Variation		**31.8**		**29.5**

of the other eight factors. The first two factors explain 31.8% of the total variation. Eight factors with eigenvalues greater than 1.0 explain 68.6% of the total variation.

When estimated percentage of belly area covered by scales, and the number of nape, cheek, opercle, breast and nape-parietal juncture scales are removed from the analysis, the factor scores change mainly in that most variables which weighed heaviest on the second factor when squamation was included, are now weighted most heavily on the first factor (Table **11**). The exceptions include the number of scales above the lateral line (LL1), the number of scales in the lateral line and the number of left and right branchiostegal rays which remain on the second factor. These characters are considered most important for detecting and identifying variation in *E. nigrum* other than the obvious differences in squamation. The first factor weights the number of dorsal rays (DR1 and DR2), anal rays (AR1 and AR2), left and right POM pores, scales in the lateral line and the pectoral rays. Pectoral rays receive their highest weighting on this factor even though the weight is considerably less than other variables. Left and right POM pores are negatively signed while the seven other variables are signed positive.

The second factor gives high weight only to the number of pores in the left and right posterior infraorbital canal system. These variables possess the same positive sign. The seven factors with eigenvalues greater than 1.0 explain 67.7% of the sample variation. The first two factors explain 29.5% of this variation.

Analysis of all 240 samples using PCA on means shows the heavy influence of squamation characters. Left and right POM pores, the number of branchiostegals and

dorsal and anal ray counts are additional important variables. The number of scales above the lateral line (both LL1 and LL2) weigh heavily with squamation characters on the first factor. The second factor heavily weights many of the same variables as when the individual specimens were used. However, the lateral line weighs heaviest on the second factor. All characters are positive except for the POM pores. The biplot of samples and the component scores for characters with squamation included is shown in Figure **10**. Samples with scaled specimens and many Ohio Basin samples are distinct. Component scores for the first two factors are given in Table **12**. A greater percentage of variation, 41.7% on the first two factors, is explained using sample means as compared to when using the individual specimens (Table **11**, **12**).

Those characters that were most heavily weighted on the second factor when squamation was included were identified on the first factor when squamation was excluded. Scaling characters associated with the lateral line were, however, not identified on the first factor (Table **12**). The second factor gives heaviest weighting to the number of scales above the lateral line (LL1), the number of posterior infraorbital pores and, to a lesser extent, right branchiostegal rays. All variables on both factors are positive except for the POM pores. Ohio Basin darters remain distinct on the second biplot but samples with scaled specimens are no longer distinct (Fig. **11**). The first two factors explain 39.16% of the variation within the samples. This is much higher than that explained using the individual specimens for PCA.

Analysis of morphometric data shows that the first factor is dominated by predorsal length. The first factor explains 38% of the variation. The second factor

Figure 10: Biplot of the first two PC axes of 240 samples of *Etheostoma nigrum* when squamation is included. Variable abbreviations are as in methods. Sample abbreviations are as given in Appendix **2**. Samples with scaled specimens are identified with an asterisk (*). The scaling factor for variables is 9.33.

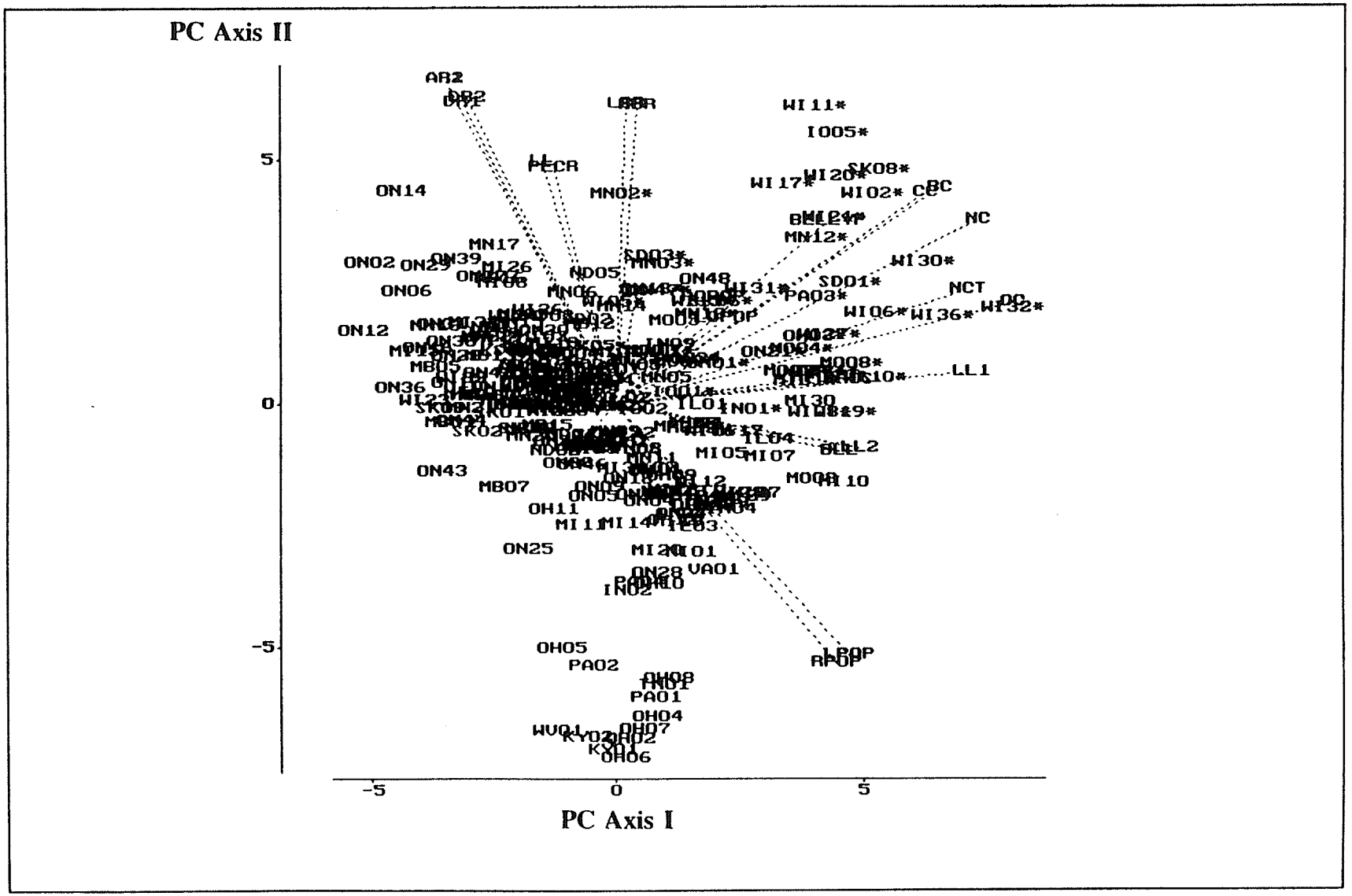
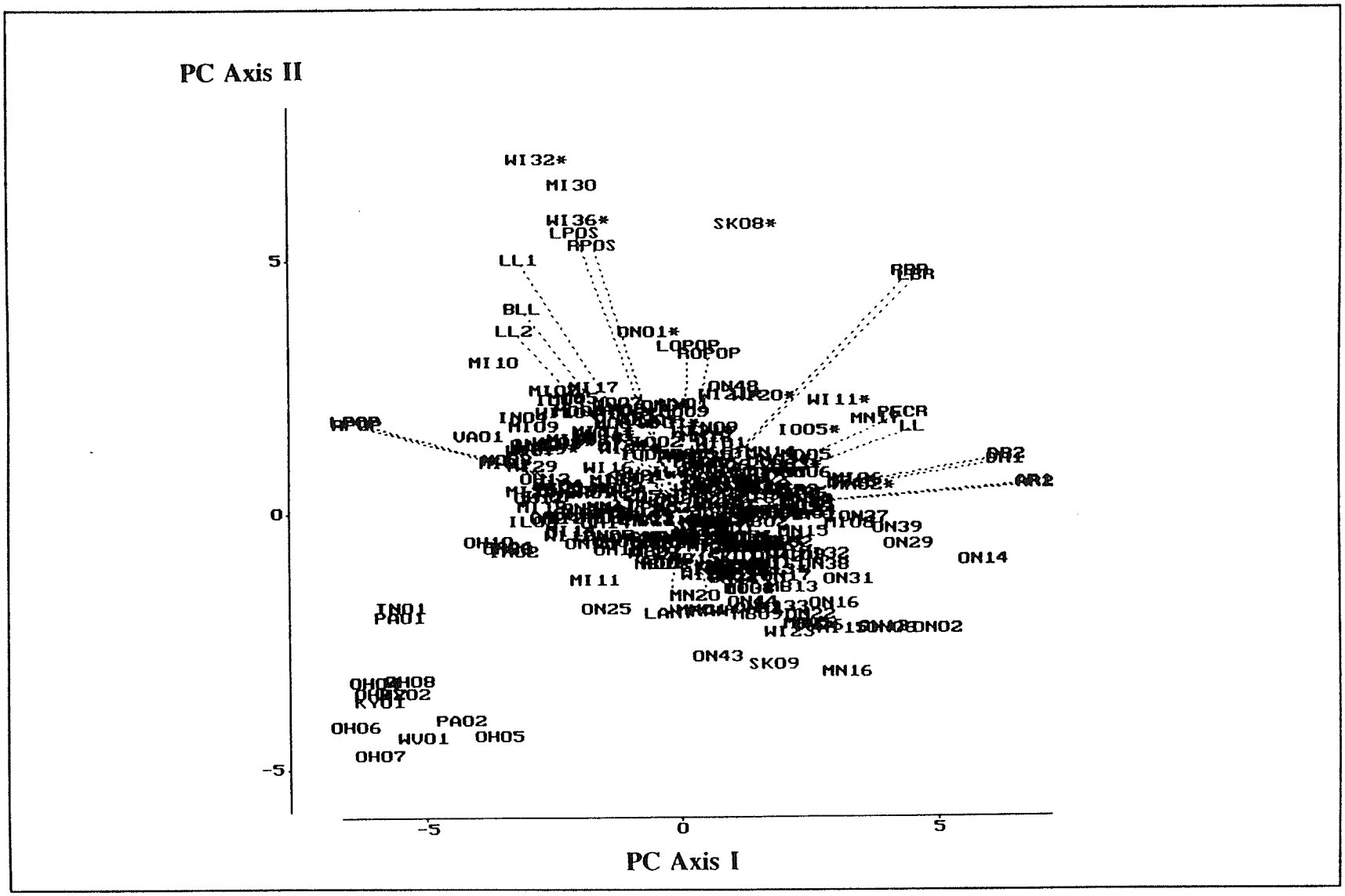

PC Axis II
PC Axis I
5
0
-5
-5
0
5
AR2
LABR
WI11*
I005*
LL
PECR
SK08*
WI20*
WI17*
WI02*
CBC
ON14
MN02*
NC
MN12*
MN17
SD03*
WI30*
ON02
ON39
MN03*
ND05
ON48
SDD1*
WI31*
PA03*
NCT
ON06
MN06
WI06*
WI36*
WI32*
ON12
MO08*
OP
MO04*
MO08*
LL1
ON36
MN05
ND01*
TL01
IN01*
MI30
WI08*9*
SK02
TL04
MI05
MI07
BLL2
ON43
MB07
MO08
MI10
OH11
MI14
TL03
ON25
MI20
TL01
ON28
VA01
IN02
OH05
PA02
RPOP
OH08
IN01
PA01
OH04
OH07
WV01
KY02
OH02
KY01
OH06

Table 12: Principal component scores from the first two factors for 240 samples of *Etheostoma nigrum* performed with squamation characters both included and excluded (heavily weighted variables in bold).

	With Scaling		Without Scaling	
VARIABLE	**PC 1**	**PC 2**	**PC 1**	**PC 2**
LL	-.1628	**.5308**	.5293	.1916
LL1	**.7735**	.0700	-.3664	**.5793**
LL2	**.5301**	-.0991	-.3771	.4172
BLL	.4839	-.1049	-.3575	.4674
DS	.0476	.2461	.1823	.0923
DR1	-.3388	**.6612**	**.7385**	.1168
DR2	-.3275	**.6714**	**.7414**	.1278
AR1	-.3738	**.7136**	**.8043**	.0641
AR2	-.3769	**.7146**	**.8073**	.0606
LBR	.0232	**.6595**	**.5401**	.5388
RBR	.0478	**.6557**	.5238	**.5498**
LPOP	.5058	**-.5527**	**-.7367**	.2093
LOPOP	.2392	.1878	.0200	.3800
RPOP	.4776	**-.5676**	**-.7357**	.2041
ROPOP	.2114	.2331	.0675	.3628
LPOS	.5034	.0526	-.2348	**.6426**
LANT	-.0805	-.0940	-.0267	-.2304
RPOS	.4355	.0539	-.1971	**.6127**
RANT	-.1063	.0164	.0789	-.2259
PECR	-.1394	**.5190**	**.5069**	.2240
NC	**.7866**	.4053		
NCT	**.7673**	.2506		
OC	**.8635**	.2211		
CC	**.6709**	.4639		
BC	**.7032**	.4749		
BELLYP	**.4575**	.4003		
% of Variation	**22.39**	**19.30**	**25.62**	**13.54**
Cumulative % of Variation		**41.70**		**39.16**

Figure 11: Biplot of the first two PC axes of 240 samples of *Etheostoma nigrum* when squamation is excluded. Variable abbreviations are as in methods. Sample abbreviations are as given in Appendix **2**. Samples with scaled specimens are identified with an asterisk (*). The scaling factor for variables is 8.60.


PC Axis II
PC Axis I
WI32*
MI30
WI36*
LPDS
RPDS
SKO8*
LL1
BLL
LL2
DNO1*
LOPOP
ROPOP
MI10
MI17
WI11*
MN17
PECR
LL
I005*
VA01
WI16
ON48
ON39
ON29
ON14
ON31
MI11
MN20
ON25
IN01
PA01
ON43
SK09
MN16
OH08
OH02
KY01
OH06
PA02
WV01
OH05
OH07
5
0
-5
-5
0
5

gives heaviest weighting to the standard length. The second factor explains 27.97% of the data variation (Table **13**). Pectoral and snout length explain little of the morphometric variation in *E. nigrum*. All factors possess eigenvalues of less than 1.0.

Meristic Data

Means, modes and standard deviations of the following characters are based on examination of 2111 *E. nigrum*. Summary statistics by major drainages and for the entire sample are presented in Table **14**.

Although the total number of samples from each drainage is vastly different, and the entire Atlantic Slope is represented by a single sample, some trends are present in Table **14**. The highest lateral line scale counts are from Lake Superior and the Hudson Bay drainages of Ontario and Manitoba-Saskatchewan. Lateral lines are largely incomplete in these areas. The Ontario Hudson Bay drainages have the lowest percentage of specimens with nape, cheek and breast squamation. These specimens also possess the least number of scales above the lateral line. Atlantic Slope and Ohio Basin samples have only a few specimens with nape, cheek or breast squamation. Atlantic Slope and Ohio Basin samples also exhibit the lowest estimated percentage area of the belly scaled. These two areas possess the greatest number of specimens with 11 or fewer dorsal rays. Samples from the Hudson Bay drainages have the lowest number of POM pores. Lake Superior, Mississippi and Missouri river samples have an intermediate number of POM pores whereas those samples to the east possess higher counts. Although these differences are rather obvious, due to the size of the

Table 13: Principal component scores from the first two factors for 2111 specimens of *Etheostoma nigrum* performed with morphometric variables (heavily weighted variables in bold).

VARIABLE	PC 1	PC 2
Snout length	.6243	.3221
Pectoral length	.4316	**.6544**
Predorsal length	**.8406**	-.1357
Standard length	-.4901	**.7539**
% of Variation	**38.07**	**27.97**
Cumulative % of Variation		**66.04**

Table 14: Means of meristic variables (SD in parentheses) and number of *Etheostoma nigrum* within major drainage divisions (Lake Ontario includes waters tributary to the St. Lawrence River; Atlantic Slope represented by one sample). See methods for variable abbreviations.

Variable	Lake Ontario	Lake Erie	Atlantic Slope
LL	45.72 (3.13)	45.79 (2.89)	43.47 (2.54)
LL1	3.48 (0.85)	3.95 (0.43)	4.05 (0.40)
LL2	5.01 (0.56)	5.44 (0.51)	5.53 (0.51)
CC	1.27 (5.09)	3.73 (9.04)	1.21 (1.08)
OC	11.32 (6.15)	14.03 (5.48)	15.11 (2.42)
NC	4.95 (11.61)	10.72 (15.70)	1.68 (2.87)
NCT	1.41 (2.02)	2.15 (2.07)	0.00 (0.00)
BC	5.48 (16.11)	13.93 (30.90)	0.42 (1.22)
BELLYP	67.07 (35.09)	81.25 (24.37)	58.42 (15.00)
BLL	6.90 (0.90)	7.28 (0.73)	7.58 (0.77)
DS	8.83 (0.62)	8.93 (0.59)	8.47 (0.70)
DR1	12.13 (0.70)	11.43 (0.80)	11.11 (0.46)
AR1	8.20 (0.74)	8.06 (0.62)	7.47 (0.51)
LBR	5.99 (0.11)	5.98 (0.18)	6.00 (0.00)
RBR	5.99 (0.11)	5.98 (0.20)	6.00 (0.00)
LPOP	8.41 (1.66)	8.53 (1.28)	9.05 (0.23)
LOPOP	6.15 (0.45)	6.01 (0.18)	6.05 (0.23)
RPOP	8.29 (0.63)	8.53 (1.26)	9.11 (0.32)
ROPOP	6.15 (0.47)	6.01 (0.08)	6.11 (0.32)
IN/UN	2.45 (1.79)	1.84 (1.28)	2.00 (0.00)
LPOS	2.23 (0.48)	2.35 (0.53)	2.95 (0.23)
LANT	4.00 (0.27)	4.06 (0.28)	4.00 (0.00)
RPOS	2.41 (0.90)	2.37 (0.53)	2.95 (0.23)
RANT	3.94 (0.51)	4.11 (0.35)	4.05 (0.23)
PECR	11.88 (0.62)	11.76 (0.54)	11.74 (0.45)
SNOUT	2.95 (0.61)	2.59 (0.93)	2.53 (0.50)
LLSTAT	1.82 (0.83)	1.90 (0.71)	1.21 (0.54)
N	**82**	**176**	**19**

Table 14: Continued.

Variable	Lake Superior	Lake Michigan	Lake Huron
LL	49.27 (2.97)	47.84 (3.07)	45.92 (2.65)
LL1	3.62 (0.69)	4.06 (0.53)	3.60 (0.72)
LL2	5.18 (0.56)	5.44 (0.56)	5.12 (0.50)
CC	2.40 (7.57)	7.36 (11.63)	0.54 (2.20)
OC	8.04 (4.23)	14.16 (5.97)	11.56 (5.00)
NC	2.54 (8.44)	16.68 (20.38)	3.41 (8.43)
NCT	0.48 (1.28)	2.51 (2.16)	1.40 (1.91)
BC	7.03 (21.25)	27.47 (36.80)	2.99 (11.31)
BELLYP	79.42 (25.67)	92.82 (13.60)	73.76 (26.75)
BLL	7.03 (0.78)	7.34 (0.75)	7.12 (0.84)
DS	8.93 (0.58)	8.85 (0.54)	8.84 (0.62)
DR1	11.95 (0.69)	11.67 (0.73)	11.37 (0.68)
AR1	8.42 (0.63)	8.17 (0.59)	8.04 (0.69)
LBR	6.00 (0.11)	5.99 (0.11)	6.02 (0.13)
RBR	6.00 (0.13)	6.01 (0.16)	6.00 (0.00)
LPOP	7.03 (1.43)	8.12 (1.59)	8.61 (1.47)
LOPOP	6.07 (0.31)	6.07 (0.26)	6.07 (0.32)
RPOP	7.04 (1.47)	8.21 (1.56)	8.42 (1.50)
ROPOP	6.08 (0.32)	6.07 (0.28)	6.03 (0.29)
IN/UN	2.76 (1.55)	1.88 (1.42)	1.65 (1.28)
LPOS	2.22 (0.47)	2.52 (0.77)	2.45 (0.80)
LANT	4.02 (0.24)	4.03 (0.41)	4.03 (0.46)
RPOS	2.22 (0.45)	2.49 (0.70)	2.48 (0.82)
RANT	4.02 (0.24)	4.03 (0.42)	4.02 (0.45)
PECR	11.98 (0.44)	11.84 (0.48)	11.82 (0.52)
SNOUT	3.23 (0.89)	3.09 (1.21)	2.84 (0.86)
LLSTAT	2.13 (0.79)	1.41 (0.61)	1.74 (0.79)
N	**395**	**316**	**121**

Table 14: Continued.

Variable	Mississippi	Ohio River	Missouri River
LL	47.78 (3.20)	45.52 (2.98)	46.80 (2.40)
LL1	4.11 (0.55)	3.86 (0.46)	3.64 (0.66)
LL2	5.50 (0.54)	5.48 (0.53)	5.29 (0.52)
CC	8.56 (14.85)	0.13 (0.79)	3.42 (11.14)
OC	14.30 (6.76)	9.94 (4.62)	11.19 (5.58)
NC	15.67 (19.98)	0.93 (4.15)	5.64 (12.33)
NCT	2.66 (2.28)	0.34 (1.13)	1.06 (1.69)
BC	29.79 (40.96)	0.34 (2.62)	13.09 (27.85)
BELLYP	94.35 (9.51)	57.92 (31.42)	86.71 (17.64)
BLL	7.36 (0.83)	7.33 (0.71)	7.19 (0.78)
DS	8.88 (0.56)	8.67 (0.56)	8.56 (0.54)
DR1	11.86 (0.71)	11.17 (0.72)	12.07 (0.73)
AR1	8.35 (0.69)	7.61 (0.68)	8.67 (0.59)
LBR	6.00 (0.13)	5.51 (0.50)	6.00 (0.00)
RBR	6.01 (0.16)	5.52 (0.50)	5.99 (0.09)
LPOP	7.09 (1.41)	9.04 (0.72)	7.07 (1.23)
LOPOP	6.05 (0.24)	6.05 (0.24)	6.06 (0.24)
RPOP	7.12 (1.42)	9.08 (0.73)	7.01 (1.16)
ROPOP	6.06 (0.24)	6.09 (0.29)	6.04 (0.23)
IN/UN	2.72 (1.64)	1.80 (1.04)	2.53 (1.55)
LPOS	2.38 (0.73)	2.11 (0.49)	2.44 (0.53)
LANT	3.98 (0.37)	4.03 (0.25)	4.01 (0.34)
RPOS	2.40 (0.74)	2.10 (0.39)	2.38 (0.50)
RANT	4.00 (0.40)	3.99 (0.09)	4.01 (0.23)
PECR	11.76 (0.50)	11.41 (0.54)	11.74 (0.51)
SNOUT	3.06 (1.06)	2.42 (0.91)	3.24 (0.89)
LLSTAT	1.65 (0.73)	2.02 (0.70)	1.46 (0.60)
N	**406**	**132**	**129**

Table 14: Continued.

Variable	Hudson Bay-Ontario	Hudson Bay-Manitoba	Entire Sample
LL	49.54 (3.34)	48.59 (3.46)	47.62 (3.33)
LL1	2.86 (0.62)	3.53 (0.57)	3.79 (0.66)
LL2	4.68 (0.47)	5.03 (0.47)	5.29 (0.57)
CC	0.00 (0.00)	2.52 (8.52)	4.15 (10.36)
OC	5.29 (2.69)	9.51 (4.93)	11.46 (6.07)
NC	0.05 (0.40)	3.80 (10.90)	8.19 (15.68)
NCT	0.11 (0.59)	0.64 (1.53)	1.46 (2.05)
BC	0.14 (0.67)	9.99 (24.27)	14.85 (30.72)
BELLYP	74.20 (23.89)	80.88 (25.30)	82.61 (24.31)
BLL	6.63 (0.73)	6.89 (0.74)	7.17 (0.80)
DS	8.98 (0.40)	8.88 (0.61)	8.85 (0.58)
DR1	12.16 (0.68)	11.79 (0.63)	11.76 (0.75)
AR1	8.61 (0.59)	8.41 (0.60)	8.27 (0.69)
LBR	6.00 (0.00)	6.01 (0.08)	5.97 (0.21)
RBR	6.00 (0.00)	6.00 (0.08)	5.97 (0.22)
LPOP	6.54 (1.22)	6.70 (1.20)	7.56 (1.57)
LOPOP	5.98 (0.23)	6.07 (0.32)	6.06 (0.28)
RPOP	6.79 (1.33)	6.76 (1.15)	7.58 (1.56)
ROPOP	6.07 (0.32)	6.10 (0.35)	6.07 (0.29)
IN/UN	2.82 (1.08)	2.99 (1.48)	2.42 (1.54)
LPOS	1.98 (0.36)	2.24 (0.43)	2.33 (0.62)
LANT	3.91 (0.29)	4.03 (0.25)	4.01 (0.33)
RPOS	2.00 (0.43)	2.23 (0.42)	2.33 (0.62)
RANT	4.02 (0.30)	4.02 (0.27)	4.02 (0.34)
PECR	11.98 (0.36)	12.03 (0.46)	11.84 (0.51)
SNOUT	2.77 (0.71)	2.92 (0.94)	2.98 (1.00)
LLSTAT	2.38 (0.80)	2.12 (0.72)	1.82 (0.78)
N	**56**	**278**	**2111**

drainages, individual samples and tertiary watersheds ought to be examined in greater detail for statistical differences.

Opercle Squamation

The modal number of scales on the opercle is ten ($\bar{x}$ = 11.480; SD = 6.052). Opercle squamation ranges from absent (11 specimens) to 36 scales (one specimen). A frequency distribution histogram (Fig. **12**) shows that 91.1% of the specimens possess 20 or fewer scales. Based on the results of correlation analysis using the 152 one-degree latitude and longitude grids, sample means and the individual specimen matrix, there is a highly significant negative correlation between the number of opercle scales and increasing latitude ($P < 0.001$). The correlation is strongest using the one-degree grids ($r = -0.4206$) and weakest with the individual specimens ($r = -0.2836$). Except for a higher number of opercle scales in some samples from the south, the number of scales on the opercle, due to its scattered distribution and variation within the examined range, does not vary in a geographically informative manner which could provide information on the biogeographic history of *E. nigrum*.

Excluding the eleven specimens which do not possess opercle scales, four main patterns of opercle squamation are evident on specimens of *E. nigrum* (Fig. **13**). These patterns appear as progressive steps in squamation from light ("A") to heavy ("C") although most specimens within samples exhibit only one or two patterns. There is no evidence to suggest that specimens progress through patterns "A" and "B" to reach pattern "C" and little evidence of clinal variation. One pattern ("D"), is a variation of "A" but exhibits scattered scales instead of clustered scales. No

Figure 12: Frequency histogram of the number of scales on the opercle enumerated on 2111 specimens of *Etheostoma nigrum*.

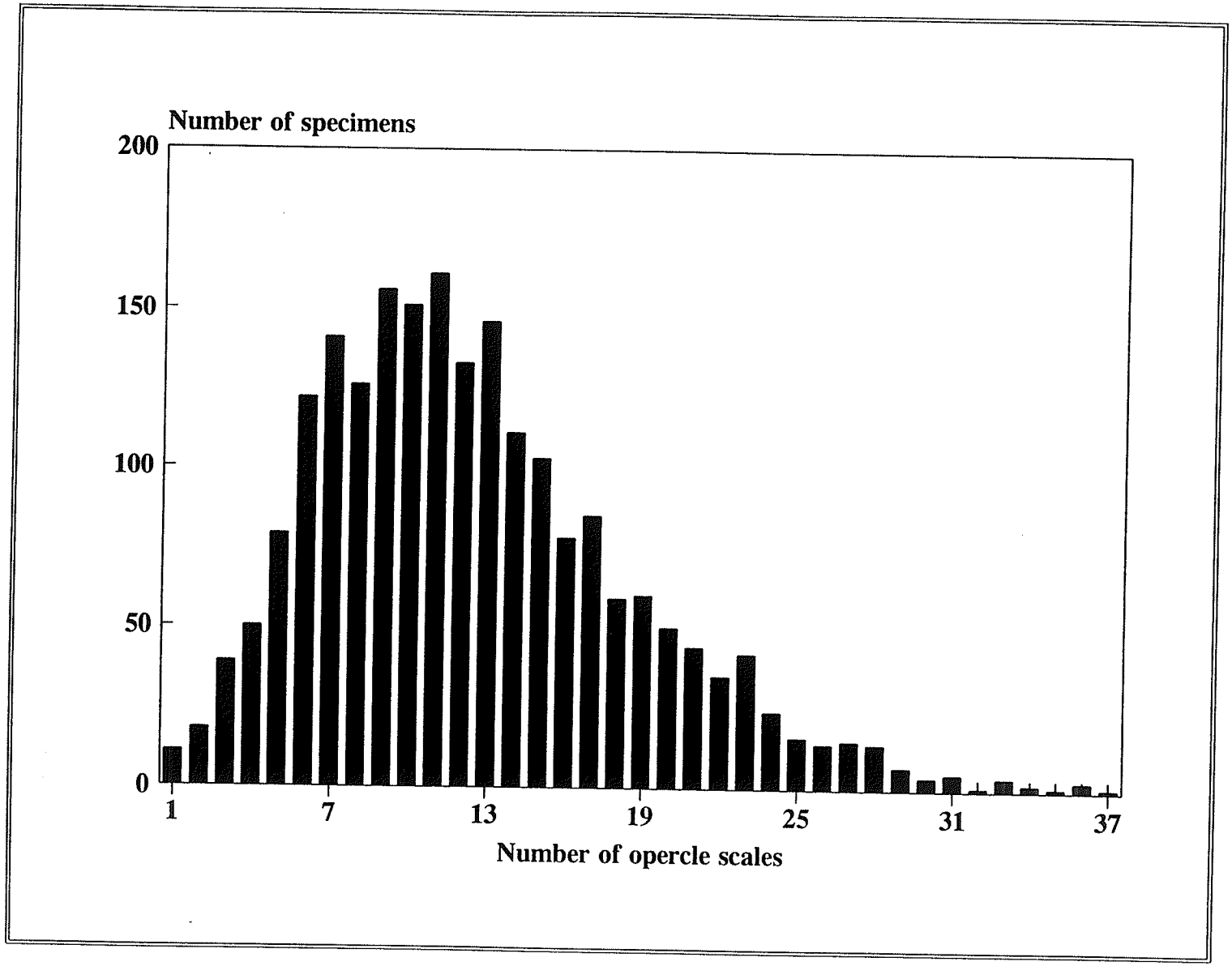
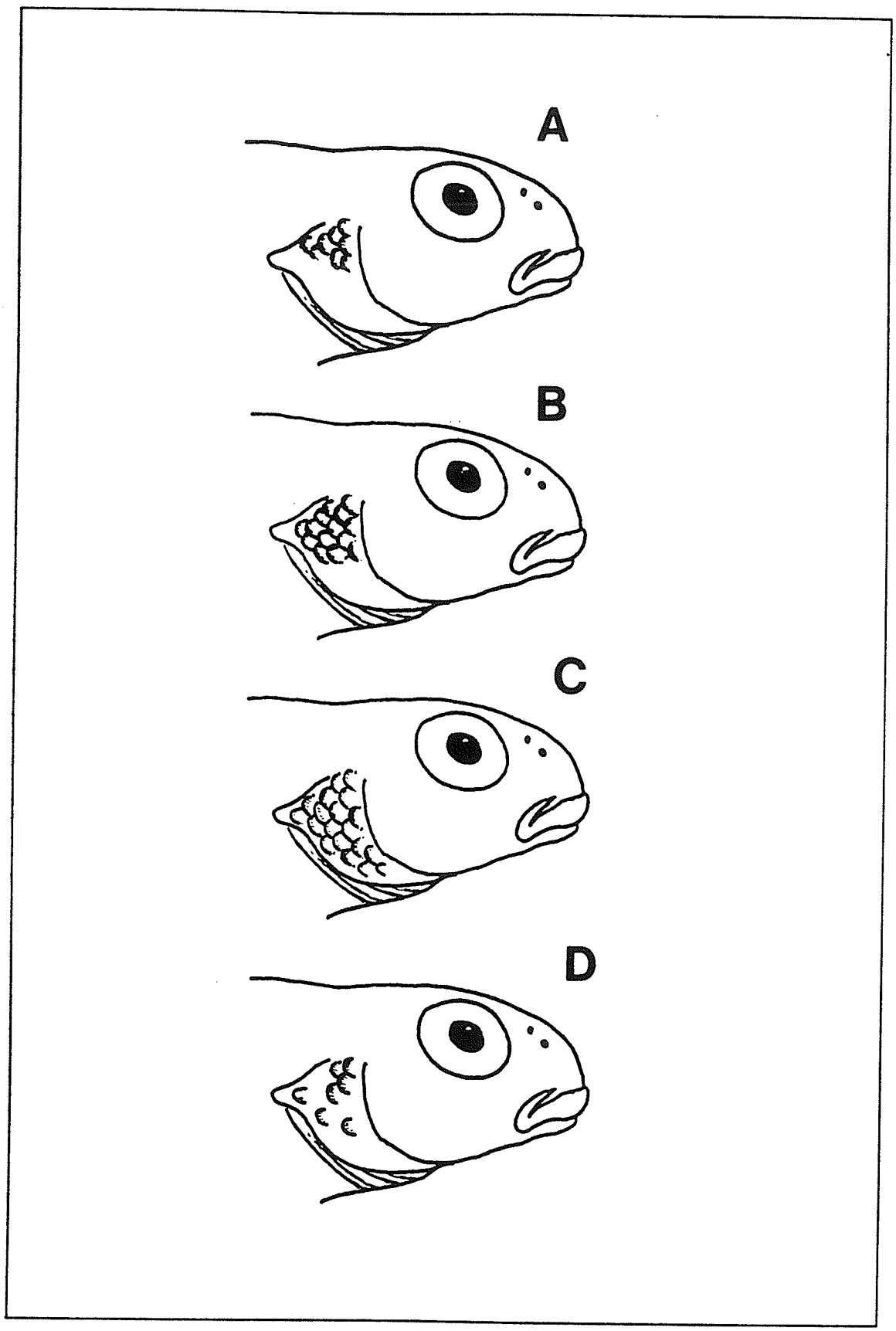

Number of specimens
200
150
100
50
0
1
7
13
19
25
31
37
Number of opercle scales

Figure 13: Patterns of opercle squamation observed on specimens of *Etheostoma nigrum*. The most heavily scaled specimens exhibit patterns "B" and "C". Specimens classified as unscaled typically exhibit patterns "A", "B" or "D".

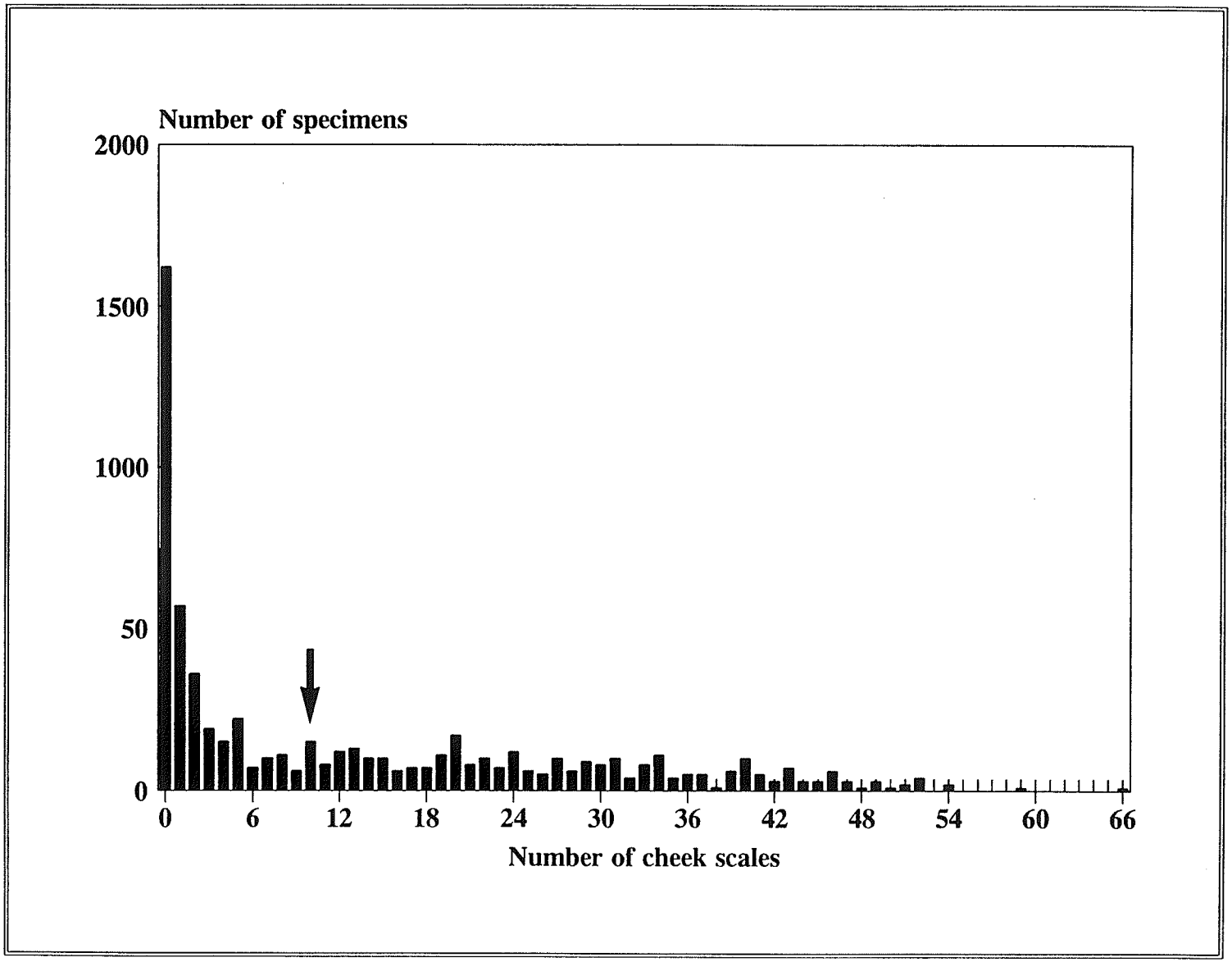

A
B
C
D

specimens exhibited opercle squamation only on the lower portion of the opercle. Specimens with the most heavily scaled nape, cheek or breast regions possess opercle patterns "B" or "C" only. Those specimens which are not as heavily scaled possess patterns "A" through "D" although full scaling of the opercle ("C") is rare on these specimens. Specimens classed as unscaled possess patterns "A" through "D" although the "C" pattern is found on less than 3.0% of these. Many specimens exhibit variation in the size of opercle scales although no geographic pattern emerges based on scale size alone.

Cheek Squamation

The modal number of cheek scales is zero ($\bar{x}$ = 4.147; SD = 10.357) with a range of from zero to 66 scales (one specimen). Cheek scaling is absent in 76.8% (1622 specimens) of the sample. The frequency distribution histogram shows a peak in the number of specimens with 20 cheek scales (Fig. **14**). This peak was used as a starting point from which the sample standard deviation was subtracted to create a grouping of 306 specimens, representing 13.8% of the sample, possessing ten or more cheek scales (arrow in Fig. **14**). A total of 183 specimens, or 9.4% of the entire sample, possess 1-10 scales. The correlation between cheek squamation and longitude using the individual specimen matrix results in a significant positive correlation ($r = 0.0670$; $P < 0.01$). The sample matrix and one-degree grids produce non-significant correlations. There are no significant correlations with latitude. When other squamation variables are considered, the distribution of samples where specimens average ten or more cheek scales seems to vary in a geographically interpretable

Figure 14: Frequency histogram of the number of scales on the cheek enumerated on 2111 specimens of *Etheostoma nigrum*. The arrow at ten scales indicates one sample standard deviation subtracted from the peak at the number of specimens possessing 20 cheek scales.


Number of specimens
2000
1500
1000
50
0
0
6
12
18
24
30
36
42
48
54
60
66
Number of cheek scales

manner.

Cheek squamation exhibits seven patterns with four of these being particularly predominant among heavily scaled and partially scaled darters. Most scaled darters exhibit patterns "C" or "D" (Fig. **15**). Specimens closest to, or meeting, the definition of scaled exhibit patterns "C", "D" or "E". Partially scaled darters usually exhibit patterns "C", "D" or "E", with lesser numbers exhibiting one of the other patterns. Those darters with only lightly scaled or naked napes or breasts exhibit the least frequently seen patterns ("A", "B", "F" and "G") of cheek squamation.

While pattern "F" can be viewed as an enlargement of pattern "B" and pattern "G" as an enlargement of pattern "A" and "B", very little intergrading, such as that seen in opercle squamation, was seen between these patterns. The failure to note intergrading patterns suggests that the patterns are discrete. When those specimens exhibiting patterns "C", "D" or "E" are considered with other squamation variables, geographic trends occur which may be useful for explaining variation in *E. nigrum*.

Breast Squamation

Breast scaling ranged from absent (1428 specimens) to 156 scales (one specimen). The modal number of breast scales is zero ($\bar{x}$ = 14.846; SD = 30.719) and represents 67.6% of the sample. There is a minor peak in the frequency distribution at the number of specimens possessing 77 breast scales (Fig. **16**). I included all specimens within one lower sample standard deviation of this peak to create a grouping of 15.0% of the sample, representing 320 specimens, which possess 47 or more breast scales (arrow in Fig. **16**). Analyzed in this way, and when other

Figure 15: Patterns of cheek squamation observed on specimens of *Etheostoma nigrum*. The most heavily scaled specimens exhibit patterns "C", "D" or "E". Lightly scaled specimens or those with naked napes or breasts exhibit the least frequently seen patterns ("A", "B", "F" and "G").

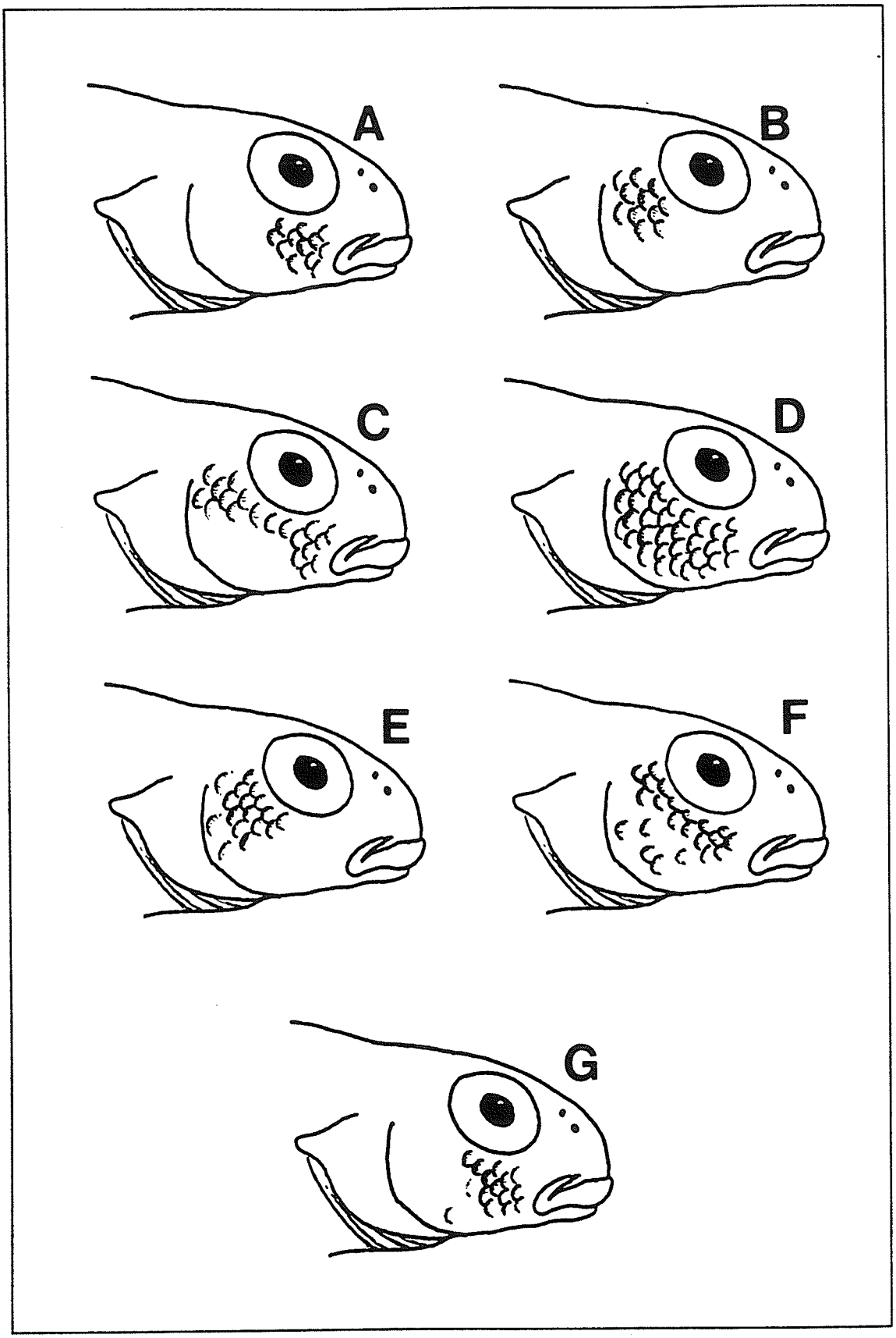
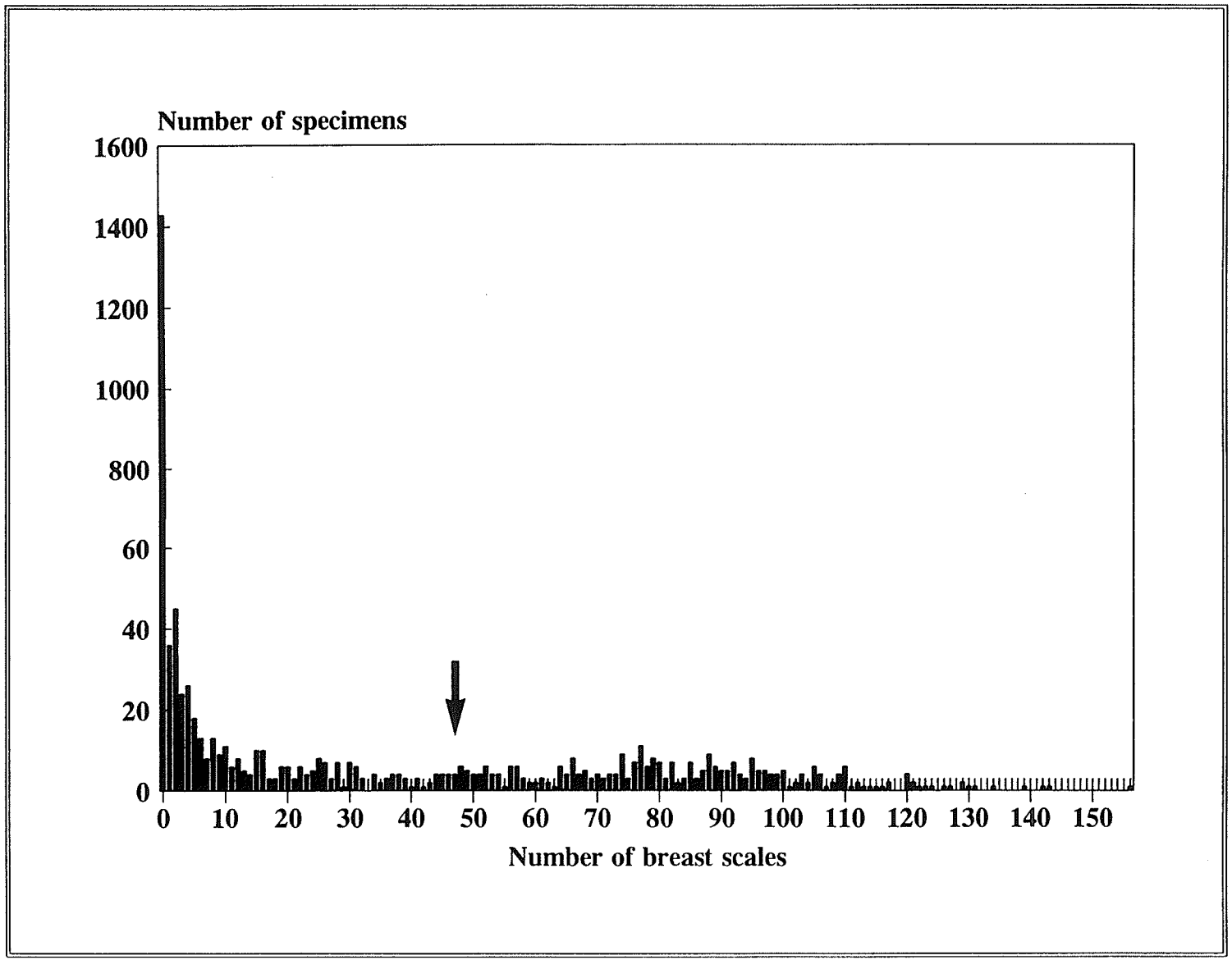

A
B
C
D
E
F
G

Figure 16: Frequency histogram of the number of breast scales enumerated on 2111 specimens of *Etheostoma nigrum*. The arrow at 47 scales indicates one sample standard deviation subtracted from the peak at the number of specimens possessing 77 breast scales.


Number of specimens
1600
1400
1200
1000
800
60
40
20
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Number of breast scales

squamation variables are considered, breast squamation varies in a geographically interpretable manner (Fig. **17a,b**). Breast squamation exhibits a highly significant positive correlation with longitude ($r = 0.0969$; $P < 0.001$) and a significant negative correlation with latitude ($r = -0.0588$; $P < 0.01$) only with the individual specimen matrix. Sample means and the one-degree grids do not produce significant correlations with either latitude or longitude.

Breast squamation can be classified into one of eight patterns (Fig. **18**) although some of these patterns are rather similar. The most heavily scaled darters typically possess patterns "E", "F" or "H". Lighter-scaled specimens possess the lesser-scaled patterns with "D" and "E" being most common. The lightest-scaled specimens typically exhibit only patterns "B" or "C". A pattern (not shown) similar to a combination of "A" and "B" and pattern "A" itself were the least frequently observed and were seen only on heavily scaled specimens. Many specimens of partially scaled darters possess imbedded or cycloid type scales on some portion of, or on the entire breast. Cycloid or imbedded scales were observed near the centre of the breast ("E" or "G") and/or near the posterior margin of the breast ("C") on partially scaled specimens. Cycloid or imbedded scales were never seen on heavily scaled specimens.

The patterns depicted in Figure **18** do not show the variation in scale size that was observed on some specimens. These patterns are discrete and, with the exception of pattern "F", few specimens were observed which would not fall into one of these patterns. Pattern "F" exhibited variation only in that some specimens exhibited a

Figure 17a: Samples of *Etheostoma nigrum* in which some specimens possess 47 or more breast scales. A square (■) equals 47 or more breast scales. A circle (●) equals fewer than 47 breast scales.

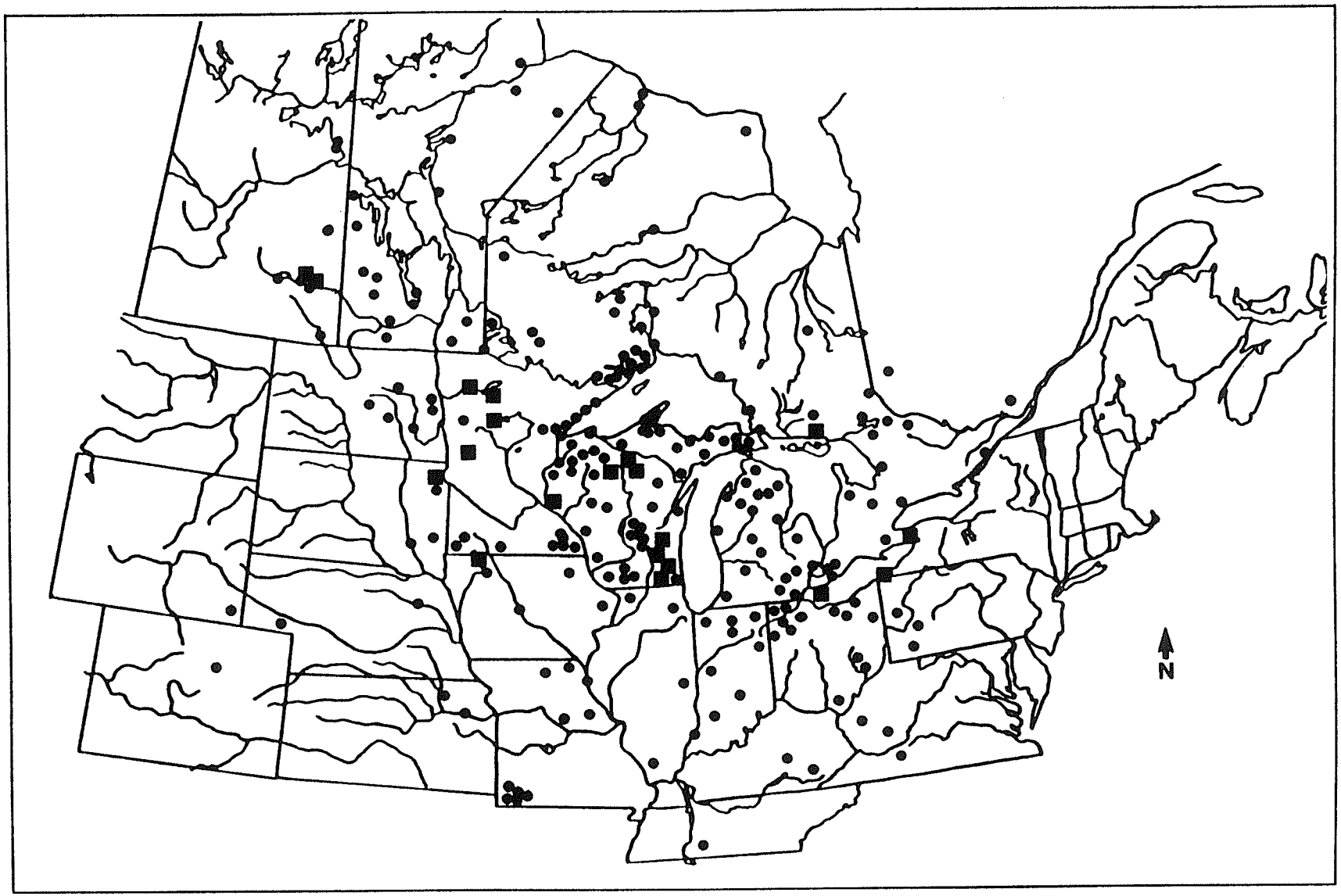

N

Figure 17b: Samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario in which some specimens possess 47 or more breast scales. A square (■) equals 47 or more breast scales. A circle (●) equals fewer than 47 breast scales.

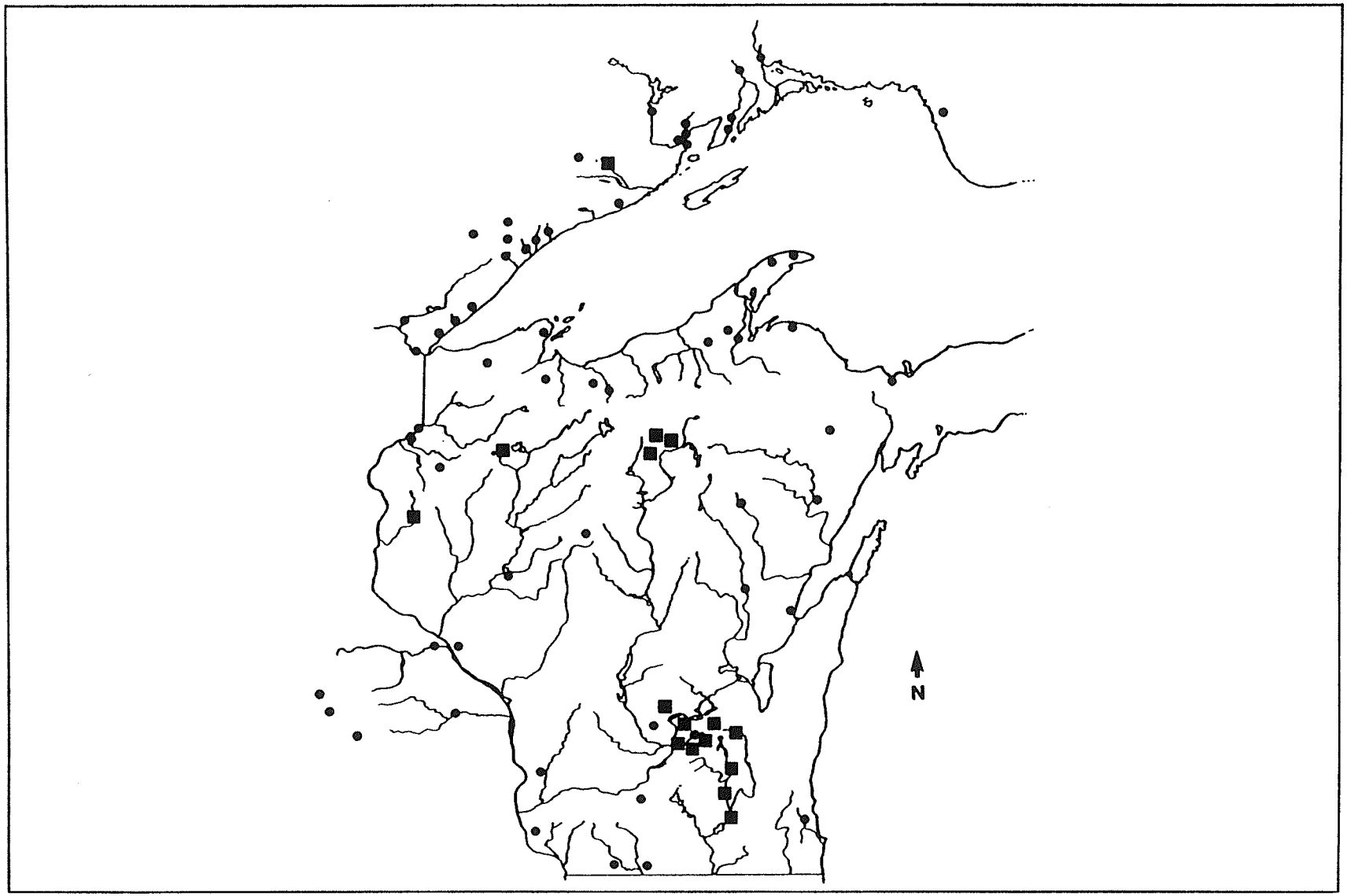
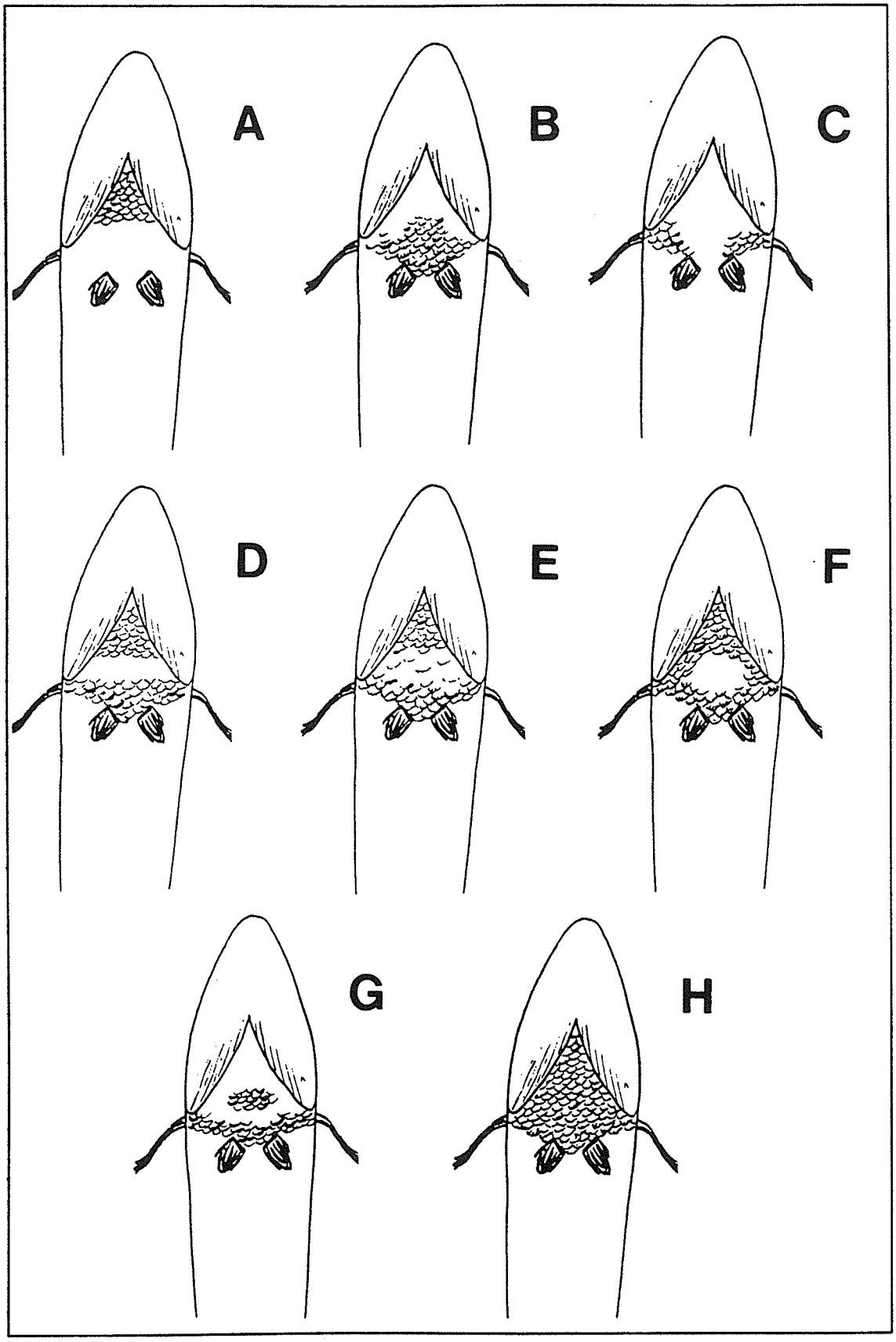

N

Figure 18: Patterns of breast squamation observed on specimens of *Etheostoma nigrum*. The most heavily scaled specimens exhibit patterns "E", "F" and "H". Lighter scaled specimens typically possess patterns "D" or "E". Very lightly scaled specimens exhibit patterns "B" and "C". Pattern "A" was infrequently observed on heavily scaled specimens.


A
B
C
D
E
F
G
H

slightly smaller or larger central unscaled area.

Nape Squamation

The number of nape scales ranges from zero to 62 with a mode of zero ($\bar{x}$ = 8.188; SD = 15.678). A majority of the sample (64.2%) representing 1355 specimens, possess a naked nape. A total of 237 specimens possess a 100% scaled nape. A small peak in the frequency histogram at the number of specimens possessing 45 scales (Fig. **19**) was used as a starting point to count back one sample standard deviation to create a grouping of 305 specimens representing 14.5% of the sample which possessed 30 or more nape scales (arrow in Fig. **19**) (Fig. **20a,b**). An additional peak is found at 35 nape scales but this second peak is included within one SD if the starting point is 45 nape scales. A total of 451 specimens possessing fewer than 30 but more than one nape scale represents 21.6% of the sample. Nape squamation varies in a geographically interpretable manner. Correlation of nape squamation with latitude and longitude generally does not produce significant results. Highly significant negative correlations ($P < 0.001$) with latitude are produced only when the means of samples ($r = -0.1966$) or the individual specimen matrix ($r = -0.1462$) are used.

Most partial nape scaling is confined to the lateral margins or posterior portions of the nape. However, some specimens exhibiting prominent anterior nape scaling, are found among samples from northern Minnesota with lesser numbers in Wisconsin, northwestern Ontario, Saskatchewan and Michigan. In the sample from Sandstone Lake, Ontario, used for biochemical analysis, 93.3% of specimens possess

Figure 19: Frequency histogram of the number of nape scales enumerated on 2111 specimens of *Etheostoma nigrum*. The arrow at 30 scales indicates one sample standard deviation subtracted from the peak at the number of specimens possessing 45 nape scales.

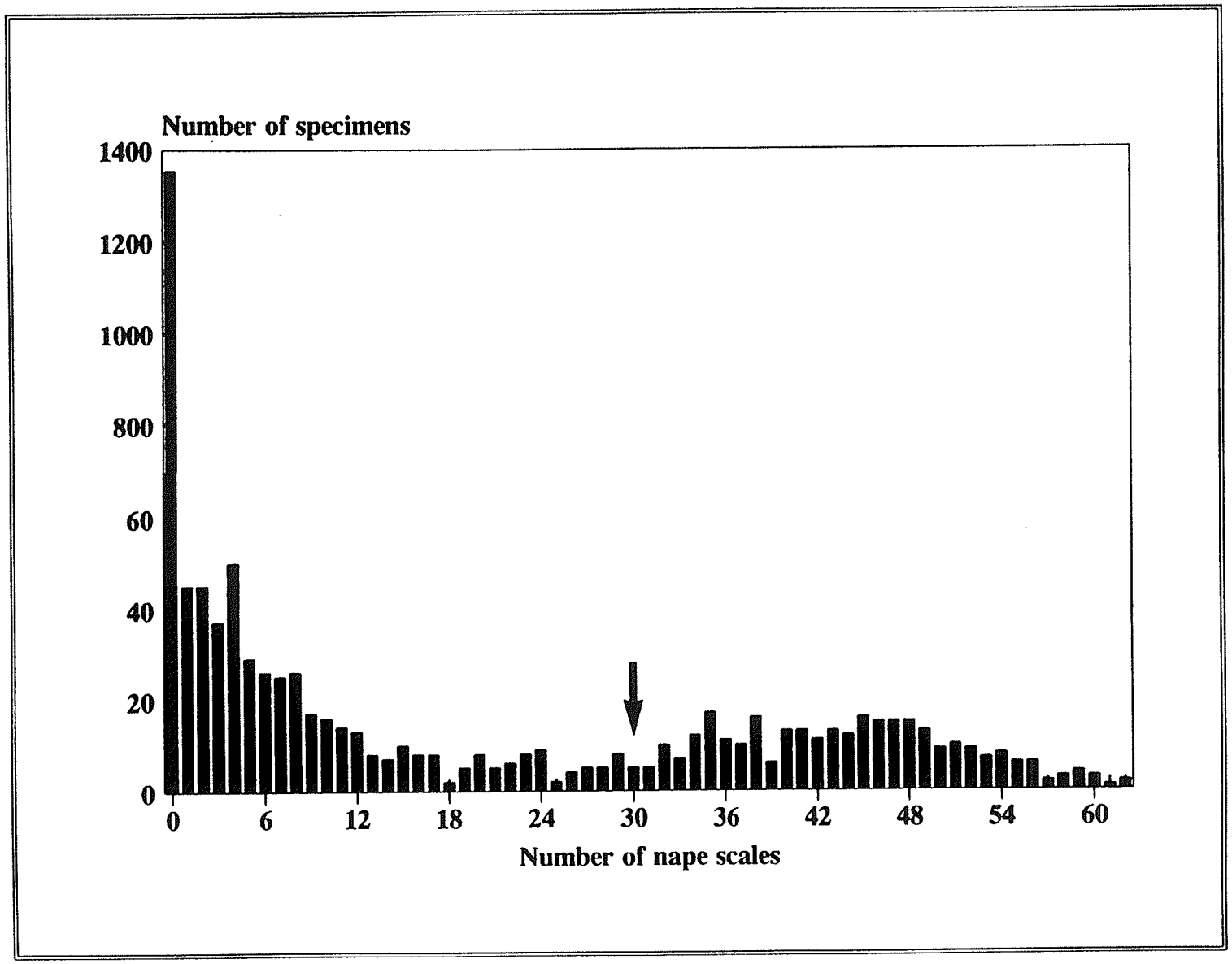

Number of specimens
1400
1200
1000
800
60
40
20
0
0
6
12
18
24
30
36
42
48
54
60
Number of nape scales

Figure 20a: Samples of *Etheostoma nigrum* where the mean number of nape scales is 30 or more. A square (■) equals 30 or more nape scales. A circle (●) equals fewer than 30 nape scales.

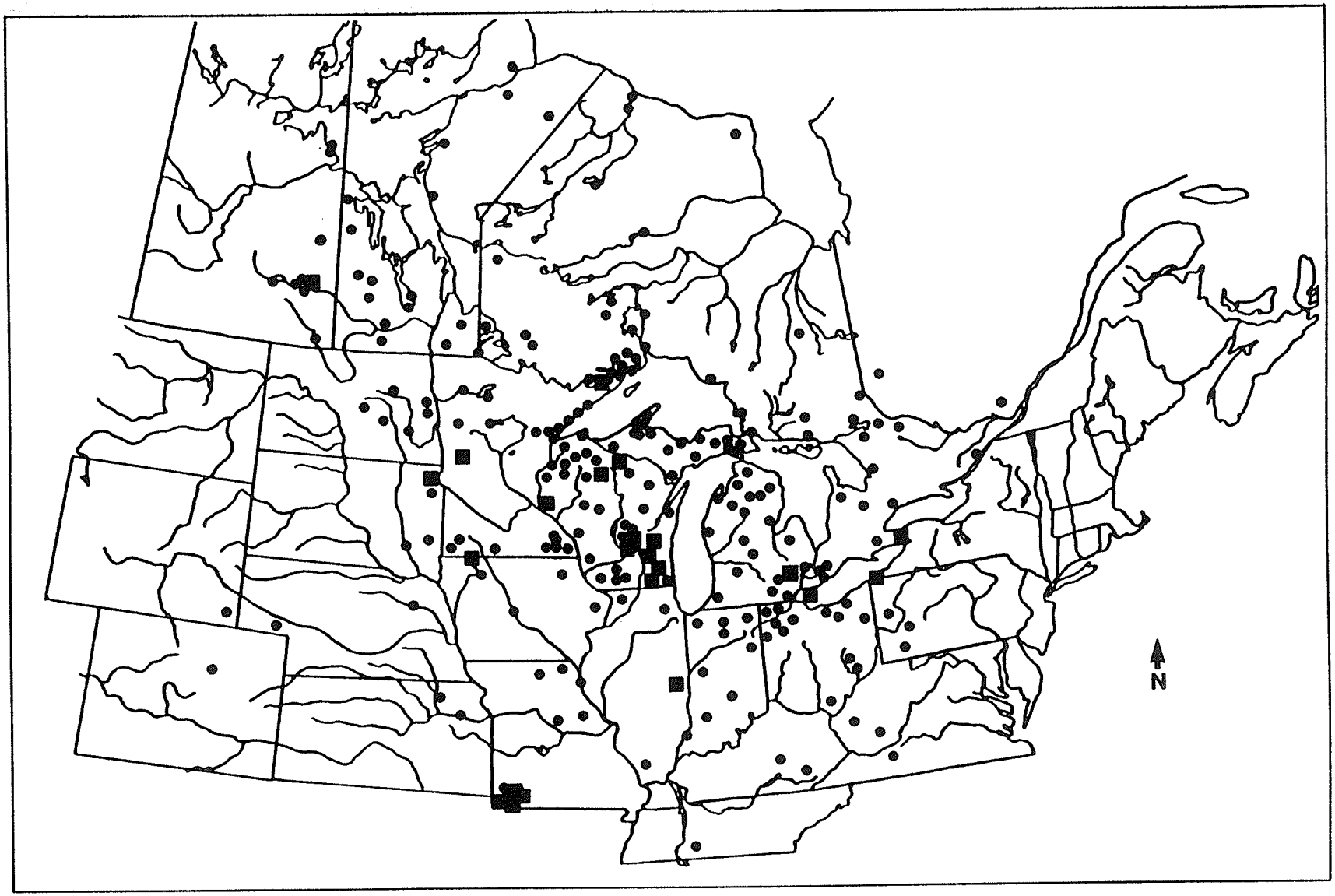
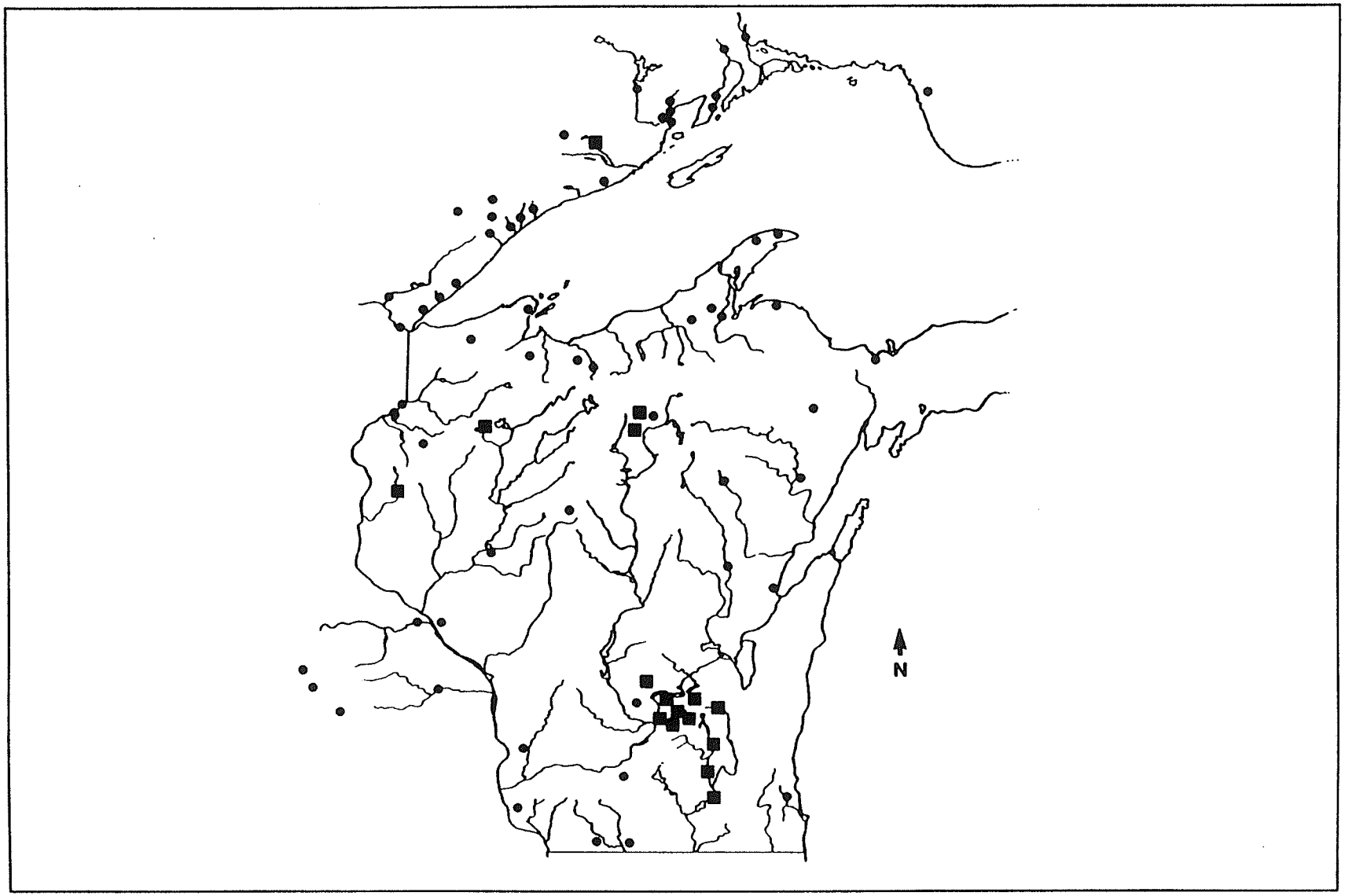

N

Figure 20b: Samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario where the mean number of nape scales is 30 or more. A square (■) equals 30 or more nape scales. A circle (●) equals fewer than 30 nape scales.


N

an anteriorly scaled nape.

Nape squamation falls into one of nine categories (Fig. **21**) although, like breast squamation, some of the patterns are somewhat similar. The most heavily scaled specimens exhibit patterns "D", "G", "H", and "I". No discernable pattern is evident for lightly scaled specimens although a pattern similar to "F" is common in some areas where *E. nigrum* is typically lightly scaled. Several northern Minnesota areas exhibit a large number of specimens with patterns "B", "H" or "G". Areas near Thunder Bay, Ontario, exhibit a larger number of specimens with patterns "C", "D" and "I". Many specimens exhibit the "A" pattern, with or without the scales at the nape-parietal juncture, and possess only one or two scales near the edge of the nape or in the posterior position.

Although some of the patterns in Figure **21** seem to show evidence of progressively increased squamation, like breast squamation, few specimens show intergradation between patterns. Except for changes in the location of the scattered scales shown in pattern "F", most of the patterns appear to be discrete.

Nape-Parietal Juncture Squamation

The number of scales at the nape-parietal juncture ranges from zero to eleven. The mode for this variable is zero ($\bar{x}$ = 1.464; SD = 2.049). There are 801 specimens possessing one or more scales at this region. Specimens from Wisconsin and portions of Michigan, Minnesota and nearby areas often possess four or more scales (Fig. **22a,b**). This trait appears to be geographically informative when considered with other squamation characters and follows the same trend as nape

Figure 21: Patterns of nape squamation observed on specimens of *Etheostoma nigrum*. The most heavily scaled specimens exhibit patterns "D", "G", "H", and "I". Northern Minnesota specimens often exhibit patterns "B", "H" and "G". Specimens from the Thunder Bay, Ontario area often exhibit patterns "C", "D" and "I". Scales at the nape-parietal juncture are shown (as in "A") but are not considered part of the nape squamation pattern. Pattern "A", with or without the scales at the nape-parietal juncture, is exhibited by many specimens.

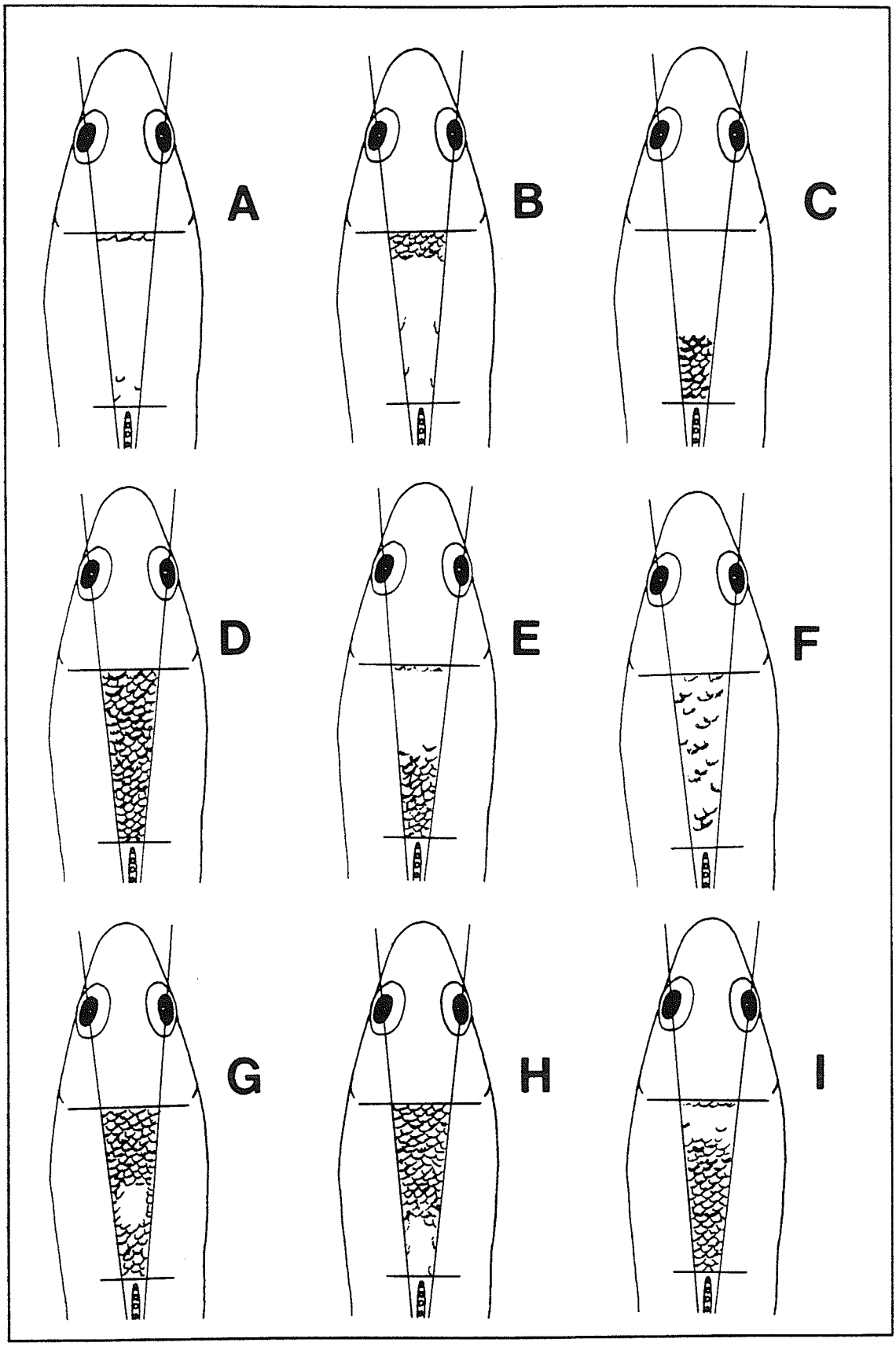

A
B
C
D
E
F
G
H
I

Figure 22a: Mean number of nape-parietal juncture scales among samples of *Etheostoma nigrum*. ● = < 2, ■ = 2-4, and ★ = 4 or more scales.

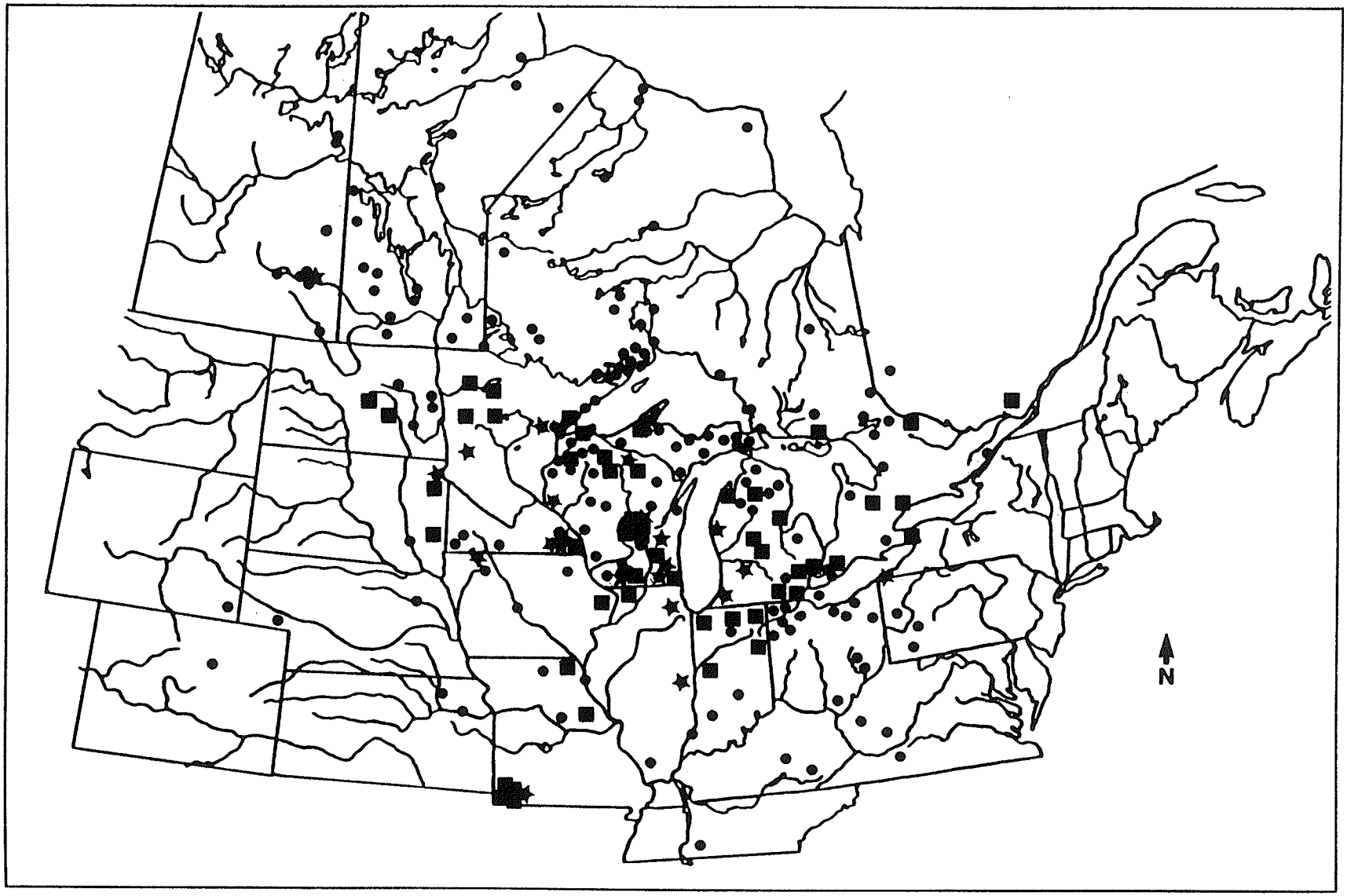
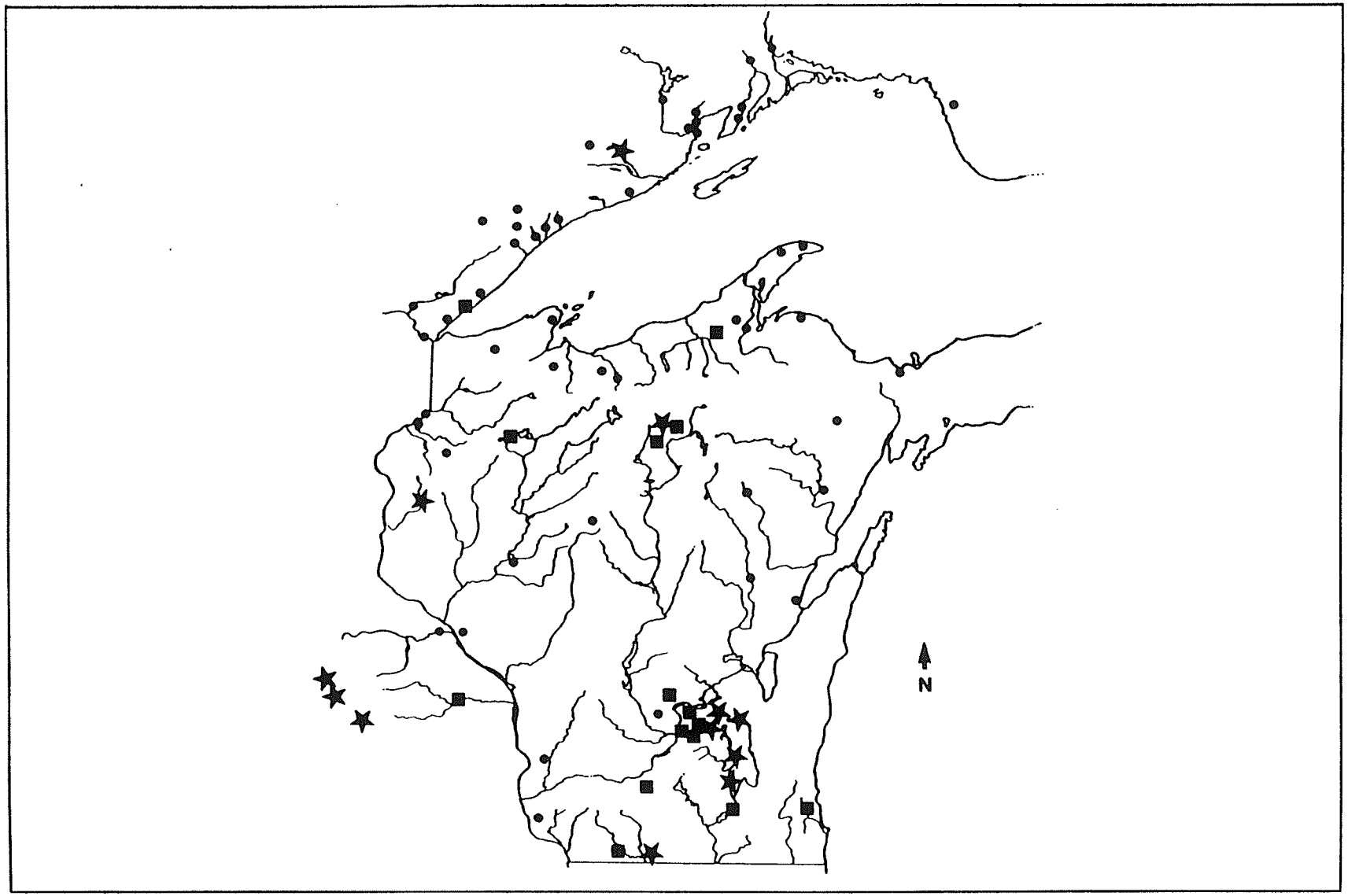

N

Figure 22b: Mean number of nape-parietal juncture scales among samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario. ● = < 2, ■ = 2-4, and ★ = 4 or more scales.


N

scaling. However, unlike nape scaling, the number of scales at the nape-parietal juncture exhibits a highly significant negative correlation ($r = -0.3325$; $P < 0.001$) with latitude when sample means are considered. The correlation is also highly significant when the individual specimen matrix is used ($r = -0.1462$; $P < 0.001$). The correlation is less significant ($r = -0.2549$; $P < 0.01$) when the 152 one-degree grids are used. There are no significant correlations with longitude. Squamation at the nape-parietal juncture is shown with nape squamation in Figure **20**.

When compared to other geographic areas, specimens from Minnesota have a higher than average number of scales at the nape-parietal juncture. Although the Minnesota sample makes up only 10.8% of all specimens examined, 25.2% of those specimens with five or more scales at the nape-parietal juncture are from Minnesota. Similarly, 41.7% and 46.7% of the specimens with six or more or seven or more scales, respectively, at the nape-parietal juncture come from Minnesota. Other states or provinces have only a few specimens with a high number of scales at the nape-parietal juncture. Additional specimens with high nape-parietal juncture scale counts are from areas adjacent to Minnesota. Nape-parietal juncture squamation may provide geographically interpretable information.

Belly Squamation

The estimated percentage of belly squamation exhibits a mode of 100.0% coverage ($\bar{x} = 82.610$; $SD = 24.311$) with a range from zero to 100.0% coverage. A total of 38.9% of the sample, or 822 specimens, possess a belly that is 100% scaled. Over 81.0% of the specimens possess 75.0% or greater squamation on the

belly. Highly significant correlations occur between belly squamation and longitude when the individual specimen matrix is used ($r = 0.1857$; $P < 0.001$). This correlation is less significant when the sample means matrix is used ($r = 0.1689$; $P < 0.01$). When considered with nape, breast and cheek squamation, the estimated percent of belly squamation may provide information on the biogeographic history of populations of *E. nigrum*. Those samples in which the mode of estimated percent of belly squamation is less than 50% are shown in Figure **23**.

Definition of the Scaled and Unscaled Phenotypes

The number of specimens exhibiting the patterns of cheek, breast, and nape squamation reported above are presented in Table **15**.

I constructed a quantifiable definition of a scaled darter using the methods given above using the number of scales on the nape, cheek, and breast areas. All scaled darters were assumed to possess a completely scaled belly for the reasons outlined above. The number of opercle scales was not included as part of the definition since, although few darters lacked any opercle scaling, opercle scaling varied widely among samples and did not vary consistently with nape, cheek or breast scaling. Because the number of specimens is approximately similar for a 47+ breast scale count (320), a 30+ nape scale count (305) and a 10+ cheek scale count (306) as well as a large number of specimens with a 100% scaled belly (822), I defined a scaled darter as one that possesses all of these traits.

There are 240 darters from 38 geographic locations that meet the requirement of "scaled". Scaled darters possess a mean of 21.471 opercle scales (SD = 5.339)

Figure 23: Samples of *Etheostoma nigrum* where the mode for the estimated percent of the belly covered in scales is 50% or less. A square (■) denotes these samples with less than 50% belly squamation. A circle (●) denotes those samples with 50% or greater belly squamation.

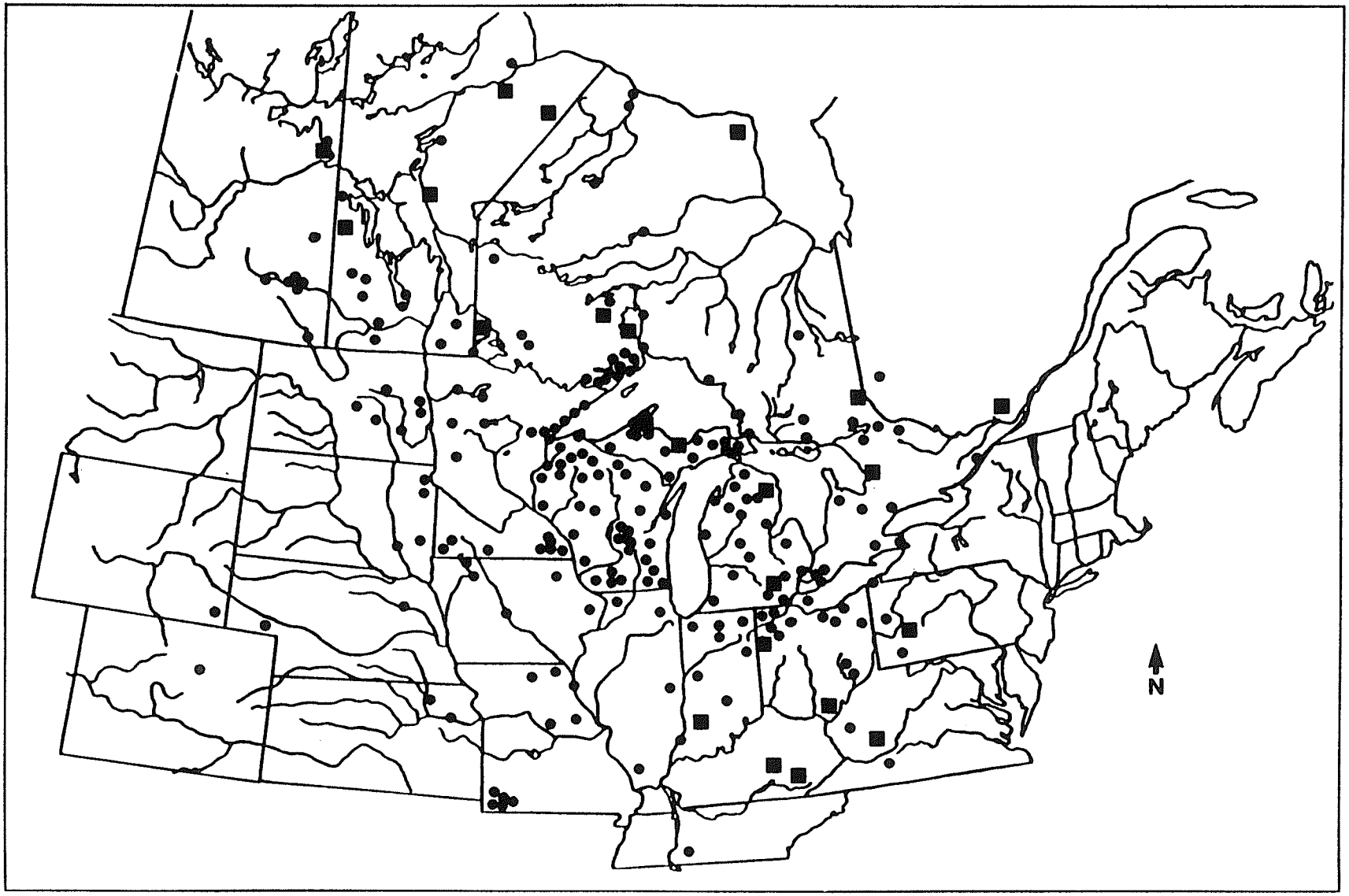

N

Table 15: Number of specimens exhibiting various patterns of nape, cheek and breast squamation. Pattern types ("A"-"G" for cheek, "A"-"H" for breast, and "A"-"I" for nape) correspond to those shown in Figs. **15**, **18** and **21**. The number of specimens not exhibiting any squamation on that body area is also given. A question mark (?) denotes those specimens that could not be satisfactorily classified using any of the described patterns.

Cheek pattern	**Naked**	**A**	**B**	**C**	**D[1]**	**E**	**F**	**G**	**?**
Specimens (N)	1622	61	101	63	44	43	87	31	59

Breast pattern	**Naked**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H[1]**	**?**
Specimens (N)	1428	12	229	24	10	12	13	187	79	117

Nape pattern	**Naked**	**A**	**B**	**C**	**D[1]**	**E**	**F**	**G**	**H**	**I**	**?**
Specimens (N)	1355	257	5	28	237	62	19	30	29	9	80

[1] = the fully scaled pattern for that body area

with a range of from 7-36 scales. Except for eight of these scaled specimens, all possess at least some scales at the nape-parietal juncture ($\bar{x}$ = 4.148 scales; SD = 1.102; range 1-7). Most of the scaled specimens (88.3%) possess four or more scales at this position. Scaled darters are found in (number of specimens; number of locations) Wisconsin (138; 17), Minnesota (21; 5), Ontario (17; 5), South Dakota (13; 2), Indiana (10; 1), Ohio (10; 1), Iowa (9; 2), Michigan (8; 2), Missouri (7; 2), Saskatchewan (4; 2) and Pennsylvannia (3; 1). Table **16** gives the locations of these collections and they are plotted in Figure **24**.

The definition of the unscaled phenotype for this study was any specimen which lacked any nape, breast or cheek squamation. Intergrading specimens are those that possess increased squamation in any or all of these three areas. However, due to the variable occurrence of nape-parietal juncture squamation in almost half of the examined specimens, it was excluded as a qualifier from the scaled definition. Similarly, for the reasons given above, opercle squamation was also excluded. Thus an unscaled darter may possess one or more scales at the nape-parietal juncture and the opercle may be variously scaled. Frequency distribution tables of squamation values for all specimens examined are presented in Appendix **7**.

Distribution of the Scaled Phenotype

In Wisconsin, the highest concentrations of scaled *E. nigrum* are found in the Rock (Mississippi River drainage) and Fox (Lake Michigan drainage) river systems. The scaled form is found in the northern lakes area tributary to the headwaters of the Wisconsin River system and headwaters of the Mississippi River (St. Croix).

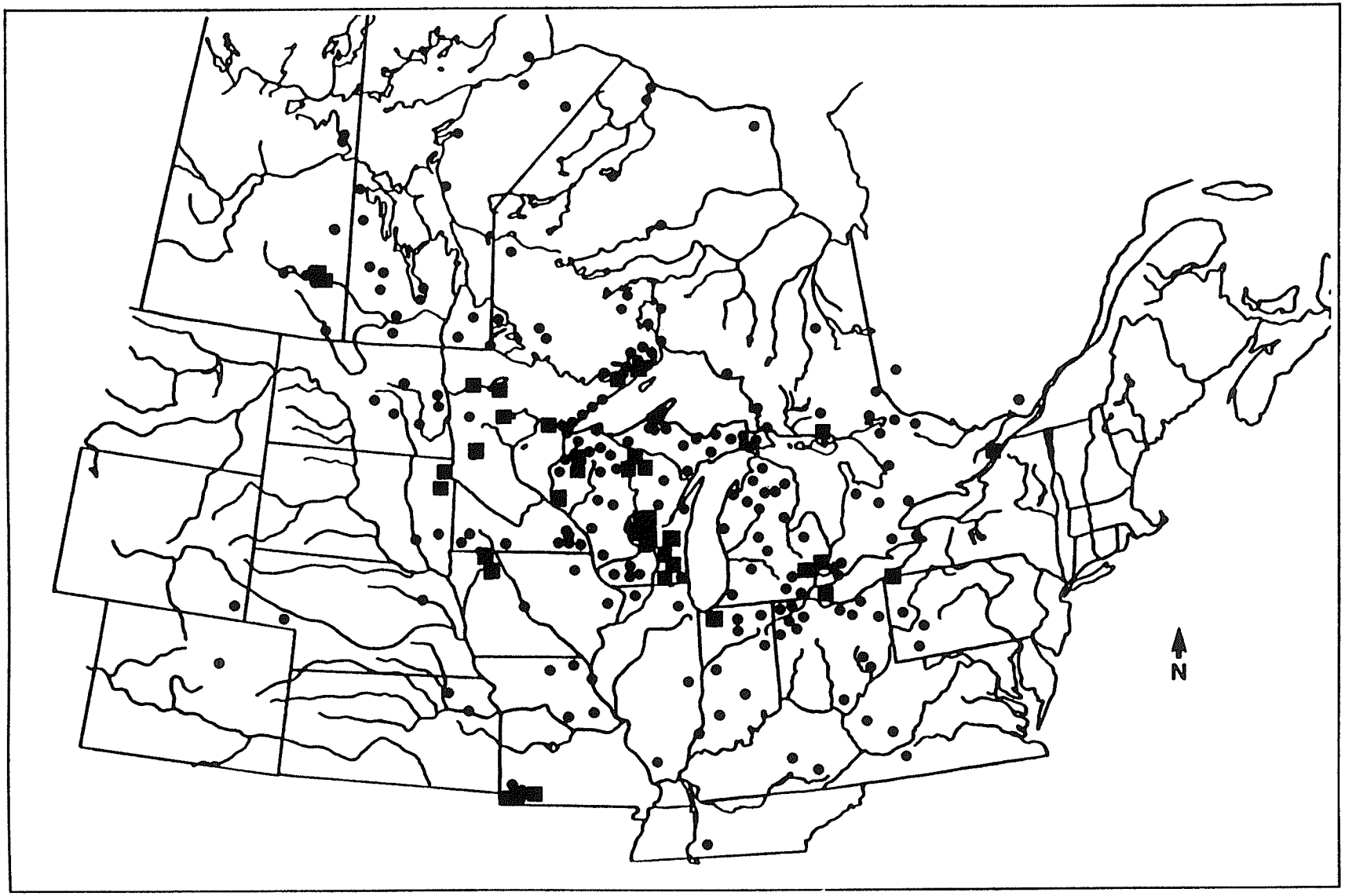
Table 16: Location, number scaled/number examined in sample, and major and tertiary drainages of 240 scaled *Etheostoma nigrum*.

Location	Scaled/Examined	Drainages	
Wisconsin			
Little Arbor Vitae L.	10/10	Mississippi	Wisconsin River
Yellow River	1 /6	Mississippi	St. Croix River
Grand River	9/10	Atlantic	Fox River
trib to Grand River	5/11	Atlantic	Fox River
Rock River	11/14	Mississippi	Rock River
Grindstone Lake	3 /3	Mississippi	St. Croix River
St. Germain Lake	5/14	Mississippi	Wisconsin River
Apple River	15/15	Mississippi	St. Croix River
Fox River	15/25	Mississippi	Rock River
French Creek	13/24	Mississippi	Fox River
Kawaguesaga Lake	8 /9	Mississippi	Wisconsin River
Spring Lake	4 /5	Atlantic	Fox River
trib to Neenah Creek	5/11	Atlantic	Fox River
Crawfish River	11/11	Mississippi	Rock River
below Park Lake	11/23	Atlantic	Fox River
Rock River	6 /6	Mississippi	Rock River
Crawfish River	6 /7	Mississippi	Rock River
	138		
Minnesota			
Mississippi River	5/10	Mississippi	Mississippi River
Mud River	2 /5	Mississippi	Mississippi River
Red Lake River	2/11	Hudson Bay	Red River
Deer Creek	1 /4	Mississippi	Mississippi River
Otter Tail River	11/11	Hudson Bay	Red River
	21		
Ontario			
Rideau Creek	1/11	Atlantic	St. Lawrence River
McGregor Bay	2 /6	Atlantic	Lake Huron
Niagara River	2 /5	Atlantic	Lake Ontario
Round Lake	11/14	Atlantic	Lake Superior
McIntyre River	1/10	Atlantic	Lake Superior
	17		
South Dakota			
Big Stone Lake	7 /8	Mississippi	Minnesota River
Whetstone Creek	6/23	Mississippi	Minnesota River
	13		
Indiana			
Coffee Creek	10/48	Atlantic	Lake Michigan
	10		

Table 16: Continued.

Location	Scaled/Examined	Drainages	
Ohio			
South Bass Island, L. Erie	10/11	Atlantic	Lake Erie
	10		
Iowa			
Meadow Creek	1 /5	Missouri	Little Sioux River
Spirit Lake	8 /8	Missouri	Little Sioux River
	9		
Michigan			
Lake St. Clair	1 /5	Atlantic	Lake Erie
Huron River	7/17	Atlantic	Lake Erie
	8		
Missouri			
Coon Creek	2 /2	Mississippi	Arkansas River
Muddy Fork, Spring R.	5 /6	Mississippi	Arkansas River
	7		
Saskatchewan			
Echo Lake	1/10	Hudson Bay	Assiniboine River
Echo Lake	2 /4	Hudson Bay	Assiniboine River
Qu'Appelle River	1 /1	Hudson Bay	Assiniboine River
	4		
Pennsylvannia			
Erie Harbour, L. Erie	3 /4	Atlantic	Lake Erie
	3		

Figure 24: Distribution of the scaled form of *Etheostoma nigrum* as determined by the definition developed for this study. Sample locations which possess some specimens meeting the definition of scaled are identified with a square (■). Sample locations at which specimens do not meet the definition of scaled are identified with a circle (●).


N

Minnesota concentrations of the scaled form are found mainly in headwaters of the Red River (Hudson Bay Manitoba drainage) and nearby central state headwaters of the Mississippi River. Ontario concentrations of the scaled form are found primarily at the international border with Minnesota (Lake Superior drainage). A few scaled specimens occur in Lake Ontario or St. Lawrence River tributaries. Scaled *E. nigrum* from Indiana are from Coffee Creek which drains into southern Lake Michigan. Scaled specimens from Iowa are from Spirit Lake with the exception of one specimen from a downstream location. Michigan collections are from Lake St. Clair and the Huron River, tributary to the northwestern end of Lake Erie. Ohio specimens come from the Bass Islands in western Lake Erie. Pennsylvannia specimens are from Erie Harbour on southeastern Lake Erie. South Dakota specimens are restricted to headwaters of the Minnesota River (Mississippi River system). Four Saskatchewan specimens are from the area of the Qu'Appelle Valley. Missouri specimens are from the southeast portion of the state in, or tributary to, the Spring River. The majority of these scaled specimens thus come from headwater lakes and streams. However, some minor scaling on the nape, cheek or breast is common within many areas of the range of *E. nigrum*. Also note that samples which possess scaled darters, as defined in this study, do not always consist solely of specimens which meet the definition (Table **16**).

If the original definition had used 20+ nape scales instead of 30+ nape scales, that is, the frequency distribution histogram peak of 35 scales rather than 45 scales had been used as a starting point (Fig. **19**), 19 additional specimens would have been

classified as scaled. However, these additional specimens would have come from eleven samples already identified as scaled using the 30+ nape scale definition (number of samples: number of specimens) (Wisconsin 4:9; Minnesota 1:2; Ontario 2:4; South Dakota 1:1; Saskatchewan 2:2; Pennsylvannia 1:1). Therefore, the original definition is likely suitable for identifying the geographic distribution of scaled specimens.

Because I did not count the number of scales on the belly, I determined how many specimens could have met the definition as scaled but did not possess a 100% scaled belly. There were only four additional specimens, two each with 90% and 95% scaled bellies, that did not qualify for inclusion as scaled. Two of the specimens are from Wisconsin samples with one each from Minnesota and Michigan. All but the Minnesota specimen (Dinner Creek, Becker Co., Hudson Bay Manitoba drainage) are from samples previously identified as possessing scaled specimens.

Adjusting the criterion developed for the definition of scaled by approximately one-half (23 breast scales, 15 nape scales, five cheek scales and a 100% scaled belly) adds an additional 67 specimens and six new locations. Approximately halving this definition again to 12 breast scales, eight nape scales, three cheek scales and a 100% scaled belly adds a further 39 specimens that includes six more new locations. However, the geographic distribution of these darters does not change by much as most new locations are very near those first identified using the 47 breast, 30 nape, and ten cheek scales definition. It is mainly specimens from the initial samples identified which add to the total number of specimens rather than new samples being

identified as the definition of "scaled" is relaxed. The locations and numbers of specimens according to each of these definitions are presented in Table 17.

A comparison of the 47 breast, 30 nape, and ten cheek scales definition with my *a priori* "type" designations shows contrasts in both the number of specimens and the geographic locations identified. Initially, I subjectively called all darters with more than 90% scaling on the nape, cheek and breast "scaled", and those with 75% or more, but less than 90% scaling on the nape, cheek or breast, "intergrades". Table 18 compares these estimates of "types" to those of the quantified definition. Using the type designations, only 70 darters were identified as scaled while a further 74 were identified as intergrades. Classification by type (based on percent estimate of squamation) thus under-estimates both the number and geographic distribution of scaled *E. nigrum* and shows that the two definitions are not compatible.

I examined all Wisconsin and Minnesota samples from rivers tributary to the Mississippi River to eliminate any possible habitat and drainage influences and divided the sample into scaled, naked and intergrade using the definitions presented earlier. This method, however, does allow specimens without scales on two areas (nape, cheek or breast) and only one scale in one of these areas to be classified as an intergrade. Similarly, a darter may have a fully scaled belly, 47+ breast and 30+ nape scales and still be classified as an intergrade if it has only nine cheek scales. In total, 146 specimens were identified from this area (50 scaled; 56 unscaled; 40 intergrades). Among 91 Minnesota riverine specimens, the number of unscaled greatly exceeds the scaled and intergrade specimens (52 unscaled; 31 intergrades; 8

Table 17: Location, number scaled/number examined in sample for three classes of scaled *Etheostoma nigrum* showing how their numbers would increase based on various definitions. The three classes are for those specimens possessing a 100% scaled belly and possessing more than or at least; **1/** 47 breast, 30 nape and 10 cheek scales **2/** 23 breast, 15 nape and 5 cheek scales or **3/** 12 breast, 8 nape and 3 or more cheek scales. Major drainages are given as; AT=Atlantic, MS=Mississippi, MO=Missouri and HB=Hudson Bay.

	Definition			
	1	**2**	**3**	**Drainage**
Wisconsin				
Little Arbor Vitae L.	10/10	10/10	10/10	MS
Yellow River	1 /6	1 /6	1 /6	MS
Grand River	9/10	10/10	10/10	AT
trib to Grand River	5/11	8/11	8/11	AT
Rock River	11/14	12/14	14/14	MS
Grindstone Lake	3 /3	3 /3	3 /3	MS
St. Germain Lake	5/14	9/14	10/14	MS
Apple River	15/15	15/15	15/15	MS
Fox River	15/25	18/25	19/25	MS
French Creek	13/24	17/24	19/24	MS
Kawaguesaga Lake	8 /9	8 /9	8 /9	MS
Spring Lake	4 /5	4 /5	4 /5	AT
trib to Neenah Creek	5/11	10/11	10/11	AT
Crawfish River	11/11	11/11	11/11	MS
below Park Lake	11/23	15/23	17/23	AT
Rock River	6 /6	6 /6	6 /6	MS
Crawfish River	6 /7	6 /6	6 /6	MS
Root River	0/14	1/14	3/14	AT
Number of specimens	**138**	**164**	**174**	
Minnesota				
Mississippi River	5/10	7/10	7/10	MS
Mud River	2 /5	3 /5	3 /5	MS
Red Lake River	2/11	4/11	6/11	HB
Deer Creek	1 /4	1 /4	1 /4	MS
Otter Tail River	11/11	11/11	11/11	HB
Sucker River	0 /6	1 /6	2 /6	AT
Number of specimens	**21**	**27**	**30**	

Table 17: Continued.

	Definition			
	1	**2**	**3**	**Drainage**
Ontario				
Rideau Creek	1/11	3/11	3/11	AT
McGregor Bay	2 /6	4 /6	4 /6	AT
Niagara River	2 /5	2 /5	2 /5	AT
Round Lake	11/14	14/14	14/14	AT
McIntyre River	1/10	4/10	6/10	AT
McVicars Creek	0/10	1/10	1/10	AT
Mission Marsh	0/12	0/12	3/12	AT
Number of specimens	**17**	**28**	**33**	
Indiana				
Coffee Creek	10/48	13/48	17/48	AT
Number of specimens	**10**	**13**	**17**	
South Dakota				
Big Stone Lake	7 /8	8 /8	8 /8	MS
Whetstone Creek	6/23	11/23	18/23	MS
Big Sioux River	0 /3	0 /3	1 /3	MS
James River	0 /6	0 /6	1 /6	MO
Number of specimens	**13**	**19**	**28**	
Ohio				
Bass Islands, L. Erie	10/11	11/11	11/11	AT
Number of specimens	**10**	**11**	**11**	
Iowa				
Meadow Creek	1 /5	1 /5	1 /5	MO
Spirit Lake	8 /8	8 /8	8 /8	MO
Number of specimens	**9**	**9**	**9**	
Michigan				
Lake St. Clair	1 /5	1 /5	1 /5	AT
Huron River	7/17	11/17	11/17	AT
Cass River	0 /7	0 /7	1 /7	AT
Number of specimens	**8**	**12**	**13**	

Table 17: Continued.

	Definition			
	1	2	3	Drainage
Missouri				
Coon Creek	2 /2	2 /2	2 /2	MS
Muddy Fork, Spring R.	5 /6	6 /6	6 /6	MS
Spring River trib.	0 /5	2 /5	5 /5	MS
N. fork Spring R.	0 /1	1 /1	1 /1	MS
Number of specimens	**7**	**11**	**14**	
Saskatchewan				
Echo Lake	1/10	3/10	4/10	HB
Echo Lake	2 /4	3 /4	4 /4	HB
Qu'Appelle River	1 /1	1 /1	1 /1	HB
Number of specimens	**4**	**7**	**9**	
Pennsylvannia				
Erie Harbour	3 /4	4 /4	4 /4	AT
Number of specimens	**3**	**4**	**4**	
Illinois				
Stony Brook	0 /2	1 /2	1 /2	MS
Number of specimens	**0**	**1**	**1**	
North Dakota				
James River	0 /8	0 /8	1 /8	MO
Goose River	0/12	0/12	1/12	HB
Number of specimens	**0**	**0**	**2**	
Total Specimens	**240**	**306**	**345**	
Total Locations	**40**	**46**	**52**	

Table 18: Locations and comparison of three methods of defining scaled darters based on the *a priori* "type" designations of 75% and 90% scaled and a quantified definition. The quantified definition refers to 47 or more breast scales, 30 or more nape scales, 10 or more cheek scales and a 100% scaled belly.

Location	90%+ Scaled "Scaled"	75%+ Scaled "Intergrades"	Quantified Definition
Indiana	3	4	10
Iowa	8	9	9
Michigan	0	1	8
Minnesota	6	18	21
Missouri	0	0	7
Ohio	4	9	10
Pennsylvannia	2	2	3
South Dakota	3	6	13
Wisconsin	36	79	138
Ontario	8	15	17
Saskatchewan	0	1	4
Number of specimens	**70**	**144**	**240**

scaled).

Results of a meristic examination shows that the 40 Wisconsin intergrades fall between the scaled and unscaled forms in about half the characters (Table **19**). Four characters are slightly lower while three characters are slightly higher in intergrades than in either of the parental forms. Squamation characters cannot be adequately examined in Table **19** and compared with the scaled form. Figure **25** shows the number of nape, cheek and breast scales on these intergrade darters. Most specimens do not possess cheek scales and much of the sample does not possess nape or breast scales. Only a few of these specimens have nape or breast scale counts which exceed the number required to be classed as scaled. In the Minnesota sample, the means and SD for squamation variables are similar to those from Wisconsin although the number of breast scales is higher ($\bar{x}$ cheek = 1.00 (SD = 1.93); $\bar{x}$ nape = 7.32 (SD = 10.47); $\bar{x}$ breast = 16.39 (SD = 24.81)).

Branchiostegal Rays

The number of left branchiostegals is six for most samples ($\bar{x}$ = 5.967; SD = 0.206). The number of right branchiostegals is approximately similar ($\bar{x}$ = 5.971; SD = 0.216). Those samples tributary to or within the mainstem Ohio River have the highest number of specimens with five branchiostegal rays (Fig. **26**). Three samples in Ohio tributaries of Lake Erie also possess specimens with this trait. However, Ontario, Wisconsin and several other areas also possess samples in which some specimens have five branchiostegal rays.

Of all darters in my samples with five branchiostegal rays, 81.3% of them (65

Table 19: Comparison of the means (SD in parentheses) of 17 meristic characters from 146 scaled, unscaled and "intergrade" specimens of *Etheostoma nigrum* from Wisconsin rivers tributary to the Mississippi River.

	Scaled	**Intergrade**	**Unscaled**
LL	46.72	46.55	46.96
	(2.46)	(3.02)	(2.87)
LL1	4.40	4.23	3.84
	(0.57)	(0.53)	(0.53)
DS	8.80	8.73	9.09
	(0.53)	(0.45)	(0.51)
DR1	11.94	11.70	11.89
	(0.77)	(0.61)	(0.71)
AR1	8.24	8.33	8.29
	(0.89)	(0.62)	(0.65)
LPOP	7.56	7.87	7.00
	(1.73)	(1.49)	(1.39)
RPOP	7.50	7.95	7.04
	(1.74)	(1.48)	(1.32)
LPOS	2.98	2.33	2.21
	(1.53)	(0.47)	(0.46)
LANT	3.76	4.02	4.00
	(0.98)	(0.16)	(0.00)
RPOS	3.08	2.48	2.18
	(1.48)	(0.64)	(0.43)
RANT	3.84	3.98	4.00
	(1.02)	(0.16)	(0.00)
PECR	11.72	11.70	11.77
	(0.45)	(0.52)	(0.50)
BELLYP	100.00	96.25	88.84
	(0.00)	(5.86)	(11.48)
CC	33.78	0.85	-
	(12.84)	(2.35)	-
NC	45.56	8.90	-
	(6.48)	(11.87)	-
BC	87.34	9.23	-
	(21.73)	(15.11)	-
$LLSTAT_1$	1.18	1.38	1.57
	(0.44)	(0.59)	(0.53)
N	**50**	**40**	**56**

$_1$ Lateral line condition; 1=complete, 2=incomplete (< 3 unpored scales), 3=very incomplete (> 3 unpored scales).

Figure 25: Frequency histogram of the number of nape, cheek and breast scales on 40 *Etheostoma nigrum* classed as intergrades from Wisconsin rivers tributary to the Mississippi River. The cutoff points for the number of cheek, nape and breast scales required to meet the definition of scaled for this study are indicated with arrows.

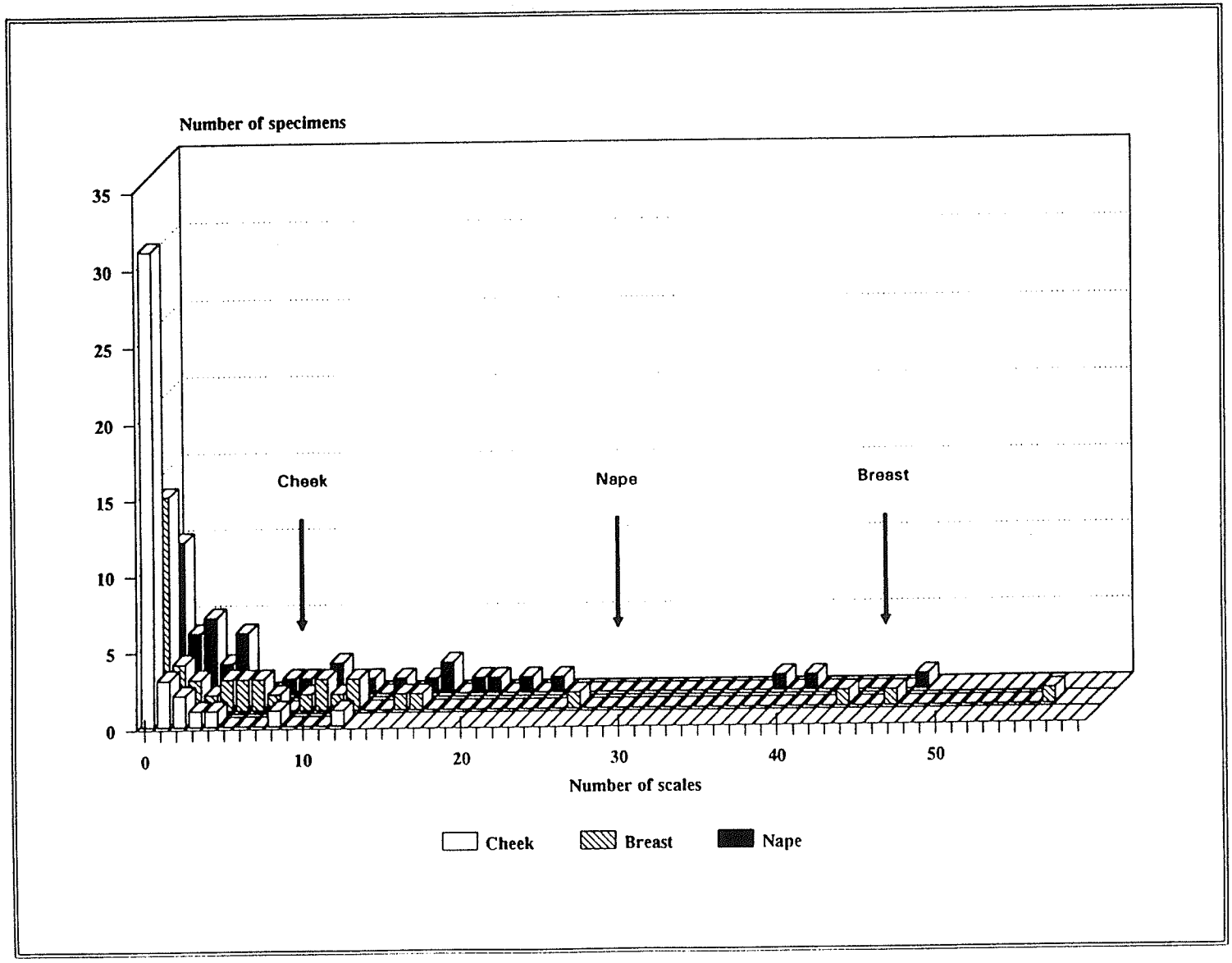
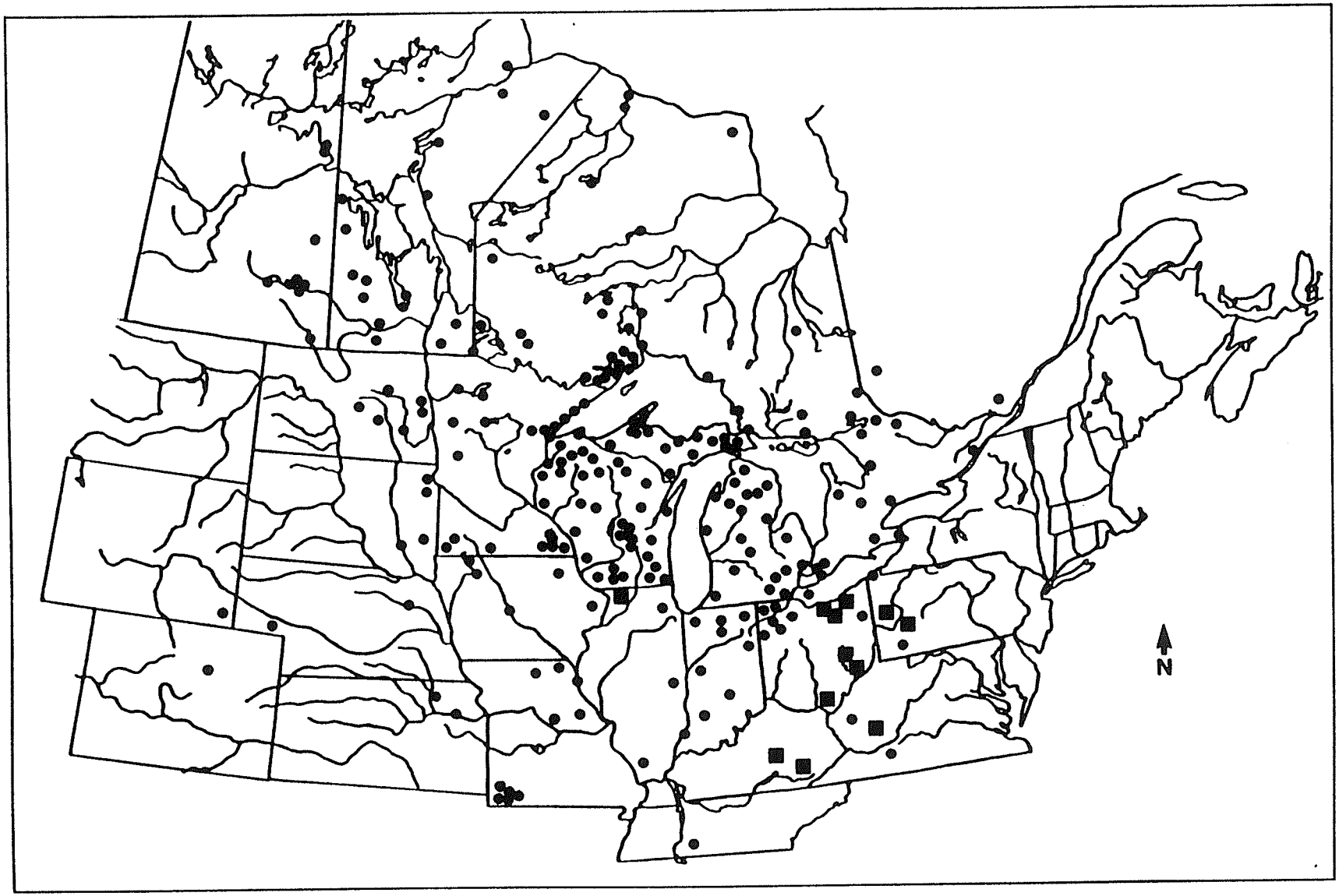

Number of specimens
35
30
25
20
15
10
5
0
Cheek
Nape
Breast
0
10
20
30
40
50
Number of scales
Cheek
Breast
Nape

Figure 26: Samples of *Etheostoma nigrum* which possess a mode of five branchiostegal rays. Samples are identified with a square (■). The Illinois sample consists of only two specimens. Samples identified with a circle (●) possess a mode of six branchiostegal rays.


N

specimens) are confined to the Ohio Basin (Table **20**). Only 18.7% are found in drainages other than the Ohio and slightly more than one-third of those occur in nearby Lake Erie tributaries. Almost half of the Ohio Basin specimens (49.2%) possesses five branchiostegal rays. The distribution of other specimens with five branchiostegal rays, mainly in the extreme upper reaches of the Ohio River or in lower reaches and tributaries of the Ohio River below Cincinnati, Ohio, due to both the low numbers and their failure to occur in any geographically interpretable manner, represent either expression of the character due to emigration of individuals from the Ohio Basin or chance. This trait is not clinal. Instead, the number of branchiostegal rays changes over a short distance between samples within and nearby, but outside the basin. It appears to be stable within the upper and portions of the middle Ohio Basin and it rarely occurs outside this area. These reasons suggest that there is a strong genetic basis for the trait.

Eight specimens (number of specimens in parentheses) from Michigan (3), Minnesota (1), South Dakota (3) and Saskatchewan (1) possess seven branchiostegal rays on both their left and right sides. Most specimens from each state are from a single location. Two of these specimens meet the definition of scaled developed for this study. An additional 15 specimens possess seven branchiostegal rays on either their left or right sides. Ten of these specimens are unscaled while five meet the definition as scaled. Nine of these 15 specimens are from collections in which at least one specimen possesses seven branchiostegal rays on both sides.

All data matrices result in highly significant ($P < 0.001$) positive correlations

Table 20: Distribution of samples of *Etheostoma nigrum* possessing specimens with five left branchiostegal rays from the Ohio and Mississippi rivers and their tributaries.

State	Location	Drainage	Number
Pennsylvannia			
	Little Bull Creek	Ohio	6/10
	Shenango River tributary	Ohio	4/4
Ohio			
	Indian Guyan Creek	Ohio	8/9
	Rocky River, W. Br.	Erie	2/2
	Rocky River, E. Br.	Erie	3/3
	Little Beaver Creek	Erie	9/9
	Walhonding River	Ohio	4/4
	Muskingum River	Ohio	5/5
Kentucky			
	Russell Fork	Ohio	9/9
	Quicksand Creek	Ohio	9/10
Tennessee			
	Eagle Creek	Ohio	4/10
West Virginia			
	New River	Ohio	4/4
Indiana			
	Clear Creek	Ohio	2/11
	Trib of Six Mile Creek	Ohio	1/6
Illinois			
	near Quincy	Mississippi	1/5
	Unnamed Creek	Mississippi	1/2

between both left and right branchiostegal rays and latitude and longitude. The highest correlation is with latitude. Branchiostegal rays exhibit meaningful geographic variation in the upper and some sections of the middle Ohio Basin.

Pectoral Rays

Left pectoral rays number twelve for the majority of samples examined ($\bar{x}$ = 11.838; SD = 0.511). Many samples from the middle and upper Ohio Basin and several nearby tributaries to Lake Erie possess a mode of eleven pectoral rays. Several samples in Michigan and Wisconsin also possess this trait although it is centred around and south of Lake Erie.

Of the specimens examined, 457 possess 11 or fewer pectoral rays representing approximately 1/5 of the sample and originating from all drainages. Most of these are from the Ohio (16.6%) or Mississippi (23.4%) basins. These percentages represent 57% of Ohio Basin samples but only 26% of Mississippi Basin samples. A total of 49 specimens possess five branchiostegal rays and 11 or fewer pectoral rays. The majority (47 specimens) are from the Ohio Basin while the two remaining specimens are from nearby Lake Erie tributaries. These specimens represent over 1/3 of the entire Ohio Basin sample. Both the sample means and individual specimen matrix produce highly significant correlations ($P < 0.001$) with latitude and longitude. Using the one-degree grid matrix results in a highly significant correlation ($r = 0.4520$; $P < 0.001$) only with latitude. Pectoral rays vary in a geographically meaningful and interpretable manner south of the Lower Great Lakes, particularly in the Ohio Basin.

Dorsal Spines

The modal number of dorsal spines on *E. nigrum* is nine ($\bar{x}$ = 8.851; SD = 0.582). Several samples possess higher (ten) and lower (eight) mean or modal counts but overall, the variation does not vary in a geographically informative manner. Correlations between the number of dorsal spines with latitude and longitude are all non-significant.

Dorsal Rays

The modal number of dorsal rays (DR1) for *E. nigrum* is twelve ($\bar{x}$ = 11.737; SD = 0.766). Samples from the middle and upper Ohio Basin and nearby areas, such as Michigan, possess a mode of ten or eleven dorsal rays. Most Saskatchewan samples and several from southwestern Manitoba possess eleven dorsal rays. There are 78 samples which possess a modal dorsal ray count of 11 or less representing 729 specimens. Dorsal rays vary in a meaningful and interpretable geographic manner in and near the Ohio Basin. Because the second dorsal ray count (DR2) does not vary much from DR1 except for generally being one ray higher ($\bar{x}$ = 12.740; SD = 0.770), there are no independent geographically informative trends shown by the DR2 count used in this study.

The number of dorsal rays (DR1) exhibits a highly significant ($P < 0.001$) positive correlation with latitude using all three matrices. The correlation coefficient is approximately similar using both the sample means and the one-degree grids ($r = 0.3454$). The individual specimen matrix also produces a highly significant correlation ($r = 0.1290$; $P < 0.001$) with longitude.

Anal Rays

Anal rays exhibit a mode of eight ($\bar{x}$ = 8.271; SD = 0.686) with a range of from six to twelve. Anal rays do not provide any useful information on the biogeographic history of *E. nigrum* due to their irregular distribution among samples. Similarly, the second anal ray count (AR2) does not reveal any geographically interpretable pattern of variation ($\bar{x}$ = 9.251; SD = 0.712). Correlation analysis of anal rays (AR1) using all matrices does produce highly significant ($P < 0.001$) correlations with both latitude and longitude indicating generally lower anal ray counts in the southeastern range of *E. nigrum*.

Infraorbital Pores

Etheostoma nigrum has a mode of two left ($\bar{x}$ = 2.329; SD = 0.619) and two right ($\bar{x}$ = 2.333; SD = 0.618) posterior infraorbital pores and four left ($\bar{x}$ = 4.014; SD = 0.326) and four right ($\bar{x}$ = 4.021; SD = 0.342) anterior infraorbital pores. Three specimens from two Wisconsin river samples possess an uninterrupted infraorbital canal on both sides of the head. One Ontario specimen has a complete infraorbital canal on the left side only. Only the individual specimen matrix produces highly significant negative correlations between the number of left and right posterior infraorbital pores and latitude ($r = -0.01340$; $P < 0.001$). However, the number of infraorbital pores does not vary in a geographically informative manner.

Preoperculomandibular Pores

There is a bimodal distribution in the number of POM pores throughout the examined range of *E. nigrum* with some samples possessing a mode of six pores and

some possessing nine or more. The modal number of POM pores is six on both the left ($\bar{x}$ = 7.563; SD = 1.572) and right ($\bar{x}$ = 7.583; SD = 1.559) sides. Most samples collected in tributaries to the Ohio River, and some nearby areas, possess a mode of nine or more POM pores. Most samples tributary to the St. Lawrence and the Ottawa rivers, or eastern Lake Ontario, as well as the Virginia sample, possess modal POM pore counts of nine to eleven (Fig. **27a,b**). Six pores are found in western or northern areas while nine pores are more common in eastern and southeastern areas. Wisconsin, Michigan and the western Lake Superior region are areas of intergradation where samples possess specimens with six or nine pores and many specimens possess seven or eight pores. Table **21** shows that the number of left POM pores gradually decreases by state or province from the southeast to the northwest. The Colorado sample does not fit this pattern. Nebraska, Kansas and Wyoming, with admittedly small samples, do follow the westward trend for decreasing left POM pore counts.

Using the latitude and longitude from the centre of each state or portion of the province presented in Table **21** results in highly significant negative correlations with both longitude ($r = -0.7806$, $P < 0.001$) and latitude ($r = -0.5582$, $P < 0.001$) with the total number of left POM pores. The negative correlations suggest that left POM pores decrease as latitude and longitude increase. Similarly, using the individual specimen matrix results in significant correlations for total left POM pores and latitude ($r = -0.3866$, $P < 0.001$) and longitude ($r = -0.3905$, $P < 0.001$). Using the 152 one-degree latitude and longitude grids and the sample means also results in

Figure 27a: Mean number of preoperculomandibular pores for samples of *Etheostoma nigrum*. ● = < 6.9, ■ = 7.0-8.0, + = 8.1-9.0 and ★ = 9.1+ scales.

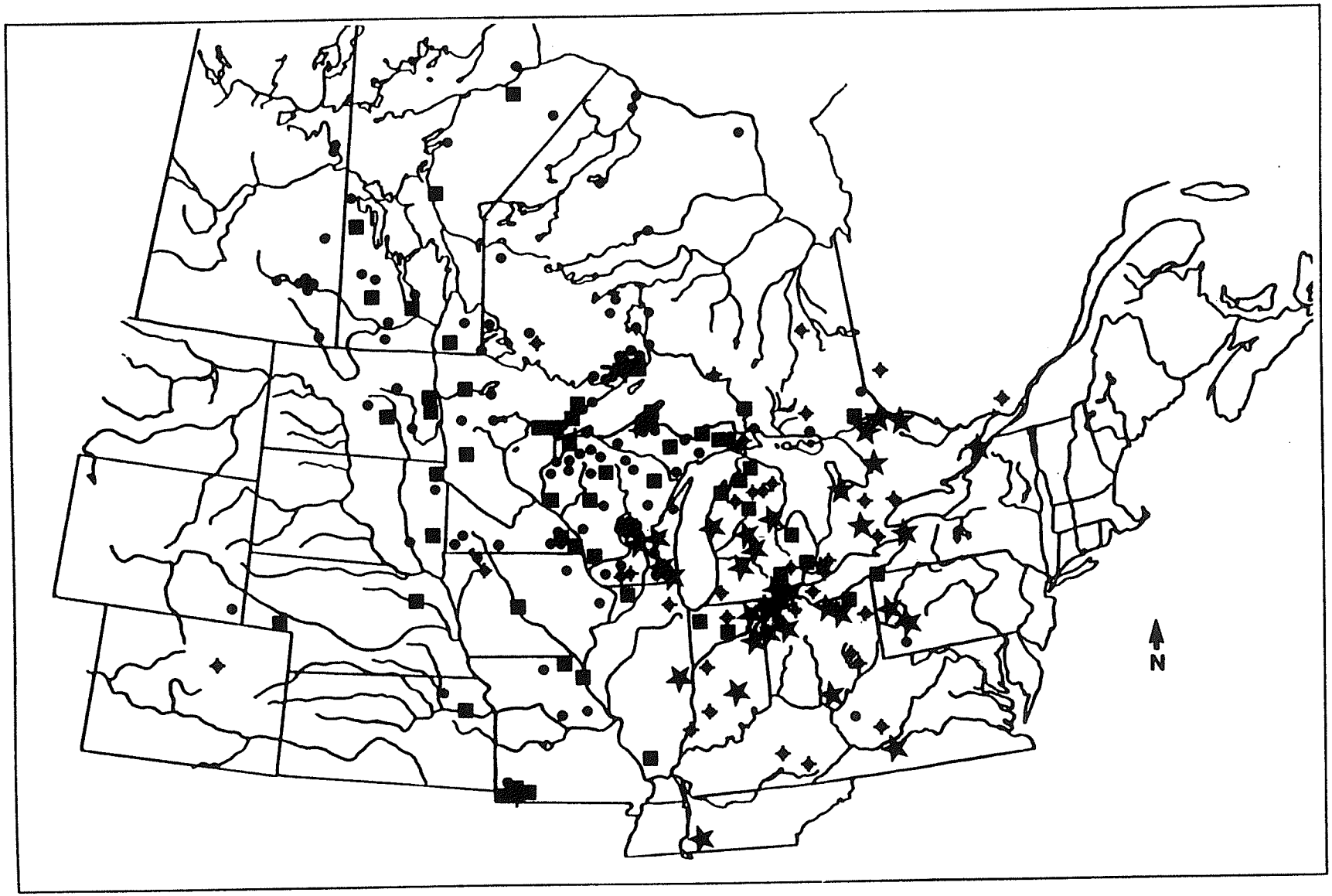

N

Figure 27b: Mean number of preoperculomandibular pores for samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario. ● = < 6.9, ■ = 7.0-8.0, + = 8.1-9.0 and ★ = 9.1+ scales.

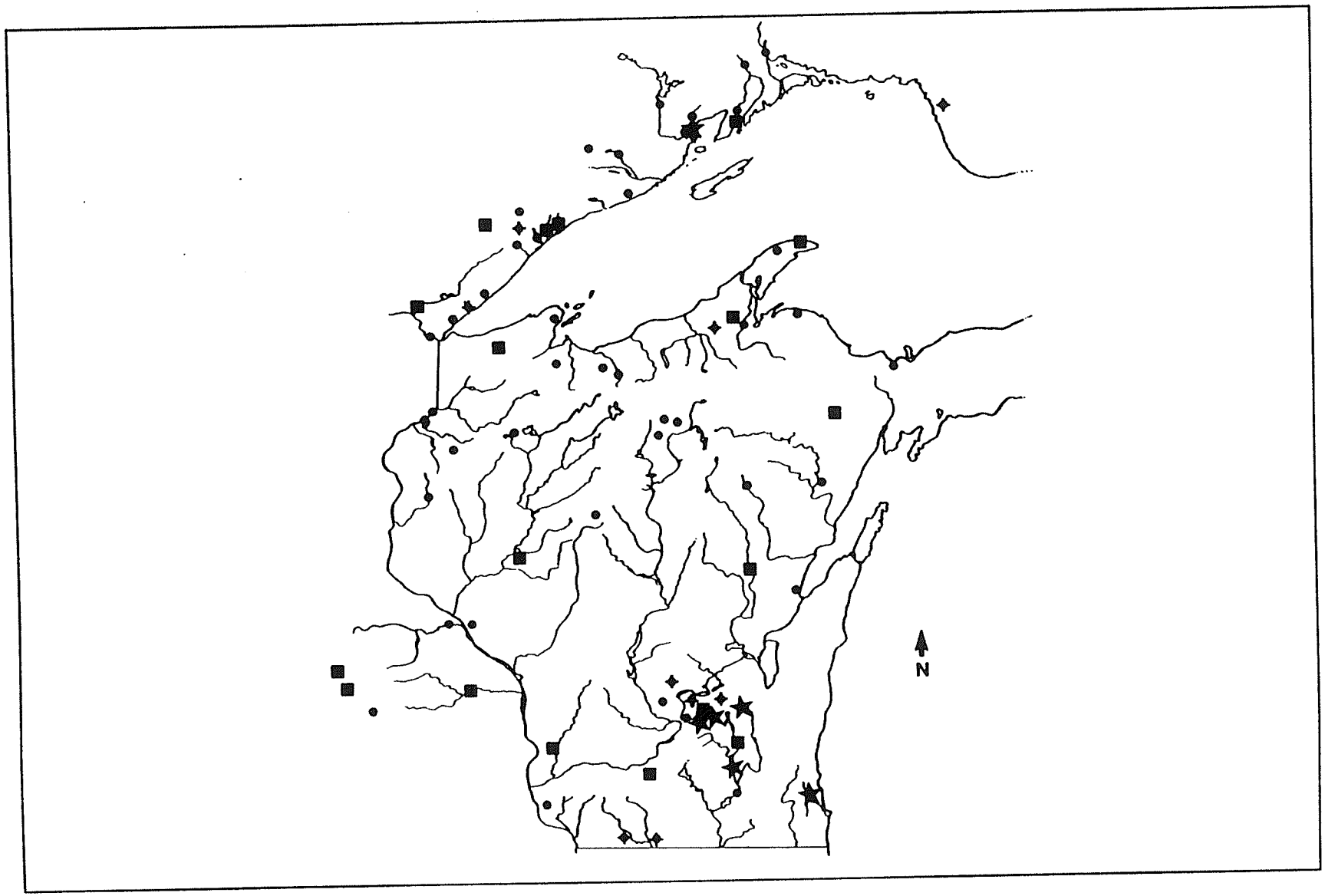

N

Table 21: Frequency distribution of the total number of left preoperculomandibular pores from samples of *Etheostoma nigrum* from selected states and provinces. Locations are roughly arranged from east to west.

	Number of pores									
Location	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Mean**
Pennsylvannia	-	2	-	2	20	7	-	-	-	8.968
Kentucky	-	-	-	4	13	2	-	-	-	8.895
Ontario$_1$	-	15	3	9	19	10	11	2	1	8.367
Ontario$_2$	1	4	1	10	23	6	-	-	-	8.511
Ohio	-	10	3	15	48	15	1	-	-	8.630
Michigan$_3$	-	28	1	42	62	41	1	-	-	8.514
Indiana	-	3	2	19	45	22	3	-	-	8.957
Illinois	-	5	-	14	8	4	-	-	-	8.194
Michigan$_4$	2	108	11	34	18	4	1	-	-	6.854
Wisconsin	2	194	11	56	76	57	1	-	-	7.553
Ontario$_5$	1	80	3	16	16	10	4	-	-	7.092
Missouri	-	22	3	7	8	2	-	-	-	7.167
Minnesota	2	142	7	46	19	13	-	-	-	6.900
Iowa	-	25	3	7	2	2	-	-	-	6.795
Manitoba	1	98	9	25	8	4	-	-	-	6.676
Kansas	-	6	-	2	-	1	-	-	-	6.889
Nebraska	-	3	-	3	-	-	-	-	-	7.000
South Dakota	-	30	1	9	-	-	-	-	-	6.475
North Dakota	-	28	2	10	7	-	1	-	-	7.000
Saskatchewan	2	23	5	-	-	1	-	-	-	6.226
Wyoming	-	6	1	-	-	-	-	-	-	6.143
Colorado	-	3	-	19	10	1	-	-	-	8.182
N	**11**	**834**	**66**	**349**	**402**	**207**	**23**	**2**	**1**	

$_1$ waters tributary to Lake Ontario-St. Lawrence River
$_2$ waters tributary to Lake Erie
$_3$ Lower Peninsula
$_4$ Upper Peninsula
$_5$ waters tributary to Lake Superior

highly significant ($P < 0.001$) negative correlations between the total number of left POM pores with increases in latitude and longitude.

Specimens from Wisconsin identified as scaled possess both high and low POM pore counts (Table **22**). Scaled and unscaled specimens from throughout the examined range, also possess high or low counts (Table **22**). Within samples, left and right POM pore counts are almost always similar.

A Mann-Whitney *U*-test reveals significant differences between habitat in the total number of left POM pores among all scaled darters (N = 240 (river = 164 ($\bar{x}$ = 8.02 pores, SD = 1.69): lake = 76 ($\bar{x}$ = 6.97 pores, SD = 1.28)), Mann-Whitney *U*-test statistic = 4003.5; $P < 0.001$). Significant differences between habitats was also observed for unscaled darters (N = 1215 (river = 833 ($\bar{x}$ = 7.54 pores, SD = 1.53): lake = 382 ($\bar{x}$ = 6.79 pores, SD = 1.28)), Mann-Whitney *U*-test statistic = 117229.5; $P < 0.001$).

The Mann-Whitney test reveals significant differences in the total number of left POM pores between habitats for the entire sample (N = 2111 (1562 = river ($\bar{x}$ = 7.80 pores): 549 = lake ($\bar{x}$ = 6.89 pores)), Mann-Whitney *U*-test statistic = 293045.5; $P < 0.001$). Forty-eight of the sixty lacustrine samples are, however, from Michigan, Wisconsin, Manitoba and northern Ontario. The majority of these drain into Lake Superior, Hudson Bay or the Upper Mississippi River. The Pearson product moment correlation coefficient shows that the occurrence of lakes and latitude are highly correlated ($P < 0.001$). Results of the Mann-Whitney test for all states and provinces are statistically significant as are all results for individual drainages

Table 22: Frequency distribution of the number of left preoperculomandibular pores among 138 scaled specimens from Wisconsin, all 240 scaled specimens and 1215 unscaled *Etheostoma nigrum*.

Scaled - Wisconsin

Value	Frequency	Percent
6	57	41.3
7	7	5.1
8	16	11.6
9	27	19.6
10	23	16.7
11	8	5.8
Totals	**138**	**100.0**

Mean 7.826
SD 1.755

Scaled - Entire sample

Value	Frequency	Percent
6	99	41.3
7	11	4.6
8	46	19.2
9	42	17.5
10	33	13.8
11	8	3.3
12	1	0.4
Totals	**240**	**100.0**

Mean 7.696
SD 1.645

Unscaled - Entire sample

Value	Frequency	Percent
5	13	1.1
6	607	50.0
7	46	3.8
8	213	17.5
9	227	18.7
10	103	8.5
11	5	0.4
13	1	0.1
Totals	**1215**	**100.0**

Mean 7.303
SD 1.495

(Table 23). Differences between the number of POM pores for Wisconsin scaled and unscaled riverine populations are large. The greatest difference between habitat and the total number of left POM pores is in Ontario, especially in the far north (Hudson Bay drainage). The Mann-Whitney tests suggest that the number of POM pores is associated with habitat differences. Figure 28 shows the total number of left POM pores as a percent of the lake and river samples.

The modal number of POM pores confined solely to the opercle is six on both the left ($\bar{x}$ = 6.059; SD = 0.282) and right ($\bar{x}$ = 6.068; SD = 0.290) sides. The range on both the left and right sides is from five to eight pores. No geographically interpretable trend is evident for this variable. Correlations between the number of pores confined to the opercle and latitude and longitude are non-significant.

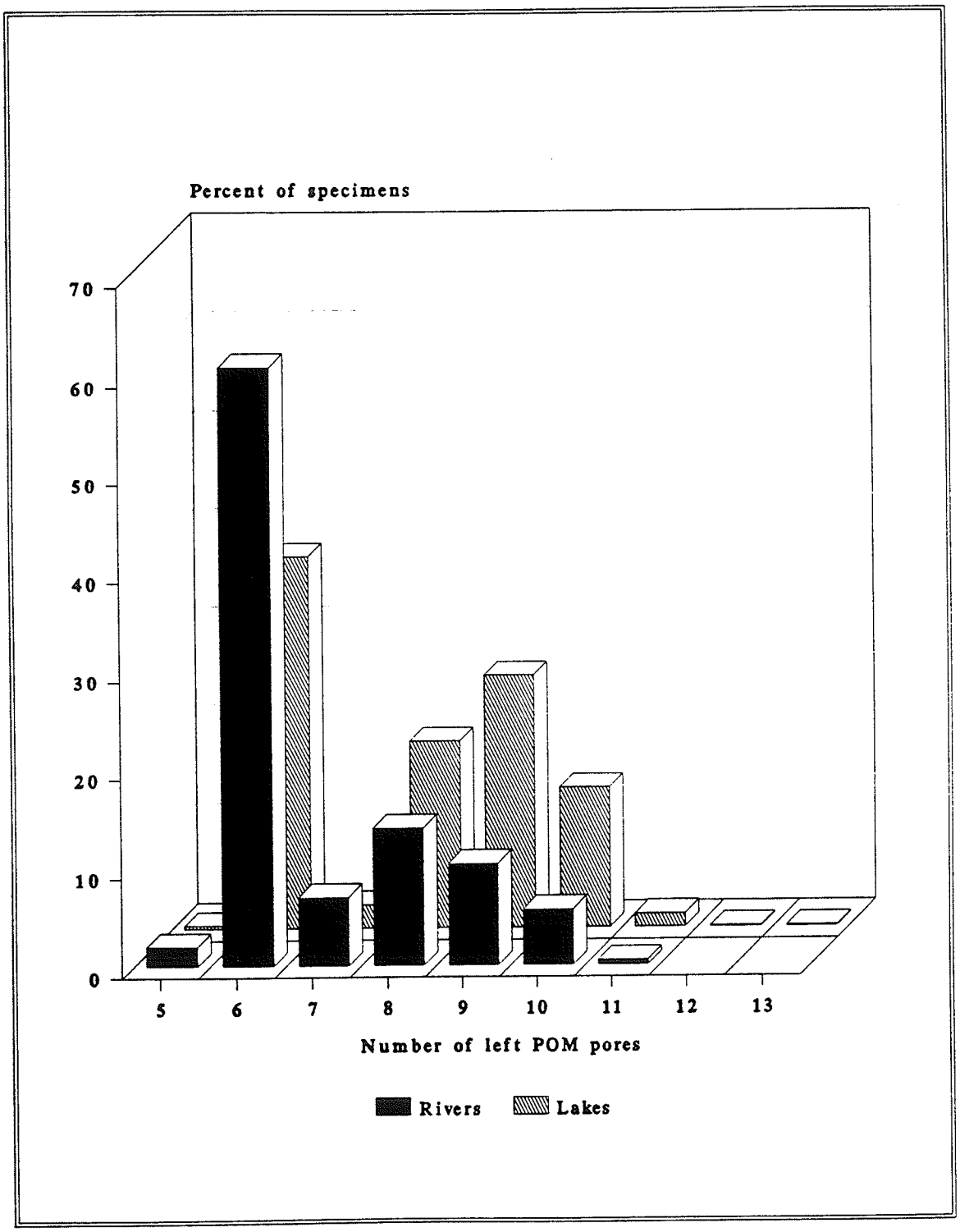
A POM system with pores confined to the opercle is most common and is the mode of all *E. nigrum* examined. This is found on 887 specimens. An interrupted POM system is found on 835 specimens. An uninterrupted POM system with more than six pores is found on 184, mainly southern, specimens. The remaining 205 specimens possess POM systems which are different on the left and right sides and are combinations of interrupted, uninterrupted or with pores occurring only on the opercle. There is a trend for an uninterrupted POM pore system south of the Lower Great Lakes, mainly within the Ohio River system.

Specimens with a POM system that is different on both sides of the head are most common in (number of specimens in parentheses) Wisconsin (45), Ontario (43) and Minnesota (39). Lesser numbers are found in Manitoba (19), Indiana, Michigan

Table 23: Comparison of the total number of left preoperculomandibular pores within samples of *Etheostoma nigrum* collected from lakes and rivers in four states or provinces and within four major drainages. Wisconsin samples are also divided into scaled, unscaled and intergrades.

	Rivers			Lakes			Mann-Whitney *U*-test statistic	
Location	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**		
Ontario	200	8.37	1.60	200	6.80	1.41	9959.5	(P < 0.001)
Manitoba	85	6.86	1.20	60	6.42	0.94	2054.5	(P = 0.0163)
Michigan	199	8.00	1.55	154	7.26	1.38	11106.0	(P < 0.001)
Wisconsin	352	7.74	1.68	55	6.33	0.92	5255.0	(P < 0.001)
Scaled	108	8.16	1.75	30	6.63	1.16	861.5	(P < 0.001)
Intergrade	105	8.31	1.50	11	5.91	0.30	125.0	(P < 0.001)
Unscaled	139	6.99	1.46	14	6.00	0.00	630.0	(P = 0.0094)
Mississippi drainage	348	7.19	1.44	58	6.45	1.01	7307.5	(P = 0.0002)
Lake Superior drainage	61	7.44	1.66	69	6.78	1.42	1654.5	(P = 0.0162)
Hudson Bay drainage (Manitoba)	156	6.87	1.28	122	6.48	1.06	8066.5	(P = 0.0081)
Hudson Bay drainage (Ontario)	12	8.17	1.53	44	6.09	0.60	74.0	(P < 0.001)

Figure 28: Frequency histogram of the total number of left preoperculomandibular pores from the total sample of 2111 *Etheostoma nigrum* divided into lake and river samples. The number of pores is given as a percent of sample.


Percent of specimens
70
60
50
40
30
20
10
0
5
6
7
8
9
10
11
12
13
Number of left POM pores
Rivers
Lakes

and Ohio (10 each), Missouri (9), Quebec and North Dakota (4 each), Nebraska, South Dakota, Tennessee and Kentucky (2 each) and Iowa, Pennsylvannia, Wyoming and Saskatchewan (1 each). For the size of the sample (Table **10**), Michigan possesses a disproportionately low number of specimens with a POM pore number that is asymmetric. Twenty-five of 240 scaled darters possess this trait; 12 of them from the 138 Wisconsin scaled specimens.

Scales above the Lateral Line

The modal number of scales above the lateral line counted at the origin of the spiny dorsal fin is four ($\bar{x}$ = 3.788; SD = 0.656). Many Wisconsin samples, specimens from the Spring River, Kansas, and one sample near Thunder Bay, Ontario, possess a mode of five. Samples to the north and west of Lake Superior often possess a mode of three scales. Samples with a mode of two scales are generally confined to the most northern areas of the range (Fig. **29a,b**). Correlation analysis using the 152 one-degree latitude and longitude grids results in a highly significant negative correlation with latitude ($r = -0.5276$; $P < 0.001$). The individual specimen matrix and the sample means also produced highly significant negative correlations with latitude. The number of scales above the lateral line varies in a geographically interpretable manner.

The modal number of scales above the lateral line counted at the posterior insertion of the spiny dorsal fin is five ($\bar{x}$ = 5.287; SD = 0.567). Scales above the lateral line at this position range from a low of three (one specimen) to a high of seven scales. Little information on geographic variation can be gained from this

Figure 29a: Modal number of scales above the lateral line counted from the anterior dorsal spine in samples of *Etheostoma nigrum*. ◆ = 2, ■ = 3, ● = 4 and ★ = 5 scales.

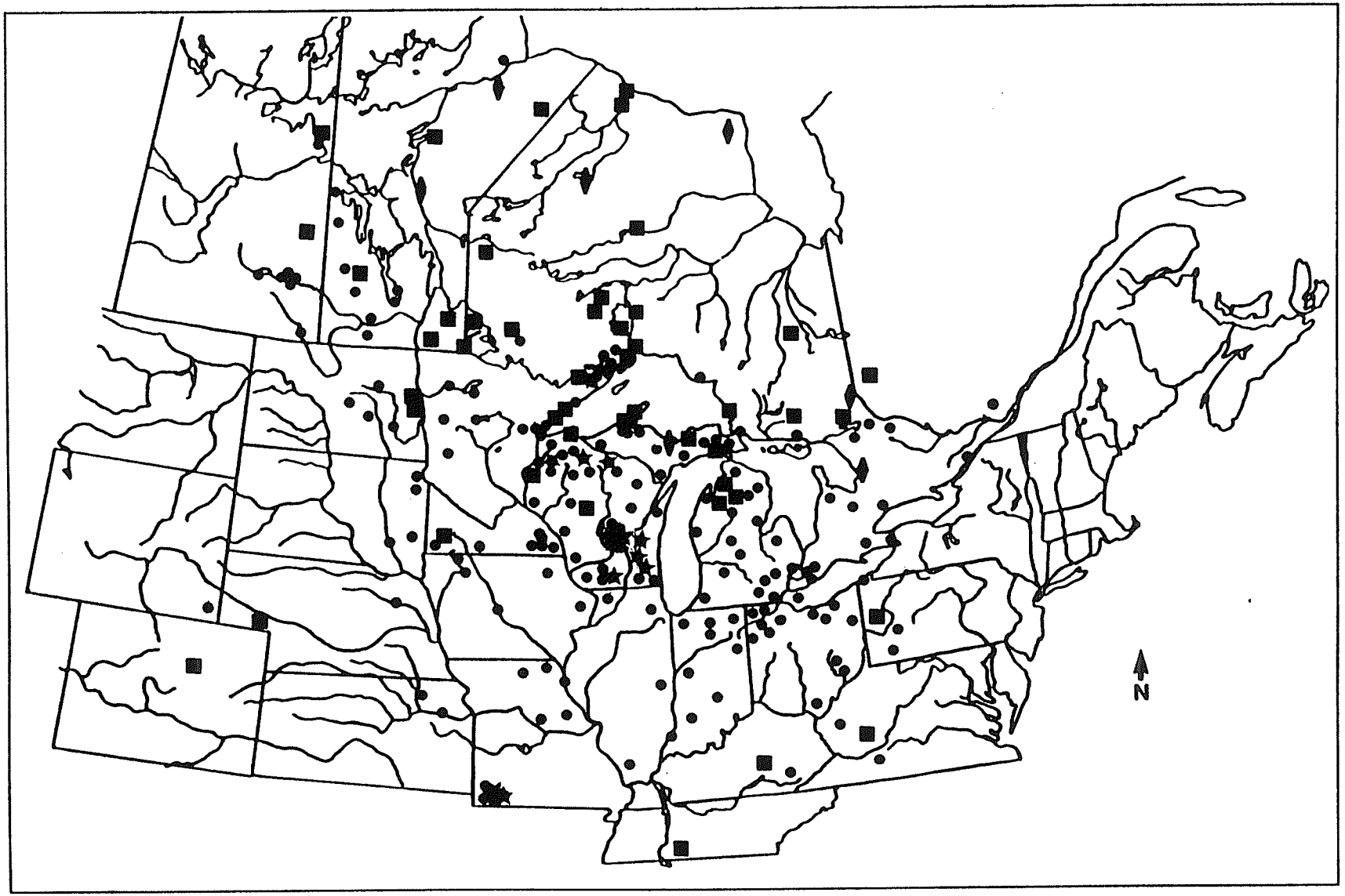
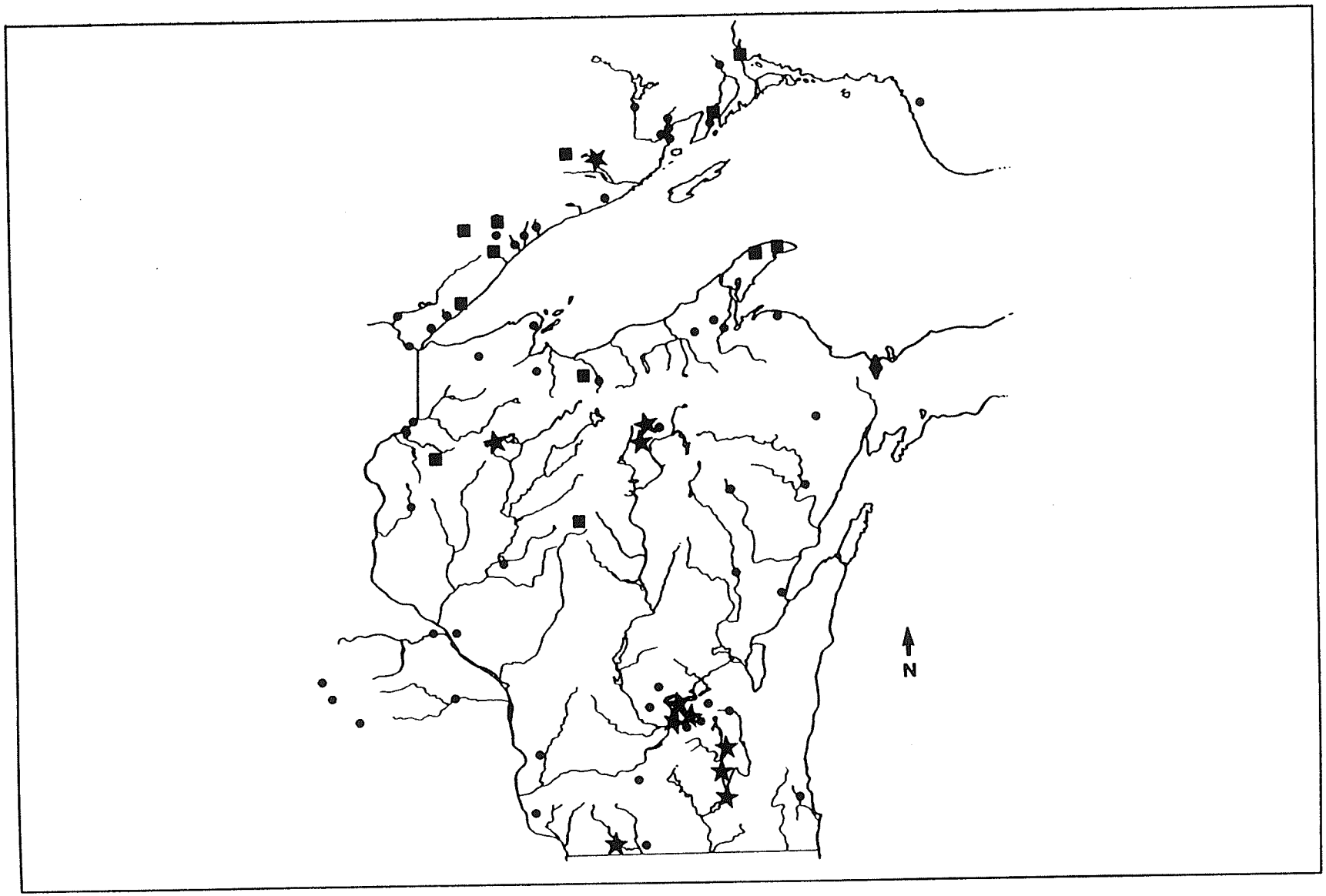

N

Figure 29b: Modal number of scales above the lateral line counted from the anterior dorsal spine in samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario. ◆ = 2, ■ = 3, ● = 4 and ★ = 5 scales.


N

variable due to its irregular distribution among samples. Correlation analysis does not produce any significant correlations with latitude or longitude and this variable.

Scales below the Lateral Line

The modal number of scales below the lateral line is seven ($\bar{x}$ = 7.166; SD = 0.804) with a range from five to ten scales. The number of scales below the lateral line is not geographically informative as it does not vary in a geographically interpretable manner. No significant correlations occur with latitude or longitude.

Lateral Line Scale Counts

Lateral line scale counts vary from a low of 38 scales among samples from both Ohio and Iowa to a high of 61 on a Michigan specimen. The modal number of lateral line scales for the entire sample is 48 ($\bar{x}$ = 47.625; SD = 3.335). There is a clinal trend for lateral line scales to increase from the southeast to the northwest (Fig. **30a,b**). Correlations between the number of lateral line scales with latitude (r = 0.2926) and longitude (r = 0.2157) are highly significant ($P < 0.001$) when all specimens are used. Using the 152 one-degree latitude and longitude grids results in even higher correlation coefficients for both latitude (r = 0.4270; $P < 0.001$) and longitude (r = 0.3211; $P < 0.001$). Utilizing sample means results in approximately similar high correlation coefficients (r = 0.3574; $P < 0.001$). Two areas appear as concentrations where the lateral line scale count is 45 or less. These areas are the Sheyenne River, North Dakota, several smaller United States and Canadian tributaries of the Red River and southwestern Wisconsin tributaries to the Wisconsin or Mississippi River. Specimens from several areas in Wisconsin and northern Michigan

Figure 30a: Mean number of scales in the lateral line in samples of *Etheostoma nigrum*. ■ = < 44.5, + = 44.6-46.5, ● = 46.6-48.5, ★ = 48.6-50.5 and ◉ = 50.6+ scales.

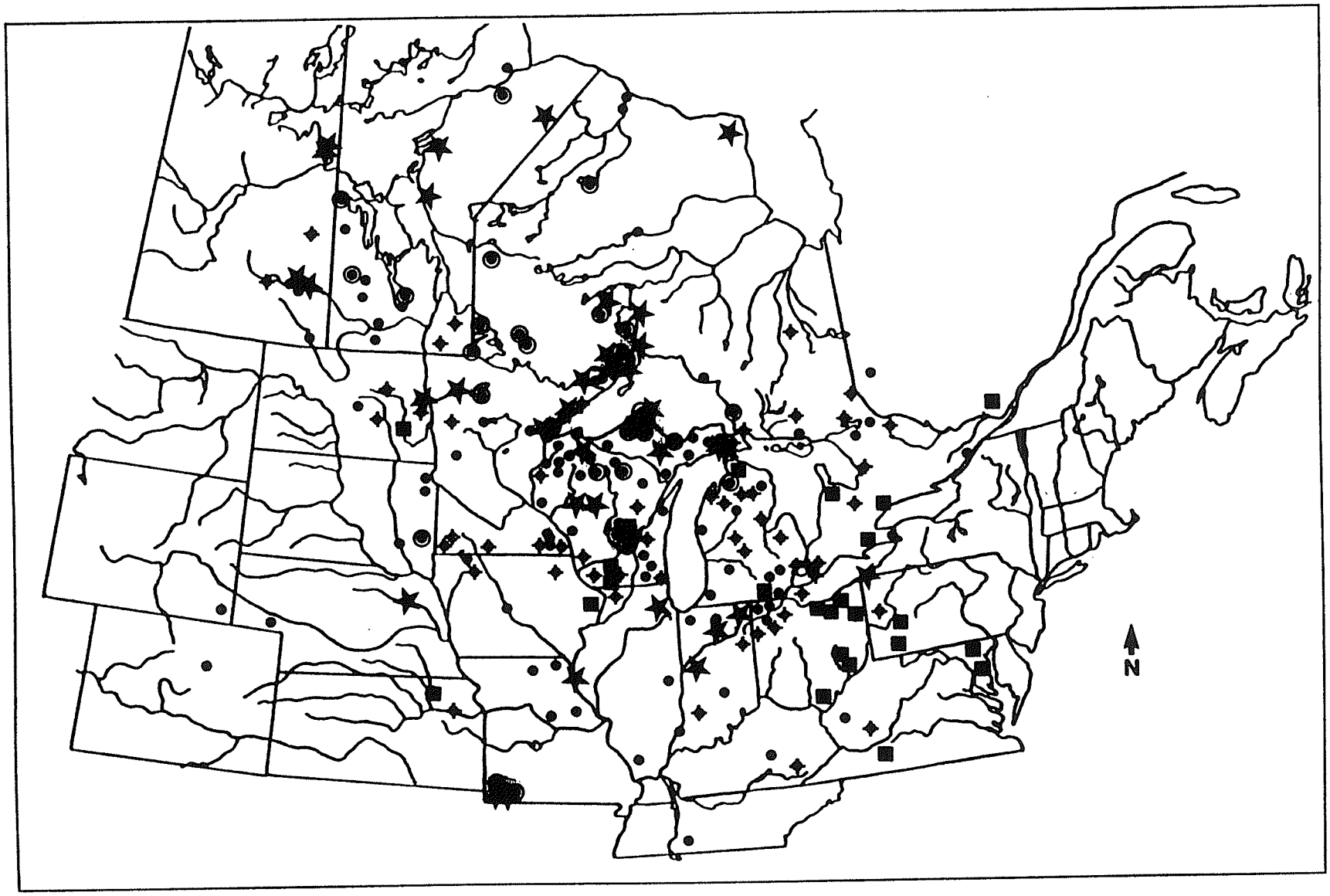
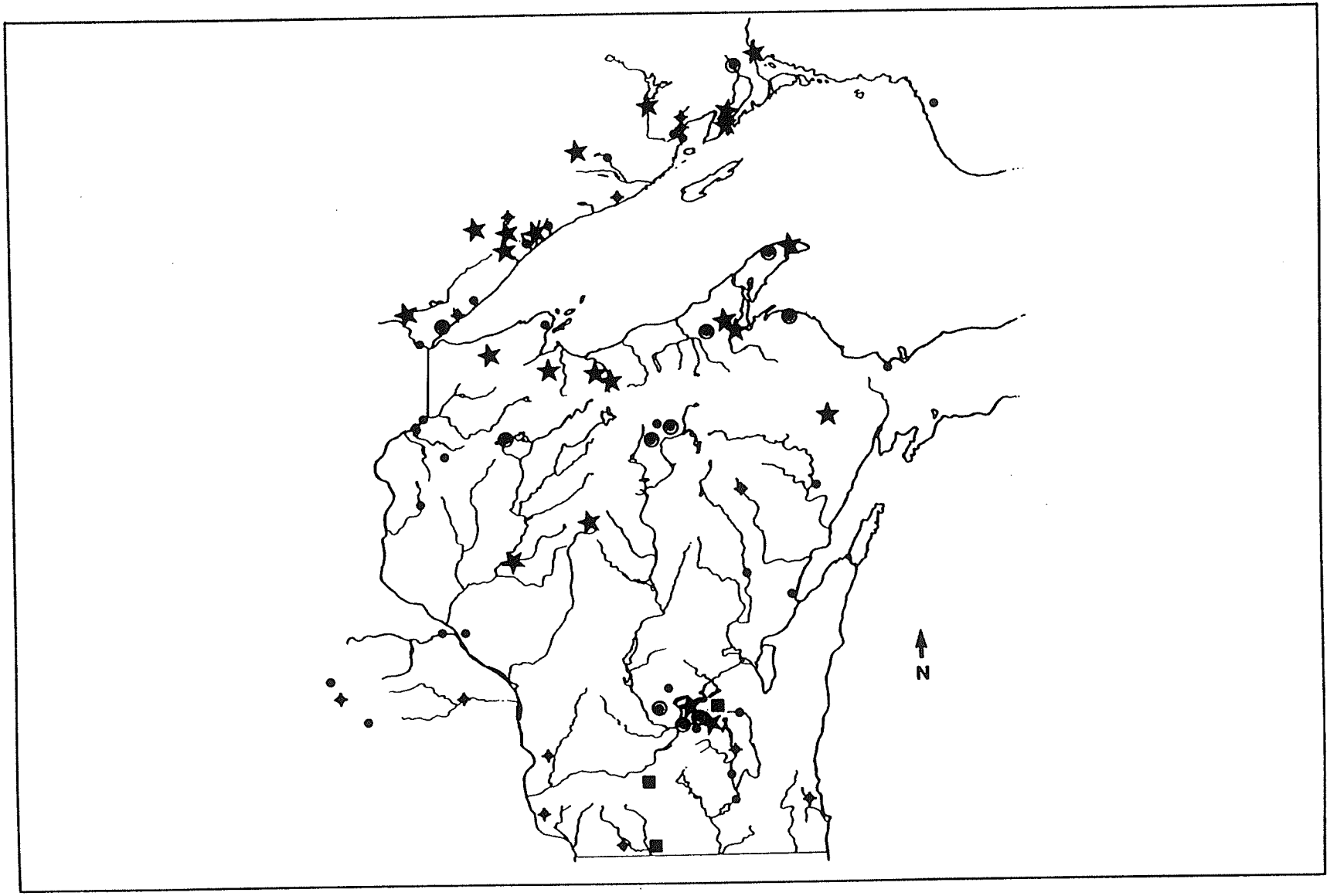

N

Figure 30b: Mean number of scales in the lateral line in samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario. ■ = < 44.5, + = 44.6-46.5, ● = 46.6-48.5, ★ = 48.6-50.5 and ⊙ = 50.6+ scales.


N

possess a higher number of lateral line scales than the regional average. Many samples north of 49°00' N latitude possess lateral line scale counts of 50 or greater. Samples immediately south of the Great Lakes generally have lateral line counts less than 50 but greater than 46. Lateral line counts may provide some information which may be interpretable in a biogeographic manner.

Completeness of the Lateral Line

The mean score for the lateral line condition is 1.824 (SD = 0.775) which, in this study, signifies a slightly incomplete lateral line (three of fewer unpored scales). Most samples possessing a very incomplete lateral line are found in tributaries of, or near, Lake Superior. The majority of *E. nigrum* classed as scaled (63.3%) possess a complete lateral line. Scaled specimens possess complete lateral lines more often than do unscaled specimens and exhibit fewer incomplete lateral lines, regardless of habitat (Table **24**). Scaled and unscaled specimens from lakes tend to have more incomplete lateral lines than specimens from rivers (Table **24**). Complete lateral lines are present in many of the most western samples (Fig. **31a,b**). Overall, 852 specimens possess a complete lateral line, 778 have a slightly incomplete lateral line and 481 possess a lateral line which is very incomplete and/or interrupted. The condition of the lateral line exhibits a highly significant positive correlation with latitude ($r = 0.2764$; $P < 0.001$) only with the matrix of individuals. The completeness of the lateral line varies in a meaningful geographic pattern within some areas and among some samples and may provide information on the biogeographic history of some populations of *E. nigrum*.

Table 24: Completeness of the lateral line among *Etheostoma nigrum* divided by the entire sample and the scaled and unscaled sample. Samples are divided by riverine and lacustrine habitats. Numbers in parentheses are percent of specimens in that habitat with that lateral line type.

Entire Sample	**River**	**Lake**
N = 2111	N = 1562 (74.0)	N = 549 (26.0)
Lateral line condition	1 = 743 (47.6)	1 = 109 (19.9)
	2 = 581 (37.2)	2 = 197 (35.9)
	3 = 238 (15.2)	3 = 243 (44.2)
	100%	100%

Scaled Sample	**River**	**Lake**
N = 240	N = 165 (68.8)	N = 75 (31.2)
Lateral line condition	1 = 126 (76.4)	1 = 26 (34.6)
	2 = 27 (16.4)	2 = 17 (22.7)
	3 = 12 (7.2)	3 = 32 (42.7)
	100%	100%

Unscaled sample	**River**	**Lake**
N = 1215	N = 833 (68.6)	N = 382 (31.4)
Lateral line condition	1 = 315 (37.8)	1 = 67 (17.5)
	2 = 367 (44.1)	2 = 124 (32.5)
	3 = 151 (18.1)	3 = 191 (50.0)
	100%	100%

1 = complete (all scales pored)
2 = incomplete (three of fewer unpored scales)
3 = very incomplete (more than three unpored scales and/or interrupted)

Figure 31a: Mode of the lateral line condition for samples of *Etheostoma nigrum*. ● = complete, ■ = slightly incomplete (1-3 unpored scales) and ★ = very incomplete (more than 3 unpored scales) and/or interrupted.

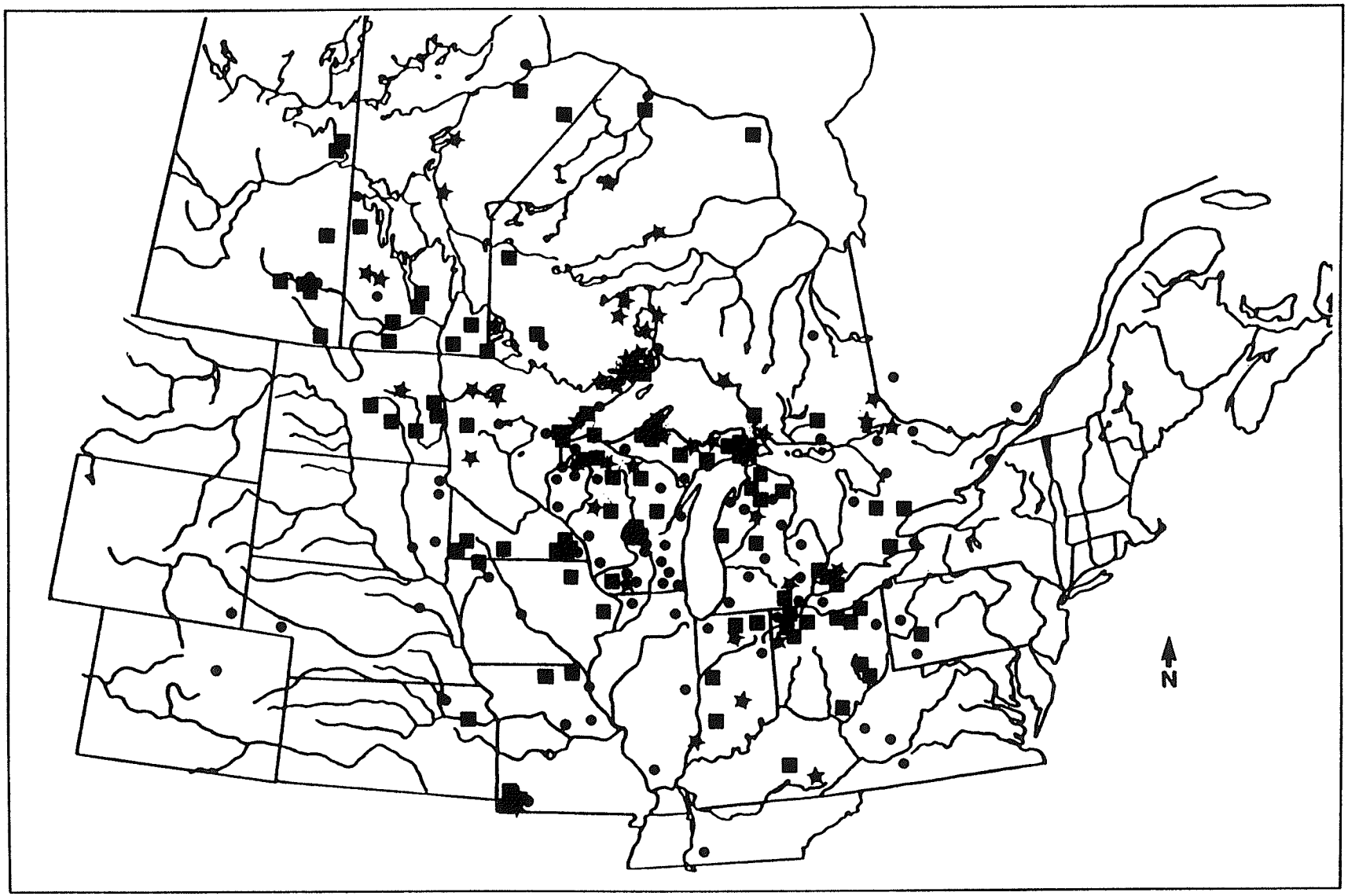
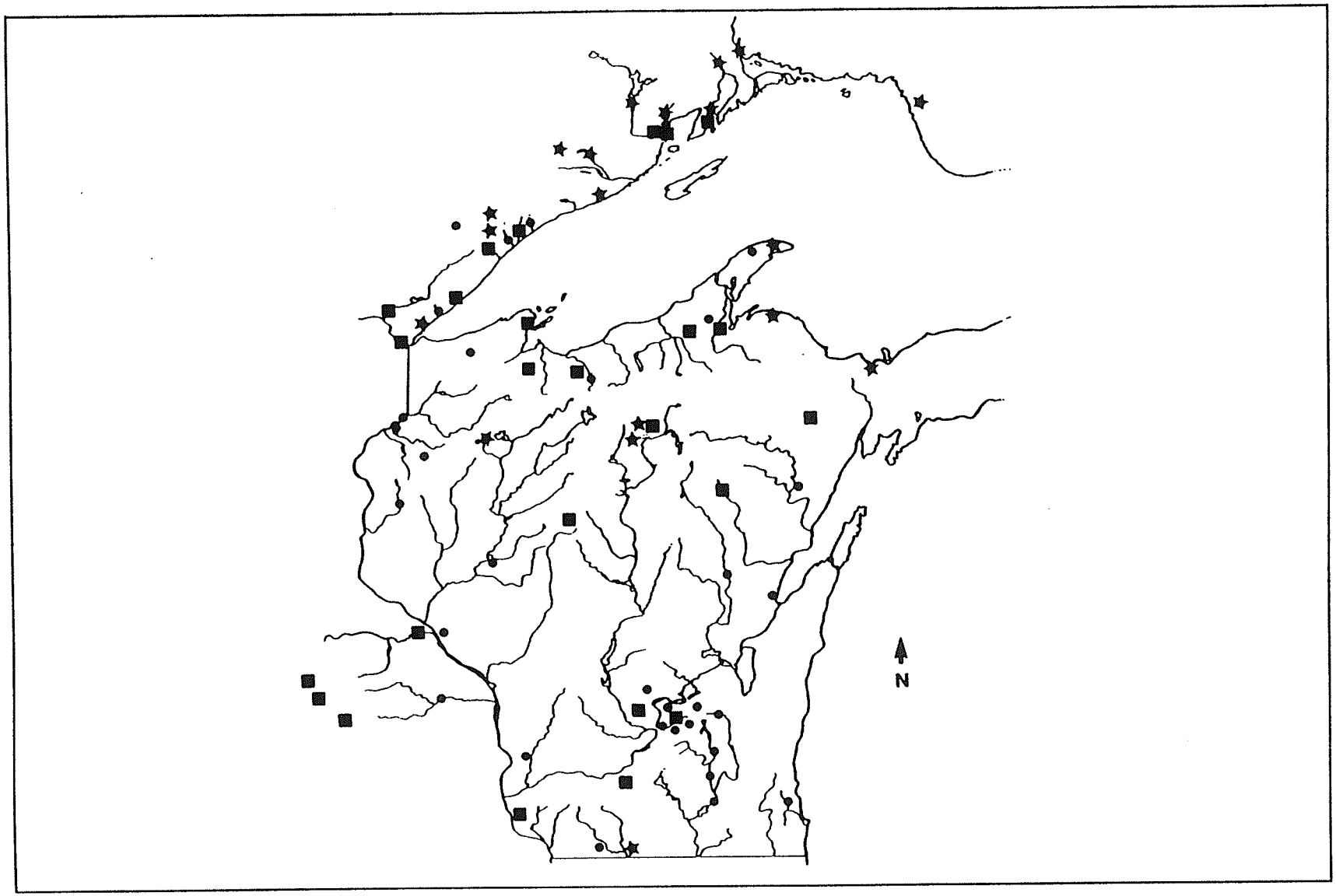

N

Figure 31b: Mode of the lateral line condition for samples of *Etheostoma nigrum* from Wisconsin, Upper Peninsula Michigan, western Minnesota and northwestern Ontario. ● = complete, ■ = slightly incomplete (1-3 unpored scales) and ★ = very incomplete (more than 3 unpored scales) and/or interrupted.


N

Other Variables

Variation in snout-type (*e.g.*, intermediate, blunt, etc.) does not vary significantly between lake and river habitats ($t_P = 0.46$). Most samples exhibit some variation in snout-type. Table **25** shows, however, that a higher than average number of specimens with subconical snouts occur in the Lake Michigan drainage. The Lake Michigan drainages also exhibits a lesser number of specimens possessing the intermediate snout-type compared to other drainages. Most samples have a mean snout score ($\bar{x} = 2.985$; SD = 1.0) which falls very near the intermediate score value (3) assigned for this study. Snout-type scores exhibit a normal distribution among males and females. Paired "*t*" tests between males and females divided by snout-types (1-5) show that statistically significant differences occur only at type 2 (transitional between blunt and intermediate) ($t_P < 0.001$) and type 4 (transitional between intermediate and subconical) ($t_P < 0.044$). Overall, there are no significant differences in snout-type between males and females. Table **26** presents the percent of the sexed samples divided by snout-type score and major drainage.

A 5x11 (snout-type by drainages) contingency table was constructed to determine if drainage and snout-type were independent. I used the data presented in Table **25**. Results of two tests, using all eleven drainages, as well as a smaller set using only the Great Lakes, resulted in significant chi-square levels for only the Lake Michigan drainage ($P < 0.01$). Snout-types are therefore statistically different in the Lake Michigan drainage and specimens from these waters tend to have more subconical snouts. The Lake Michigan drainage samples are comprised of 146 males,

Table 25: Number of specimens in drainage (N), percent of total sample from drainage with that snout-type score, number of specimens from drainage with that snout-type (in parentheses) and percent of that snout-type score in each drainage for 2111 specimens of *Etheostoma nigrum* (1=blunt, 3=intermediate, 5=subconical).

		Snout profile score				
Drainage	**Drainage N**	**1**	**2**	**3**	**4**	**5**
Mississippi River		17.9	20.0	17.8	19.2	28.7
number of specimens	405	(33)	(71)	(187)	(68)	(46)
% snout score in drainage		8.1	17.5	46.2	16.8	11.4
Ohio River		15.2	8.5	6.2	2.8	0
number of specimens	133	(28)	(30)	(65)	(10)	(0)
% snout score in drainage		21.1	22.6	48.9	7.5	0
Missouri River		1.1	4.8	7.0	5.6	9.8
number of specimens	129	(2)	(17)	(74)	(20)	(16)
% snout score in drainage		1.6	13.2	57.4	15.5	12.4
Atlantic Slope		0	2.5	0.9	0	0
number of specimens	19	(0)	(9)	(10)	(0)	(0)
% snout score in drainage		0	47.4	52.6	0	0
Hudson Bay (ON)		2.2	2.8	3.5	1.4	0
number of specimens	56	(4)	(10)	(37)	(5)	(0)
% snout score in drainage		7.1	17.9	66.1	8.9	0
Hudson Bay (MB-SK)		13.0	13.0	14.3	11.8	9.1
number of specimens	278	(24)	(46)	(151)	(42)	(15)
% snout score in drainage		8.6	16.5	54.3	15.1	5.4
Lake Ontario*		2.2	1.1	6.4	1.7	0.6
number of specimens	82	(4)	(4)	(67)	(6)	(1)
% snout score in drainage		4.9	4.9	81.7	7.3	1.2
Lake Erie		14.1	13.5	7.2	7.0	0.6
number of specimens	176	(26)	(48)	(76)	(25)	(1)
% snout score in drainage		14.8	27.3	43.2	14.2	0.6
Lake Huron		3.8	8.5	6.0	4.8	2.4
number of specimens	121	(7)	(30)	(63)	(17)	(4)
% snout score in drainage		5.8	24.8	52.1	14.0	3.3

Table 25: Continued.

Drainage	Drainage N	Snout profile score 1	2	3	4	5
Lake Michigan		22.3	15.2	9.0	24.2	24.4
number of specimens	316	(41)	(54)	(95)	(86)	(40)
% snout score in drainage		13.0	17.1	30.1	27.2	12.7
Lake Superior		8.2	10.1	21.7	21.4	25.0
number of specimens	396	(15)	(36)	(228)	(76)	(41)
% snout score in drainage		3.8	9.1	57.6	19.2	10.4
Total sample %		8.7	16.8	49.9	16.8	7.8
Total specimens	2111	(184)	(355)	(1053)	(355)	(164)

* includes St. Lawrence River tributaries

Table 26: Number of specimens examined by major drainage and percent of samples of *Etheostoma nigrum* divided by sex (N=Males:Females) and classified according to snout-type (1=blunt, 3=intermediate, 5=subconical; 2 and 4 are intermediate categories).

Location	N	Males					Females				
		1	2	3	4	5	1	2	3	4	5
	♂:♀										
St. Lawrence[1]	34:42	6	9	73	9	3	5	2	86	7	0
Mississippi	185:194	9	16	48	15	12	7	19	45	19	10
Hudson Bay[2]	19:30	16	16	52	16	0	3	20	70	7	0
Ohio	56:70	20	13	62	5	0	24	30	39	7	0
Missouri	56:63	2	11	62	18	7	2	14	54	14	16
Atlantic	7:12	0	71	29	0	0	0	33	67	0	0
Hudson Bay[3]	140:119	8	19	52	16	5	10	16	56	13	5
Erie	90:82	15	32	39	13	1	15	23	47	15	0
Superior	173:185	4	10	58	23	5	3	7	61	18	11
Huron	54:58	7	31	52	6	4	4	17	53	22	4
Michigan	146:153	13	18	31	26	12	14	17	28	28	13
TOTAL	**N=1968**	9.2	17.7	49.7	16.9	6.6	8.7	16.2	50.1	17.2	7.8

[1] Includes St. Lawrence River and Lake Ontario tributaries.
[2] Hudson Bay-James Bay Ontario drainage.
[3] Hudson Bay Manitoba-Saskatchewan drainage.

153 females and 17 specimens that cannot be sexed.

Only two samples of *E. nigrum* possess specimens with a darkly pigmented suborbital bar (Neebing-McIntyre River, Ontario, and Temperance River, Minnesota). Approximately half the specimens from these samples possess this trait. Therefore, the distribution of this trait is not geographically informative for describing variation in *E. nigrum*. This trait, as noted earlier, seems valuable for describing *E. olmstedi*.

Specimens with a bifurcation of the distal most portion of the soft dorsal rays are found in 61 samples of *E. nigrum*. The appearance of the double bifurcation is sporadic in all samples and often appears only on the largest specimens. Therefore, this trait does not vary in a geographically informative manner and may be a function of size.

Correlation with Abiotic Characters

Most meristic characters are not closely correlated with any abiotic characters examined when the distribution of meristic characters is overlain with maps of the abiotic variables. Only the number of scales in the lateral line increases from the southeast to the northwest, with a decrease in the number of degree-days above 5.6°C and/or length of growing season (Phillips and McCulloch 1972; Court 1974).

Morphometric Data

Three characters were examined for morphometric analysis. Principal component analysis suggests that morphometric variables are relatively unimportant in describing overall geographic variation in *E. nigrum* except, perhaps, for the predorsal length variation noted above. In reporting morphometric differences

between males and females, it should be noted that 960 of the specimens examined were male, 1008 were female and 143 could not be sexed. None of the variables exhibit any significant correlation with changes in latitude or longitude and none exhibits interpretable geographic variation.

Predorsal length is, on average, 34.04% of standard length (SD = 1.291) with a range of from 25.2% to 43.1%. Predorsal length is not significantly different between males and females.

Pectoral length is, on average, 25.28% of standard length (SD = 1.552). The range is from 18.7% to 30.8%. Pectoral length varies significantly between males and females ($t_P < 0.05$) with males possessing slightly longer fins.

The mean snout length is 5.26% of standard length (SD = 0.509). Snout length ranged from 3.26% to 6.99% of standard length. Snout length is significantly different between males and females ($t_P < 0.005$) although the distribution of snout length is normal for both males and females.

Pearson Product Moment Correlation Coefficients

Many of the Pearson product moment correlation coefficients calculated between variables for 2111 *E. nigrum* were significant. Most of the highly significant correlations (*e.g.*, squamation variables) were previously identified by high weighting on the first or second PC axes and thus provide little useful, new information. The correlation matrix does show the relationship between variables weighted heavily on the first two PC factors. The percentage of an area estimated to be covered with scales and the scale counts themselves are highly positively correlated ($r > 0.85$; P

< 0.001, in all cases). The nape, cheek and breast scale counts are all highly positively correlated with each other ($r > 0.88$; $P < 0.001$, in all cases). Thus, a darter with a high number of cheek and breast scales also typically possesses a high number of nape scales. Since correlations between the number of scales and the percentage estimate of the area scaled on the cheek, nape, breast and opercle are high, the estimated percent of belly scaled is considered to have been accurately estimated and likely to provide information on geographic variation somewhat similar to an actual count. Right and left branchiostegal rays are highly correlated ($r > 0.87$; $P < 0.001$) as are the number of pores on the left and right opercle ($r > 0.84$; $P < 0.001$). The first and second dorsal and anal ray counts are very highly correlated ($r > 0.97$; $P < 0.001$) suggesting there are few specimens in which the last dorsal or anal ray is not or does not appear to be joined to the second last ray. Correlations between other characters were low (always $r < 0.50$) although many are significant ($P < 0.01$) or highly significant ($P < 0.001$) (Table **27**).

Comparison of Scaled and Unscaled *Etheostoma nigrum*

With the meristic and morphometric data analyzed and a suitable definition of a scaled darter, it is possible to compare the two forms. Table **28** shows the means of 21 variables from all scaled and unscaled *E. nigrum* as well as means for samples from Wisconsin and Minnesota. These two states were chosen for comparison because a relatively high number of scaled *E. nigrum* occurs in them. Table **28** suggests that, except for the differences in squamation and lateral line related characters, there are few differences between scaled and unscaled darters. In many

Table 27: Pearson product moment correlation coefficient matrix of meristic and squamation characters (abbreviations as in methods) based on 2111 specimens of *Etheostoma nigrum* from 240 locations in Canada and the United States. Correlations in bold are significant at $P < 0.001$.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. LL	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2. LL1	-.03	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3. LL2	.03	**.46**	*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4. CC	.05	**.32**	**.13**	*	-	-	-	-	-	-	-	-	-	-	-	-	-
5. CP	.04	**.32**	**.12**	**.97**	*	-	-	-	-	-	-	-	-	-	-	-	-
6. OP	**-.14**	**.45**	**.24**	**.41**	**.40**	*	-	-	-	-	-	-	-	-	-	-	-
7. OC	-.06	**.49**	**.28**	**.64**	**.61**	**.85**	*	-	-	-	-	-	-	-	-	-	-
8. NP	.04	**.41**	**.18**	**.79**	**.78**	**.47**	**.66**	*	-	-	-	-	-	-	-	-	-
9. NC	.06	**.41**	**.19**	**.83**	**.81**	**.47**	**.67**	**.97**	*	-	-	-	-	-	-	-	-
10. NCT	-.04	**.43**	**.22**	**.48**	**.47**	**.44**	**.56**	**.64**	**.62**	*	-	-	-	-	-	-	-
11. BP	**.08**	**.37**	**.15**	**.86**	**.85**	**.42**	**.62**	**.89**	**.90**	**.57**	*	-	-	-	-	-	-
12. BC	**.09**	**.37**	**.16**	**.89**	**.86**	**.42**	**.64**	**.88**	**.90**	**.56**	**.97**	*	-	-	-	-	-
13. BELLYP	.01	**.42**	**.13**	**.27**	**.28**	**.35**	**.38**	**.36**	**.35**	**.36**	**.35**	**.34**	*	-	-	-	-
14. DS	**.09**	.01	-.04	.05	.05	.02	.02	.04	.05	.01	.05	.06	.05	*	-	-	-
15. DR1	**.15**	-.04	-.06	.03	.01	**-.14**	-.06	-.03	-.02	-.03	.01	.01	**.09**	.03	*	-	-
16. AR1	**.17**	**-.08**	-.06	.02	.01	**-.13**	-.05	-.02	-.01	-.01	.01	.02	**.11**	**.07**	**.37**	*	-
17. LBR	**.16**	.01	-.04	**.08**	**.08**	.03	**.09**	**.10**	**.09**	**.12**	**.10**	**.09**	**.21**	**.07**	**.13**	**.20**	*

Table 27: Continued.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
18. RBR	.15	.02	-.03	.09	.08	.05	.11	.12	.11	.14	.12	.11	.21	.08	.12	.18	.87
19. LPOP	-.19	.17	.12	.02	.05	.32	.24	.12	.11	.17	.04	.02	.02	-.06	-.18	-.25	-.15
20. LOPOP	.10	.06	.02	.11	.12	.03	.05	.08	.08	.02	.09	.08	.01	-.01	.02	-.01	.04
21. RPOP	-.16	.16	.13	.01	.03	.30	.21	.12	.11	.16	.04	.03	.02	-.04	-.17	-.26	-.15
22. ROPOP	.13	.07	.01	.09	.11	.02	.03	.06	.07	.02	.09	.09	.03	.03	.02	.02	.02
23. INUN	.08	-.06	-.06	.01	.01	-.16	-.13	-.05	-.04	-.07	-.01	-.01	-.03	.03	.09	.15	.06
24. LPOS	-.08	.13	.07	.13	.14	.21	.23	.18	.18	.23	.14	.14	.15	-.04	.01	-.02	.09
25. LANT	.04	.01	.02	-.01	.01	.01	.01	-.03	-.02	-.01	-.01	-.01	.01	.02	-.02	-.01	-.01
26. RPOS	-.10	.15	.06	.16	.17	.23	.25	.20	.20	.23	.15	.16	.14	-.03	.01	-.01	.09
27. RANT	.06	.01	.01	.01	.01	.01	.01	-.01	-.01	.01	.01	-.01	.02	.03	.01	-.01	.01
28. PECR	.24	-.02	-.03	.04	.04	-.03	.01	.05	.05	-.01	.06	.05	.08	.06	.14	.14	.21
29. LLSTAT	.14	-.16	-.12	-.11	-.11	-.21	-.21	-.19	-.17	-.18	-.14	-.13	-.19	.07	.08	.04	-.04
30. BLL	-.04	.35	.42	.06	.05	.22	.22	.12	.12	.14	.08	.09	.06	-.02	-.05	-.04	-.01

Character	18	19	20	21	22	23	24	25	26	27	28	29	30
18. RBR	*	-	-	-	-	-	-	-	-	-	-	-	-
19. LPOP	-.15	*	-	-	-	-	-	-	-	-	-	-	-
20. LOPOP	.04	.21	*	-	-	-	-	-	-	-	-	-	-
21. RPOP	-.15	.84	.10	*	-	-	-	-	-	-	-	-	-
22. ROPOP	.02	.07	.34	.20	*	-	-	-	-	-	-	-	-
23. INUN	.05	.48	-.01	-.49	.05	*	-	-	-	-	-	-	-
24. LPOS	.10	.21	.04	.20	.01	-.09	*	-	-	-	-	-	-
25. LANT	-.02	.06	.04	.06	.01	-.03	-.29	*	-	-	-	-	-
26. RPOS	.09	.23	.05	.19	.01	-.07	.70	-.25	*	-	-	-	-
27. RANT	.01	.04	.04	.05	.03	-.02	-.23	.43	-.30	*	-	-	-
28. PECR	.19	-.06	.08	-.05	.07	.07	.05	.01	-.03	.01	*	-	-
29. LLSTAT	-.05	-.24	-.04	-.20	.01	.13	-.15	-.02	-.15	.01	.01	*	-
30. BLL	-.01	.11	.03	.13	.02	-.08	.15	.01	.14	.02	.01	-.07	*

Table 28: Comparison of the means (SD in parentheses) of meristic characters among scaled and unscaled *Etheostoma nigrum* from the examined range (total scaled sample or total unscaled sample), and geographic subsets from Wisconsin and Minnesota. Character abbreviations are as in methods. Bilateral data are presented for the left side only due to close agreement between sides.

Character	Location					
	SCALED			UNSCALED		
	Scaled Sample	Wisconsin	Minnesota	Unscaled Sample	Wisconsin	Minnesota
LL	48.23	48.36	48.52	47.85	47.83	47.56
	(3.18)	(3.29)	(3.47)	(3.45)	(3.18)	(2.38)
LL1	4.38	4.49	3.90	3.53	3.76	3.69
	(0.54)	(0.54)	(0.44)	(0.64)	(0.60)	(0.54)
LL2	5.50	5.56	5.00	5.19	5.34	5.04
	(0.54)	(0.55)	(0.32)	(0.56)	(0.58)	(0.51)
CC	28.75	29.71	30.62	0.0	0.0	0.0
	(11.9)	(12.2)	(11.7)	(0.0)	(0.0)	(0.0)
OC	21.47	22.08	18.67	8.57	8.10	9.06
	(5.40)	(5.27)	(5.88)	(4.16)	(3.98)	(3.11)
NC	44.67	46.18	40.57	0.0	0.0	0.0
	(7.51)	(7.21)	(7.18)	(0.0)	(0.0)	(0.0)
NCT	4.16	4.18	4.86	0.37	0.71	0.89
	(1.10)	(0.79)	(0.96)	(0.37)	(1.41)	(1.85)
BC	88.55	90.63	86.86	0.0	0.0	0.0
	(20.0)	(21.0)	(14.9)	(0.0)	(0.0)	(0.0)
BELLYP	100.0	100.0	100.0	72.80	84.64	84.38
	(0.0)	(0.0)	(0.0)	(26.9)	(20.4)	(16.5)
BLL	7.31	7.75	6.57	7.04	7.10	6.97
	(0.84)	(0.80)	(0.87)	(0.76)	(0.70)	(0.73)
DS	8.92	8.93	9.14	8.84	9.06	8.75
	(0.58)	(0.59)	(0.57)	(0.59)	(0.49)	(0.51)
DR1	11.73	11.83	11.81	11.76	11.88	11.96
	(0.85)	(0.85)	(0.51)	(0.74)	(0.62)	(0.58)
AR1	8.29	8.25	8.43	8.28	8.42	8.55
	(0.70)	(0.74)	(0.60)	(0.70)	(0.58)	(0.63)
LBR	6.01	6.00	6.00	5.95	5.99	5.99
	(0.09)	(0.0)	(0.0)	(0.24)	(0.11)	(0.09)
LPOP	7.70	7.83	6.81	7.30	6.90	6.75
	(1.65)	(1.75)	(1.17)	(1.50)	(1.42)	(1.22)
LOPOP	6.13	6.12	6.05	6.05	6.02	6.04
	(0.36)	(0.32)	(0.22)	(0.28)	(0.18)	(0.28)
LPOS	2.55	2.63	2.48	2.22	2.19	2.47
	(0.88)	(1.05)	(0.51)	(0.51)	(0.41)	(0.58)

Table 28: Continued.

Character	Location					
	SCALED			UNSCALED		
	Scaled Sample	Wisconsin	Minnesota	Unscaled Sample	Wisconsin	Minnesota
LANT	4.00	3.96	4.00	4.01	4.00	4.01
	(0.53)	(0.64)	(0.0)	(0.27)	(0.11)	(0.21)
PECR	11.88	11.83	11.86	11.83	11.81	11.78
	(0.51)	(0.48)	(0.48)	(0.51)	(0.44)	(0.55)
SNOUT[1]	2.99	2.96	3.19	2.98	3.16	3.02
	(1.07)	(1.10)	(1.29)	(0.97)	(1.05)	(0.83)
LLSTAT[2]	1.55	1.48	2.33	1.97	1.48	2.28
	(0.79)	(0.77)	(0.73)	(0.77)	(0.54)	(0.65)
N	240	138	21	1215	153	113

[1] 1=blunt, 3=intermediate, 5=subconical, 2 or 4=transitional forms

[2] 1=complete, 2=slightly incomplete (3 or fewer unpored scales), 3=incomplete and/or interrupted (more than 3 unpored scales)

instances, however, as the scaled samples from Minnesota exhibit a directional change in a character, the same direction and degree of change is observed in the unscaled samples. Thus, both scaled and unscaled *E. nigrum* from Minnesota exhibit the highest number of scales at the nape parietal juncture and the most incomplete lateral lines. The greatest differences between scaled and unscaled *E. nigrum* from Minnesota are confined to squamation characters alone.

I/ Character Index Analysis

Examination of meristic and other characters from scaled, unscaled and intergrade specimens from the Fox River drainage in Wisconsin reveals some distinct differences between the scaled and unscaled form. Most of the characters of the intergrades fall halfway between the two forms (Table **29**). Because the Fox River flows mainly from west to east for only 150 km, latitudinal and longitudinal differences are small between sampled areas. However, differences in habitat due to substrate, current and associated species may be great. All but five of the 133 specimens are from streams or rivers. Paired "*t*" tests suggest significant differences ($t_P < 0.05$) occur in all squamation characters, canal pore characters, number of dorsal rays, snout-type and the number of scales in and above the lateral line. Few significant differences, except those associated with squamation, are found between the scaled and intergrades or the unscaled and intergrades.

The frequency distribution for the character index made by summing eight characters which varied significantly in "*t*" tests (number of scales in and above the lateral line (LL1 and LL2), number of dorsal rays, total number of POM pores and

Table 29: Comparison of the means of characters (SD in parentheses) from scaled, unscaled and intergrade specimens of *Etheostoma nigrum* from the Fox River (Lake Michigan drainage) in central Wisconsin. Bilateral data are presented for the left side only due to close agreement between sides.

Character	Scaled	Intergrade	Unscaled
LL	48.77	48.09	46.12 *
	(3.31)	(2.75)	(2.97)
LL1	4.50	4.16	3.85 *
	(0.54)	(0.42)	(0.46)
LL2	5.65	5.53	5.27 *
	(0.52)	(0.50)	(0.45)
CC	24.85	9.40	0.0
	(8.93)	(9.83)	(0.0)
OC	20.19	14.20	8.19 *
	(4.50)	(5.97)	(2.79)
NC	44.97	24.60	0.0
	(7.16)	(18.30)	(0.0)
NCT	4.24	3.51	1.08 *
	(0.64)	(1.53)	(1.72)
BC	84.74	42.76	0.0
	(16.52)	(28.62)	(0.0)
BELLYP	100.0	98.44	80.19
	(0.0)	(3.34)	(19.67)
BLL	7.52	7.40	7.35
	(0.76)	(0.75)	(0.56)
DS	8.81	8.76	8.92
	(0.54)	(0.53)	(0.48)
DR1	11.50	11.71	12.00 *
	(0.86)	(0.63)	(0.49)
AR1	8.18	8.18	8.27
	(0.64)	(0.49)	(0.45)
LBR	6.00	6.00	5.96
	(0.0)	(0.0)	(0.20)
LPOP	8.53	8.44	7.15 *
	(1.66)	(1.50)	(1.59)
LOPOP	6.10	6.09	5.96 *
	(0.30)	(0.29)	(0.20)
LPOS	2.58	2.38	2.27 *
	(0.56)	(0.53)	(0.45)
LANT	4.11	4.02	4.00
	(0.32)	(0.26)	(0.0)

Table 29: Continued.

Character	Scaled	Intergrade	Unscaled
PECR	11.82 (0.50)	12.00 (0.52)	11.85 (0.46)
SNOUT[1]	3.05 (1.31)	3.24 (1.28)	3.69 * (0.93)
LLSTAT[2]	1.19 (0.44)	1.24 (0.48)	1.35 (0.49)
IN/UN[3]	1.94 (1.71)	2.02 (1.82)	2.85 * (1.85)
N	62	45	26

[1] 1=blunt, 3=intermediate, 5=subconical, 2 or 4=transitional forms
[2] 1=complete, 2=slightly incomplete, 3=incomplete and/or interrupted
[3] 1=interrupted, 2=uninterrupted, 3=POM pores confined to opercle
* significant differences ($t_P < 0.05$) between scaled and unscaled

those confined to the opercle, and the number of infraorbital pores) shows that scaled and unscaled *E. nigrum* in the Fox River system can be separated based on these characters alone more than 65.0% of the time (Table **30**). The character index separates 69.0% of the scaled and unscaled specimens (mean scaled CI + mean unscaled CI ÷ 2) between 88 and 89. If opercle squamation (number of scales) were included in Table **30**, the separation of the scaled and unscaled form would be near complete (90.90%). Scales at the nape-parietal juncture were not included due to a high correlation with nape squamation ($r > 0.62$) among the scaled sample. Snout-type and estimated percent of belly scaled were considered somewhat subjective and similarly excluded from the character index. Possible problems with coding of the condition of the POM pore canal also resulted in its exclusion from the character index. Most often, unscaled specimens found within scaled samples possess some of the highest character index values among those classified as unscaled. Similarly, when the scaled and unscaled forms occur together, the CI value for the scaled form is reduced.

II/ <u>Discriminant Analyses</u>

Discriminant analysis, performed with squamation characters (BC, NC, CC, OC, NCT) and estimated percent of belly scaled (BELLYP) included, successfully separated all scaled (240) and unscaled (1215) darters. Darters which were not included in the analyses to establish the discriminant function were classified using these results. These were classified as 107 scaled and 549 unscaled. Additional scaled darters were selected from samples in (number of new locations: number of

Table 30: Character indices for *Etheostoma nigrum* from the Fox River, Wisconsin. Characters include number of dorsal rays, preoperculomandibular pores, infraorbital pores and the number of scales in and above the lateral line (LL1 and LL2). The best line of separation occurs between 88 and 89 and separates 69.0% of the scaled and unscaled specimens.

Location	Character Index																			
	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	Mean
Scaled																				
Grand R.	-	-	-	-	-	-	1	-	1	2	-	1	1	1	1	1	-	-	-	90.7
Grand R. trib.	-	2	1	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	83.0
Fox R.	-	-	-	-	1	-	-	2	2	-	2	1	1	-	1	4	-	-	1	91.3
French Cr.	-	-	-	-	-	-	1	2	1	-	1	3	1	-	1	-	1	2	-	91.3
Spring L.	-	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	1	-	-	92.5
Neenah Cr.	-	-	-	-	-	-	-	-	2	-	-	2	-	-	-	-	-	1	-	91.0
below Park L.	-	-	-	-	-	1	-	-	-	-	-	2	3	2	1	1	-	1	-	92.3
N = 62	-	2	1	-	2	1	2	5	6	2	4	9	8	3	4	6	2	4	1	90.8
N as % of sample	19 (31%) | 43 (69%)																			

Location	Character Index																			
	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	Mean
Unscaled																				
Grand R. trib.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	91.0
Fox R.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	-	-	-	92.5
French Cr.	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	93.0
Mill Cr.	2	3	5	3	1	-	1	2	1	-	2	1	-	-	-	-	-	-	-	84.1
N = 26	2	3	5	3	1	-	1	2	1	-	3	2	-	2	-	1	-	-	-	85.7
N as % of sample	18 (69%) | 8 (31%)																			

new specimens in parentheses) Illinois (2:2), Michigan (4:4), Minnesota (2:4), Missouri (3:12), Ohio (2:2), Wisconsin (2:4) and Ontario (1:1). These 16 new locations account for 29 of the 107 additional specimens classified as scaled. Thus 78 of the darters classified as scaled, or almost 73% of the new specimens, come from samples already identified as possessing scaled specimens. Eight variables were selected in the stepwise discriminant analysis which include: the number of nape scales, number of scales at the nape-parietal juncture, number of breast scales, completeness of the lateral line, number of opercle scales, estimated percent of belly scaled, number of dorsal rays (DR1) and the number of scales below the lateral line. The discriminant function is:

$$\mathbf{Y = -0.12439(OC) + 0.76905(NC) + 0.21519(NCT) + 0.40612(BC) + 0.07313(BELLYP) + -0.06118(BLL) + -0.07466(DR1) + -0.09434(LLSTAT).}$$

This function produces Y values of 13.37376 for the scaled form and -2.65274 for the unscaled form. Several of the squamation variables used to define scaled *E. nigrum* are given the highest weighting by this function (*e.g.*, NC and BC). The frequency histograms for this function are shown in Figure **32**.

Discriminant analysis performed with squamation excluded, but including the estimated percent of belly scaled, successfully separated 81.7% of the two forms (216 of 240 scaled and 974 of 1215 unscaled). Unclassified darters were classified as 416 scaled and 240 as unscaled. The six discriminating characters selected were: the number of scales above the lateral line (LL1), estimated percent of belly scaled, number of right posterior infraorbital pores, completeness of the lateral line, number

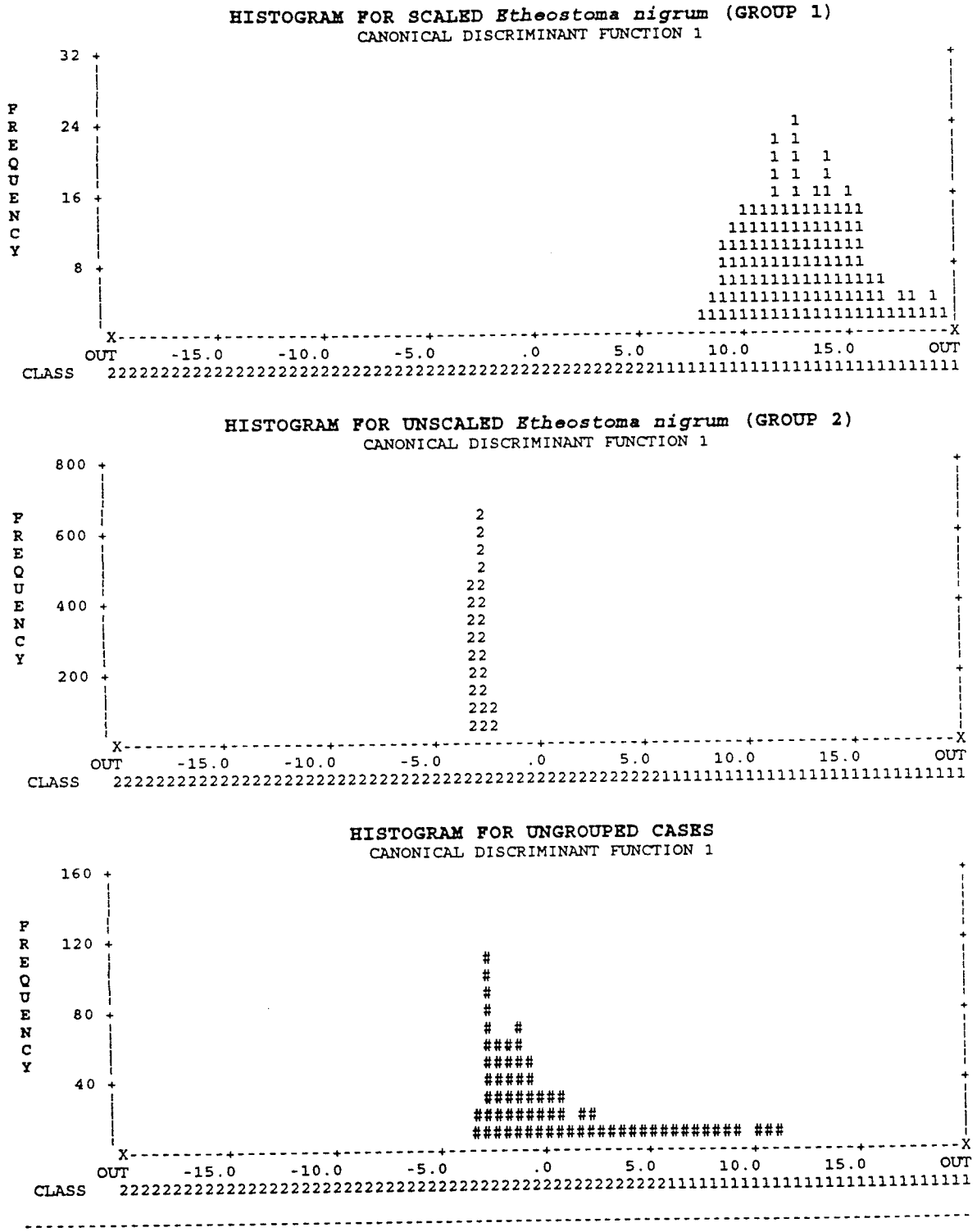


Figure 32: Frequency histograms from discriminant functions for scaled and unscaled *Etheostoma nigrum* and unclassified specimens from the examined range. The discriminant function is based on eight variables including squamation.

of left POM pores confined to the opercle and the number of scales in the lateral line. The discriminant function produced is:

$$\mathbf{Y = 0.12616(LL) + 0.64053(LL1) + 0.40788(BELLYP) + 0.12577(LOPOP) + 0.31136(RPOS) + -0.21070(LLSTAT).}$$

This function produces a Y value of 1.40455 for the scaled form and -0.27860 for the unscaled form. This function gives the heaviest weight to the number of scales above the lateral line and the extent of belly squamation. Specimens may be misclassified within a very narrow range (Fig. 33).

The 125 new sample locations, representing the 416 "scaled" specimens identified without squamation characters in the analysis, were often geographically distant from previously identified scaled samples although the majority still occurred in Wisconsin, Minnesota and Ontario. Discriminant analysis without squamation characters shows both the importance of squamation characters for discrimination and that there are few meristic differences between the two forms.

TEMPORAL VARIATION ANALYSIS

A discriminant analysis performed on each sample utilizing the characters with a non-zero variance from both the historic and recent collections resulted in the Crawfish River samples being completely discriminated into historic and recent samples. The Crawfish River samples were discriminated by the number of cheek scales, scales below the lateral line and number of left posterior infraorbital pores. Two variables (dorsal spines and number of right POM pores) separated 77.3% of the Whitefish Lake samples. Three variables (number of scales above the lateral line

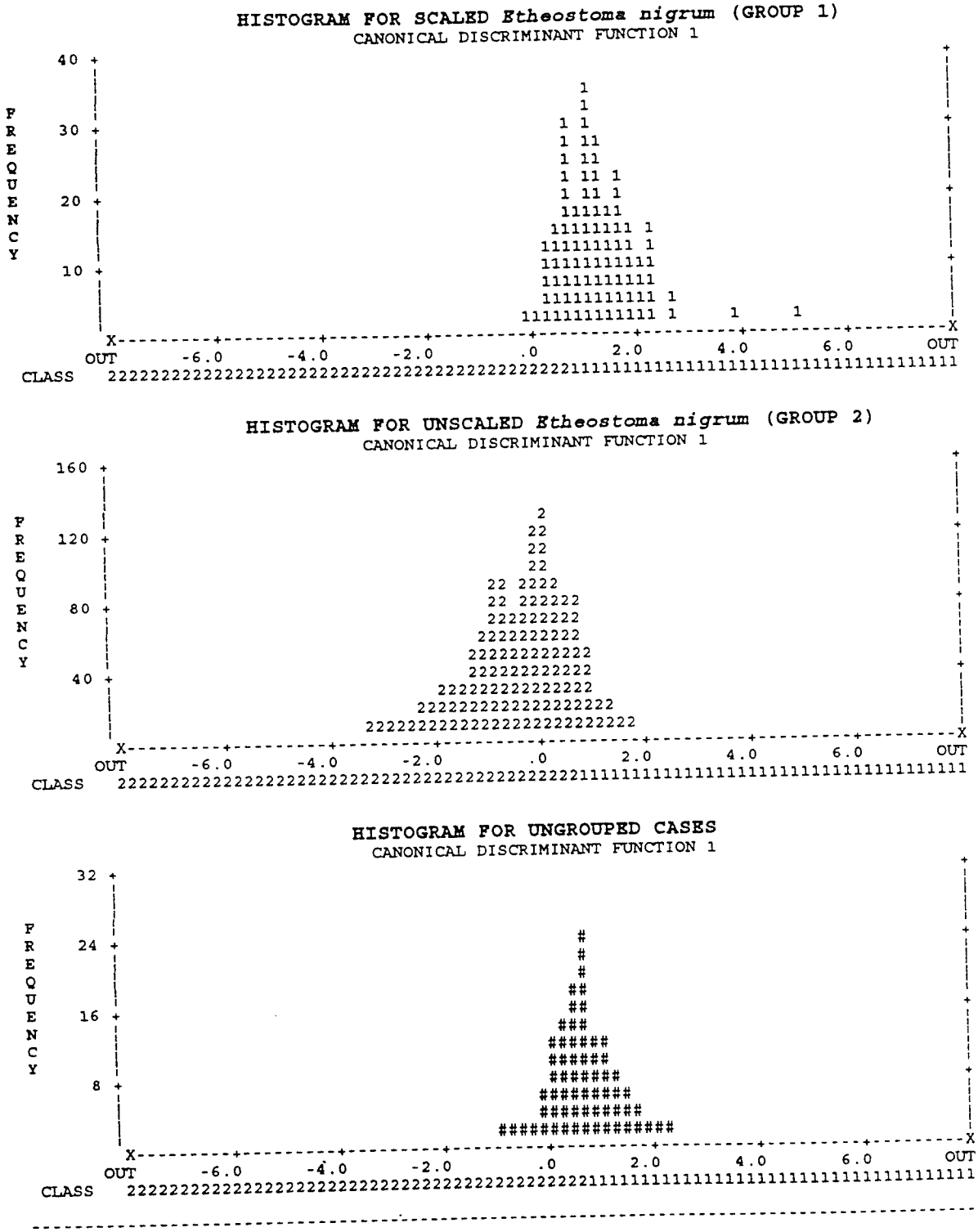


Figure 33: Frequency histograms from discriminant functions for scaled and unscaled *Etheostoma nigrum* and unclassified specimens from the examined range. The discriminant function is based on six variables excluding squamation.

(LL1), number of cheek scales and total number of right POM pores) provide 90.0% discrimination of the Rock River samples. Four variables (nape scales, scales below the lateral line, number of right posterior infraorbital pores and number of pectoral rays) provide for 96.2% discrimination of the Flambeau River samples. Five variables (scales above the lateral line (LL1), number of opercle, cheek and nape scales and the number of dorsal rays) discriminated 71.9% of the Big Arbor Vitae Lake samples. Because all samples were separated to some extent, the results of these analyses suggest there have been significant changes in some characters from historic to recent sampling periods.

Summary statistics for all variables examined from the five sampled populations are presented in Table **31**. Results from MANOVA are summarized in Table **32**. Snout-type, lateral line condition and the condition of the POM canal system (IN/UN) were not used in MANOVA due to difficulties in coding or their subjective nature. These variables were used in univariate "*t*" tests.

The Flambeau River sample has the greatest number of statistical differences (5) based on univariate "*t*" tests. Multiple analysis of variance shows that most of the variation is due to variation in characters other than squamation, which has exhibited only minor changes. The three squamation characters that did exhibit some differences were not significant, as the multivariate test shows. There was a decrease in the number of lateral line scales, the number of scales below and above the lateral line (both LL1 and LL2), and a decrease in the number of right posterior infraorbital pores. One specimen is heavily scaled and does not seem to fit in with the rest of the

Table 31: Sample means (SD in parentheses) and variables used for MANOVA or univariate analysis of five samples of *Etheostoma nigrum* from Wisconsin. The year of collection is given above the sample means.

	Whitefish L.		Flambeau R.		Big Arbor Vitae L.	
	1928	1992	1928	1992	1925	1992
LL	51.27	51.29	50.70	47.63*	48.57	47.73
	(3.24)	(2.06)	(2.21)	(1.78)	(2.71)	(1.89)
LL1	4.067	4.429	4.400	3.813*	4.067	4.237
	(0.26)	(0.54)	(0.52)	(0.40)	(0.36)	(0.47)
LL2	5.133	5.143	5.600	4.875*	5.033	5.085
	(0.35)	(0.38)	(0.52)	(0.34)	(0.18)	(0.28)
BLL	7.267	7.429	7.900	6.625*	7.100	7.153
	(0.59)	(0.79)	(0.57)	(0.62)	(0.55)	(0.45)
DS	9.133	9.429	8.800	8.875	9.400	9.339
	(0.35)	(0.54)	(0.42)	(0.34)	(0.50)	(0.51)
DR	11.53	11.57	12.40	12.00	12.50	12.20*
	(0.52)	(0.54)	(0.52)	(0.37)	(0.51)	(0.61)
AR	8.400	7.857	8.600	8.625	8.333	8.203
	(0.63)	(0.69)	(0.52)	(0.50)	(0.55)	(0.52)
PECR	11.93	11.86	11.80	11.75	12.23	12.20
	(0.26)	(0.38)	(0.63)	(0.45)	(0.43)	(0.48)
LPOP	6.000	6.714	6.200	6.625	6.567	6.593
	(0.00)	(1.25)	(0.42)	(0.96)	(1.22)	(1.19)
LOPOP	6.000	6.000	6.200	6.000	6.000	6.017
	(0.00)	(0.00)	(0.42)	(0.00)	(0.00)	(0.13)
RPOP	6.133	7.143	6.200	6.750	6.733	6.576
	(0.52)	(1.34)	(042)	(1.00)	(1.41)	(1.07)
ROPOP	6.000	6.429	6.200	6.000	6.000	6.017
	(0.00)	(0.54)	(0.42)	(0.00)	(0.00)	(0.13)
IN/UN	3.200	2.429	3.000	2.563	3.100	2.763
	(0.78)	(0.98)	(0.00)	(1.32)	(1.47)	(1.17)
LPOS	2.067	2.000	2.200	2.000	2.133	2.034
	(0.26)	(0.00)	(0.42)	(0.00)	(0.35)	(0.26)
LANT	4.000	4.000	4.000	4.000	3.900	4.000
	(0.00)	(0.00)	(0.00)	(0.00)	(0.31)	(0.00)
RPOS	2.067	2.000	2.500	2.063*	2.067	2.051
	(0.26)	(0.00)	(0.53)	(0.25)	(0.37)	(0.22)
RANT	4.000	4.000	4.000	4.000	3.967	4.000
	(0.00)	(0.00)	(0.00)	(0.00)	(0.18)	(0.00)
SNOUT	3.200	3.143	1.500	3.125*	2.733	3.068*
	(0.56)	(0.38)	(0.71)	(0.34)	(0.69)	(0.45)

Table 31: Continued.

	Whitefish L.		Flambeau R.		Big Arbor Vitae L.	
	1928	1992	1928	1992	1925	1992
LLSTAT	3.000	3.000	3.000	2.563	2.967	2.814
	(0.00)	(0.00)	(0.00)	(0.63)	(0.18)	(0.47)
CC	28.33	33.00	0.000	1.875	14.77	11.66
	(18.9)	(12.6)	(0.00)	(7.50)	(10.1)	(12.7)
OC	20.87	18.43	6.700	7.813	21.23	16.71*
	(7.94)	(4.12)	(3.74)	(3.69)	(5.07)	(6.10)
NC	23.67	32.57	1.300	4.125	39.50	28.58*
	(21.2)	(13.4)	(2.79)	(10.4)	(14.9)	(15.1)
NCT	3.000	4.000	1.300	2.500	4.367	4.119
	(1.60)	(0.00)	(1.64)	(1.97)	(0.56)	(0.46)
N	**15**	**7**	**10**	**16**	**30**	**59**

* $t_P < 0.05$

Table 31: Continued.

	Rock R.		Crawfish R.	
	1927	1992	1927	1992
LL	46.57	46.33	46.36	46.67
	(3.35)	(2.02)	(1.12)	(1.51)
LL1	4.286	4.933*	4.727	4.500
	(0.61)	(0.26)	(0.47)	(0.55)
LL2	5.571	5.733	5.545	5.500
	(0.65)	(0.46)	(0.69)	(0.55)
BLL	7.071	7.867*	7.818	7.167
	(0.62)	(1.06)	(0.60)	(0.98)
DS	8.857	8.933	8.727	8.500
	(0.36)	(0.46)	(0.79)	(0.55)
DR	11.57	11.60	12.36	12.00
	(0.85)	(0.74)	(0.81)	(0.00)
AR	8.000	8.333	7.818	8.500
	(0.68)	(0.72)	(0.51)	(0.55)
PECR	11.86	11.47	11.64	11.50
	(0.36)	(0.64)	(0.51)	(0.55)
LPOP	9.571	9.200	7.273	8.833*
	(0.85)	(0.56)	(1.27)	(0.41)
LOPOP	6.214	6.200	6.000	6.000
	(0.43)	(0.41)	(0.00)	(0.00)
RPOP	9.786	9.067*	6.909	9.000
	(0.80)	(0.46)	(1.34)	(0.00)
ROPOP	6.214	6.267	6.091	6.167
	(0.43)	(0.80)	(0.30)	(0.41)
IN/UN	1.143	1.133	3.091	2.000
	(0.36)	(0.35)	(2.12)	(0.00)
LPOS	2.643	2.933	2.727	4.667
	(0.50)	(0.60)	(0.65)	(2.16)
LANT	4.071	4.133	4.000	3.333
	(0.27)	(0.35)	(0.00)	(1.63)
RPOS	2.857	3.067	2.727	4.667
	(0.66)	(0.70)	(0.47)	(2.16)
RANT	4.071	4.000	4.000	3.500
	(0.27)	(0.00)	(0.00)	(1.76)
SNOUT	2.929	2.933	2.000	3.333*
	(0.27)	(0.26)	(0.76)	(0.52)

Table 31: Continued.

	Rock R.		Crawfish R.	
	1927	1992	1927	1992
LLSTAT	1.357 (0.75)	1.400 (0.74)	1.455 (0.52)	1.000 (0.00)
CC	19.57 (14.0)	35.73* (13.5)	42.55 (11.9)	20.50* (15.3)
OC	20.43 (6.42)	25.53* (3.96)	27.73 (5.26)	22.17 (4.88)
NC	42.07 (7.93)	43.93 (6.72)	49.82 (6.03)	42.00 (11.5)
NCT	4.214 (0.58)	4.067 (0.26)	4.546 (0.69)	4.333 (0.52)
BC	-	-	94.64 (24.6)	60.17* (21.3)
N	**14**	**15**	**11**	**7**

* $t_p < 0.05$

Table 32: Results of MANOVA on meristic and squamation characters and three, four and/or five squamation characters from five recent and historic samples of *Etheostoma nigrum* from Wisconsin. See text for characters used.

Location	F	Significance[1]
Whitefish Lake		
12 characters	3.00468	0.0530
3 squamation characters	1.25754	0.3190
Flambeau River		
14 characters	3.66422	0.0180*
3 squamation characters	0.81811	0.4980
Big Arbor Vitae Lake		
16 characters	3.69747	0.0001*
4 squamation characters	5.86007	0.0001*
Rock River		
19 characters	1.65121	0.2230
4 squamation characters	2.61443	0.0610
Crawfish River		
16 characters	329.67122	0.0430*
4 squamation characters	2.32978	0.1111
5 squamation characters	1.86954	0.1740

[1] * = highly significant

sample. However, even removing this specimen from the sample and performing the same univariate and multivariate tests results in a similar number of significant results for the same variables. Removing this specimen from discriminant analysis lowers the successful separation of recent and historic samples from 96.2% to 92.3%.

The Whitefish Lake samples do not exhibit statistically significant changes for either the 12 variables (P = 0.053) or the four squamation variables (P = 0.31). When individual variables are examined, only the scales at the nape-parietal juncture exhibit a significant change, due to an increase in the number of scales in this area. However, because the recent sample exhibits a zero variance for this character, the number of scales at the nape-parietal juncture was not included in MANOVA.

The Big Arbor Vitae Lake samples are the largest available of the five recent/historic collections and exhibit highly significant differences in the multivariate analysis of all 16 characters and the four squamation characters. Squamation characters thus play an important role in determining the highly significant results of MANOVA. The results of "*t*" tests run on individual variables show a significant decrease in the number of nape and opercle scales and the number of dorsal rays.

The Rock River samples exhibit a significant increase in the number of both cheek and opercle scales when univariate "*t*" tests are performed with these variables. The number of scales above (LL1) and below the lateral line also increase while the number of pores on the right opercle decrease. However, MANOVA suggests no significant changes occurring between these two samples when all 19 variables are included in the analysis (P = 0.223). Because the number of nape scales and scales

at the nape-parietal juncture are nearly identical between samples, squamation characters alone do not result in a statistically significant difference being reached.

The Crawfish River samples exhibit significant differences only when all 16 variables are analyzed with MANOVA. However, statistical significance is not reached when either the four or five squamation characters are analyzed. Therefore, although there have been significant changes over time within these samples, they cannot be attributed directly to changes in squamation. Univariate "*t*" tests suggest that the number of scales on both the cheek and breast is significantly lower on the more recent sample. The number of pores on both the left and right opercle has noticeably increased.

Consideration of squamation characters alone suggests few significant changes among samples except in Big Arbor Vitae Lake. Examination of Table **31** shows that when squamation means are different between recent and historic samples, in most cases, one standard deviation encompasses the earlier or later mean. There are, however, more significant changes detected using the univariate "*t*" tests. The riverine samples are, overall, more variable than lake samples (Table **31**).

Table **33** presents the number and percent of scaled specimens in the five samples using the definition for this study. All historic collections and the recent Crawfish River sample identified the same specimens and thus possess a similar number of scaled specimens irrespective of which of the methods given above was used to define them (*i.e.*, breast scales included or excluded). Only in the recent Flambeau River sample were scaled specimens detected when the historic sample

Table 33: Comparison of the number of scaled specimens as a total number and as a percent of the total sample for five recent and historic collections of *Etheostoma nigrum* from Wisconsin.

Location	N	N Scaled	% Scaled	% Change
Whitefish Lake				
1992	7	5	71	+18
1928	15	8	53	
Flambeau River				
1992	16	1	6	+6
1928	10	0	0	
Big Arbor Vitae Lake				
1992	59	24	41	-22
1925	30	19	63	
Rock River				
1992	15	15	100	+22
1927	14	11	78	
Crawfish River				
1992	7	6	86	-14
1927	11	11	100	

failed to show any. This is due to the occurrence of a single, heavily scaled specimen.

The percent of scaled specimens relative to the entire sample (Table **33**) shows that the percent of scaled specimens has both increased and decreased at specific locations since the historic samples. The average change for the five sample locations is approximately 16%. Excluding the Flambeau River sample, this figure is 19%.

CONSISTENCY OF SQUAMATION AND MERISTIC CHARACTERS

I/ Squamation Characters

Examination of specimens from Wisconsin samples shows that most variation in the number of scales required to completely cover the nape (*i.e.*, 100% scaled) is found within rather than between samples. One hundred and thirty-two Wisconsin specimens were identified as possessing a 100% scaled nape. These fish come from 17 locations, although four of these locations, while geographically distinct, occur within two rivers. The average number of scales on a 100% scaled nape on *E. nigrum* in Wisconsin is 47 (SD = 6.75). Ranges, means and standard deviations for all samples examined are given in Table **34**. Table **34** also provides an estimate of the number of age classes examined based on the mean standard length-at-age reported by Becker (1983) for Wisconsin specimens. Because aging was not performed, no statistical analyses could be performed to examine differences between age classes. In each sample examined, however, both large and small specimens, presumably representing different age classes, exhibited little variation in the number of nape scales.

Table 34: Location of collection, number of specimens in collection (N), number of specimens in collection with a 100% scaled nape (n), range, mean, standard deviation (SD), and number of age classes likely examined (Age) for the number of scales on the nape for Wisconsin *Etheostoma nigrum*.

Location	N	n	Range	Mean	SD	Age
Little Arbor Vitae Lake	10	10	49-56	52.30	2.58	2
Kawaguesaga Lake	9	7	51-61	57.14	3.97	3
Grand River	10	9	39-60	47.77	5.73	2
Grand River tributary	11	7	30-53	40.00	8.10	3
Rock River	14	12	38-58	44.25	5.78	1
Grindstone Lake	3	1	50	50.00	0.00	1
Black Earth Creek	3	1	38	38.00	0.00	1
Root River	14	2	34-50	42.00	11.31	2
Apple River	15	10	37-53	45.00	4.98	3
Fox River	25	19	35-62	47.52	6.78	2
French Creek	24	12	29-52	42.58	6.71	3
Spring Lake	5	4	38-55	46.25	7.36	2
Neenah Creek tributary	11	3	45-53	48.66	4.04	2
Crawfish River	11	11	43-62	49.82	6.03	2
below Park Lake	23	12	42-55	48.58	4.62	2
Rock River	6	6	41-59	47.00	7.18	2
Crawfish River	7	6	43-50	47.16	2.78	2
TOTALS	**201**	**132**	**29-62**	**47.17**	**6.75**	**3**

The Black Earth Creek and Kawaguesaga Lake specimens do not fall within one standard deviation of the 132 fish sample (Table **34**). Little Arbor Vitae Lake and Kawaguesaga Lake, both in the Tomahawk River system, possess a high mean number of nape scales. These two samples generally exhibit statistically significant differences in the number of nape scales compared to other locations. The Little Arbor Vitae Lake specimens fall within one sample standard deviation of the mean whereas Kawaguesaga Lake specimens exceed this. Black Earth Creek specimens fall below one standard deviation of the mean. Lacustrine samples possess the highest nape scale counts.

Comparison with other areas where specimens possess a 100% scaled nape show that most fall near the sample mean or within one standard deviation of Wisconsin specimens. The additional samples examined for nape scaling include the Niagara River, ON ($\bar{x}$ = 44.75, SD = 8.53; 4 of 5 specimens), Roundtable Lake, ON ($\bar{x}$ = 42.75, SD = 4.03; 8 of 14 specimens), Coffee Creek, IN ($\bar{x}$ = 48.45, SD = 3.95; 11 of 48 specimens), Spirit Lake, IA ($\bar{x}$ = 54.8, SD = 3.56; 5 of 8 specimens), Otter Tail River, MN ($\bar{x}$ = 44.33, SD = 7.19; 9 of 11 specimens), Muddy Fork of Spring River, MO ($\bar{x}$ = 43.67, SD = 5.08; 6 of 6 specimens), South Bass Island, OH ($\bar{x}$ = 40.72, SD = 5.25; 11 of 11 specimens), Erie Harbour, PA ($\bar{x}$ = 46.67, SD = 3.21; 3 of 4 specimens) and Big Stone Lake, SD ($\bar{x}$ = 48.00 SD = 4.76; 4 of 8 specimens). Only Spirit Lake, Iowa, and the South Bass Islands, Ohio, fall slightly above or below, respectively, of one standard deviation of the Wisconsin sample mean. Therefore, there is a high degree of consistency in the number of

scales which completely cover a nape from sample to sample. The latitudinal and longitudinal distribution of these fish is from approximately 37.0° to 48.5° north latitude and approximately 80.0° to 97.0° west longitude.

The amount of scaling covering a body area does vary considerably within some samples. Several specimens from some samples also possess more scales when the nape or breast is incompletely scaled than when it is completely scaled. Certain body areas also show large amounts of variation in the number of scales when that portion is completely scaled. Table **35** presents the number of breast scales for the same Wisconsin samples used in Table **34**. Several specimens, while possessing completely scaled napes, do not have completely scaled breasts. The amount of variation is similar for both the nape and breast (range is equal to the lower limit). As with nape squamation, lacustrine samples generally possess the greatest number of breast scales. Specimens of different sizes from the same sample possess a similar number of breast scales.

Comparison with other areas where specimens possess a 100% scaled breast show that most fall near the sample mean or within one standard deviation of that of Wisconsin specimens (Table **35**). Additional areas which possess specimens with a 100% scaled breast include Coffee Creek, IN, (109; 1 of 48 specimens), Spirit Lake, IA ($\bar{x}$ = 108.25, SD = 12.68; 8 of 8 specimens), Mississippi River, MN (73; 1 of 10 specimens), Mud River, MN (82; 1 of 5 specimens), Otter Tail River, MN ($\bar{x}$ = 108,SD = 22.61; 3 of 11 specimens), South Bass Island, OH ($\bar{x}$ = 81.91, SD = 11.23; 11 of 11 specimens), Erie Harbour, PA ($\bar{x}$ = 106, SD = 5.65; 2 of 4

Table 35: Location of collection, number of specimens in collection (N), number in collection with a 100% scaled breast (n), range, mean, standard deviation (SD) and probable number of age classes examined (Age) for the number of scales on the breast for Wisconsin *Etheostoma nigrum*.

Location	N	n	Range	Mean	SD	Age
Little Arbor Vitae Lake	10	9	105-143	120.22	14.12	2
Kawaguesaga Lake	9	3	121-139	127.00	10.39	1
Grand River	10	2	91-109	100.00	12.73	2
Grand River tributary	11	2	97-100	98.50	2.12	2
Rock River	14	1	97	97.00	0.00	1
Grindstone Lake	3	1	110	110.00	0.00	1
Apple River	15	4	99-110	103.50	4.79	3
Fox River	25	1	96	96.00	0.00	1
French Creek	24	2	72-98	85.00	18.38	1
Spring Lake	5	1	127	127.00	0.00	1
Neenah Creek tributary	11	1	115	115.00	0.00	1
Crawfish River	11	3	110-130	123.00	11.27	2
Rock River	6	4	88-131	111.75	18.91	2
TOTALS	**154**	**34**	**72-143**	**111.91**	**16.03**	**3**

specimens), Big Stone Lake, SD ($\bar{x}$ = 90.5, SD = 6.36; 2 of 8 specimens), Rideau Creek, ON (47, 1 of 11 specimens), McGregor Bay, ON ($\bar{x}$ = 69.5, SD = 10.61; 2 of 6 specimens), Niagara River, ON (95, 1 of 5 specimens), Roundtable Lake, ON ($\bar{x}$ = 96.36, SD = 10.45; 11 of 14 specimens) and Mission Marsh, ON (104, 1 of 12 specimens).

The latitudinal range of specimens exhibiting complete belly scaling is less than that of complete nape scaling (41.5° to 48.5° N latitude) representing a range decrease of approximately 500 km. This range decrease is due to the lack of any of the Missouri specimens possessing a 100% scaled breast. Although many of these samples fall near the Wisconsin mean or within one standard deviation of the mean, several samples in the Lake Erie-Lake Ontario area and west of Wisconsin possess counts which fall far below. There is, thus, a trend for fewer scales on a 100% scaled breast to the east and west of Wisconsin.

Table **36** shows the frequency distribution for the percent estimate of scaled body area for all 240 scaled darters. There is variation in the estimated percent of coverage for all body regions among scaled *E. nigrum*. The estimated percent of the cheek area covered by scales is the most variable of all. This is largely due to the variable size of the cheek scales.

The highest correlations in Wisconsin darters with a 100% scaled nape are found between the number of nape scales and the number of cheek scales (r = 0.3030), the first dorsal ray count (r = 0.3180) and the complete state of the lateral line (r = 0.3707). These correlations are highly significant ($P < 0.001$). Thirty-

Table 36: Frequency distribution of the estimated percent coverage of the cheek, opercle, nape and breast among 240 scaled *Etheostoma nigrum*.

	Percent Estimate of Squamation										
	10	**20**	**30**	**40**	**50**	**60**	**75**	**80**	**90**	**95**	**100**
Cheek	1	10	33	16	25	16	30	30	29	11	39
Opercle	-	-	2	1	2	9	26	48	70	25	57
Nape	-	-	-	1	1	-	2	5	14	26	191
Breast	-	-	-	-	1	9	20	37	54	45	74

four Wisconsin specimens with a 100% scaled breast possess few significant correlations with other meristic variables. The number of breast scales correlates most strongly with the percent estimate of opercle scaling ($r = 0.6141$), the number of scales below the lateral line ($r = 0.4833$), a complete lateral line ($r = 0.4735$) and the number of right POM pores ($r = -0.4978$). These correlations are highly significant ($P < 0.001$).

Table **37** shows the length of the nape versus the number of nape scales and the Pearson product moment correlation coefficient for 23 specimens from three samples from Wisconsin, Minnesota and Ontario with completely scaled napes. Correlations are weak ($r = 0.1424$) and non-significant for the entire sample and are only slightly higher, but still non-significant, for the individual samples. The coefficient of determination is near zero ($r^2 = 0.02028$) suggesting no linear relationship between nape length and the number of nape scales.

The width of scales taken from the centre of the nape on these 23 specimens varies by approximately 45-64%. The differences in scale length is approximately similar. The amount of variation within these few samples thus suggests approximately 20% variation in the size of nape scales on fully scaled specimens.

Correlation between the number of nape scales and the predorsal length on 132 Wisconsin specimens possessing a 100% scaled nape is weak ($r = 0.1340$) and non-significant. The coefficient of determination ($r^2 = 0.01796$) suggests no linear relationship between the number of scales on the nape and the predorsal length. There is no relationship between the length of the nape and the number of scales

Table 37: Length of nape (as a percentage of standard length) versus the number of nape scales of specimens with a 100% scaled nape in samples of *Etheostoma nigrum* from the Otter Tail River, Minnesota, the Crawfish River, Wisconsin, and Roundtable Lake, Ontario. The Pearson product moment correlation coefficient and the coefficient of determination of the number of scales versus the nape length are also given.

Location	# of Scales	Nape Length	Correlation
Otter Tail River, MN			
	41	15.06	
	42	15.50	
	36	16.33	
	51	16.48	
	44	15.98	
	40	15.71	
	40	15.01	
	45	16.05	
	60	16.39	
	---	---	
Mean	**44.33**	**15.83**	**r = 0.5169**
SD	**7.19**		
Crawfish River, WI			
	45	14.86	
	48	14.78	
	47	13.85	
	43	14.78	
	50	15.36	
	50	16.29	
	---	---	
Mean	**47.17**	**14.99**	**r = 0.5270**
SD	**2.79**		
Roundtable Lake, ON			
	44	16.72	
	40	15.81	
	46	15.53	
	37	16.34	
	43	16.39	
	38	14.00	
	48	15.49	
	46	15.17	
	---	---	
Mean	**42.75**	**15.68**	**r = 0.0892**
SD	**4.03**		
Grand Means	**44.52**	**15.56**	**r = 0.1424**
Grand SD	**5.36**		**r^2 = 0.0203**

covering it.

Table **38** shows the frequency distribution for the percent estimate of nape squamation for some samples which possess scaled darters. In some samples, there is great variation in the estimated percent of nape squamation. Most lacustrine samples exhibit a relatively stable amount of nape squamation. Riverine samples generally exhibit the greatest variation in the estimated percent of nape squamation.

Seventy-five of the 240 scaled *E. nigrum* identified in this study are from lakes while 164 are from rivers or streams. However, only 60 of the 240 samples examined, representing 549 specimens, were from lakes. As a percent of sample, the lacustrine samples represent 26.01% of the 2111 specimens examined. Therefore, scaled specimens from lakes, 31.3% of the entire scaled sample, are over represented from lakes. Chi-square tests reveal highly significant differences in the occurrence of the scaled form in lakes and rivers both within the entire sample of scaled specimens ($\chi^2 = 33.14$; $P < 0.005$) and within Wisconsin alone ($\chi^2 = 43.28$; $P < 0.005$).

Figure **34** shows the number of nape scales plotted against the estimated percent of nape coverage for 407 Wisconsin darters. There are many instances in which a darter with an estimated 95% and, occasionally, a 90% coverage possesses more scales than one which was called 100% scaled.

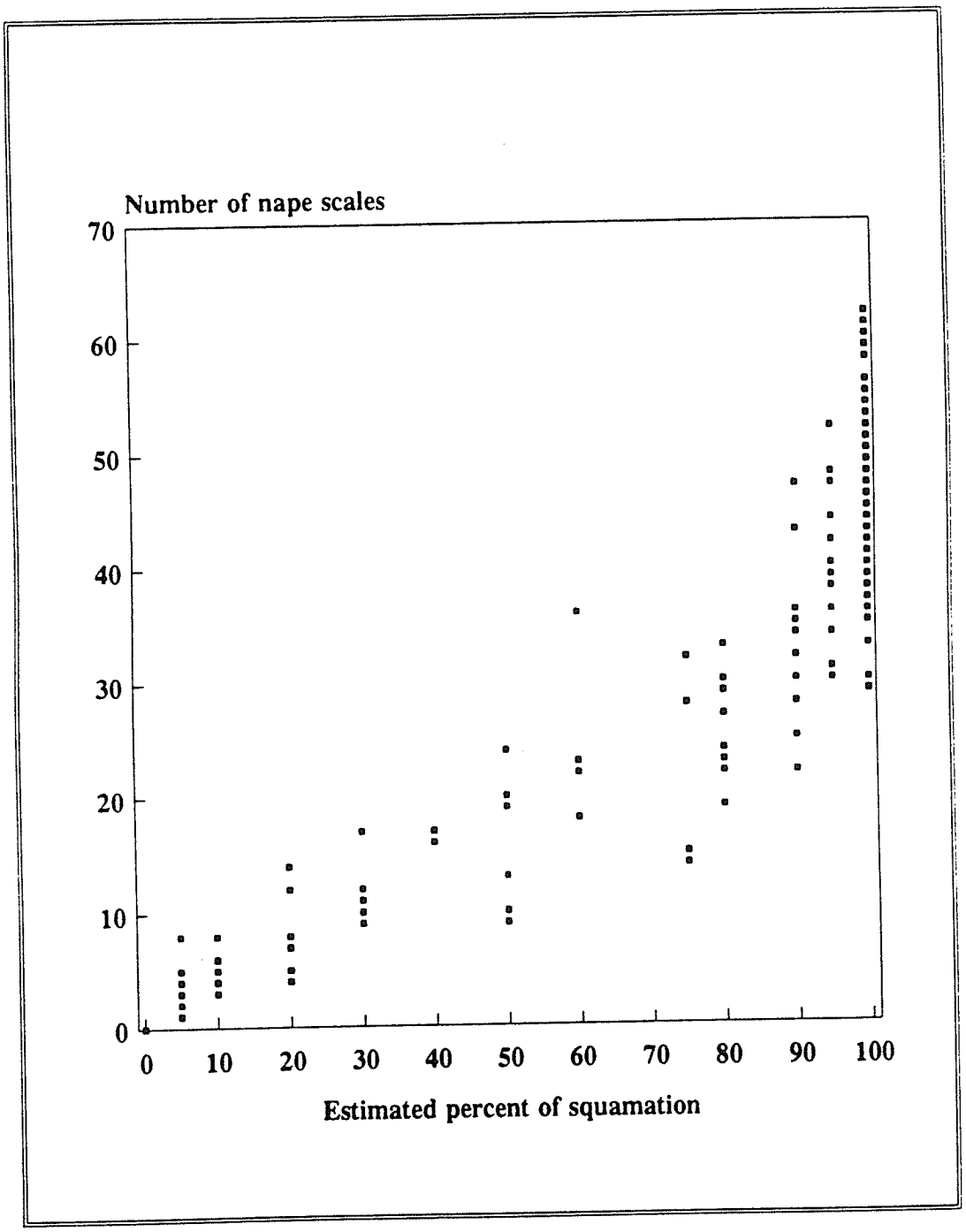
II/ <u>Meristic Characters</u>

Paired "*t*" tests performed between 107 river or stream and 30 lacustrine specimens of scaled *E. nigrum* from Wisconsin detected many significant differences in the means of meristic variables. Of these, the most significant is the higher

Table 38: Frequency distribution table of the estimated percent of squamation on the nape for 316 *Etheostoma nigrum* from 24 locations.

% Estimate Scaled	0	5	10	20	30	40	50	60	75	80	90	95	100	N
Little Arbor Vitae, WI	-	-	-	-	-	-	-	-	-	-	-	-	10	10
Kawaguesaga Lake, WI	-	-	-	1	-	-	-	-	-	-	-	1	7	8
Grand River, WI	-	-	-	-	-	-	-	-	-	1	-	-	9	10
Grand River trib, WI	-	2	-	-	-	-	-	1	-	-	-	1	7	11
Rock River, WI	-	-	-	-	-	-	-	-	-	1	1	-	12	14
Rock River, WI	-	-	-	-	-	-	-	-	-	-	-	-	6	6
Grindstone Lake, WI	-	-	-	-	-	-	-	-	-	1	-	1	1	3
Black Earth Creek, WI	-	-	-	-	-	1	-	1	-	-	-	-	1	3
Root River, WI	-	-	4	2	3	-	-	-	-	-	3	-	2	14
Apple River, WI	-	-	-	-	-	-	-	-	-	-	-	5	10	15
Fox River, WI	-	4	-	-	-	-	2	-	-	-	-	-	19	25
French Creek, WI	1	2	-	-	-	-	2	-	1	3	1	2	12	24
Spring Lake, WI	-	-	-	1	-	-	-	-	-	-	-	-	4	5
Neenah Creek, WI	-	-	-	1	-	-	-	1	-	2	2	2	3	11
Crawfish River, WI	-	-	-	-	-	-	-	-	-	-	-	-	11	11
Crawfish River, WI	-	-	-	-	-	-	-	-	-	1	-	-	6	7
below Park Lake, WI	1	3	-	-	-	-	-	-	2	-	2	3	12	23
Roundtable Lake, ON	-	-	-	-	-	1	1	-	-	-	3	1	8	14
Niagara River, ON	-	-	-	-	1	-	-	-	-	-	-	-	4	5
Erie Harbor, PA	-	-	-	-	-	-	-	-	-	-	-	1	3	4
Coffee Creek, IN	5	5	12	5	3	1	-	-	1	2	1	1	12	48
Spirit Lake, IA	-	-	-	-	-	-	-	-	-	1	2	-	5	8
Otter Tail River, MN	-	-	-	-	-	-	-	-	-	-	1	1	9	11
Big Stone Lake, SD	-	-	-	-	-	-	2	-	-	-	-	2	4	8
South Bass Island, OH	-	-	-	-	-	-	-	-	-	-	-	-	11	11
Muddy Fork, MO	-	-	-	-	-	-	-	-	-	-	-	-	6	6

Figure 34: Scatterplot of the number of nape scales versus the estimated percent of the nape scaled enumerated on 407 *Etheostoma nigrum* from Wisconsin. Each symbol may represent more than one specimen.


Number of nape scales
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Estimated percent of squamation

number of breast and nape scales found in lacustrine samples as opposed to those from rivers and streams. The number lateral line scales is statistically higher in lacustrine samples. These samples also possess a greater number of dorsal spines, dorsal rays, anal rays and pectoral rays. Other significant results ($t_P \leq 0.05$) are presented in Table **39**. Riverine samples possess a more complete lateral line, more posterior infraorbital pores and a greater number of POM pores than lacustrine samples. The completeness of the POM canal is significantly different between lakes and rivers ($P < 0.001$) with riverine specimens typically possessing an uninterrupted canal. Specimens from lakes possess pores only on the opercle. Thus there is evidence of differences between the riverine and lacustrine scaled form of *E. nigrum*.

The frequency distribution of some meristic variables for all scaled specimens of are shown in Tables **40-45**. The specimens have been separated by major drainages and geographic areas and make comparisons with all unscaled specimens. Only a few areas allow for comparison between lake and river specimens as most scaled specimens are from rivers. Because "*T*" tests suggest differences between the meristics of scaled *E. nigrum* from lakes and rivers, these tables were constructed to see if wider, geographic trends are evident among meristic variables between geographic areas and between lacustrine and riverine habitats.

Table **40** shows the variation in lateral line scale counts. Lateral line scale counts cannot be used to ascertain the history of scaled darters and provide limited information due to their wide range within and between sample areas. Little variation exists between scaled samples from lakes and rivers and so they are not presented.

Table 39: Comparison of group means and standard deviations of 30 lake and 108 river specimens of scaled *Etheostoma nigrum* from Wisconsin. Abbreviations used are as in methods section.

Location	Variable	Mean	SD	t_P
River	LL	47.7196	3.083	0.001
Lake		50.6333	3.079	
River	NC	45.3364	6.729	0.005
Lake		49.7333	7.428	
River	BC	84.8411	18.516	0.001
Lake		111.000	16.595	
River	DS	8.7944	0.527	0.001
Lake		9.4333	0.504	
River	DR1	11.6822	0.853	0.001
Lake		12.3333	0.606	
River	AR1	8.1776	0.762	0.039
Lake		8.4667	0.629	
River	LPOP	8.1776	1.747	0.001
Lake		6.6333	1.159	
River	RPOP	8.1402	1.724	0.001
Lake		6.8000	1.157	
River	LPOS	2.7664	1.138	0.003
Lake		2.1333	0.346	
River	RPOS	2.8318	1.120	0.002
Lake		2.1667	0.379	
River	PECR	11.7664	0.487	0.007
Lake		12.0333	0.414	
River	IN/UN_1	2.1495	1.571	0.015
Lake		2.9333	1.413	
River	$LLSTAT_2$	1.1963	0.444	0.001
Lake		2.5000	0.820	

[1] 1=interrupted, 2=uninterrupted, 3=POM pores confined to opercle only

[2] 1=complete, 2=slightly incomplete (1-3 unpored scales), 3=incomplete (4 or more unpored scales) and/or interrupted

Table 40: Frequency distribution table of the number of scales in the lateral line among 240 scaled specimens of *Etheostoma nigrum* divided by major drainages and geographic areas. The percent frequency distribution of 1215 unscaled specimens is also given. The percent frequency distribution of unscaled specimens does not equal 100% as four lower and higher scale counts which total 0.5% of the sample have been excluded.

Lateral line scales	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59
Mississippi River (WI)	-	1	-	1	7	10	10	9	9	4	9	3	5	4	2	1	-	-	1	-
Mississippi River (MN)	-	-	-	-	-	2	-	1	1	1	-	1	-	-	1	-	-	1	-	-
Mississippi River (SD)	-	-	-	-	-	1	1	-	5	2	-	2	1	1	-	-	-	-	-	-
Fox River (WI)	1	1	1	1	1	5	3	7	11	3	6	10	6	2	3	-	-	1	-	-
Lake Michigan	-	-	-	1	1	-	1	2	3	1	-	-	1	-	-	-	-	-	-	-
Hudson Bay (MN)	-	-	-	1	-	2	2	1	1	2	1	2	-	1	-	-	-	-	-	-
Hudson Bay (SK)	-	-	-	-	-	1	-	-	1	-	2	-	-	-	-	-	-	-	-	-
Lake Superior tributaries	-	-	-	2	1	1	2	2	-	2	2	-	-	-	-	-	-	-	-	-
Lake Erie and tributaries	-	-	2	3	1	3	-	1	2	3	3	2	1	-	-	-	-	-	-	-
Lake Ontario and tributaries	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-
Lake Huron and tributaries	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Missouri River (IA)	-	-	-	-	-	1	1	2	1	-	3	1	-	-	-	-	-	-	-	-
Spring River (MO)	-	-	-	-	-	-	-	-	1	-	2	1	1	1	1	-	-	-	-	-
Scaled total	1	2	3	9	11	26	20	27	37	18	28	22	15	9	7	1	1	2	1	0
Percent of scaled	0.4	0.8	1.3	3.8	4.6	10.8	8.3	11.2	15.4	7.5	11.6	9.2	6.3	3.7	2.9	0.4	0.4	0.8	0.4	0.0
Percent of unscaled	0.5	1.3	3.1	4.8	7.7	8.6	9.5	10.6	11.8	10.0	10.7	7.5	5.2	3.1	2.0	1.2	0.8	0.7	0.4	0.1

Table 41: Frequency distribution table of the number of dorsal rays (DR1) among 240 scaled specimens of *Etheostoma nigrum* divided by major drainages and geographic areas. Sampled areas consist only of riverine specimens except for those areas separated into lacustrine and riverine samples. The percent frequency distribution of 1215 unscaled specimens is also given.

Dorsal rays	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Mississippi River (WI) **Total**	-	-	-	16	37	22	1
Rivers	-	-	-	15	24	10	1
Lakes	-	-	-	1	13	12	-
Mississippi River (MN)	-	-	-	2	5	1	-
Mississippi River (SD) **Total**	1	-	-	4	4	4	-
Rivers	-	-	-	1	2	3	-
Lakes	1	-	-	3	2	1	-
Fox River (WI) **Total**	1	-	4	24	28	5	-
Rivers	1	-	4	23	25	5	-
Lakes	-	-	-	1	3	-	-
Lake Michigan	-	-	-	5	4	1	-
Hudson Bay (MN)	-	-	-	3	10	-	-
Hudson Bay (SK) **Total**	-	-	-	2	2	-	-
Rivers	-	-	-	1	-	-	-
Lakes	-	-	-	1	2	-	-
Lake Superior tributaries **Total**	-	-	1	9	1	-	1
Rivers	-	-	-	-	-	-	1
Lakes	-	-	1	9	1	-	-
Lake Erie and tributaries **Total**	-	1	-	12	8	-	-
Rivers	-	-	-	6	1	-	-
Lakes	-	1	-	6	7	-	-
Lake Ontario and tributaries	-	-	-	-	2	1	-
Lake Huron and tributaries	-	-	-	-	2	-	-
Missouri River (IA) **Total**	-	-	-	1	6	2	-
Rivers	-	-	-	-	1	-	-
Lakes	-	-	-	1	5	2	-
Spring River (MO)	-	-	-	6	1	-	-
Scaled total (N)	2	1	5	84	110	36	2
Percent of scaled	0.8	0.4	2.0	35.0	45.8	15.0	0.8
Percent of unscaled	0.0	0.1	4.0	29.3	53.5	12.8	0.4

Table 42: Frequency distribution table of the number of scales above the lateral line (LL1) among 240 scaled specimens of *Etheostoma nigrum* divided by major drainages and geographic areas. Sampled areas consist only of riverine specimens except for those areas separated into lacustrine and riverine samples. The percent frequency distribution of 1215 unscaled specimens is also given.

Scales above the Lateral Line (LL1)	**2**	**3**	**4**	**5**	**6**
Mississippi River (WI) **Total**	-	1	38	36	1
Rivers	-	1	29	19	1
Lakes	-	-	9	17	-
Mississippi River (MN)	-	1	7	-	-
Mississippi River (SD) **Total**	-	-	8	5	-
Rivers	-	-	3	3	-
Lakes	-	-	5	2	-
Fox River (WI) **Total**	-	-	32	29	1
Rivers	-	-	31	26	1
Lakes	-	-	1	3	-
Lake Michigan	-	-	9	1	-
Hudson Bay (MN)	-	2	10	1	-
Hudson Bay (SK) **Total**	-	-	4	-	-
Rivers	-	-	1	-	-
Lakes	-	-	3	-	-
Lake Superior tributaries **Total**	-	-	3	8	1
Rivers	-	-	1	-	-
Lakes	-	-	2	8	1
Lake Erie and tributaries **Total**	-	-	20	1	-
Rivers	-	-	6	1	-
Lakes	-	-	14	-	-
Lake Ontario and tributaries	-	-	1	2	-
Lake Huron and tributaries	-	-	2	-	-
Missouri River (IA) **Total**	-	-	8	1	-
Rivers	-	-	1	-	-
Lakes	-	-	7	1	-
Spring River (MO)	-	-	2	5	-
Scaled total (N)	0	4	144	89	3
Percent of scaled	0.0	1.7	59.2	37.0	1.3
Percent of unscaled	6.7	34.7	57.0	1.6	0.0

Table 43: Frequency distribution table of the number of scales below the lateral line among 240 scaled specimens of *Etheostoma nigrum* divided by major drainages and geographic areas. Sampled areas consist only of riverine specimens except for those areas separated into lacustrine and riverine samples. The percent frequency distribution of 1215 unscaled specimens is also given.

Scales below the Lateral line	**5**	**6**	**7**	**8**	**9**	**10**
Mississippi River (WI) **Total**	-	10	32	29	4	1
Rivers	-	9	23	15	3	-
Lakes	-	1	9	14	1	1
Mississippi River (MN)	-	6	2	-	-	-
Mississippi River (SD) **Total**	-	2	7	4	-	-
Rivers	-	1	4	1	-	-
Lakes	-	1	3	3	-	-
Fox River (WI) **Total**	-	3	31	21	7	-
Rivers	-	3	29	20	6	-
Lakes	-	-	2	1	1	-
Lake Michigan	-	-	4	5	1	-
Hudson Bay (MN)	1	4	6	1	1	-
Hudson Bay (SK) **Total**	-	-	2	2	-	-
Rivers	-	-	1	-	-	-
Lakes	-	-	1	2	-	-
Lake Superior tributaries **Total**	-	-	7	5	-	-
Rivers	-	-	-	1	-	-
Lakes	-	-	7	4	-	-
Lake Erie and tributaries **Total**	-	6	13	2	-	-
Rivers	-	2	3	2	-	-
Lakes	-	4	10	-	-	-
Lake Ontario and tributaries	-	-	3	-	-	-
Lake Huron and tributaries	-	-	2	-	-	-
Missouri River (IA) **Total**	-	3	5	1	-	-
Rivers	-	-	1	-	-	-
Lakes	-	3	4	1	-	-
Spring River (MO)	-	-	-	4	2	1
Scaled total (N)	1	34	114	74	15	2
Percent of scaled	0.4	14.0	47.5	30.8	6.3	0.8
Percent of unscaled	1.1	20.7	53.3	22.6	2.1	0.2

Table 44: Frequency distribution table of the number of preoperculomandibular pores confined to the left opercle (LOPOP) among 240 scaled specimens of *Etheostoma nigrum* divided by major drainages and geographic areas. Sampled areas consist only of riverine specimens except for those areas separated into lacustrine and riverine samples. The percent frequency distribution of 1215 unscaled specimens is also given.

Left POM Pores on Opercle (LOPOP)	**5**	**6**	**7**	**8**
Mississippi River (WI) **Total**	-	66	10	-
Rivers	-	43	7	-
Lakes	-	23	3	-
Mississippi River (MN)	-	8	-	-
Mississippi River (SD) **Total**	-	13	-	-
Rivers	-	6	-	-
Lakes	-	7	-	-
Fox River (WI) **Total**	-	56	6	-
Rivers	-	53	5	-
Lakes	-	3	1	-
Lake Michigan	-	8	2	-
Hudson Bay (MN)	-	12	1	-
Hudson Bay (SK) **Total**	-	3	1	-
Rivers	-	-	1	-
Lakes	-	3	-	-
Lake Superior tributaries **Total**	-	11	1	-
Rivers	-	-	1	-
Lakes	-	11	-	-
Lake Erie and tributaries **Total**	-	19	2	-
Rivers	-	6	1	-
Lakes	-	13	1	-
Lake Ontario and tributaries	-	1	1	1
Lake Huron and tributaries	-	2	-	-
Missouri River (IA) **Total**	-	6	3	-
Rivers	-	1	-	-
Lakes	-	5	3	-
Spring River (MO)	-	6	-	1
Scaled total (N)	0	211	27	2
Percent of scaled	0.0	87.9	11.3	0.8
Percent of unscaled	1.2	93.0	5.4	0.3

Table 45: Frequency distribution table of the number of right posterior infraorbital pores (RPOS) among 240 scaled specimens of *Etheostoma nigrum* divided by major drainages and geographic areas. Sampled areas consist only of riverine specimens except for those areas separated into lacustrine and riverine samples. The percent frequency distribution of 1215 unscaled specimens is also given.

Right Posterior Infraorbital Pores	**0-1**	**2**	**3**	**4**	**5**	**8**	**9**
Mississippi River (WI) **Total**	-	39	29	5	-	2	1
Rivers	-	17	25	5	-	2	1
Lakes	-	22	4	-	-	-	-
Mississippi River (MN)	-	7	1	-	-	-	-
Mississippi River (SD) **Total**	-	6	6	1	-	-	-
Rivers	-	4	2	-	-	-	-
Lakes	-	2	4	1	-	-	-
Fox River (WI) **Total**	-	28	31	3	-	-	-
Rivers	-	25	30	3	-	-	-
Lakes	-	3	1	-	-	-	-
Lake Michigan	-	2	6	2	-	-	-
Hudson Bay (MN)	-	3	10	-	-	-	-
Hudson Bay (SK)	-	4	-	-	-	-	-
Rivers	-	1	-	-	-	-	-
Lakes	-	3	-	-	-	-	-
Lake Superior tributaries **Total**	-	6	6	-	-	-	-
Rivers	-	-	1	-	-	-	-
Lakes	-	6	5	-	-	-	-
Lake Erie and tributaries **Total**	-	9	10	2	-	-	-
Rivers	-	1	4	2	-	-	-
Lakes	-	8	6	-	-	-	-
Lake Ontario and tributaries	-	1	1	1	-	-	-
Lake Huron and tributaries	-	2	-	-	-	-	-
Missouri River (IA) **Total**	-	5	4	-	-	-	-
Rivers	-	1	-	-	-	-	-
Lakes	-	4	4	-	-	-	-
Spring River (MO)	-	7	-	-	-	-	-
Scaled total (N)	0	119	104	14	0	2	1
Percent of scaled	0.0	49.6	43.3	5.8	0.0	0.8	0.4
Percent of unscaled	0.8	78.1	19.8	1.0	0.1	0.0	0.2

Table **41** shows that scaled darters possess approximately equal numbers of dorsal rays when compared to unscaled darters. Many specimens from Lake Superior tributaries and the Spring River, Missouri, possess a lower number of dorsal rays than do most other scaled samples. A majority of specimens from Mississippi River tributaries in Wisconsin possess a higher than average number of dorsal rays.

Table **42** shows that although both scaled and unscaled *E. nigrum* typically possess four scales above the lateral line, many scaled specimens possess five scales. These higher counts are most prominent in Lake Superior tributaries and the Spring River, Missouri, where specimens with five scales above the lateral line outnumber those with four more than 2:1. There are no larger trends within the entire scaled sample. Similarly, Table **43** shows that there are no interpretable geographic trends for this character among scaled samples from different geographic areas.

While Table **44** shows that scaled *E. nigrum* are more likely to possess seven POM pores on the opercle than is the unscaled form, there is no geographic trend for this condition. Most scaled darters possess a similar number of POM pores confined to the opercle regardless of geographic area and habitat.

Table **45** shows that scaled *E. nigrum* generally possess a greater number of posterior infraorbital pores than does the unscaled form. However, those specimens with two or three or more pores are roughly equally distributed among samples and there are no widespread, geographic trends which might provide additional biogeographic information on the scaled form. Riverine specimens may possess slightly more pores than lacustrine specimens.

Therefore, like squamation variables, most scaled *E. nigrum* possess similar meristic characters between geographic locations and within different habitats. Although not as consistent as the number of nape or breast scales, these meristic characters may provide additional minor information on the scaled form and help lead to a biogeographic hypothesis explaining their present distribution. Some of the variation seen in these characters may be due to regional environmental affects.

CLUSTER ANALYSES

Clustering all 240 samples using variables identified on the first PC axis when squamation variables were included provides little new information. Except for the clustering of a large number of samples with scaled specimens from Wisconsin and other areas identified earlier, there is little in the way of geographic trends. Results are somewhat similar regardless of whether the clustering is based on characters identified on the first PC axis using the means of 240 samples (Table **12**) or the PCA on 2111 specimens (Table **11**). Both methods cluster the majority of samples with scaled specimens in a similar order. Two main groups of samples with scaled specimens are formed: one with predominantly Wisconsin, eastern and southern samples and one with Minnesota, western and northern samples.

Clustering based on seven variables (PCA on individuals) clustered 37 of 41 samples previously identified as possessing scaled specimens (Table **16**). Five additional samples (MO-03, MO-05, MO-07, ON-34 and ON-48), not identified as possessing scaled specimens were included in this cluster. Samples with scaled specimens make up a very distinct and final cluster on the far right of the dendrogram

(Cluster 4; Fig. **35**). Four samples identified as possessing scaled specimens (WI-05, ON-01, IA-01 and SK-05) failed to cluster with these scaled samples but clustered with samples possessing specimens with heavy squamation. These samples are in a group which contains those samples that would be identified as possessing scaled specimens as the conditions of the definition are relaxed (Table **17**).

Four large clusters are recognized using this method (Fig. **35**). These clusters include samples containing scaled specimens (Cluster 4), a large group of samples with mainly unscaled specimens (Cluster 3), a group of heavily scaled fish including a few of which contain scaled specimens (Cluster 2), and, a small group of 19 samples representing both unscaled and lightly scaled samples (Cluster 1). Variables other than squamation have little to do with the clustering and most clusters are uninformative with respect to geography, habitat or dispersal history. Table **46** gives the sample membership within the clusters shown in Figure **35**.

The dendrogram created using eight variables identified through the PCA on sample means, clustered 36 of the 41 samples identified as possessing scaled specimens (Cluster 3; Fig. **36**). Four additional samples not identified as possessing scaled specimens (IL-01, MO-03, MO-05 and MO-07) are clustered within this group. The samples with scaled specimens cluster with an adjacent group of samples which possess some scaled specimens and heavily scaled specimens (Cluster 4). Four main clusters are again formed using this method. Using the samples means, those samples with specimens meeting the definition of scaled are more closely clustered with those samples possessing specimens with heavy squamation. Table **47** gives the

Figure 35: Dendrogram of 240 samples of *Etheostoma nigrum* clustered using the means of seven variables identified on the first PC axis when squamation variables were included. Clustering variables were identified using PCA on individuals. Those samples possessing specimens which meet the study definition of scaled are marked with an asterisk (*). Those samples with the most heavily scaled specimens are at the extreme right. The vertical scale shows variance of clusters. Numbers denoting the main clusters correspond with those shown in Table **46**.

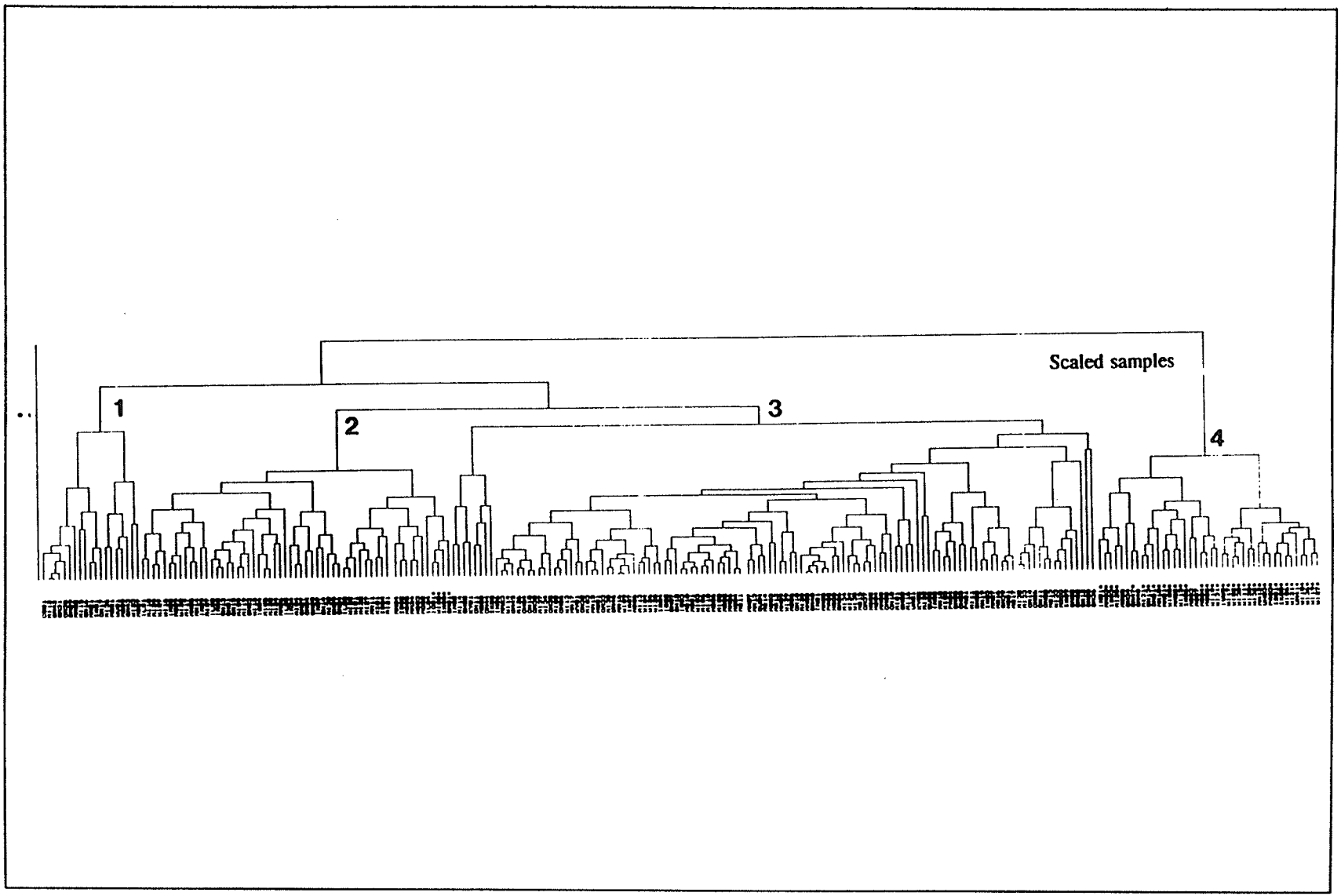

Scaled samples
1
2
3
4

Table 46: Cluster membership of 240 samples of *Etheostoma nigrum*. Clustered samples are based on the dendrogram in Figure **35** and are presented in sequence from left to right of the dendrogram. Samples in bold denote those samples with specimens meeting the definition of scaled.

Cluster 1

C0-01 MI-12 0N-42 ON-31 0N-38 0N-29 MN-22 MI-15 ON-16 MN-21 SK-01 ON-17 MI-36 MB-01 WV-01 MB-09 ON-43 WI-23 MB-07

Cluster 2

IL-01 MI-10 MI-29 WI-07 WI-16 IN-05 MO-09 WI-28 MI-17 MN-05 MN-14 IN-09 MI-07 IL-03 IN-03 OH-13 OH-01 ON-15 MI-09 MI-19 ON-23 WI-12 IA-04 MI-05 MI-22 MI-24 MN-06 IL-05 IN-04 MI-04 ON-27 ON-04 ON-09 IN-07 OH-14 MI-30 OH-09 ON-24

IL-02 WI-22 WI-01 MI-23 MI-27 MN-11 KS-01 SD-04 MI-21 MN-04 SD-02 ND-05 MN-24 ND-03 MO-02 IL-04 **IA-01 WI-05 SK-05 ON-01**

Cluster 3

IN-02 MI-14 OH-02 KY-01 OH-11 MB-08 ON-44 SK-02

IN-06 MN-23 WI-14 ON-45 MI-26 OH-04 OH-10 ON-30 MI-37 WI-34 ON-35 MI-02 OH-06 WI-13 MN-17 WY-01 ON-32 MI-03 MN-15 NB-01 ON-33 MI-35 ON-46 MN-16 WI-09 SK-07 ND-06 MI-13 WI-04 WI-26 WI-25 SK-04 IA-02 OH-07 OH-12 IA-03 MB-10 MO-01 WI-03 MI-06 MI-18 MB-06 MI-08 MB-02 MN-26 MB-04 MB-11 MN-07 MN-13 QU-04 MB-14 MB-03 MB-12 MN-09 WI-29 MO-06 ON-18 MI-28 MN-20 ON-40 TN-01 MN-25 PA-02 NB-02 ON-26 MI-34 WI-24 WI-35 MB-15 ON-37 ND-02 ND-04 MN-08 ON-28 ON-10 MI-01 OH-08 MI-16 VA-01 KY-02 MI-20 QU-05 IN-08 MN-18 KS-02 PA-04 ON-05 OH-05 PA-01 MI-11 ON-08 MN-19 SK-03 MN-27 MB-16 ON-39 ND-01 MB-17 MI-32 MI-33 ON-14 ON-06 ON-11 ON-20 MB-13 WI-33 MB-05 SK-09 ON-02 ON-22 ON-12 ON-25 ON-36

Cluster 4 - Scaled samples

IN-01 MN-03 SD-03 MI-25 MO-05 ON-34 **ON-47** ON-48 **MI-31 ON-13 ON-21 MN-10 MN-01 WI-08 MN-02 WI-15 SK-06** MO-03 MO-07 **MO-04 MO-08 WI-18 WI-31**

IA-05 WI-17 WI-02 WI-30 WI-11 WI-20 WI-32 MN-12 OH-03 PA-03 SD-01 SK-08 WI-06 WI-21 ON-41 WI-10 WI-36 WI-19 WI-27

Figure 36: Dendrogram of 240 samples of *Etheostoma nigrum* clustered using the means of eight variables identified on the first PC axis when squamation variables were included. Clustering variables were identified using PCA on sample means. The most heavily scaled specimens are in samples found near the right hand side of the dendrogram. Those samples possessing specimens which meet the study definition of scaled are marked with an asterisk (*). The vertical scale shows variance of clusters. Numbers denoting the main clusters correspond with those shown in Table **47**.

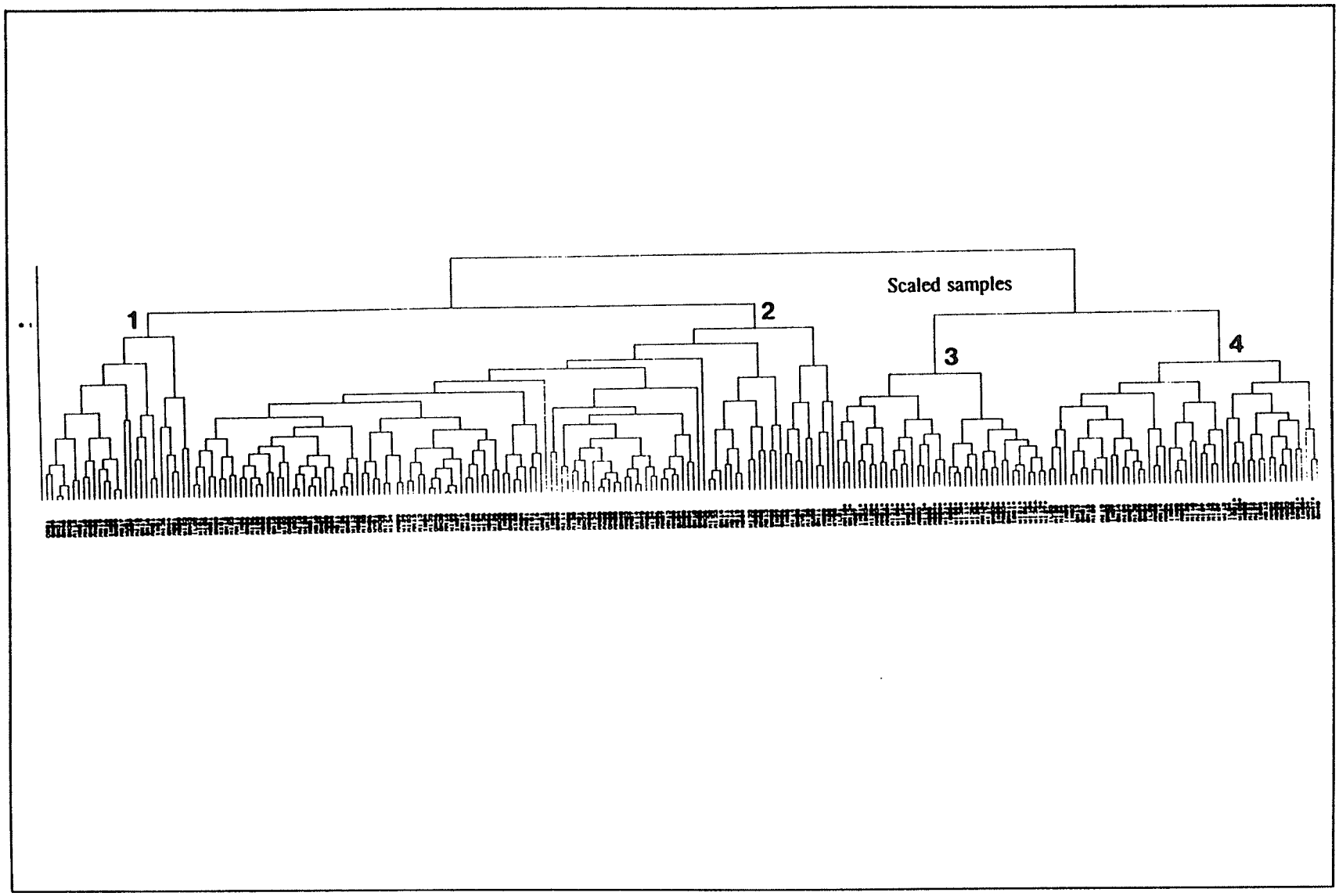

Scaled samples
1
2
3
4

Table 47: Cluster membership of 240 samples of *Etheostoma nigrum*. Clustered samples are based on the dendrogram in Figure **36** and are presented in sequence from left to right of the dendrogram. Samples in bold denote those samples with specimens meeting the definition of scaled.

Cluster 1

CO-01 ON-29 ON-31 ON-42 ON-38 MI-12 ND-01 MN-18 OH-05 MN-22 MN-19 MB-16 SK-03 ON-39 MB-17 WV-01 MB-07 MI-15 ON-17 ON-16 MN-21 SK-01 MI-36 ON-44 MB-01 MB-09 WI-23 ON-43

Cluster 2

IL-02 WI-01 MI-05 NB-01 ON-33 KS-01 OH-12 MI-23 MN-11 IN-06 ON-26 MI-06 MI-08 MB-02 MO-06 ON-18 IA-02 WI-03 OH-08 IA-03 MI-18 WI-22 MO-01 MB-10 OH-07 MB-03 MB-04 MB-11 MI-26 OH-04 WI-13 NB-02 MI-02 OH-06 WY-01 ON-32 MI-37 WI-34 MN-08 WI-29 MI-03 MN-15 MI-13 SK-04 MI-35 ON-46 ON-30 MN-16 WI-09 SK-07 MN-17 OH-10 WI-25 WI-04 WI-35 ND-06 WI-26 ON-35 IN-08 ON-05 KS-02 PA-04 PA-01 MI-01 MI-16 VA-01 ON-12 IA-04 ON-09 MI-11 ON-08 MI-28 TN-01 ON-37 ND-02 MI-34 MN-20 PA-02 ON-20 MN-25 ON-40 MN-23 MN-27 WI-14 ON-45 MN-26 WI-24 MB-06 MN-07 MN-13 MN-08 QU-04 ON-28 ON-04 ON-10 ON-25 ON-36 MI-32 MI-33 ON-14 ON-11 MB-14 MB-15 ND-04 WI-33 MB-05 ON-22 MB-13 ON-02 SK-09 ON-06 IN-02 MI-14 OH-02 KY-01 MI-20 QU-05 OH-11 MB-08 SK-02 KY-02

Cluster 3 - Scaled samples

IL-01 **MN-10 ON-13** MO-07 **IN-01 SD-03 MI-25 MN-03 MN-01 MN-02 MI-31 WI-08 WI-18 ON-21 SK-06** MO-03 **WI-15** MO-05 **MO-04 MO-08 WI-31**

IA-05 WI-02 WI-30 WI-11 WI-32 WI-20 MN-12 WI-17 OH-03 SK-08 PA-03 ON-41 SD-01 WI-21 WI-06 WI-10 WI-36 WI-19 WI-27

Cluster 4

IL-03 MI-22 MI-24 IN-09 IN-03 OH-13 OH-01 ON-15 MI-09 MI-19 ON-23 IN-07 OH-14 MI-04 ON-27 MI-30 OH-09 ON-24 IL-05 MN-05 MN-14 MN-06 IN-04 IN-05 MO-09 WI-28 MI-10 MI-17 MI-07 WI-16 MI-29 WI-07 WI-12

IL-04 **IA-01 WI-05** MO-02 MI-21 MI-27 SD-04 MB-12 MN-04 ND-05 MN-24 ND-03 SD-02 **ON-01 SK-05** ON-34 **ON-47** ON-48

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membership within each of these four clusters. Other clusters formed by this method are not biogeographically informative.

Dendrograms produced utilizing squamation characters and additional meristic variables (Tables **11** and **12**) from the second PC axis still exhibited the influence of squamation characters in the clustering. Most samples identified as possessing scaled specimens were grouped in a similar manner to that shown in Figures **35** and **36**. However, there was more agreement between the two dendrograms in the clustering of the samples with scaled specimens (36 of 41 similar to both groups) and both dendrograms looked similar. Although both methods broadly clustered scaled darters into three smaller groups (Wisconsin; Wisconsin, south and east; Minnesota, west and north), there is little, additional interpretable geographic information provided by these other clusters.

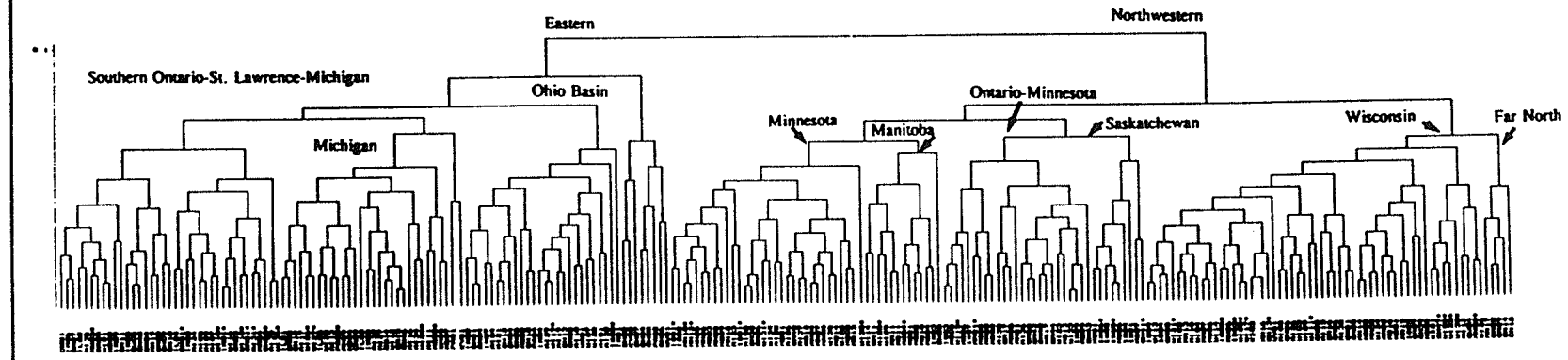
In the final clusters on the right side of both dendrograms, 53 of the samples were similar. These samples represented Ohio River samples and those in close proximity to the Ohio Basin. Using the PCA of sample means grouped 68 samples whereas the PCA on individuals grouped 62 samples in this final cluster. The same samples are clustered in several areas on both dendrograms. Nearby, discrete geographic areas tended to cluster together although considerable chaining occurred within some clusters.

Cluster analysis using eight variables (based on PCA of both sample means and individuals) identified on the first PC axis with squamation variables removed produced dendrograms difficult to interpret. Both methods used seven characters

common to each while one (PCA on individuals) used the number of lateral line scales while the other (PCA on sample means) used the number of left branchiostegal rays. Both methods produced three somewhat similar main groupings and scattered those samples identified as possessing scaled specimens. Some pairing of samples with scaled specimens occurred but in many cases these samples were geographically distant. The last cluster in both dendrograms, predominantly Ohio Basin and nearby samples, agreed on 46 of 49 (PCA on individuals) or 51 (PCA on sample means) samples. Thirty-nine of these samples were from east of Illinois, Lake Michigan or the Michigan Upper Peninsula. The Upper Ohio Basin samples again formed a clear grouping on the extreme right in both dendrograms. Many geographically adjacent samples clustered together, such as many from the northern areas of the range. However, the overall information from both of these dendrograms is limited as most clusters are not geographically informative.

The two dendrograms produced utilizing ten (PCA on individuals) or 12 (PCA on sample means) variables from the first two PC axes exhibit many similarities. Using the PCA of sample means produces two main groups while the PCA of individuals produces three main groups. Samples identified as possessing scaled specimens are distributed throughout the dendrogram although some close clustering of scaled samples occur. Sample means create a distinct northern and western group on the right while the left is largely eastern samples, including those from the Upper Ohio Basin and those east of Illinois, Lake Michigan and the Michigan Upper Peninsula (Fig. **37**).

Figure 37: Dendrogram of 240 samples of *Etheostoma nigrum* clustered using the means of twelve variables identified on the first two PC axes using means of samples when squamation variables were excluded. Those samples possessing specimens which meet the study definition of scaled are marked with an asterisk (*). The vertical scale shows variance of clusters. Labels denoting the main clusters correspond with those shown in Table **48**.


Eastern
Northwestern
Southern Ontario-St. Lawrence-Michigan
Ohio Basin
Ontario-Minnesota
Minnesota
Manitoba
Saskatchewan
Wisconsin
Far North
Michigan

Examining the right hand portion of the dendrogram created with sample means shows that the left hand portion of this cluster can be divided into five groups (from left to right): Minnesota, Manitoba, Ontario-Minnesota, Saskatchewan and, a Wisconsin group which also has a side cluster containing samples from the far north. The areas are not mutually exclusive. The main areas of overlap are northern Wisconsin, central and northern Minnesota and the southern portion of northwestern Ontario. Table **48** shows the order of samples in this dendrogram.

Half of the Manitoba and Minnesota clusters of 45 samples group together the southern portion of Manitoba and most of Minnesota, northwestern Ontario, northern Wisconsin and a large portion of the Michigan upper and lower Peninsula. A few samples from Ohio, Iowa and Missouri make up the remainder of this group. The Ontario-Minnesota and Saskatchewan portion of this large cluster, comprised of 34 samples, includes most Saskatchewan samples, several from central Manitoba, the southern portion of northwestern Ontario and the northern portions of Minnesota and Wisconsin. Additional samples within the Ontario-Minnesota and Saskatchewan clusters are from the area roughly parallel to the Mississippi River extending as far south as Missouri. The extreme right portion of the cluster exhibits considerable chaining but samples are predominantly from Wisconsin. This cluster includes 60 samples from the northern-most portion of Ontario and the area surrounding Lake Superior, portions of southern Manitoba, many eastern and western Minnesota samples, the greater portion of Wisconsin (except the southeast) and most Missouri River locations.

Table 48: Cluster membership of 240 samples of *Etheostoma nigrum*. Clustered samples are based on the dendrogram in Figure **37**. Samples in bold denote those samples with specimens meeting the definition of scaled.

Southern Ontario-St. Lawrence-Michigan
CO-01 IN-06 ON-27 IL-02 MI-35 **MN-03** ON-30 WI-01 ND-03 ON-11 IN-05 QU-05 ON-48 ON-34 MN-19 ON-20 OH-09 ON-10

IL-04 **ON-01** IN-03 MI-24 MI-05 MI-09 MI-07 **WI-10 IN-01** WI-16 ON-23 **WI-18 ON-21 MI-31** MN-24 **OH-03** MI-30 **WI-36**

Michigan
IL-01 ON-05 ON-18 MI-17 **WI-06 WI-27** IL-05 WI-25 MI-13 **PA-03** MN-09 ND-04 ON-09

IL-03 IN-08 MI-22 MI-27 OH-14 WI-12 ON-15 ON-46 MI-16 MI-34 **WI-08** MI-11 **MO-08** ON-08 MB-07 PA-01 KY-01

Ohio Basin
IN-02 OH-01 ON-24 IN-07 MI-04 MI-21 OH-12 ON-04 ON-25 MO-03 OH-10 PA-04 IN-04 MI-29 **WI-19** MI-19 OH-13 ON-28 MI-10 VA-01 WI-07 WI-28 MI-01 **SK-08** TN-01

OH-02 KY-02 OH-07 OH-04 OH-08 PA-02 OH-06 WV-01

Minnesota
IA-01 IA-02 **MN-10 MN-12** MN-25 MN-26 MB-03 MN-05 MO-09 MN-18 MI-23 **SD-01**

MI-02 ON-32 ON-26 **WI-31** WI-34 MI-28 MB-01 MI-06 ON-45 MI-37 MB-12 MN-04 MN-27 MN-08 WI-35 MB-14 WI-22 MB-08 MB-15 **WI-32**

Manitoba
MI-14 OH-11 **MI-25** WI-29 MN-11 MB-11 **MO-04** NB-01 MB-02 MB-10 MB-04 SK-03 OH-05

Ontario-Minnesota
IA-03 MO-05 **WI-15** MB-06 SK-07 **MN-01** MO-02 MN-20 ND-02 MI-33 ON-17 MB-05 MN-21 MI-36 ON-22 ON-42 ON-40 MB-17 ON-44 WI-23 MB-13 WI-33 SK-09 MB-09 ON-43

Saskatchewan
MO-01 **SK-06** SK-02 **SK-05** ND-06 SK-04 SK-01 MO-07 **ON-41**

Wisconsin
IA-04 WI-13 SD-04 ON-35 **WI-21** MI-03 MN-13 MI-18 WI-04 MN-07 MO-06 WI-14 **IA-05** MN-06 **SD-03 WI-05 WI-17** KS-02 MN-16 **ON-13**

WY-01 MI-08 ON-06 MI-26 WI-03 WI-09 **WI-11** ON-33 MI-15 ON-16 ON-36 MI-32 ON-31 ON-38 MN-14 ND-05 MN-22 WI-24 MN-23 MB-16 ND-01 MN-15 SD-02 ON-37 ON-39 NB-02

KS-01 **ON-47 WI-02 WI-20 WI-30** MI-12 QU-04 **MN-02** WI-26

Far North
MN-17 ON-02 ON-29 ON-14 ON-12

The left cluster is predominantly Ohio Basin and Lower Great Lakes samples. The area east of Illinois, Lake Michigan and the Michigan Upper Peninsula seem to be the best descriptor of the area involved. The samples with five branchiostegal rays are clustered at the extreme right of this cluster and form a distinct branch. The remaining three clusters exhibit little chaining and can best be described (from left to right) as southern Ontario-St. Lawrence-Michigan, Michigan and those with Ohio Basin affinities (Fig. **37**). While many samples in geographic proximity cluster together, scaled darters and the samples they come from are not distinctive.

The dendrogram created from PCA on individuals placed the Ohio Basin darters and those with eastern affinities on the right hand portion of the dendrogram. This cluster contained 52 samples. Western and northern samples were found largely on the left hand side of the dendrogram. However, geographic patterns were not as distinctive as those produced using the characters identified through PCA on sample means and this cluster was not considered informative. As with the PCA on sample means, none of the clusters were mutually exclusive. Samples with scaled darters were widely scattered, although some samples were adjacent to each other. Large, geographic areas were clustered rather than adjacent samples.

Clustering using the variables identified through PCA of individuals does not produce results as interpretable as those produced using PCA of sample means. While ineffective in clustering scaled samples when squamation characters are removed, this clustering does produce geographically based groupings of samples which may provide some information on the biogeographic history of *E. nigrum*.

Clustering all 152 one-degree longitude and latitude grids produced a dendrogram which provides interpretable, geographically based information. Table **49** shows the percent contribution of samples from each drainage contributing to the 152 one-degree grids. Some drainages are over represented due to the wide geographic distribution of samples from that drainage.

Four major clusters are formed using these one-degree drainage grids. Seven of the upper or middle Ohio Basin samples cluster together on the far right hand side of the dendrogram and form a distinctive group (Fig. **38**). A single Lake Erie sample is included in this cluster. This cluster of samples can be referred to as the Upper Ohio group. The order of the samples on this dendrogram are given in Table **50**.

At the left hand side of the dendrogram are 27 samples grids. Over 51.0% of these are from the Hudson Bay drainages of Manitoba and Ontario. Other areas with northern affinities in this cluster include six Lake Superior and three Mississippi River samples from near the Wisconsin Driftless Area. Two Missouri River samples and a single Lake Erie sample appear out of place. However, this first cluster largely represents northern samples and can be referred to as the Hudson Bay cluster. This cluster can be sub-divided into a Missouri-Mississippi group on the left and a Hudson Bay Manitoba group.

The second cluster from the left is the largest of all (72 sample grids) and exhibits considerable chaining of smaller clusters. The majority of this cluster (77.7%) is made up of Mississippi River (19), Hudson Bay (Manitoba) (14), Lake Superior (12) and Missouri River (11) drainage samples. Additional samples from the

Table 49: Number of samples (one-degree grids) from drainage, percent contribution of samples to 152 drainage samples and percent of specimens in drainage from total sample of 2111 specimens of *Etheostoma nigrum*. Lake Ontario and St. Lawrence River samples are grouped under St. Lawrence.

Drainage	Number of samples	% of drainage samples	% of total specimens
Hudson Bay (Manitoba-Saskatchewan)	27	17.8	13.2
Hudson Bay (Ontario)	7	4.6	2.7
Lake Superior	18	11.8	18.7
Lake Michigan	16	10.5	15.0
Lake Huron	11	7.2	5.7
Lake Erie	9	5.9	8.3
St. Lawrence	6	3.9	3.9
Mississippi R.	26	17.1	19.2
Missouri River	13	8.6	6.1
Ohio River	18	11.8	6.3
Atlantic	1	0.07	0.09

Figure 38: Dendrogram of the relationship of 152 samples of *Etheostoma nigrum* divided by one-degree longitude and latitude grids and separated by eleven major drainages. Clustering used the means of 12 variables identified by the first two PC axes created using sample means when squamation variables were excluded. Drainage abbreviations are; OH=Ohio, MO=Missouri, MI=Mississippi, LS=Lake Superior, LH=Lake Huron, LM=Lake Michigan, LE=Lake Erie, HM=Hudson Bay-Manitoba drainages, HO=Hudson Bay-Ontario drainages, AT=Atlantic Slope and SL=Lake Ontario and St. Lawrence River. The vertical scale shows variance of clusters. Numbers below the dendrogram correspond to clusters shown in Table **50**.

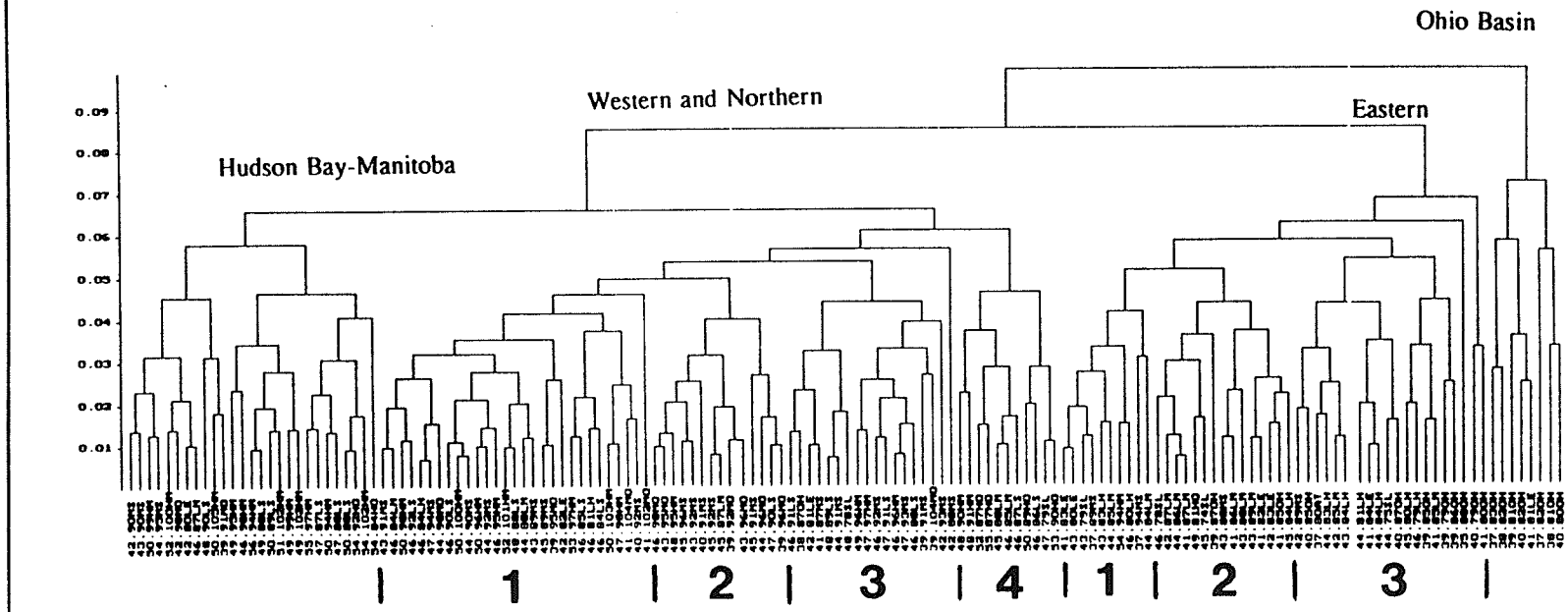

Ohio Basin
Western and Northern
Eastern
Hudson Bay-Manitoba
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
1
2
3
4
1
2
3

Table 50: Cluster membership formed using 152 one-degree longitude and latitude grids of 240 samples of *Etheostoma nigrum*. Order of samples follows those presented in Figure **38**. Drainage abbreviations are; OH=Ohio, MO=Missouri, MI=Mississippi, LS=Lake Superior, LH=Lake Huron, LM=Lake Michigan, LE=Lake Erie, HM=Hudson Bay-Manitoba-Saskatchewan drainages, HO=Hudson Bay-Ontario drainages, AT=Atlantic Slope and SL=Lake Ontario and St. Lawrence River. The first two digits for the one-degree latitude/longitude grid are shown.

HUDSON BAY-MANITOBA
42-90-MS 43-90-MS 50-99-HM 44-95-MS 52-100-HM 42-98-MO 42-80-LE 46-87-LM 48-90-LS 50-105-HM 39-91-MO 49-95-HM 46-98-HM 56-94-HM 49-88-LS 50-89-LS 51-102-HM 49-99-HM 49-102-HM 55-97-HM 47-87-LS 50-94-HM 47-88-LS 50-88-LS 54-92-HO 54-102-HM 54-84-HO

WESTERN AND NORTHERN
1/ Upper Mississippi-Lake Superior-Hudson Bay
43-91-MS 46-84-LM 50-98-HM 46-92-LS 46-84-LH 47-94-MS 44-98-MO 46-90-LS 50-100-HM 44-90-MS 50-96-HM 44-92-MS 46-95-HM 52-101-HM 48-88-LS 44-88-LM 43-89-MS 45-89-MS 39-95-MO 42-82-LE 53-97-HM 46-85-LS 46-81-LH 46-84-LS 50-103-HM 47-98-HM 41-104-MO 40-92-MS 41-102-MO

2/ Mississippi-Missouri
46-98-MO 43-95-MO 48-95-HM 45-96-MS 43-92-MS 40-91-MS 45-92-MS 45-87-LM 39-92-MO 43-96-MO 45-91-MS 44-96-MO 47-90-LS 39-96-MO

3/ Upper Mississippi
46-91-LS 38-87-OH 42-81-LE 41-87-MS 48-89-LS 44-91-MS 48-78-SL 49-96-HM 47-97-HM 46-92-MS 47-91-LS 46-96-HM 47-93-MS 46-88-LS 39-91-MS 39-104-MO 42-93-MS 42-88-MS

4/ Northern Ontario-Hudson Bay
48-90-HM 48-91-HM 52-87-HO 55-87-HO 45-88-LM 46-85-LM 46-87-LS 50-89-HO 46-86-LS 47-79-SL 53-90-HO

EASTERN
1/ Lake Erie-Huron
41-83-LE 43-80-LE 43-79-SL 37-89-MS 43-83-LH 44-85-LM 54-94-HM 46-80-LH 37-94-MS 44-84-LM

2/ Lake Michigan
46-78-SL 42-87-LM 44-86-LM 41-87-LM 49-81-HO 45-74-SL 39-87-OH 43-88-MS 41-86-LM 43-88-LM 43-89-LM 41-82-LE 42-83-LE 41-85-OH 45-84-LH

3/ Ohio East
42-89-MS 40-85-OH 37-80-AT 44-83-LH 42-85-LM 43-84-LH 44-81-LH 41-84-LE 44-84-LH 44-75-SL 40-87-OH 45-80-LH 46-79-LH 39-85-OH 41-85-LM 39-79-OH 39-86-OH 35-88-OH 40-79-OH 41-80-OH

OHIO BASIN
37-83-OH 38-82-OH 39-81-OH 40-82-OH 41-81-LE 37-82-OH 38-81-OH 40-80-OH

Lake Michigan (5), Hudson Bay (Ontario) (4), Lake Erie (2) and Ohio River (1) drainages complete this cluster. This cluster largely represents the mid-west and northern area of the range. Four broad sub-clusters can be identified within this cluster (from left to the right) as the Upper Mississippi-Lake Superior-Hudson Bay, Mississippi-Missouri, Upper Mississippi, and Northern Ontario Hudson Bay.

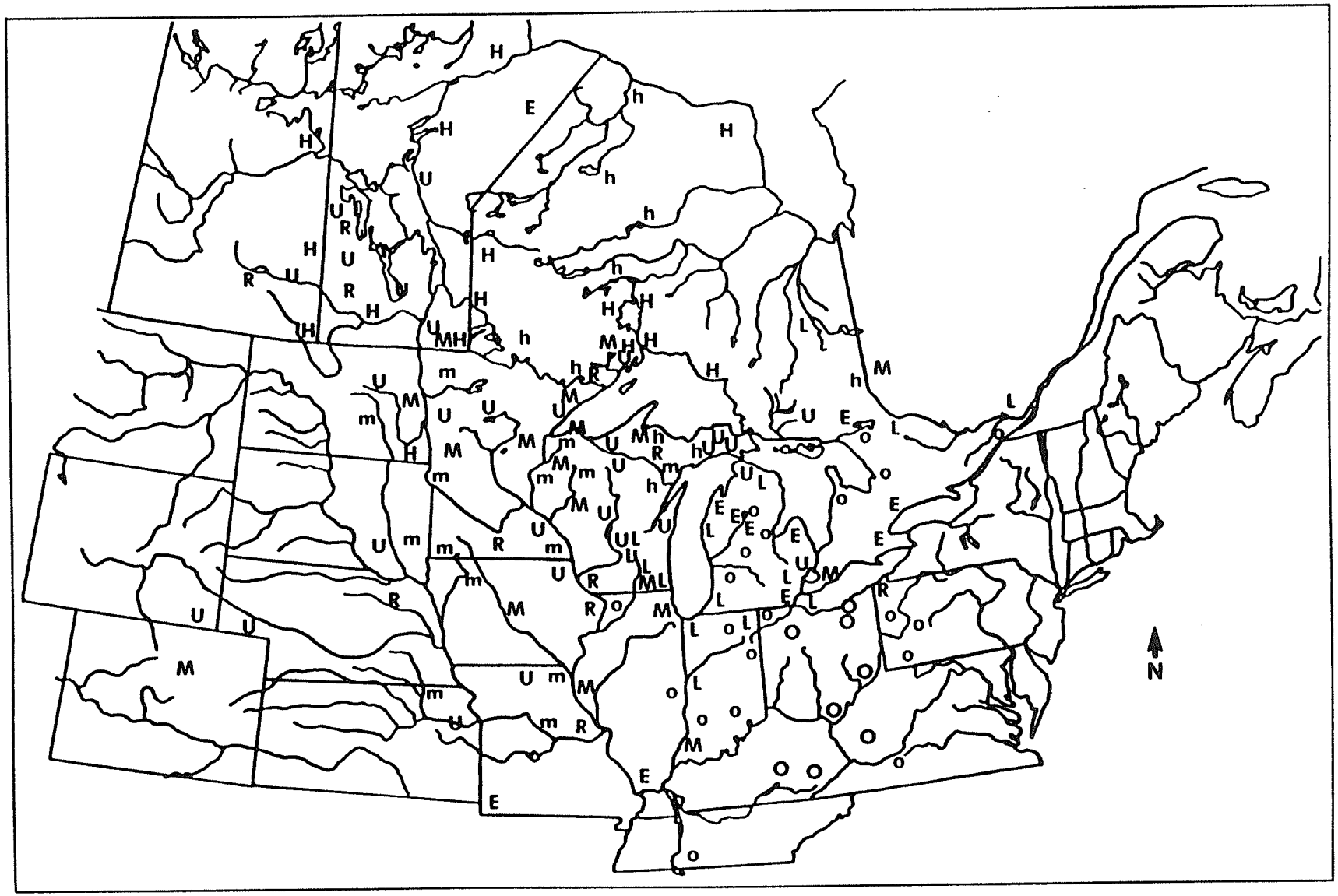
The third major cluster is predominantly (84.4%) formed with samples east of Illinois, Lake Michigan and the Michigan Upper Peninsula. These samples show definite eastern affinities and include the majority of the samples from the St. Lawrence (4 of 6), Lake Erie (5 of 9), Lake Michigan (10 of 16) the Ohio River (10 of 18) as well as the single Atlantic Slope sample. Three sub-clusters are identified from the left to right within this cluster as Lake Erie-Huron, Lake Michigan and Ohio-East.

The results of the clustering produced using one-degree grids is shown in Figure **39**. By mapping the relationships of all clusters, it is easier to see the relationship between areas. Most clusters are formed based on geographic area and/or major drainages and therefore provide interpretable information on the biogeographic history of *E. nigrum*.

LATERAL ASYMMETRY

Scaled darters do not make up a disproportionate number of those specimens exhibiting asymmetry. Of 373 fish possessing a different number of left and right posterior infraorbital pores, 50 of these, or 13.4% of the total, were scaled. The percent of scaled specimens possessing other dissimilar bilateral characters are

Figure 39: Results of clustering based on 152 one-degree longitude and latitude grids of *Etheostoma nigrum* separated by major drainages utilizing 12 variables. Abbreviations are; **R**=Missouri-Mississippi, **H**=Hudson Bay Manitoba, **U**=Upper Mississippi-Lake Superior-Hudson Bay, **m**=Mississippi-Missouri, **M**=Upper Mississippi, **h**=Northern Ontario Hudson Bay, **E**=Lake Erie-Huron, **L**=Lake Michigan, **o**=Ohio-East, and **O**=Upper Ohio.


N

approximately similar (branchiostegal rays = 8.3%; anterior infraorbital pores = 10.9%; POM pores confined to opercle = 13.2%; total number of POM pores = 12.9%). Scaled darters comprised 11.4% of the specimens examined for this study.

Darters lacking any squamation on the nape, cheek or breast made up the majority of individuals exhibiting lateral asymmetry (branchiostegal rays = 50.0%; anterior infraorbital pores = 52.9%; posterior infraorbital pores = 53.6%; POM pores confined to opercle = 57.4%; total number of POM pores = 52.8%). As with scaled darters, the percent occurrence of asymmetry in each character is approximately equal. Darters lacking squamation accounted for 57.5% of the specimens examined.

Specimens not meeting the definition of scaled or unscaled accounted for 31.1% of the entire sample. These specimens exhibit asymmetry approximately similar to their total contribution to the entire sample (branchiostegal rays = 41.7%; anterior infraorbital pores = 36.2%; posterior infraorbital pores = 33.0%; POM pores confined to opercle = 29.4%; total number of POM pores = 34.3%).

Branchiostegal rays were asymmetric in 18 samples. Two samples, Mill Creek, Wisconsin, and Whetstone Creek, South Dakota, possessed two and three specimens, respectively, with an asymmetrical number of branchiostegal rays. All other samples possessed only one specimen with an asymmetrical number of branchiostegal rays. Except for South Dakota and Missouri, asymmetry in branchiostegal rays was mainly confined to those states and provinces having the largest samples (Table **10**). Asymmetry in branchiostegal rays occurs in five

Wisconsin, four Michigan, three Ontario and two Minnesota samples as well as one each from Ohio, Missouri, South Dakota and Manitoba. Specimens with asymmetric branchiostegal rays are not found in the Ohio Basin where a majority of the upper or middle Ohio Basin *E. nigrum* possess five branchiostegals. As there is little asymmetry in branchiostegal rays, it will not be considered further.

The most asymmetric of the examined characters are presented in descending order (number of specimens exhibiting asymmetry in parentheses): the total number of POM pores (676), posterior infraorbital pores (373), anterior infraorbital pores (221), and the number of POM pores confined to the opercle (204). The state or provincial contribution to the total number of specimens exhibiting asymmetry is approximately equal to their percent contribution to the total sample. Therefore, no single state or province is over represented by asymmetric individuals.

Table **51** presents the fluctuating asymmetry values for all samples possessing at least 20% asymmetry for at least one of the four characters. Although a few locations possess fluctuating asymmetry values of 1.0 or greater for some characters, only one Manitoba sample (Mukatawa River; UMMZ 180547) was comprised of specimens which all exhibited asymmetry in one character. However, asymmetry in one character does not always mean asymmetry in another and the amount of asymmetry among characters is only weakly correlated among samples from all areas. Asymmetry in POM pores confined to the opercle is slight and few samples contain a large number of specimens with asymmetry in this character.

Among all samples presented in Table **51**, there is a negative correlation

Table 51: Fluctuating asymmetry values for samples of *Etheostoma nigrum*. Direction of asymmetry is given as R = right, L = left and - = no discernible direction. State or provincial means and standard deviation (in parentheses) are also given. Refer to Appendix **2** for locations identified by location collection numbers.

Location	N	Total POM Pores	Posterior Infraorbital	Anterior Infraorbital	Opercular pores
Colorado					
CO-1	33	0.2727**L**	0.2121**L**	0.3030-	0.0
Illinois					
IL-2	5	1.0000**L**	0.4000**R**	0.0	0.0
IL-5	19	0.3684**R**	0.0526**L**	0.0526**R**	0.0
Mean		0.68(0.45)	0.23(0.25)	0.03(0.04)	-
Indiana					
IN-1	48	0.6667**R**	0.1616**L**	0.1042-	0.0
IN-3	6	0.3333**R**	0.6667**L**	0.0	0.0
IN-6	7	0.5714-	0.0	0.0	0.2857-
IN-7	10	0.3636**R**	0.1000**L**	0.0	0.0
IN-8	5	0.6000**R**	0.0	0.0	0.2000**R**
Mean		0.51(0.15)	0.19(0.28)	0.02(0.05)	0.09(0.13)
Iowa					
IA-1	5	0.4000**L**	0.2000**L**	0.0	0.0
IA-2	11	0.6364**L**	0.2727**L**	0.0909**R**	0.0
IA-4	5	0.4000**L**	0.0	0.2000**R**	0.0
IA-5	8	0.2500**L**	0.3750**L**	0.1250**L**	0.2500**L**
Mean		0.42(0.16)	0.21(0.16)	0.10(0.08)	0.06(0.13)
Kentucky					
KY-1	10	0.2000-	0.1000**R**	0.0	0.0
Michigan					
MI-1	5	0.4000**R**	0.4000**R**	0.0	0.2000**L**
MI-2	11	0.6363**R**	0.0909**R**	0.0909**L**	0.0
MI-3	17	0.4117**R**	0.2941**R**	0.0588**L**	0.0
MI-4	11	0.3636-	0.3636**R**	0.2727**R**	0.0
MI-6	7	0.8571**R**	0.1428**L**	0.2857**R**	0.5714-
MI-8	5	0.2000**L**	0.0	0.2000**R**	0.2000**L**
MI-9	6	0.1670**L**	0.3333**L**	0.0	0.0

Table 51: Continued.

Location	N	Total POM Pores	Posterior Infraorbital	Anterior Infraorbital	Opercular pores
Michigan					
MI-10	6	0.3333R	0.0	0.1667L	0.0
MI-11	10	0.6000R	0.0	0.0	0.0
MI-12	6	0.6667R	0.0	0.3333-	0.0
MI-13	10	0.1000R	0.2000R	0.3000R	0.0
MI-14	12	0.2500L	0.1616-	0.2500R	0.0
MI-16	13	0.6154R	0.4615L	0.2308L	0.0
MI-17	8	0.5000R	0.1250R	0.0	0.0
MI-18	7	0.1428R	0.2857-	0.0	0.0
MI-19	8	0.2500R	0.5000R	0.2500-	0.0
MI-20	11	0.4545L	0.1800L	0.2727L	0.0
MI-23	10	1.0000R	0.0	0.3000L	0.0
MI-24	5	0.2000L	0.2000R	0.2000R	0.0
MI-26	10	0.1000R	0.2000-	0.0	0.0
MI-27	7	0.4286-	0.0	0.0	0.0
MI-29	19	0.1579R	0.1053-	0.1579R	0.0
MI-31	17	0.5294R	0.2353-	0.0588L	0.0
MI-32	12	0.3333-	0.1616R	0.0833L	0.0
MI-33	21	0.4286-	0.0952-	0.0	0.0
MI-34	17	1.0000R	0.0588L	0.0	0.0
MI-35	15	0.5333L	0.2000L	0.0	0.0
MI-36	20	0.3000L	0.1000L	0.1000-	0.2500L
MI-37	19	0.4737R	0.4211R	0.1579R	0.0
Mean		0.44(0.24)	0.18(0.15)	0.13(0.12)	0.41(0.12)
Minnesota					
MN-2	5	0.6000-	0.2000L	0.2000R	0.0
MN-3	11	0.7273R	0.1818-	0.0	0.0
MN-4	13	0.3846-	0.1538-	0.3077-	0.0
MN-5	14	0.4286R	0.2857-	0.0	0.0
MN-7	11	0.7273R	0.3636L	0.0909R	0.0
MN-8	12	0.4167R	0.2500R	0.0	0.0
MN-9	12	0.9167L	0.2500L	0.0	0.0
MN-11	10	0.6000R	0.0	0.0	0.0
MN-12	11	0.3636R	0.2727R	0.0909R	0.0
MN-13	12	0.3333-	0.3333-	0.0833R	0.0
MN-14	12	0.8333-	0.1667L	0.0833R	0.0
MN-21	5	0.8000-	0.6000L	0.0	0.2000R
MN-23	13	0.6923R	0.5385K	0.1538-	0.0
MN-24	6	0.8333-	0.1667R	0.0	0.0
MN-25	11	1.0000R	0.3636R	0.0909R	0.0
MN-26	17	0.5294L	0.5294L	0.0588R	0.0

Table 51: Continued.

Location	N	Total POM Pores	Posterior Infraorbital	Anterior Infraorbital	Opercular pores
Minnesota					
MN-27	16	0.3750**R**	0.3750**L**	0.1250-	0.0
Mean		0.61(0.21)	0.30(0.16)	0.08(0.09)	0.12(0.05)
Missouri					
MO-1	5	0.8000-	0.0	0.0	0.0
MO-3	10	0.4000**R**	0.0	0.3000**R**	0.2000**L**
MO-6	7	0.5714-	0.0	0.0	0.0
MO-8	6	1.3333**R**	0.1667**L**	0.1667**R**	0.5000**R**
MO-9	7	1.1429-	0.1429**L**	0.0	0.0
Mean		0.85(0.39)	0.06(0.09)	0.09(0.14)	0.14(0.22)
North Dakota					
ND-3	8	0.5000**L**	0.1250**L**	0.1250**R**	0.0
ND-4	12	0.5000**L**	0.2500**R**	0.0	0.0
ND-5	11	0.5454**R**	0.5454-	0.1818-	0.3636**R**
Mean		0.52(0.03)	0.31(0.22)	0.10(0.09)	0.12(0.21)
Ohio					
OH-1	10	0.5000-	0.2000-	0.0	0.0
OH-2	9	0.0	0.2222-	0.1111**L**	0.0
OH-3	11	0.2727**R**	0.1818-	0.1818**L**	0.0
OH-6	9	0.2222-	0.1111**L**	0.1111**L**	0.0
OH-8	5	0.2000**R**	0.0	0.2000**L**	0.2000**R**
OH-9	5	0.8000**L**	0.2000**R**	0.0	0.2000**L**
OH-11	9	0.8889**R**	0.0	0.0	0.0
OH-13	8	0.2500-	0.3750**L**	0.2500-	0.0
OH-14	11	0.1818-	0.0909**L**	0.2727**R**	0.0
Mean		0.37(0.30)	0.15(0.12)	0.13(0.11)	0.04(0.09)
Pennsylvannia					
PA-1	9	0.0	0.2222-	0.0	0.0
PA-4	13	0.4615**L**	0.0	0.0	0.0
Mean		0.23(0.33)	0.11(0.16)	-	-
South Dakota					
SD-1	8	0.6250-	0.2500**R**	0.0	0.2500**R**
SD-3	23	0.1304-	0.2174**L**	0.0435**R**	0.0
Mean		0.38(0.35)	0.23(0.02)	0.02(0.03)	0.13(0.17)

Table 51: Continued.

Location	N	Total POM Pores	Posterior Infraorbital	Anterior Infraorbital	Opercular pores
Wisconsin					
WI-1	11	0.5454-	0.1818-	0.0909L	0.0
WI-2	10	0.4000R	0.0	0.0	0.0
WI-3	10	0.3000R	0.2000L	0.1000L	0.0
WI-4	14	0.8571R	0.2143R	0.0	0.0
WI-5	6	0.3333L	0.5000R	0.0	0.0
WI-6	10	0.5000L	0.4000R	0.1000L	0.0
WI-7	7	0.2857R	0.1429R	0.1429L	0.0
WI-8	11	0.3636R	0.3636L	0.0	0.0
WI-10	14	0.3571R	0.2143R	0.0	0.0
WI-13	5	0.4000L	0.2000L	0.0	0.0
WI-15	14	0.2857R	0.0	0.0714R	0.0
WI-16	14	0.5714-	0.1429-	0.1429R	0.0
WI-17	15	0.1333L	0.2000R	0.0667R	0.0
WI-18	25	0.5600R	0.3600R	0.3200L	0.0
WI-19	24	0.3333L	0.2500L	0.1666L	0.0
WI-20	9	0.4444R	0.2222R	0.0	0.2200-
WI-21	5	0.2000L	0.2000L	0.0	0.0
WI-25	28	0.5000-	0.1786L	0.0357R	0.0
WI-27	11	0.6364L	0.0909L	0.0909R	0.0
WI-28	11	0.5454L	0.0	0.0	0.0
WI-29	18	0.3333L	0.0	0.0	0.0
WI-30	11	0.7273L	0.1818-	0.0	0.0
WI-31	23	0.6957L	0.1304L	0.3943L	0.3043R
WI-32	6	0.3333R	0.0	0.1666R	0.0
WI-34	24	0.5417-	0.2083R	0.4167R	0.0
WI-35	9	0.4444R	0.1111R	0.0	0.0
WI-36	7	0.4286-	0.1429L	0.2857R	0.2857-
Mean		0.45(0.17)	0.18(0.13)	0.10(0.13)	0.03(0.09)
Quebec					
QU-4	13	0.6923L	0.3846R	0.0769L	0.0
QU-5	9	0.3333R	0.2222-	0.1111L	0.0
Mean		0.51(0.25)	0.30(0.11)	0.09(0.02)	-
Ontario					
ON-1	11	0.7272L	0.1818R	0.0909L	0.0
ON-4	12	0.5833L	0.1666R	0.0833L	0.2500R
ON-5	11	0.1818R	0.0909L	0.0	0.0
ON-8	13	0.6154L	0.0769L	0.0769L	0.4615-
ON-9	11	1.0000L	0.2727R	0.1818R	0.0
ON-11	5	0.0	0.0	0.2000L	0.0

Table 51: Continued.

Location	N	Total POM Pores	Posterior Infraorbital	Anterior Infraorbital	Opercular pores
Ontario					
ON-13	6	0.6667L	0.0	0.0	0.0
ON-15	10	0.6000R	0.0	0.2000R	0.0
ON-16	10	0.3000R	0.0	0.2000R	0.3000R
ON-17	10	0.8000R	0.2000-	0.2000-	0.0
ON-18	10	0.6000-	0.1000R	0.1000L	0.0
ON-20	9	0.6667R	0.6667R	0.1111R	0.0
ON-21	5	0.6000L	0.0	0.0	0.2000L
ON-22	10	0.8000L	0.2000L	0.1000R	0.4000R
ON-23	7	0.4286L	0.7143R	0.0	0.0
ON-24	10	0.2000R	0.4000-	0.1000L	0.0
ON-25	10	0.6000L	0.1000R	0.0	0.0
ON-26	10	1.1000L	0.0	0.0	0.0
ON-27	14	0.2143R	0.2857-	0.1429R	0.0
ON-28	11	0.3636R	0.2727R	0.1818R	0.0
ON-30	10	0.5000L	0.1000R	0.2000-	0.0
ON-32	7	0.7143-	0.0	0.1428L	0.2857L
ON-33	7	0.1428R	0.0	0.4286R	0.0
ON-34	10	0.6000L	0.1000R	0.5000L	0.0
ON-35	10	0.3000L	0.1000L	0.0	0.0
ON-36	16	0.4286L	0.0625R	0.0625R	0.0
ON-37	10	0.4000R	0.2000R	0.2000L	0.2000R
ON-38	12	0.3333R	0.2500R	0.2500R	0.0
ON-39	8	0.3750R	0.2500R	0.5000-	0.0
ON-40	10	0.4000R	0.1000R	0.1000R	0.3000R
ON-41	14	0.2143L	0.4286R	0.0	0.0
ON-42	8	0.6250R	0.0	0.1250R	0.0
ON-43	9	0.8750R	0.0	0.1250R	0.2500-
ON-44	10	0.2000L	0.0	0.0	0.0
ON-45	8	0.6250-	0.1250L	0.2500-	0.0
ON-46	5	0.4000R	0.0	0.0	0.0
ON-47	10	0.8000R	0.2000-	0.2000R	0.2000-
ON-48	12	0.0	0.2500L	0.2500L	0.5000R
Mean		0.50(0.26)	0.16(0.17)	0.14(0.13)	0.09(0.15)
Manitoba					
MB-1	5	1.4000R	0.2000R	0.2000L	0.4000-
MB-2	10	0.4000R	0.0	0.2000-	0.0
MB-4	12	0.1667L	0.4167L	0.1667R	0.0
MB-5	10	0.9000R	0.0	0.0	0.2000R
MB-8	10	0.8000L	0.0	0.1000L	0.4000L
MB-9	5	0.2000L	0.0	0.4000L	0.0
MB-11	11	1.0000-	0.2727L	0.1818-	0.0

Table 51: Continued.

Location	N	Total POM Pores	Posterior Infraorbital	Anterior Infraorbital	Opercular pores
Manitoba					
MB-12	12	0.5000R	0.2500L	0.0833R	0.5833R
MB-13	11	0.0909R	0.6364L	0.2727L	0.0
MB-14	12	0.1667R	0.2500R	0.1667L	0.0
MB-15	12	0.5000-	0.3333R	0.1667-	0.0
MB-16	14	0.4286R	0.1428R	0.2143R	0.3571R
MB-17	11	0.3636L	0.0909L	0.0	0.0
Mean		0.53(0.39)	0.20(0.19)	0.17(0.11)	0.15(0.21)
Saskatchewan					
SK-5	10	0.5000R	0.0.0	0.1000R	0.0

between asymmetry in posterior infraorbital pores and total POM pores (r = -0.0718) and between asymmetry in anterior infraorbital pores and total POM pores (r = -0.1082). There is no correlation between asymmetry in anterior and posterior infraorbital pores. Correlations between asymmetry in the POM pores on the opercle and anterior (r = - 0.0823) or posterior (r = 0.1181) infraorbital pores is small. A comparison of the correlation between body areas for all lake and river samples combined shows little difference. None of these correlations is significant. The only significant correlation occurs for river samples between the total number of POM pores and those confined to the opercle ($r = 0.2303$; $P < 0.01$). The asymmetry in total POM pores and anterior infraorbital pores is -0.1652 lake: -0.0819 rivers, total POM pores and posterior infraorbital pores is -0.2637 lake: -0.0294 rivers, anterior and posterior infraorbital pores is 0.1516 lake: -0.0509 rivers, POM pores on opercle and total POM pores is 0.1427 lake: 0.3468 rivers, POM pores on opercle and anterior infraorbital pores is 0.0529 lake: 0.1532 rivers, and POM pores on opercle and posterior infraorbital pores is 0.0670 lake: -0.1147 rivers.

There are only eight specimens exhibiting asymmetry in left and right total POM pores, POM pores confined to the opercle and anterior and posterior infraorbital pores. There are no significant correlations for specimens to exhibit similar asymmetry levels in several characters.

Within all samples there is no significant correlation for asymmetry to be more prevalent in lakes than in rivers. In total, 48 lake and 113 riverine samples are presented in Table **51**. However, many samples did not qualify for inclusion in this

table due to there being less than five specimens in the sample or exhibiting less than 20% asymmetry for any character. For all samples exhibiting asymmetry, 55 are from lakes and 163 from rivers. Therefore, 91.5% of the lake and 90.6% of the river samples exhibit asymmetry. The average fluctuating asymmetry values are: total POM pores - 0.5032 (SD = 0.2757) for rivers and 0.4583 (SD = 0.2309) for lakes; anterior infraorbital pores - 0.1089 (SD = 0.1128) for rivers and 0.1229 (SD = 0.1248) for lakes; posterior infraorbital pores - 0.2045 (SD = 0.1635) for rivers and 0.1461 (SD = 0.1492) for lakes; and, POM pores confined to opercle - 0.0405 (SD = 0.1055) for rivers and 0.1124 (SD = 0.1726) for lakes. For the entire sample, the average fluctuating asymmetry values are 0.4898 (SD = 0.2633) for total POM pores, 0.1131 (SD = 0.1163) for posterior infraorbital pores, 0.1871 (SD = 0.1612) for anterior infraorbital pores and 0.619 (SD = 0.1328) and 0.0619 (SD = 0.1328) for POM pores confined to the opercle.

Among all states or provinces, samples from Missouri exhibit the highest asymmetry values for POM pores (0.85). The highest asymmetry values for anterior infraorbital pores are from North Dakota (0.31). The highest asymmetry values for posterior infraorbital pores are from Manitoba (0.17). Michigan exhibits the highest asymmetry values for POM pores confined to the opercle (0.41). Overall, the lowest asymmetry values for all examined characters occur in the east while slightly higher values are found in the northwest.

BIOCHEMISTRY

Johnny darters were polymorphic at 16 of the 20 allozyme loci examined.

Forty-one presumptive alleles were identified. Four of these polymorphisms were for rare alleles (*AAT-1,2*106*, *ME-1*124*, *IDH*64*, *IDH*76*) and were seen in only a few of the 644 examined specimens. Most samples analyzed were monomorphic for the majority of enzymes. Notable exceptions include (percentage of heteroallelic loci in parentheses) McVicars Creek (57.9%) and the Neebing-McIntyre River (42.15%), Ontario, and Puckaway Lake (26.3%), Upper St. Croix Lake (26.3%) and the Flambeau (21.1%) and Rock rivers (21.1%), Wisconsin. Boulevard Lake (N=92) and Roundtable Lake (N=7), Ontario, had no polymorphic loci. The mean number of alleles per locus, proportion of loci polymorphic, and the observed and expected number of heterozygotes are presented in Tables **52** and **53**. Statistically significant departures from Hardy-Weinberg expected frequencies were found at one locus for *sAAT-3,4** for Silver Falls Creek, Ontario, and the Flambeau River, Wisconsin, with $P < 0.016$ and $P < 0.036$ respectively, due to an excess of heterozygotes. All other loci exhibited frequencies expected through Hardy-Weinberg.

Since the Boulevard Lake sample had no polymorphic loci, utilizing specimens collected in two years had no influence on the results. I used the univariate "*T*" test to check for significant differences in allele frequencies between years for the McVicars Creek, Ontario, samples. As I did not find any significant differences, I combined the results of both years and treated them as one sample.

No variation was exhibited by xanthine dehydrogenase (*XDH**). Only one pattern was observed; that of a single band in the same position for all specimens examined. The quaternary structure of this enzyme could not be determined.

Table 52: Estimates of genetic variability in 15 geographic samples of *Etheostoma nigrum* from Ontario and Wisconsin using no statistical criterion to define polymorphic loci.

Locality	N	Number of alleles per locus (Mean ± SD)	Percent of polymorphic* loci	Proportion of heterozygotes	
				Observed (Mean ± SD)	Expected** (Mean ± SD)
McVicars Creek, ON	51	1.7 ± 0.2	57.9	.133 ± .047	.132 ± .046
Neebing-Mcintyre River, ON	56	1.5 ± 0.1	42.1	.109 ± .040	.114 ± .042
Boulevard Lake, ON	97	1.0	0.0	.000	.000
Little Dog Lake, ON	68	1.1 ± 0.1	5.3	.002 ± .002	.002 ± .002
Nipigon River, ON	66	1.1 ± 0.1	5.3	.002 ± .022	.002 ± .022
Roundtable Lake, ON	10	1.0	0.0	.000	.000
Silver Falls Creek, ON	9	1.2 ± 0.1	15.8	.082 ± .054	.060 ± .037
Prelate Lake, ON	52	1.1 ± 0.1	5.3	.012 ± .012	.013 ± .013
Sandstone Lake, ON	92	1.1 ± 0.1	5.3	.001 ± .001	.001 ± .001
Upper St. Croix Lake, WI	34	1.2 ± 0.1	21.1	.062 ± .038	.059 ± .036
Big Arbor Vitae Lake, WI	59	1.2 ± 0.1	15.8	.038 ± .028	.037 ± .027
Rock River, WI	15	1.3 ± 0.1	21.1	.063 ± .041	.053 ± .033
Puckaway Lake, WI	15	1.2 ± 0.1	21.1	.049 ± .028	.050 ± .029
Flambeau River, WI	16	1.3 ± 0.1	26.3	.072 ± .039	.072 ± .038
Whitefish Lake, WI	7	1.2 ± 0.1	15.8	.053 ± .031	.058 ± .036

* A locus is considered polymorphic if more than one allele was detected
** Unbiased estimate (see Nei 1978)

Table 53: Estimates of genetic variability in 15 geographic samples of *Etheostoma nigrum* from Ontario and Wisconsin using a 0.95 statistical criterion to identify polymorphic loci.

Locality	N	Number of alleles per locus (Mean ± SD)	Percent of polymorphic* loci	Proportion of heterozygotes	
				Observed (Mean ± SD)	Expected** (Mean ± SD)
McVicars Creek, ON	51	1.7 ± 0.2	36.8	.133 ± .047	.132 ± .046
Neebing-McIntyre River, ON	56	1.5 ± 0.1	26.3	.109 ± .040	.114 ± .042
Boulevard Lake, ON	97	1.0	0.0	.000	.000
Little Dog Lake, ON	68	1.1 ± 0.1	0.0	.002 ± .002	.002 ± .002
Nipigon River, ON	66	1.1 ± 0.1	0.0	.002 ± .002	.002 ± .002
Roundtable Lake, ON	10	1.0	0.0	.000	.000
Silver Falls Creek, ON	9	1.2 ± 0.1	15.8	.082 ± .054	.060 ± .037
Prelate Lake, ON	52	1.1 ± 0.1	5.3	.012 ± .012	.013 ± .013
Sandstone Lake, ON	92	1.1 ± 0.1	0.0	.001 ± .001	.001 ± .001
Upper St. Croix Lake, WI	34	1.2 ± 0.1	10.5	.062 ± .038	.059 ± .036
Big Arbor Vitae Lake, WI	59	1.2 ± 0.1	10.5	.038 ± .028	.037 ± .027
Rock River, WI	15	1.3 ± 0.1	10.5	.063 ± .041	.053 ± .033
Puckaway Lake, WI	15	1.2 ± 0.1	15.8	.049 ± .028	.050 ± .029
Flambeau River, WI	16	1.3 ± 0.1	21.1	.072 ± .039	.072 ± .038
Whitefish Lake, WI	7	1.2 ± 0.1	15.8	.053 ± .031	.058 ± .036

* A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

** Unbiased estimate (see Nei 1978).

Phosphoglucomutase (*PGM**) was determined to be a monomer based on the presence of heterozygotes with two bands. Two alleles are present for the *PGM-1** locus. Only McVicars Creek and the Neebing-McIntyre River, Ontario, exhibit the *PGM-1*109* allele (Fig. **40**). Three alleles are encoded at the *PGM-2** locus (Figs. **41-43**). The Neebing-McIntyre River and McVicars Creek, Ontario, samples exhibit the *PGM-2*130* allele. One specimen from the Rat River, Manitoba, also possessed the *PGM-2*130* allele. The Upper St. Croix Lake, Wisconsin, sample possesses the *PGM-2*60* allele. Because white muscle gels were found to replicate *PGM-2**, both *PGM-1** and *PGM-2** were read from liver gels.

Chi-square tests showed significant differences ($P < 0.001$) among all locations sampled for both *PGM-1** and *PGM-2**. There are no significant differences ($P < 0.20$) for *PGM-1** and *PGM-2** between the Neebing-McIntyre River and McVicars Creek, Ontario, samples.

In *E. nigrum*, phosphogluconate dehydrogenase (*PGDH**) heterozygotes exhibit a dimeric pattern. The single locus has 2 alleles (Fig. **44**). The Rock River, Wisconsin, sample is fixed for the *PGDH*150* allele (Fig. **45**). Heterozygous specimens from McVicars Creek and Neebing-McIntyre River, Ontario, and the Flambeau River and Whitefish Lake, Wisconsin possess the *PGDH*150* allele. All of these samples possess specimens with increased squamation. This enzyme was read from liver gels.

Glycerol-3-phosphate dehydrogenase (*G3PDH**) is encoded by two alleles at one locus. This enzyme was best read from liver gels. The dimeric structure of this

Figure 40: Banding patterns at the *PGM-1** locus in *Etheostoma nigrum* from Wisconsin and Ontario read from liver gels. Genotype and allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

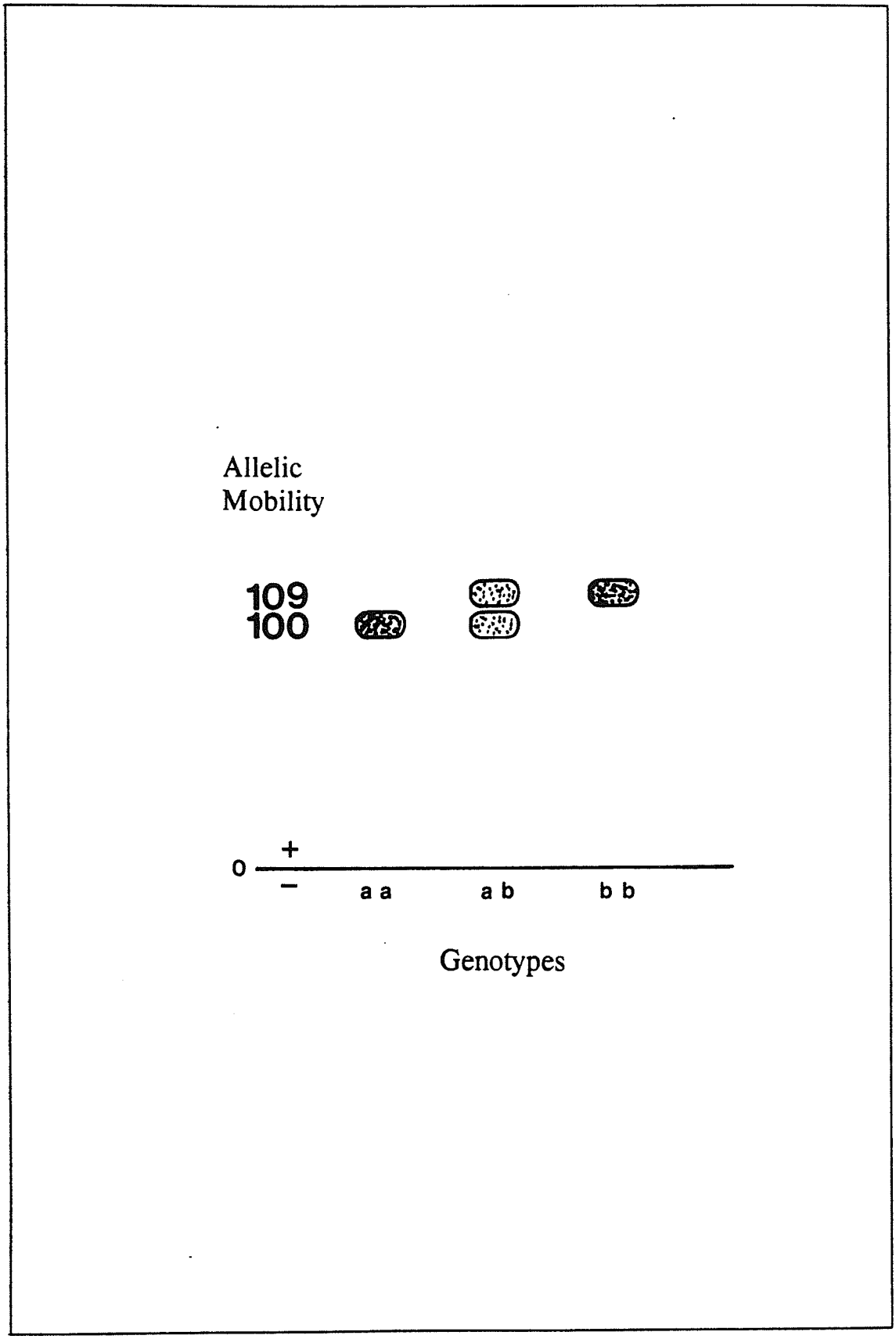

Allelic
Mobility
109
100
0
+
−
a a
a b
b b
Genotypes

Figure 41: Banding patterns at the *PGM-2** locus in *Etheostoma nigrum* from Wisconsin and Ontario read from liver gels. Genotype and allelic mobility is shown. The "bc" and "cc" patterns were not observed in this study. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

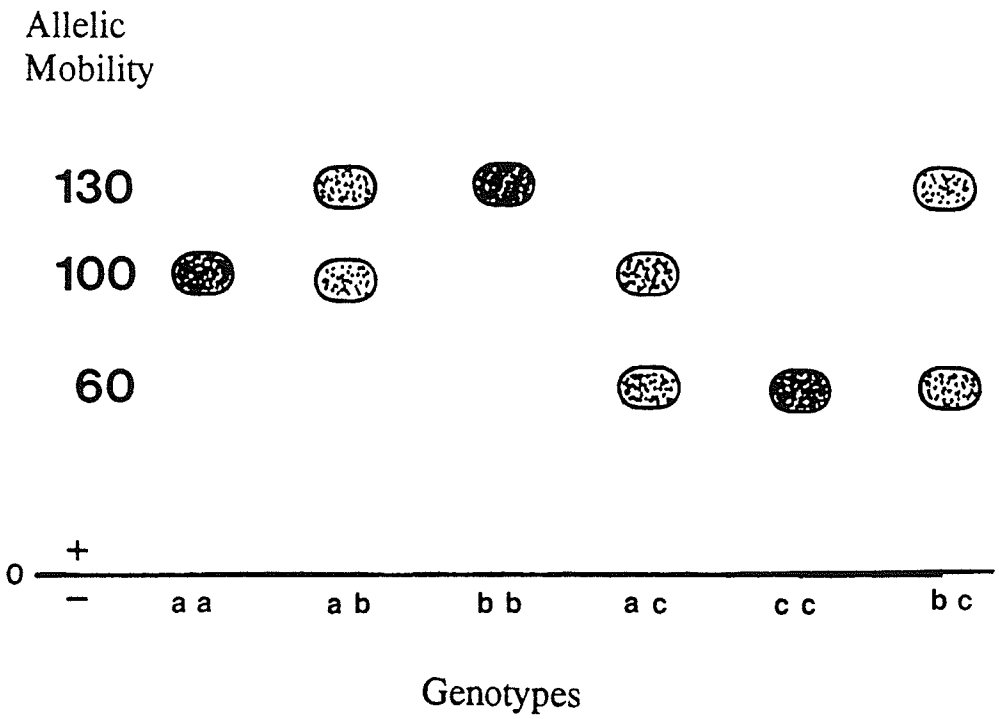

Allelic
Mobility
130
100
60
0
+
−
a a
a b
b b
a c
c c
b c
Genotypes

Figure 42: Photograph showing the *PGM-2*130* allele (arrows) in two Ontario specimens of *Etheostoma nigrum* from the Neebing-McIntyre River. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

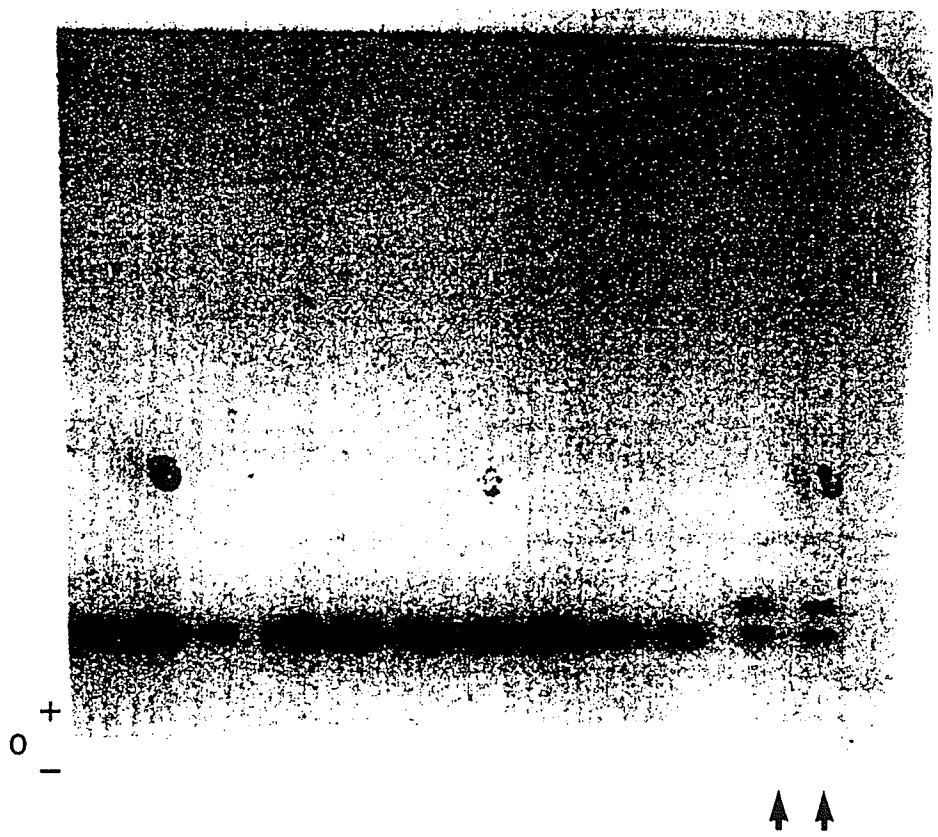

+
0
–

Figure 43: Photograph showing the *PGM-2*60* allele (arrows) in two Wisconsin specimens of *Etheostoma nigrum* from Upper St. Croix Lake. Allelic mobility is shown. Warping has occurred on the gel. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

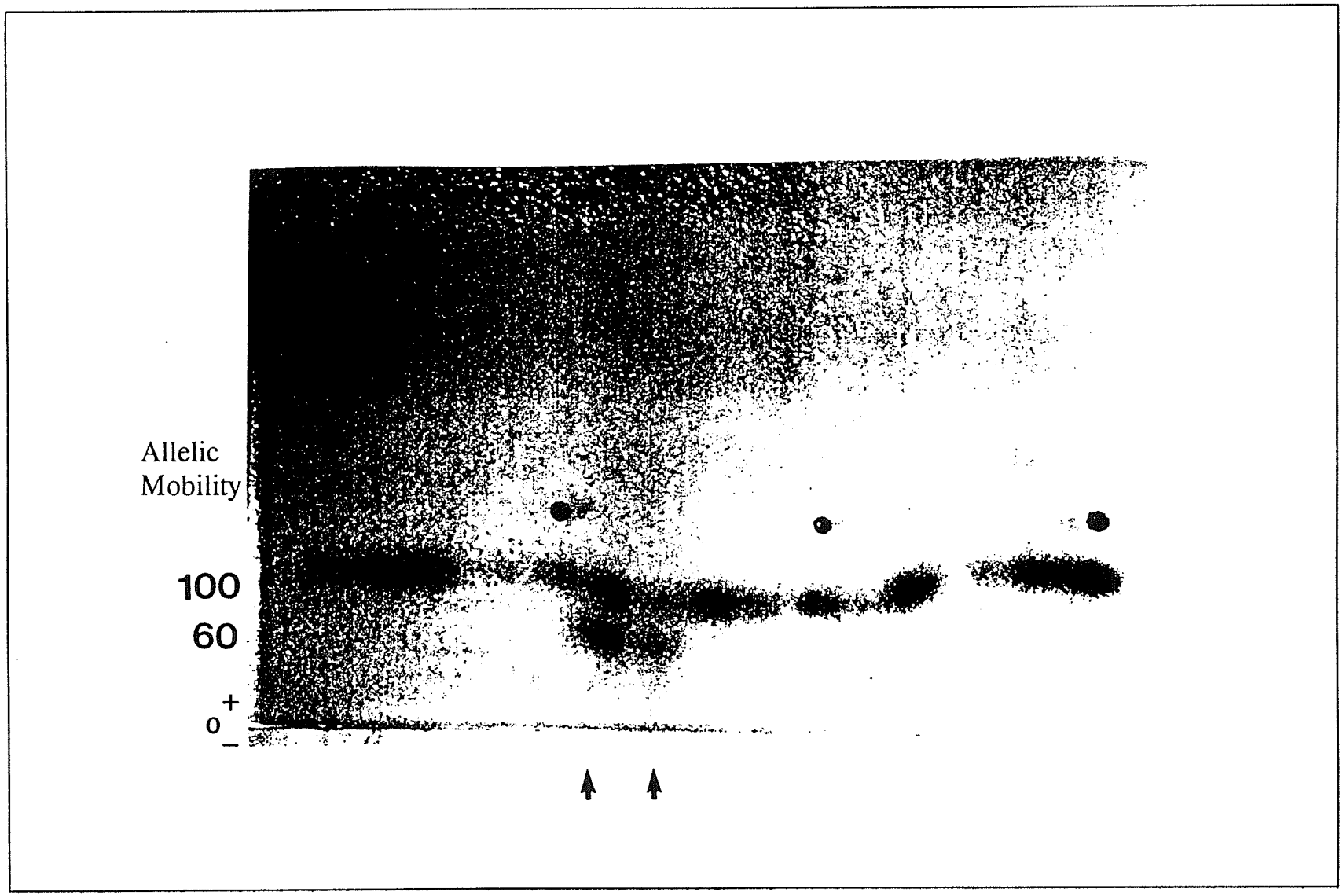

Allelic
Mobility
100
60
+
o
–

Figure 44: Banding patterns at the *PGDH** locus observed in specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Genotype and allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

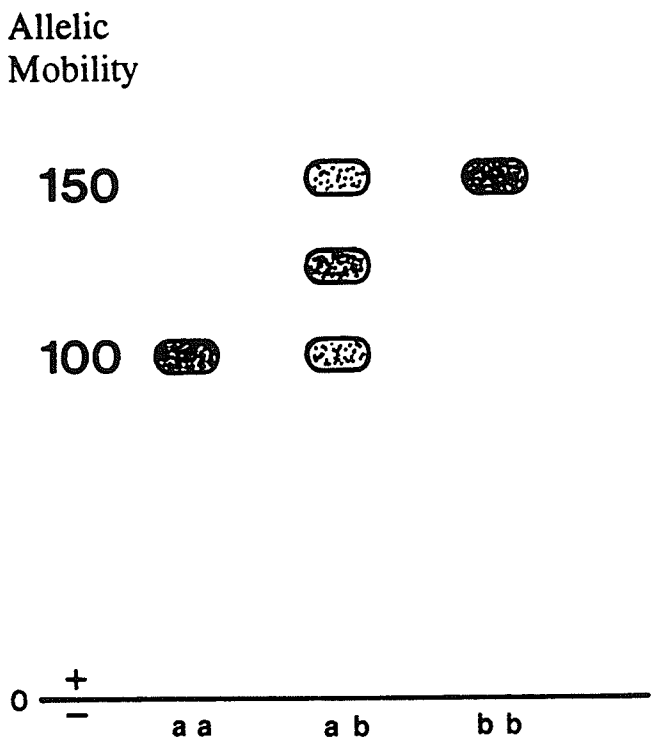
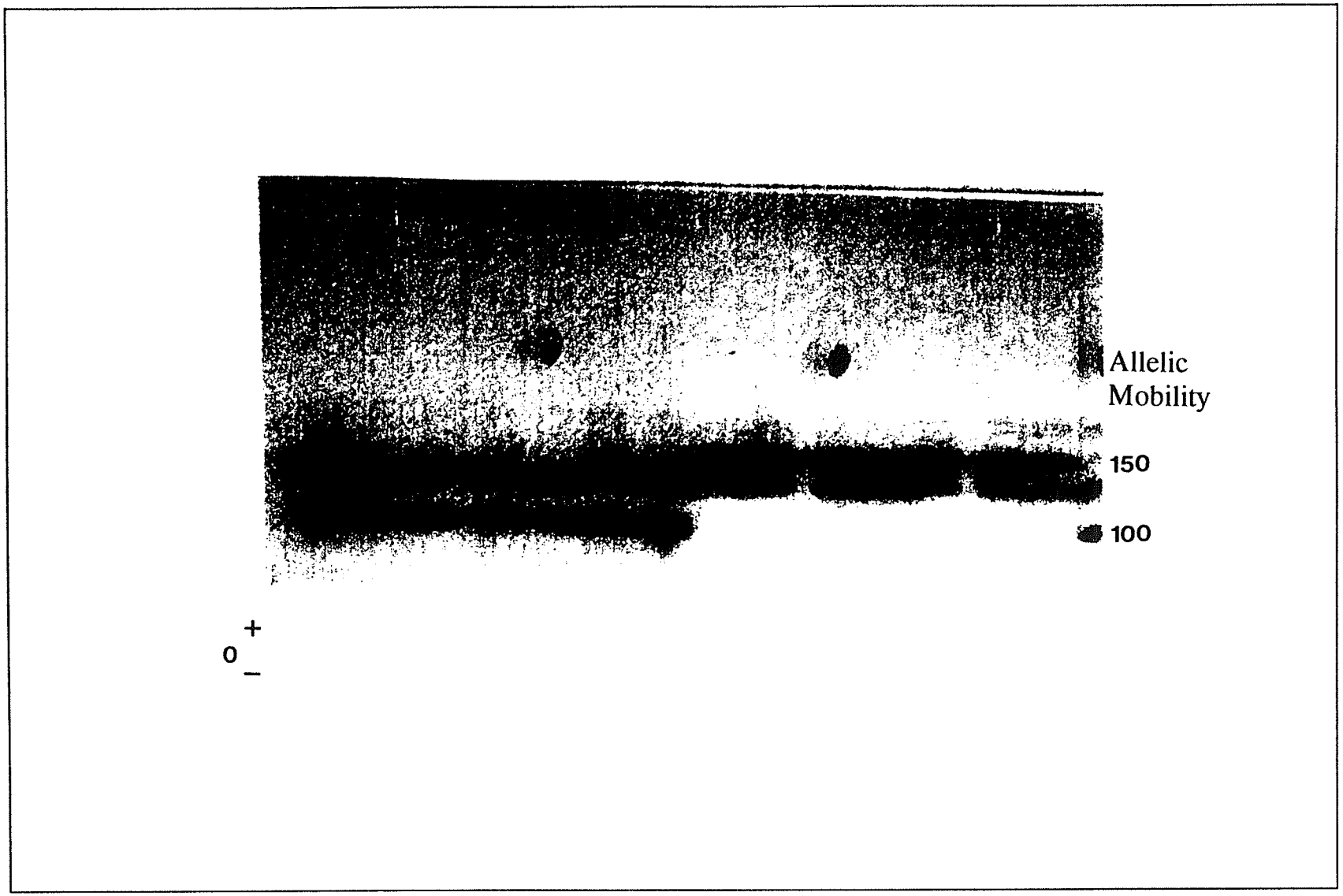

Allelic
Mobility
150
100
0
+
−
a a
a b
b b
Genotypes

Figure 45: Photograph of liver extract gel with *PGDH** (bottom) and *IDH** (top) from specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Allelic mobility is shown for the *PGDH** allele. The Rock River specimens exhibit the *PGDH*150* allele and is located just above the *IDH** locus. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).


Allelic
Mobility
150
100
0
+
−

enzyme is suggested by the presence of three bands in heterozygotes. Three specimens from the Neebing-McIntyre River and one from McVicars Creek, Ontario, exhibited the three banded pattern due to the presence of the *G3PDH*50* allele (Fig. **46**).

Isocitrate dehydrogenase (*IDH**), read from liver gels (denoted as *IDH-L**), exhibits three alleles at one locus (Fig. **47**). Heterozygotes are present in the Nipigon River and McVicars Creek, Ontario, (*IDH-L*122*) and exhibit the typical dimeric banding pattern (Fig. **48**). A single specimen from Puckaway Lake, Wisconsin, possesses a variant *IDH-L*76* allele.

Isocitrate dehydrogenase, read from white muscle samples (denoted as *IDH-W**), exhibits three alleles at one locus (Fig. **49**). This locus is clearly different from that exhibited on liver gels. Both McVicars Creek, Ontario, and Big Arbor Vitae Lake, Wisconsin, exhibit the *IDH-W*50* allele (Fig. **50**). McVicars Creek, Ontario, exhibited a variant allele in one specimen (*IDH-W*64*) (Fig. **51**).

Lactate dehydrogenase (*LDH**) is present at two loci in *E. nigrum*. The *LDH-B** locus exhibited tetrameric bands in samples from McVicars Creek, Ontario, and the Rock River and Puckaway Lake, Wisconsin (*LDH-B*150*) (Fig. **52**). Identification of the *LDH-B** locus was made easier using specimens of *Percina maculata* due to its highly anodal *LDH-B_4* mobility. A single *LDH-A** allele is present in all samples except for Upper St. Croix and Big Arbor Vitae lakes, Wisconsin, and Little Dog Lake, Ontario, which exhibit the tetramere bands moving cathodally (*LDH-A* -47*) (Fig. **53**). Liver gels stained for *LDH** activity generally lacked the resolution seen in

Figure 46: Banding patterns observed at the *G3PDH** locus in specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Genotype and allelic mobility is shown. The "bb" pattern was not observed in this study. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

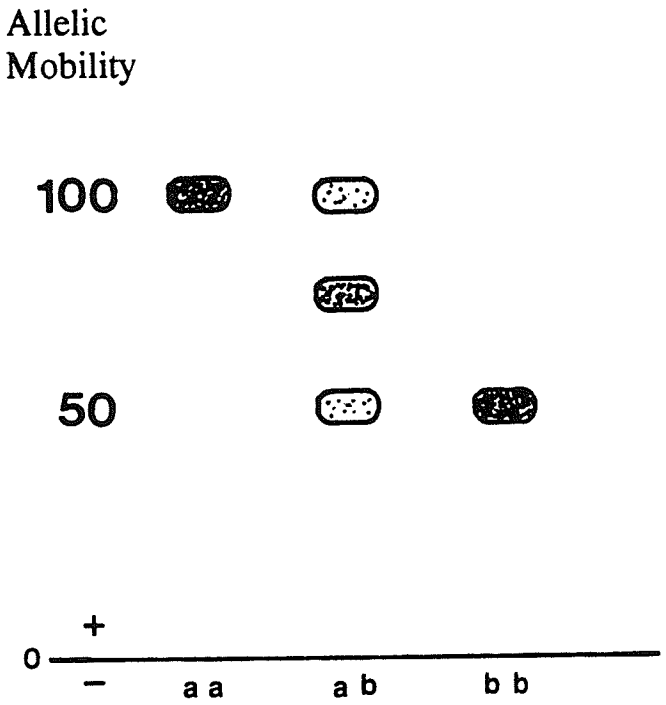

Allelic
Mobility
100
50
0
+
−
a a
a b
b b
Genotypes

Figure 47: Banding patterns observed in *IDH** read from liver gels from specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Genotype and allelic mobility is shown. The "bb", "cc" and "bc" patterns are assumed but were not observed in this study. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

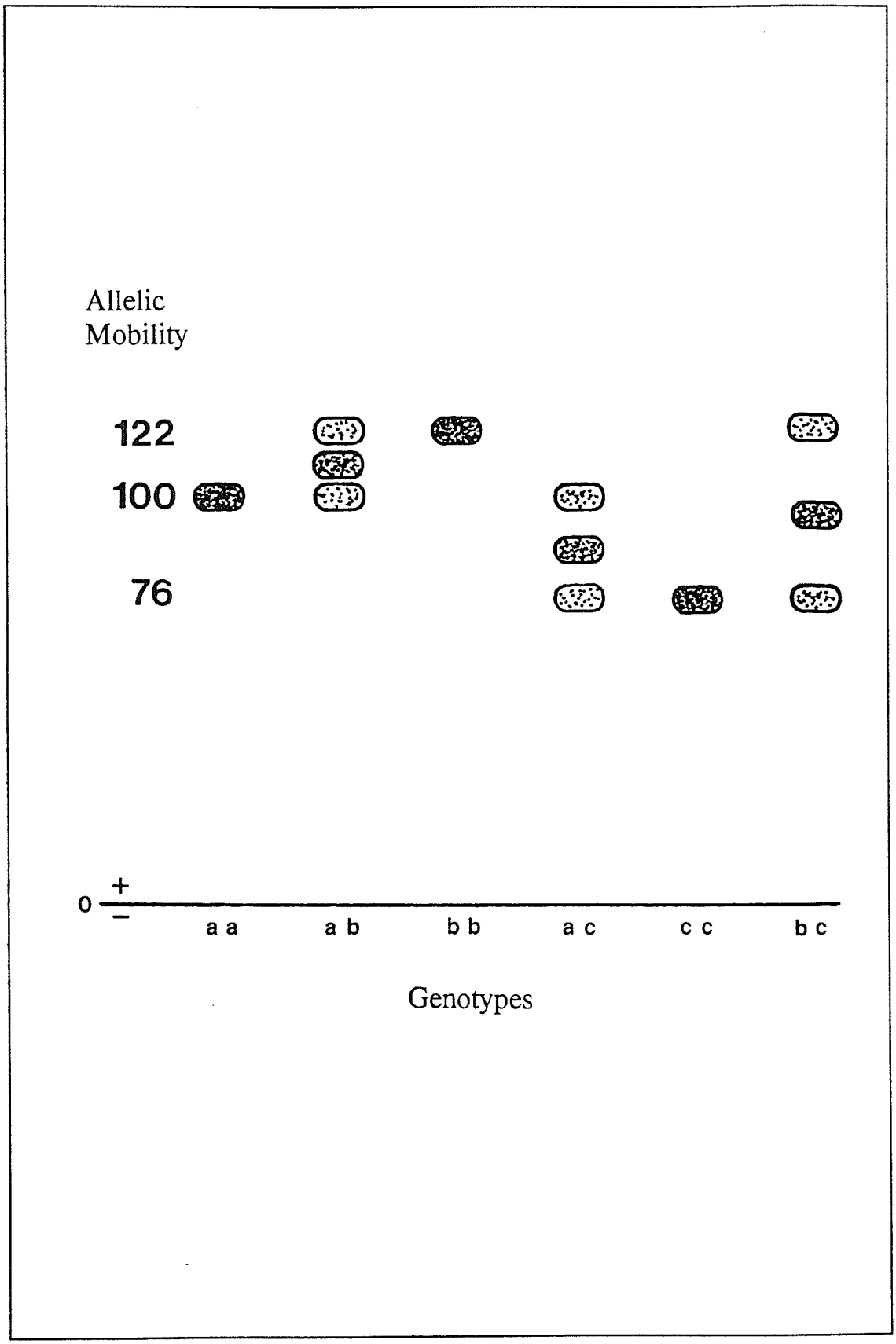

Allelic
Mobility
122
100
76
0
+
−
a a
a b
b b
a c
c c
b c
Genotypes

Figure 48: Photograph of liver extract gel showing banding patterns of *IDH** from specimens of *Etheostoma nigrum* from McVicars Creek, Ontario. Allelic mobility is shown. The *IDH-L*122* allele is evident in two specimens (arrows) on the left side of the gel. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

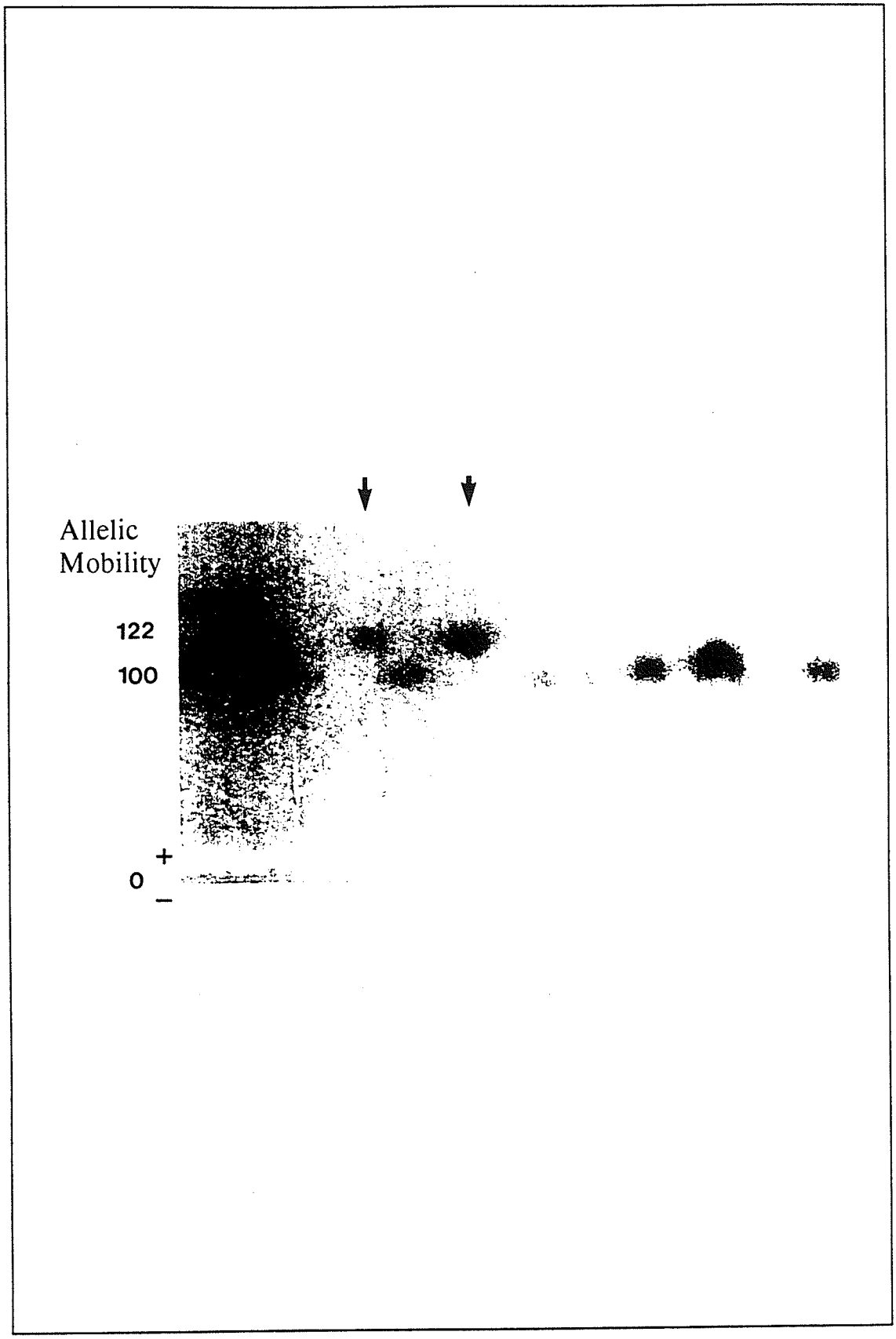

Allelic
Mobility
122
100
0
+
−

Figure 49: Banding patterns observed in *IDH** read from white muscle gels of specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Genotype and allelic mobility is shown. The "bb", "cc" and "bc" patterns were not observed in this study. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

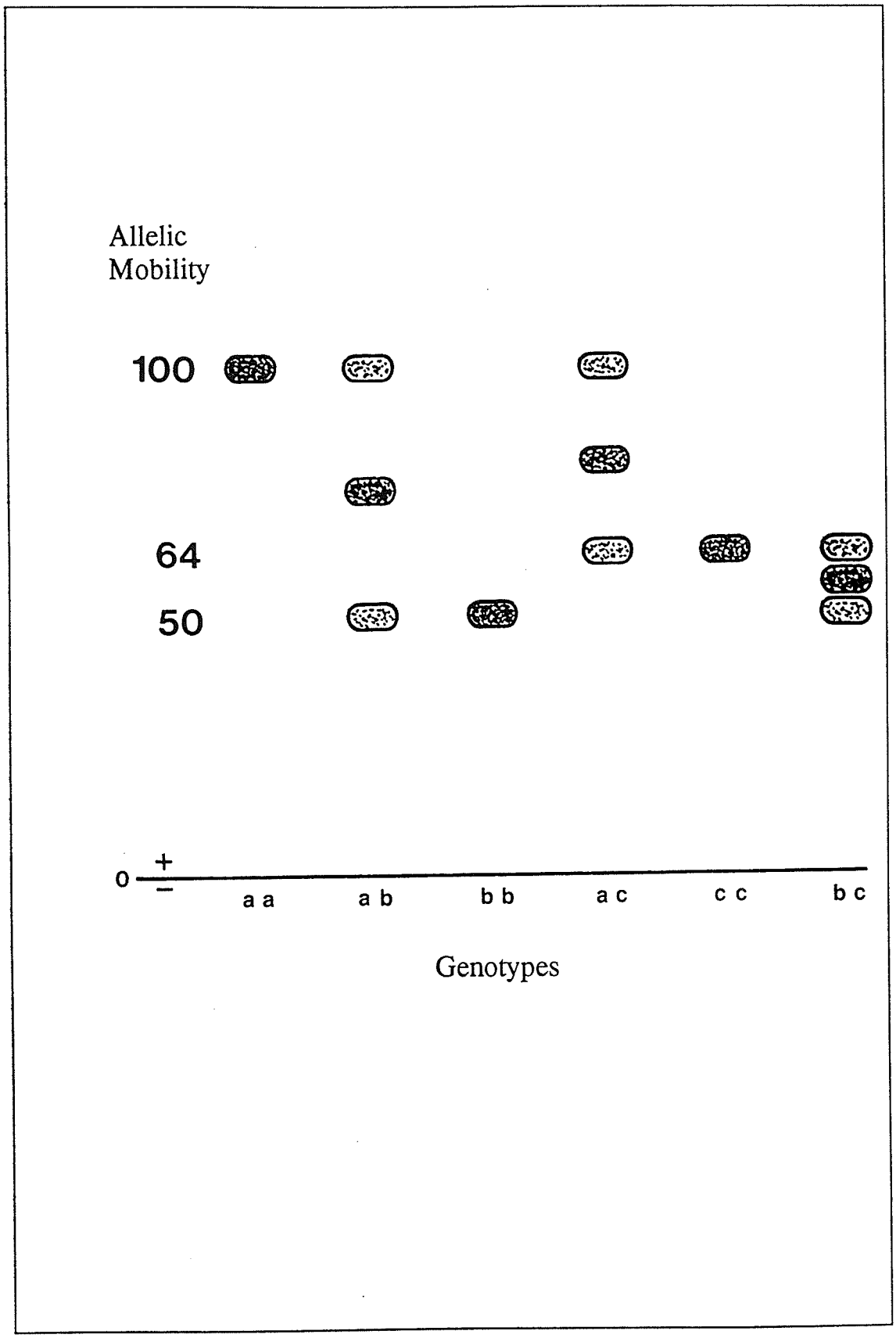

Allelic
Mobility
100
64
50
0
+
−
a a
a b
b b
a c
c c
b c
Genotypes

Figure 50: Photograph showing the *IDH-W*50* allele from a white muscle gel in a specimen of *Etheostoma nigrum* from McVicars Creek, Ontario. Allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

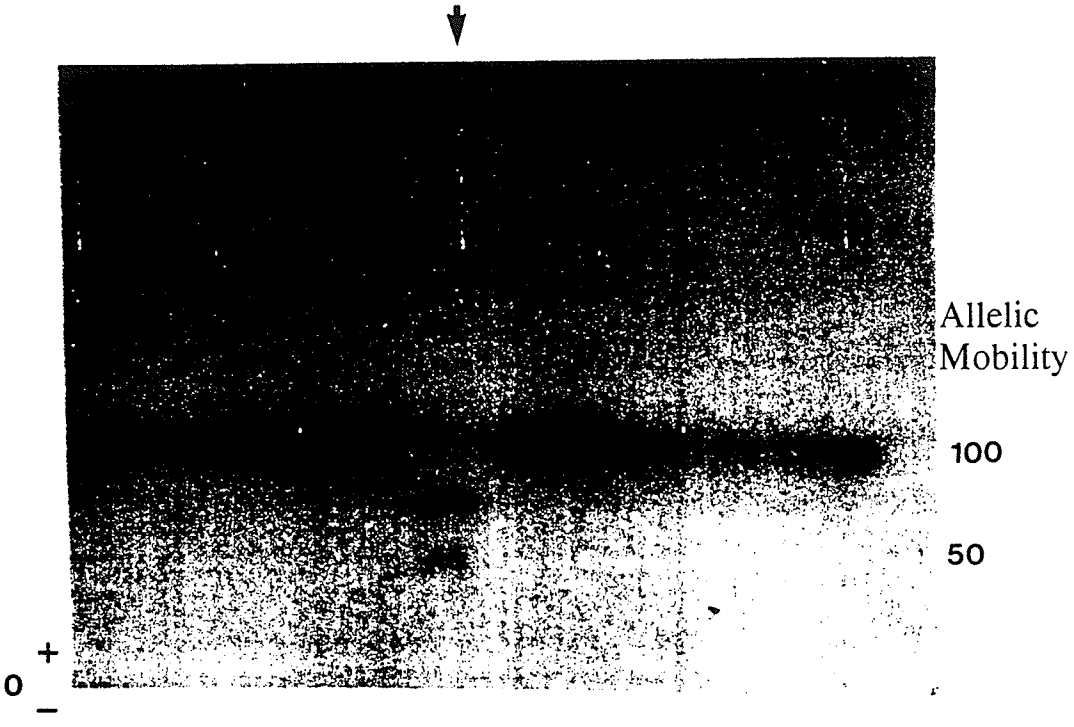

Allelic
Mobility
100
50
0
+
−

Figure 51: Photograph of the variant *IDH-W*64* allele on a white muscle gel from a specimen of *Etheostoma nigrum* from McVicars Creek, Ontario. Allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

Allelic
Mobility
100
64
0
+
−

Figure 52: Photograph showing the *LDH-B*150* allele in two specimens of *Etheostoma nigrum* from Big Arbor Vitae Lake and the Rock River, Wisconsin. A specimen of *Percina maculata* is also shown which exhibits the highly anodal *LDH-B_4* mobility. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

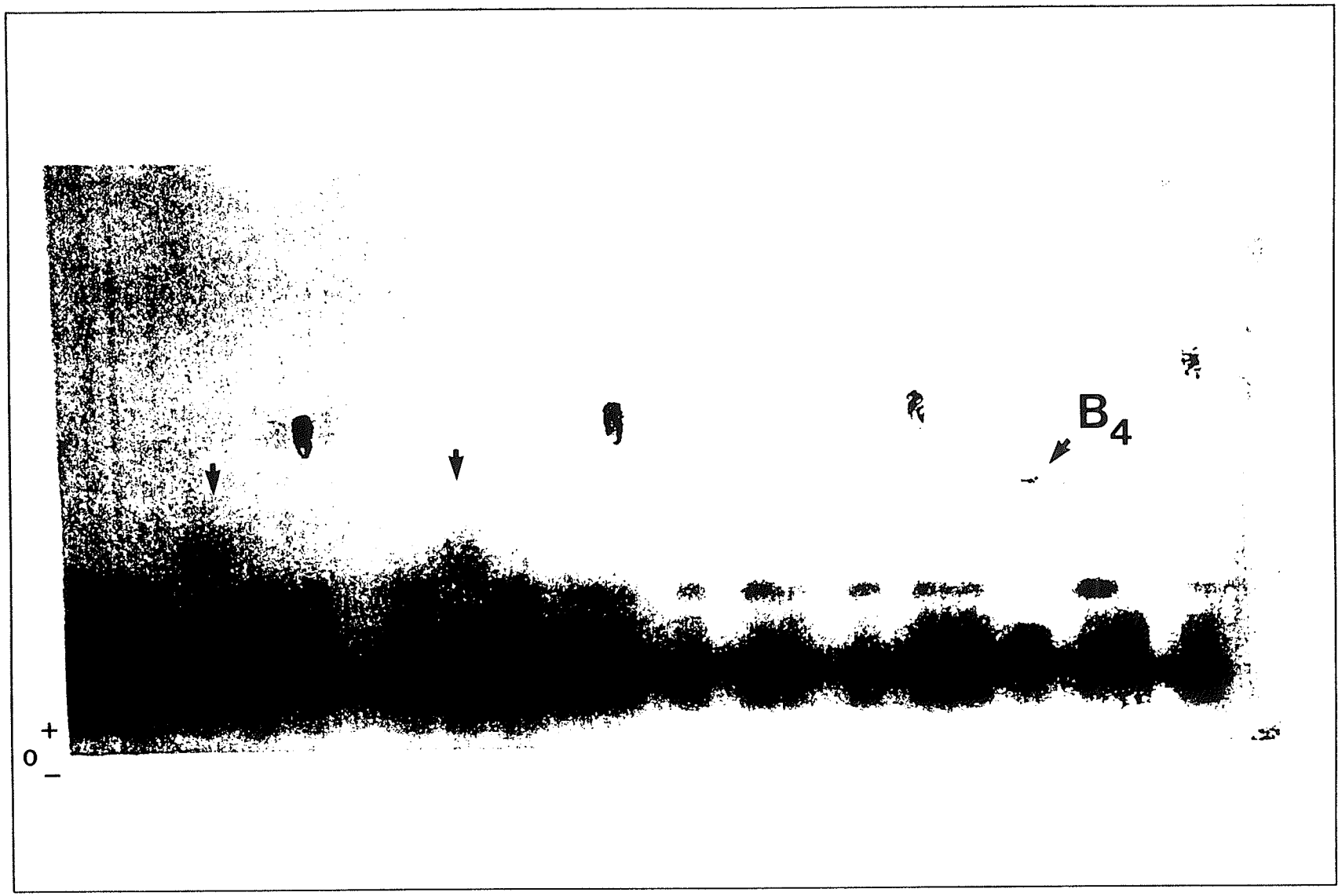
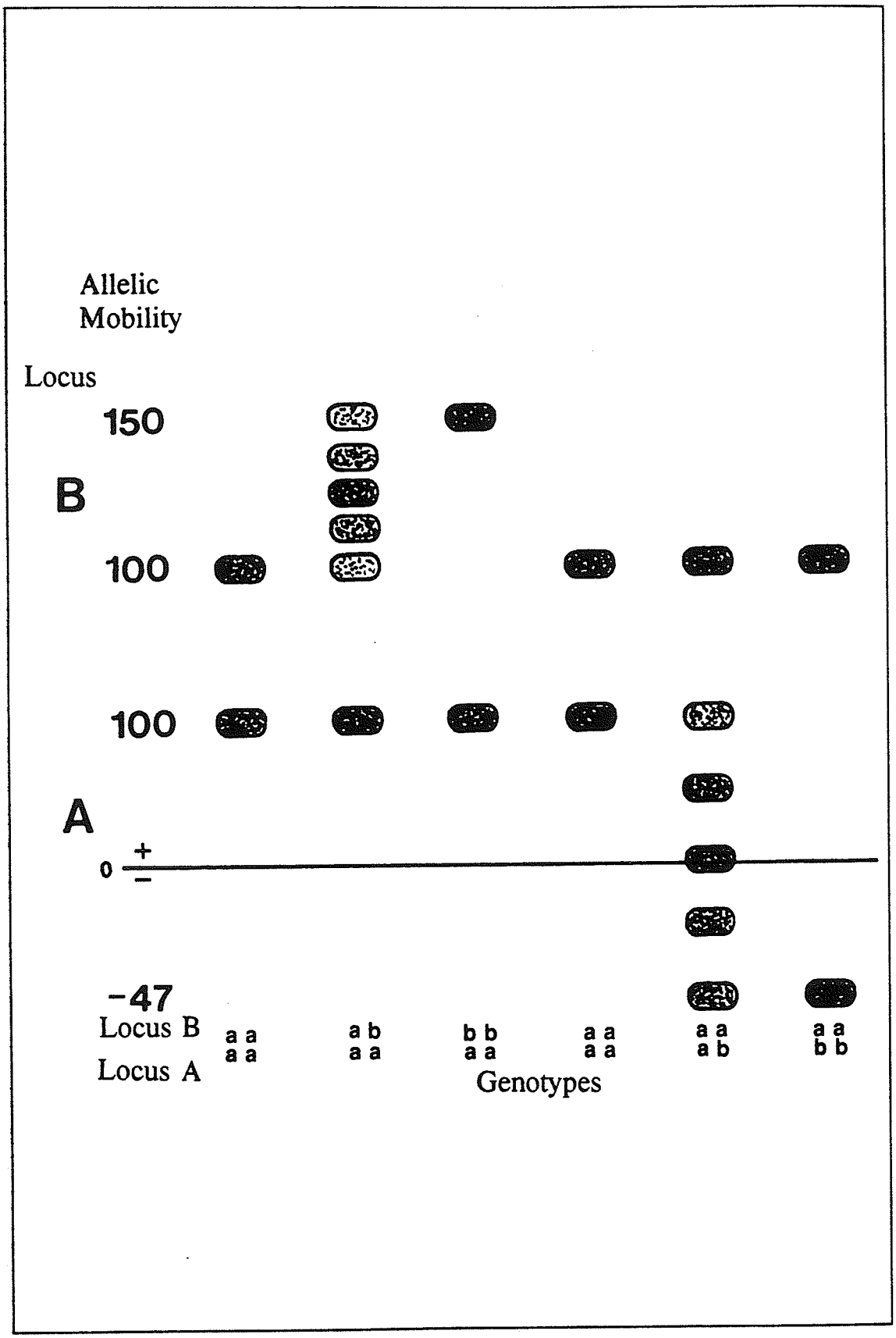

B4
O
+
−

Figure 53: Banding patterns observed in *LDH** read from white muscle gels of specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Loci, genotype and allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).


Allelic
Mobility
Locus
150
B
100
100
A
0
+
−
-47
Locus B
Locus A
a a
a a
a b
a a
b b
a a
a a
a a
a a
a b
a a
b b
Genotypes

muscle gels.

The *GPI-A** (glucose-6-phosphate isomerase) locus is encoded by three alleles (**100*, **91*, **82*). The *GPI-A** locus is variable only in McVicars Creek and the Neebing-McIntyre River, Ontario, and samples from the Rock and Flambeau rivers, Wisconsin. All areas but the Flambeau River possess specimens which exhibit a great deal of squamation. Specimens heterozygous at this locus possess three bands either at mobilities of 100-95.5-91, 91-86.5-82 or 100-91-82. The *GPI-AB** bands are known to be hybrids formed from polypeptides from both the *GPI-A** and *GPI-B** loci (Dando 1974). Specimens heterozygous at the *GPI-A** locus, but homozygous at the *GPI-B** locus, always possess two bands in the hybrid zone although the location of these bands was variable (Fig. **54**). Most specimens are monomorphic at the *GPI-B** locus, the only exception being two specimens from the Flambeau River, Wisconsin, which exhibit a three-banded pattern *GPI-B*152*. For these two fish, the AB hybrid band consists of three bands as opposed to the usual one or two bands seen in all other fish (Fig. **55**). An unexplained three-banded pattern was present below the *GPI-B** locus for one Nipigon River, Ontario, and one Flambeau River, Wisconsin, fish. Additionally, a single band appeared in the same area for one fish each from Boulevard Lake and the Neebing-McIntyre River, Ontario. The single band was located at the lowest position of the three bands seen in the Nipigon and Flambeau River fish. Because *GPI** patterns were replicated on white muscle and liver gels but these extra bands appeared only on liver gels, their presence cannot be explained as a new allele. These bands may be due to including some viscera during dissection or

Figure 54: Banding patterns observed at the *GPI** loci read from liver gels of specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Numbers 1-7 refer to observed genotypes. Genotype 1 is most common. Genotypes 2-6 were observed in McVicars Creek and the Neebing-McIntyre River, Ontario. Genotype 7 was observed in two specimens from the Flambeau River, Wisconsin. Hybrid bands between loci are denoted as "HY". Loci and allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

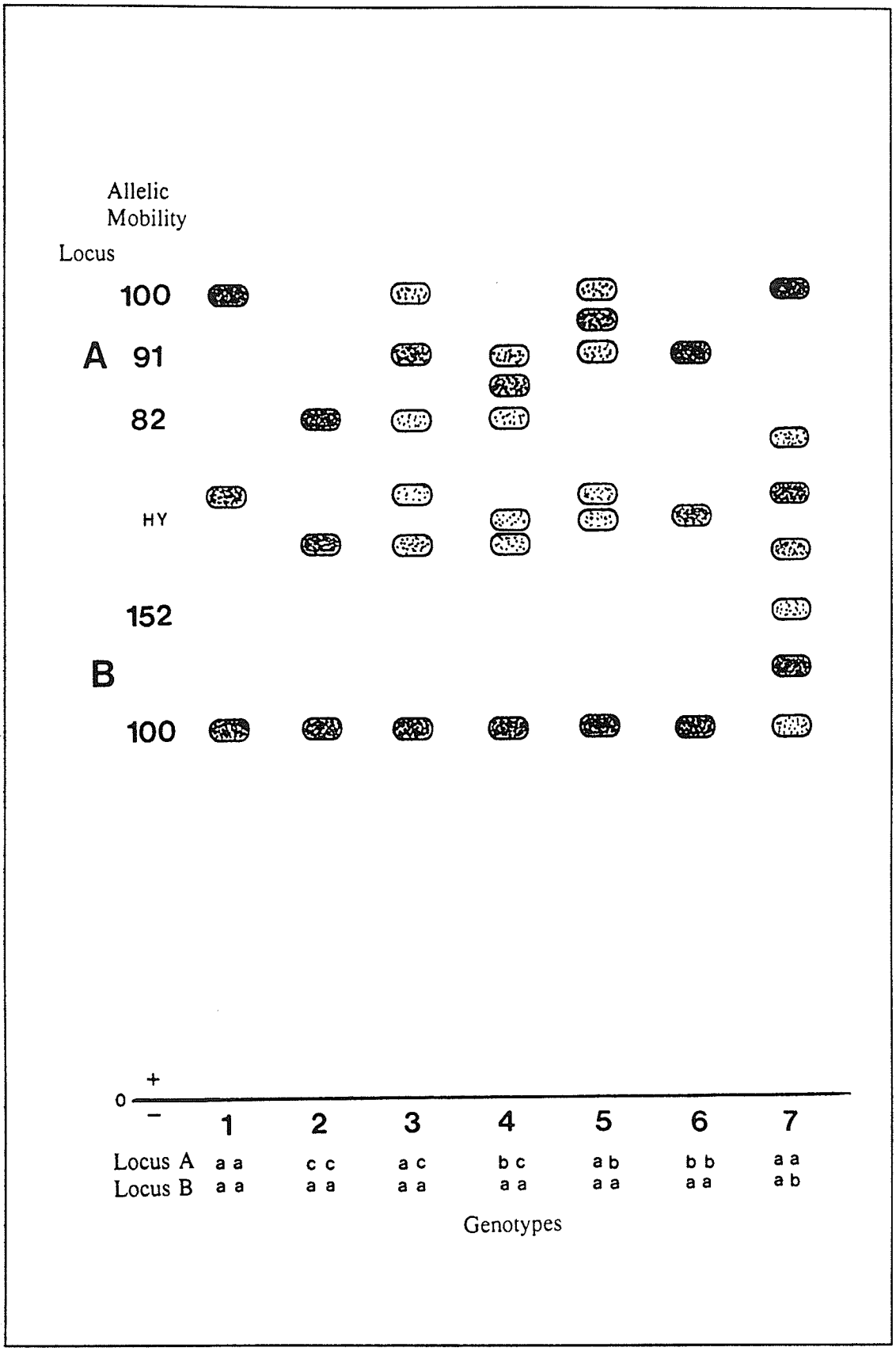
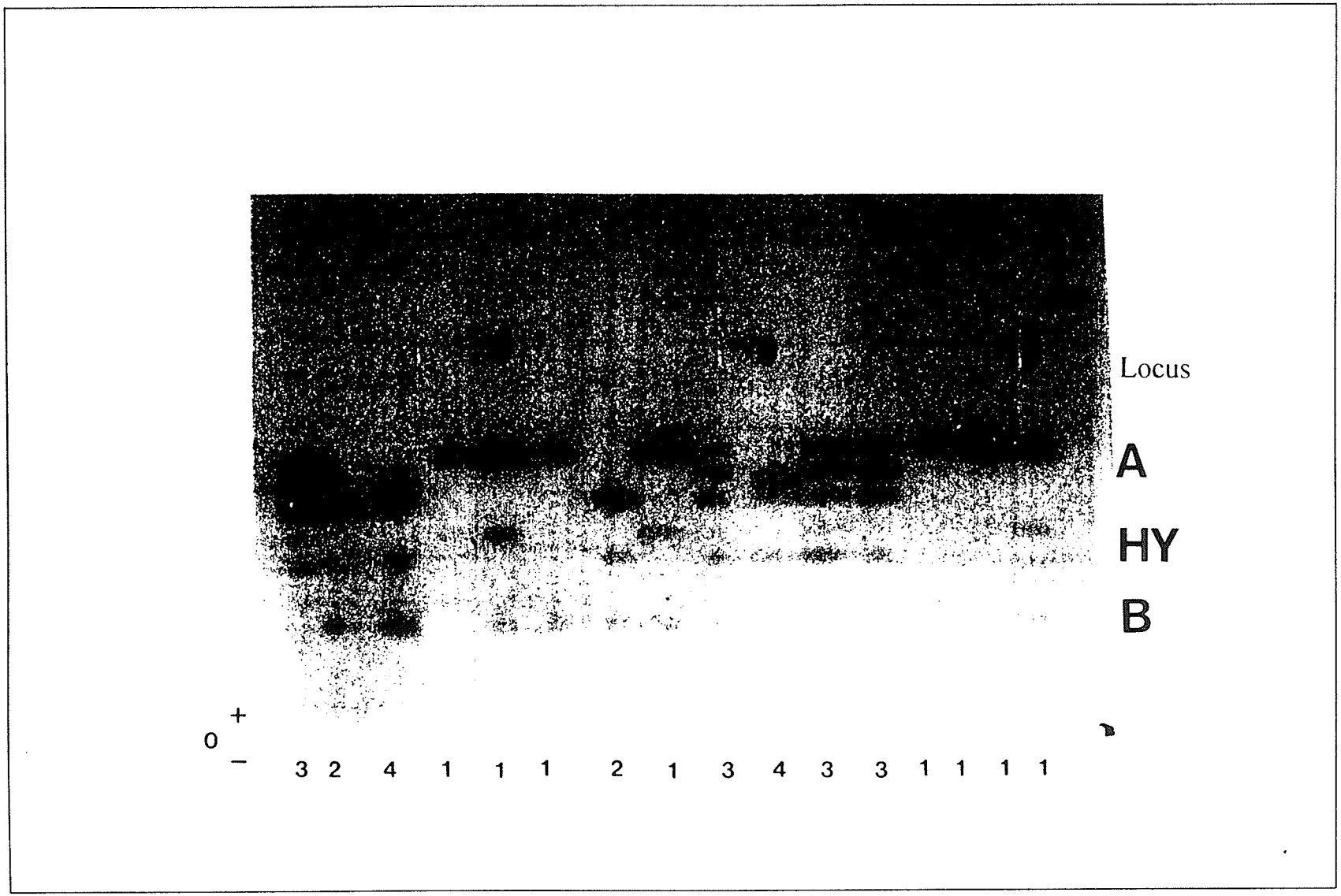

Allelic
Mobility
Locus
100
A
91
82
HY
152
B
100
+
0
−
1
2
3
4
5
6
7
Locus A a a c c a c b c a b b b a a
Locus B a a a a a a a a a a a a a b
Genotypes

Figure 55: Photograph showing the variation in *GPI** alleles common to *Etheostoma nigrum* from the Neebing-McIntyre River and McVicars Creek, Ontario. Hybrid bands are denoted as "HY". The "A" and "B" loci are identified. Numbers below samples refer to genotypes shown in Figure **54**. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).


Locus
A
HY
B
+
0
−
3 2 4 1 1 1 2 1 3 4 3 3 1 1 1 1

simply "ghost" bands. Although *GPI-A** could be read from white muscle or liver gels, it was best expressed on liver gels. The *GPI-B** locus was slightly easier to read from white muscle gels. Figure **55** shows the large number of heterozygotes for *GPI** common to the Neebing-McIntyre River and McVicars Creek, Ontario.

Esterases (*EST**) are present at three loci. The presence of heterozygotes between loci suggest that esterases are monomeric in *E. nigrum*. The *EST-2** locus is monomorphic. The *EST-1*105* locus is variable in McVicars Creek, Neebing-McIntyre River, Silver Falls Creek and Sandstone Lake, Ontario, and in the Rock River, Wisconsin. All areas but Silver Falls Creek possess specimens with increased squamation. Only McVicars Creek and the Neebing-McIntyre River, Ontario exhibit the *EST-3*90* allele (Figs. **56-57**). Esterases were read from liver gels.

Malic enzyme (*ME**) is encoded by two loci in *E. nigrum*. Approximately the same results appear whether using muscle or liver gels although the white muscle gel is somewhat easier to read. Except for one specimen from the Rock River, Wisconsin, *ME-1** is monomorphic in all samples. The presence of the *ME-1*125* allele suggests that *ME** is tetrameric in *E. nigrum*. Specimens heterozygous at the *ME-2** locus are present in McVicars Creek, the Neebing-McIntyre River and Silver Falls Creek, Ontario and Upper St. Croix, Big Arbor Vitae, Puckaway and Whitefish lakes, Wisconsin. The Rock River sample is fixed for the *ME-2*125* allele (Fig. **58**). The Flambeau River, Upper St. Croix Lake and Silver Falls Creek samples exhibit only minor squamation.

Aspartate aminotransferase occurs in both mitochondrial (*mAAT-1,2**) and

Figure 56: Banding patterns observed at *EST** loci read from liver gels of specimens of *Etheostoma nigrum* from Wisconsin and Ontario. Loci and allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

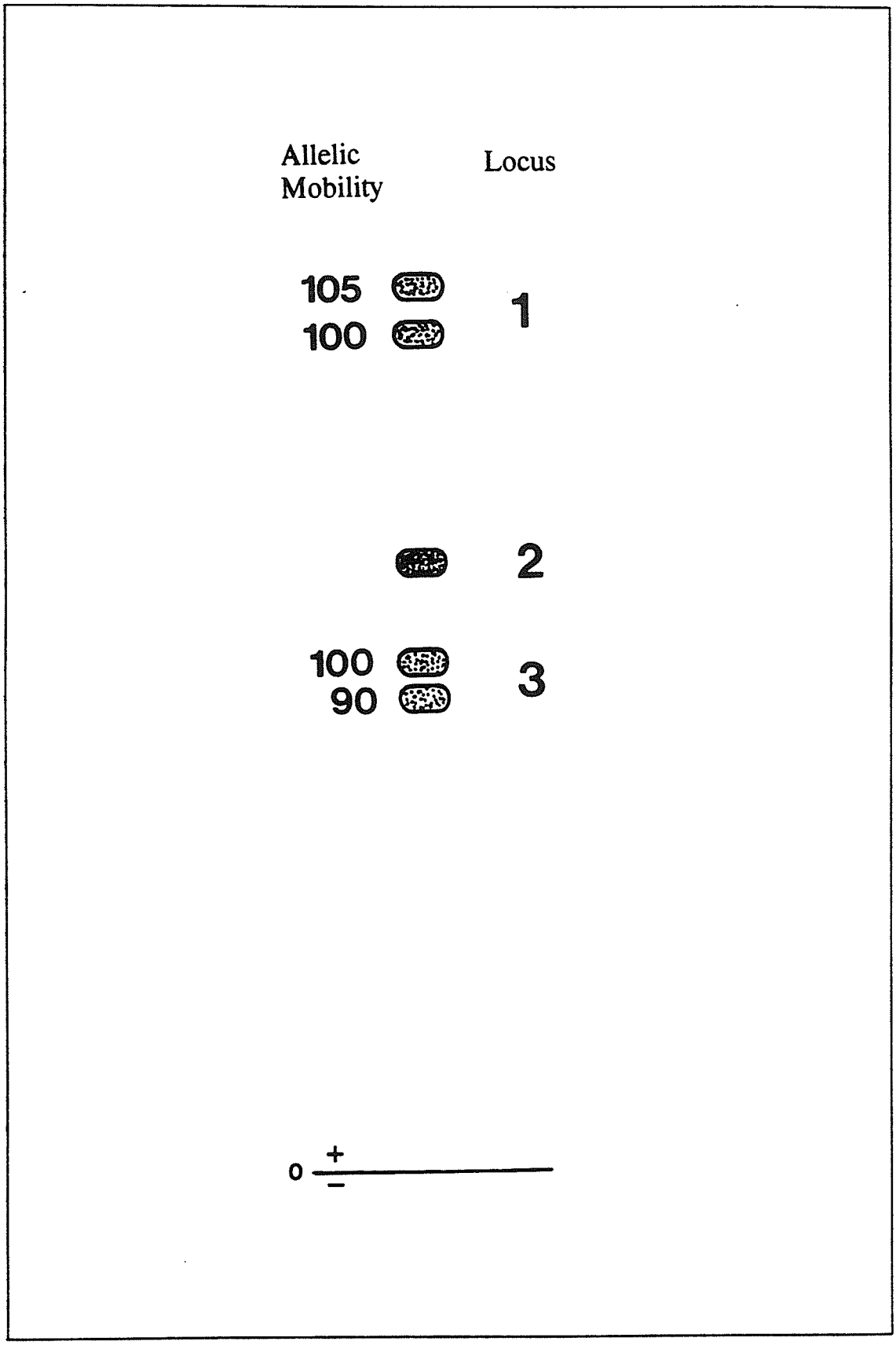

Allelic Mobility
Locus
105
100
1
2
100
90
3
0
+
−

Figure 57: Photograph of liver gel showing patterns of *EST** variation in Ontario specimens of *Etheostoma nigrum*. Loci and allelic mobility is shown. The *EST-3*90* allele is evident in two specimens (bottom arrows) from McVicars Creek. The *EST-1*105* allele is present in one specimen (top arrow). The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

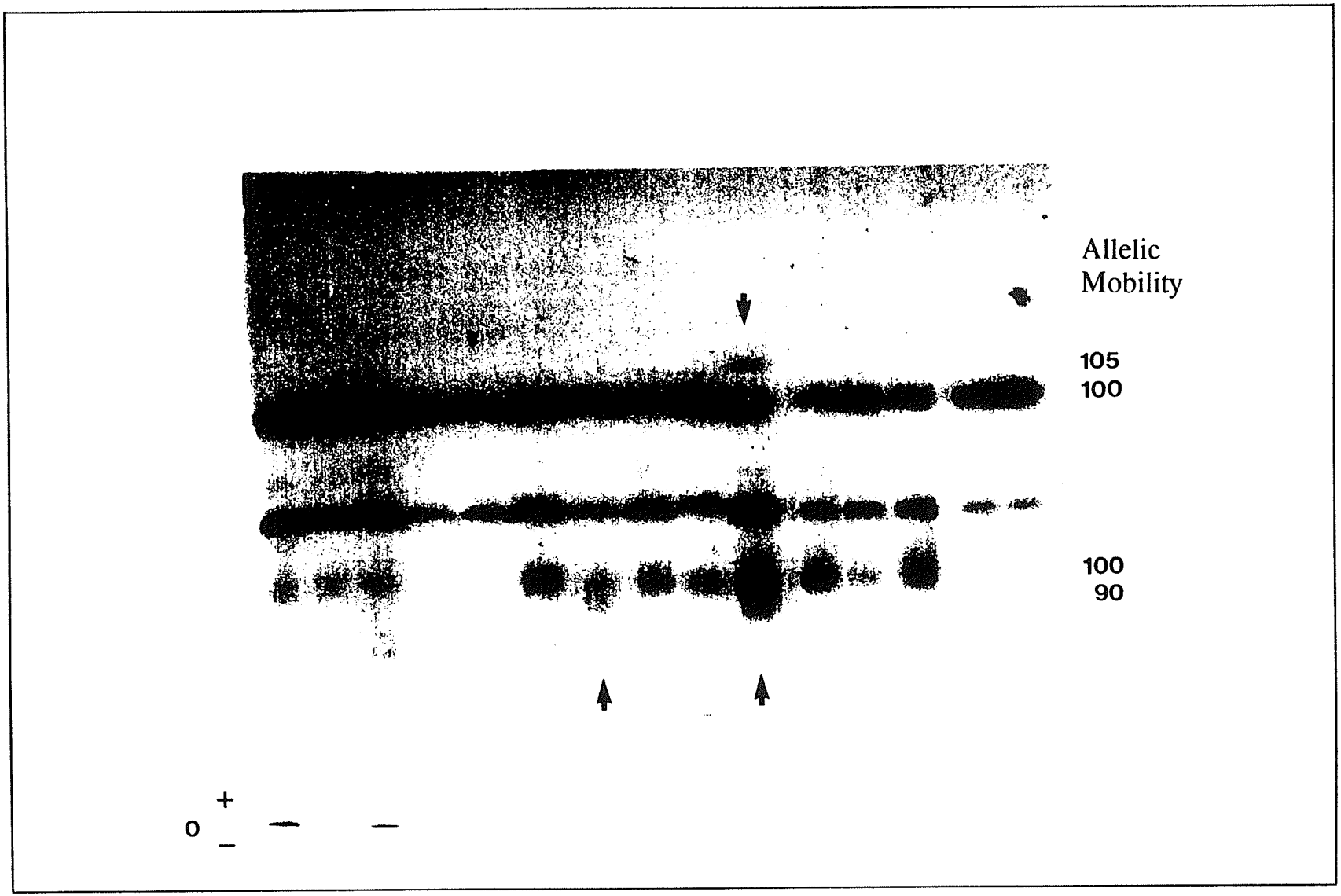
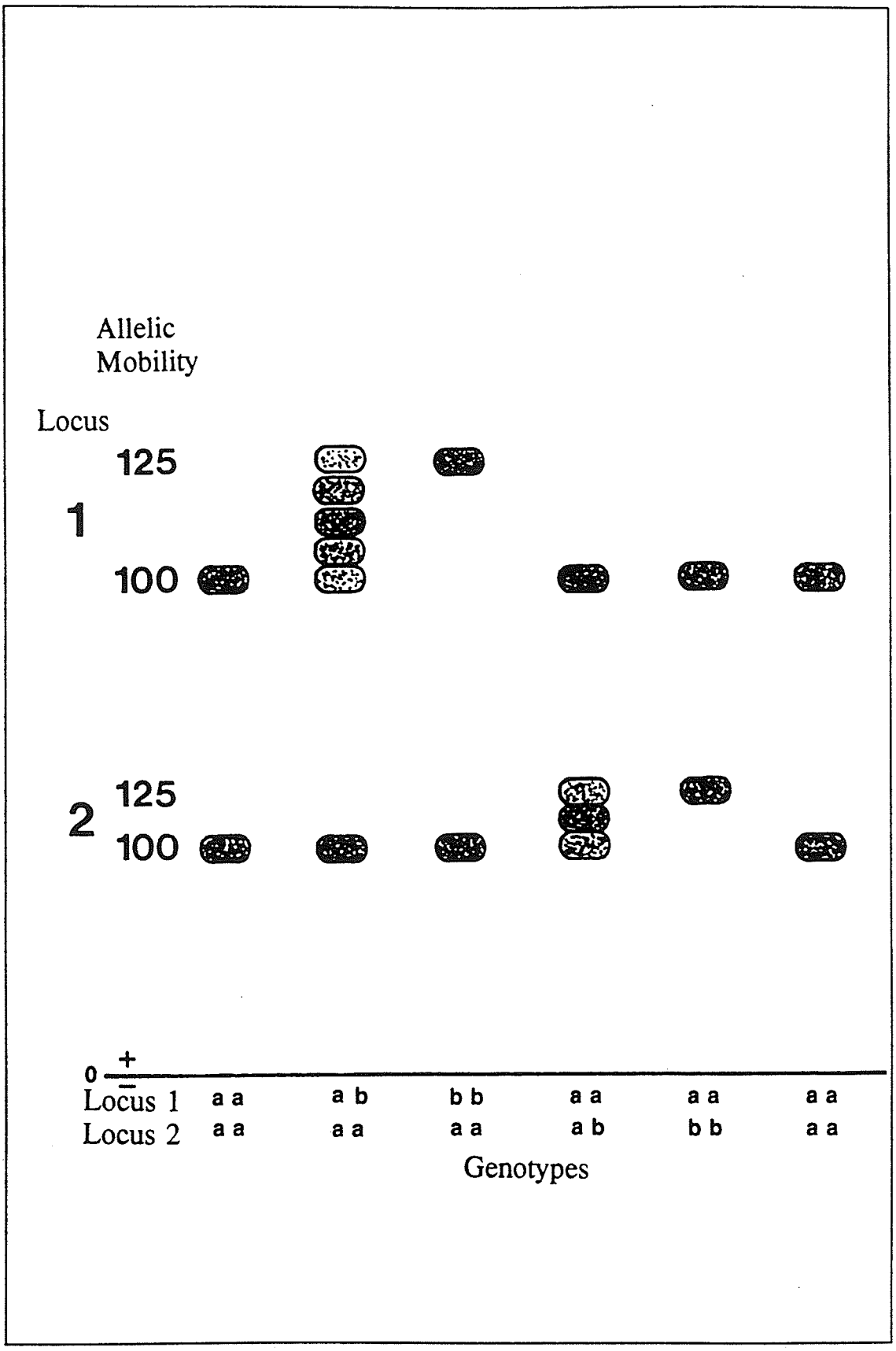

Allelic
Mobility
105
100
100
90
+
0
–

Figure 58: Banding patterns observed at the *ME-1** and *ME-2** locus read from white muscle gels among samples of *Etheostoma nigrum* from Ontario and Wisconsin. Loci, genotype and allelic mobility is shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).


Allelic
Mobility
Locus
125
1
100
125
2
100
0
+
−
Locus 1 a a a b b b a a a a a a
Locus 2 a a a a a a a b b b a a
Genotypes

cytoplasmic forms (*sAAT-3,4**). The mitochondrial form is most common in muscle tissues. The mitochondrial form (*mAAT-1,2**) is monomorphic in all samples except for one specimen from the Flambeau River, Wisconsin, which exhibits the three-banded pattern typical of heterozygotes and a dimeric enzyme (*mAAT-1,2*108*). Three or five bands conforming to dimeric and tetrameric banding are exhibited below the *mAAT-1,2** locus and yet *AAT** is known to be a dimer. This pattern corresponds to two loci encoding the same enzyme in a mitochondrial and cytoplasmic form in the same tissue, or an isolocus (Utter *et al.* 1987). Because it is difficult to determine the genotypes when isoloci are detected, it is typically most convenient to assign the variation to one locus (Utter *et al.* 1987). One locus appears fixed for the *sAAT-3,4*100* allele while the other locus varied in six samples (Fig. **59**). Variation in *sAAT-3,4** is found in Upper St. Croix Lake, Puckaway Lake, Flambeau River and Whitefish Lake, Wisconsin, and in Silver Falls Creek and Prelate Lake, Ontario. Three alleles are present with the "a" allele present in all samples at the first loci. Coupled with the "b" allele from the second locus, specimens monomorphic at this locus exhibit three bands just below the *mAAT-1,2** locus (Fig. **59**). The presence of the "c" allele in the second locus results in a three banded pattern on the lower portion of the gel (Fig. **59**). The presence of both the "b" and "c" allele at the second locus results in a five banded pattern similar to that of a tetrameric enzyme. An alternative to this interpretation is discussed below. Some samples were incompletely tested for *AAT** variation as problems occurred with the buffer during stain preparation. Figure **60** shows the variation exhibited by *AAT**.

Figure 59: Banding patterns observed in *mAAT-1,2** and *sAAT-3,4** in samples of *Etheostoma nigrum* from Ontario and Wisconsin. Loci, genotype and allelic mobility is shown. The *mAAT-1,2***108* allele was not observed in a homozygous state in this study. The *sAAT-3,4** alleles are identified based on their relative mobilities. All possible genotypes are not shown. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).

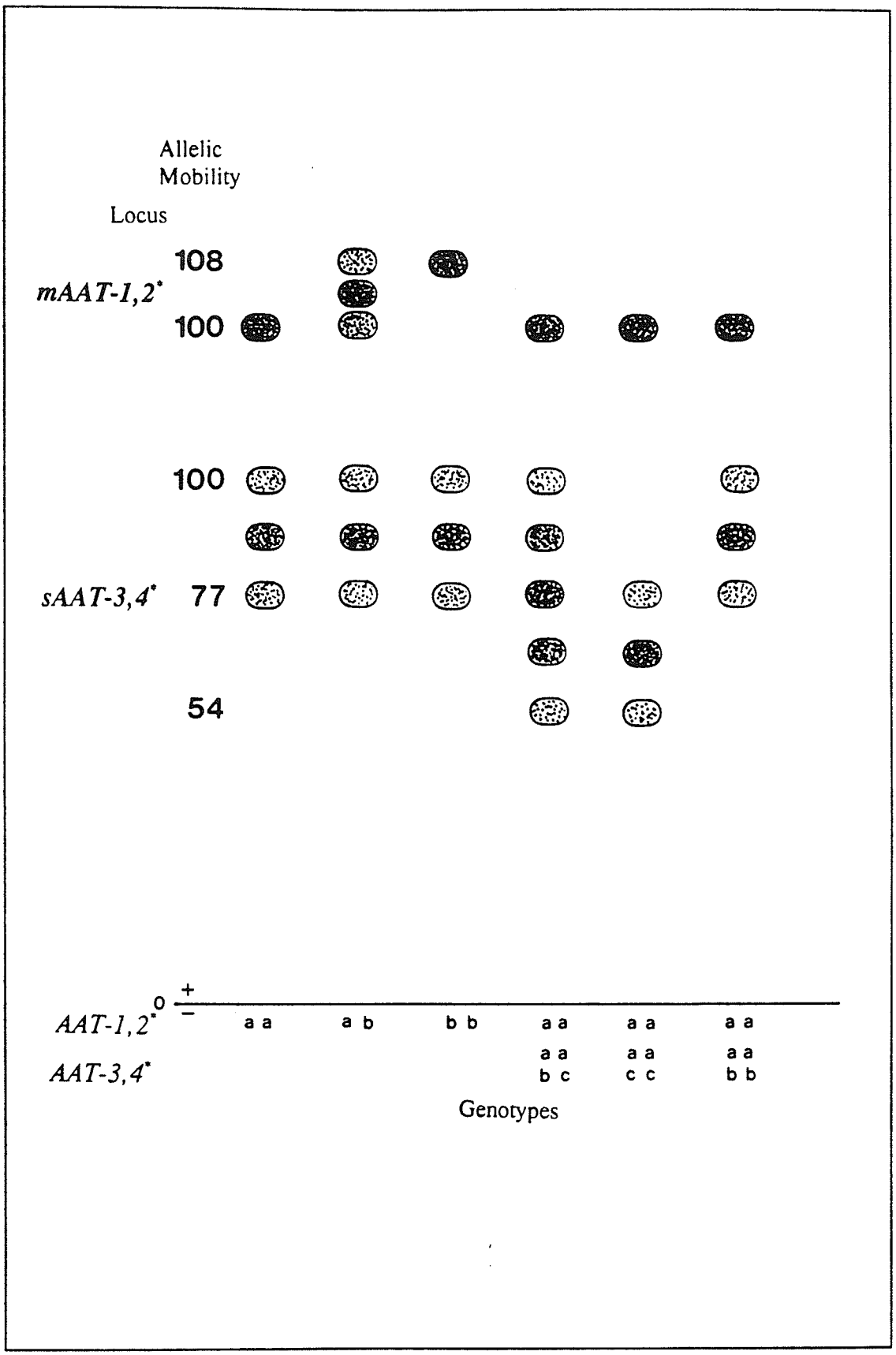
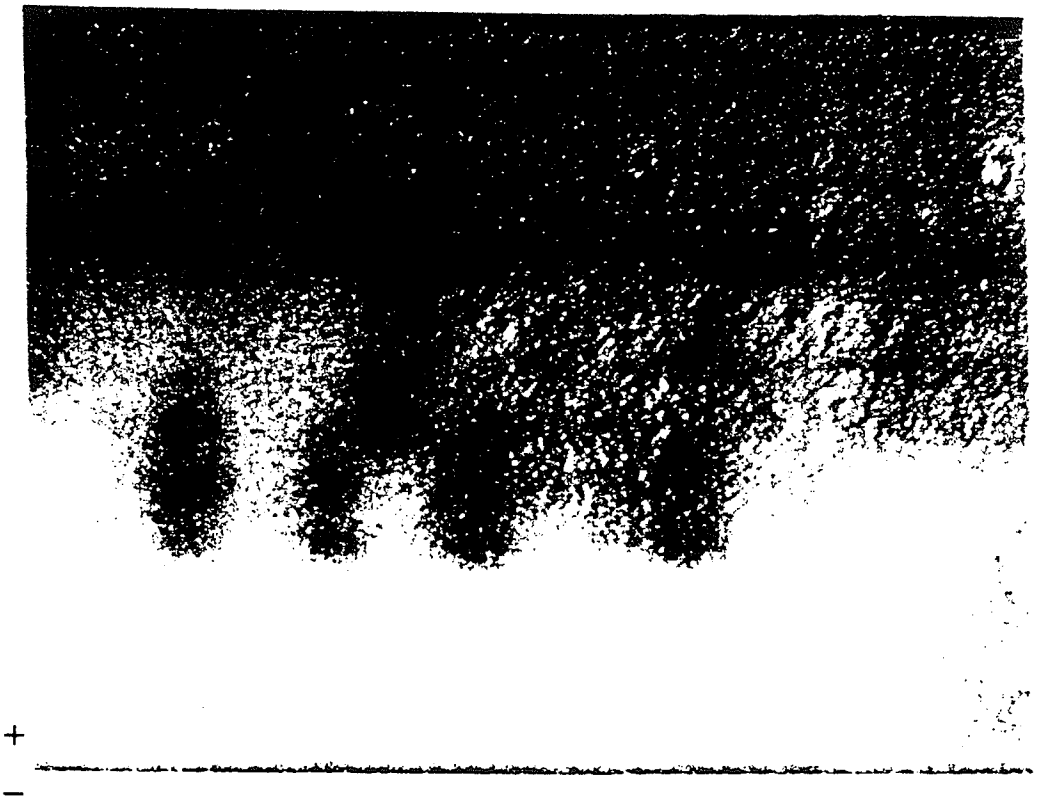

Allelic
Mobility
Locus
108
mAAT-1,2*
100
100
sAAT-3,4*
77
54
+
0
−
AAT-1,2*
AAT-3,4*
a a
a b
b b
a a
a a
a a
a a
b c
a a
c c
a a
b b
Genotypes

Figure 60: Photograph of white muscle gel stained for the activity of *AAT** in specimens of *Etheostoma nigrum* from Ontario and Wisconsin. The plus (+) sign denotes the anodal portion of the gel while the negative (-) sign denotes the cathodal portion. The origin is designated with a zero (0).


+
0
−

Significant heterogeneity occurs in the 15 samples. The chi-square value across all loci is highly significant (χ^2 = 3958.777 with 294 df, P < 0.001) (Table **54**). Within sample divergence at all loci is not high, as indicated by a relatively low F_{IS} (Table **55**). The negative value (F_{IS} = -0.068) for this statistic indicates an excess of heterozygotes within some samples. The F_{IT} value of 0.374 for all samples at all loci indicates moderate divergence of the samples.

Among all samples, genetic divergence is relatively high (F_{ST} = 0.414) (Table **55**). Excluding *AAT** from these calculations, due to incomplete analysis increases, the F_{ST} value to 0.433. Genetic divergence between neighbouring McVicars Creek and the Neebing-McIntyre River, Ontario, samples is minimal (F_{ST} = 0.024). Similarly, genetic divergence between Sandstone and Roundtable Lakes, Ontario, (with the former flowing into the latter), is very small (F_{ST} = 0.003). Divergence between the eight samples exhibiting increased squamation (McVicars, Neebing-McIntyre, Roundtable and Sandstone, Ontario and Big Arbor Vitae, Rock, Puckaway and Whitefish, Wisconsin) is high (F_{ST} = 0.399) and only slightly lower than for the seven samples without squamation (F_{ST}=0.336). Wisconsin (F_{ST} = 0.432) and Ontario (F_{ST} = 0.304) samples exhibit high genetic divergences among samples. The chi-square value across all loci for Ontario samples is highly significant (χ^2 = 2169.067 with 128 df, P < 0.001). The chi-square value for all Wisconsin samples is also highly significant (χ^2 = 780.322 with 75 df, P < 0.001).

Samples within both Wisconsin and Ontario came from two drainages. The F_{ST} for Ontario changes slightly from 0.304 to 0.303 when Prelate Lake in the

Table 54: Contingency chi-square analysis at all polymorphic loci for samples of *Etheostoma nigrum* collected from Ontario and Wisconsin. Enzyme abbreviations are as in text. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

Locus	No. of alleles	Chi-square	D.F.	P
*PGM-1**	2	71.344	14	0.00001
*PGM-2**	3	205.901	28	0.00001
*LDH-B**	2	52.988	14	0.00001
*LDH-A**	2	18.727	14	0.17565
*G3PDH**	2	25.110	14	0.03350
*PGDH**	2	656.719	14	0.00001
*GPI-A**	3	883.968	28	0.00001
*GPI-B**	2	78.622	14	0.00001
*EST-1**	2	122.525	14	0.00001
*EST-3**	2	272.665	14	0.00001
*IDH-L**	3	56.214	28	0.00121
*IDH-W**	3	106.208	28	0.00001
*ME-1**	2	41.966	14	0.00013
*ME-2**	2	550.787	14	0.00001
*mAAT-1,2**	2	33.030	14	0.00285
*sAAT-3,4**	3	782.003	28	0.00001
(Totals)		3958.777	294	0.00001

Table 55: Summary F statistics at all polymorphic loci for the combined samples of *Etheostoma nigrum* collected from Ontario and Wisconsin. Variation within samples is given by F_{IS}, variation between samples given by F_{IT} and the measure of genetic divergence between samples is given by F_{ST}. Enzyme abbreviations are as in text. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

Locus	F_{IS}	F_{IT}	F_{ST}
*PGM-1**	0.114	0.165	0.058
*PGM-2**	-0.069	0.045	0.106
*LDH-B**	-0.054	-0.007	0.044
*LDH-A**	-0.019	-0.004	0.015
*G3PDH**	-0.023	-0.002	0.020
*PGDH**	0.071	0.641	0.614
*GPI-A**	-0.103	0.466	0.515
*GPI-B**	-0.067	-0.004	0.059
*EST-1**	-0.144	-0.017	0.112
*EST-3**	-0.018	0.205	0.219
*IDH-L**	-0.025	-0.003	0.022
*IDH-W**	-0.095	-0.012	0.076
*ME-1**	-0.034	-0.002	0.031
*ME-2**	-0.060	0.403	0.437
*mAAT-1,2**	-0.032	-0.002	0.029
*sAAT-3,4**	-0.160	0.232	0.338
Mean	-0.068	0.374	0.414

Hudson Bay drainage (second Ontario drainage) is excluded suggesting most of the variation is due to Atlantic drainage samples. When Puckaway Lake in the Lake Michigan drainage is excluded (second Wisconsin drainage), the F_{ST} for the Wisconsin sample increases from 0.432 to 0.457 suggesting both great variability within the remaining samples and that the Puckaway Lake sample is similar to at least one of the remaining sample areas. When the Rock River (Mississippi drainage) sample is also excluded, F_{ST} values drop to 0.214 suggesting that much of the variation within the Wisconsin samples is due to the Rock River sample.

Allele frequency and genetic variability measures for the 13 samples examined which exhibited variability are presented in Tables **56-68**. Roundtable and Boulevard lakes, Ontario, are not presented as they lack polymorphic loci. I excluded *XDH** from the tables due to a lack of polymorphism. Allele frequencies are taken from the actual counts of the sample (Appendix **8**). All nine decimal places were used until the final figures were reached. All calculations were performed by hand and are similar to output produced via BIOSYS-1 (Swofford and Selander 1989).

Pairwise comparison of allele frequencies between samples showed that the majority of the samples do not vary in a statistically significant manner at most loci. Highly significant differences occur in the frequency of alleles at the *ME-2** locus between the Rock River and all samples except for McVicars and Silver Falls creeks, Ontario, and Upper St. Croix and Big Arbor Vitae lakes, Wisconsin. There are no significant differences in the frequency of alleles at this locus between these last four locations. The Rock River exhibits a highly significant difference with the Neebing-

Table 56: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from McVicars Creek, Ontario. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	51	51	51	51	51	51	51	51	51
Allele									
a	.922	.912	.990	1.000	.990	.510	.392	1.000	.990
b	.078	.088	.010	.000	.010	.490	.069	.000	.010
c	.000	.000	.000	.000	.000	.000	.539	.000	.000
H	.145	.161	.019	.000	.019	.500	.551	.000	.019
H(unb)	.146	.162	.020	.000	.020	.505	.556	.000	.020
H(D.C.)	.118	.176	.020	.000	.020	.471	.569	.000	.020

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	51	51	51	51	51	51	19	19	19
Allele									
a	1.000	.745	.990	.912	1.000	.510	1.000	1.000	1.000
b	.000	.255	.010	.078	.000	.490	.000	.000	.000
c	.000	.000	.000	.010	.000	.000	.000	.000	.000
H	.000	.380	.019	.162	.000	.500	.000	.000	.000
H(unb)	.000	.384	.020	.164	.000	.505	.000	.000	.000
H(D.C.)	.000	.392	.020	.176	.000	.549	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.130 (S.E. 0.045)

Mean heterozygosity per locus (unbiased estimate) = 0.132 (S.E. 0.046)

Mean heterozygosity per locus (direct-count estimate) = 0.133 (S.E. 0.047)

Mean number of alleles per locus = 1.68 (S.E. 0.15)

Percentage of loci polymorphic (0.95 criterion) = 36.84

Percentage of loci polymorphic (0.99 criterion) = 36.84

Percentage of loci polymorphic (no criterion) = 57.89

Table 57: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from the Neebing-McIntyre River, Ontario. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	56	56	56	56	56	56	56	56	56
Allele									
a	.964	.839	1.000	1.000	.973	.696	.652	1.000	.991
b	.036	.161	.000	.000	.027	.304	.027	.000	.009
c	.000	.000	.000	.000	.000	.000	.321	.000	.000
H	.069	.270	.000	.000	.052	.423	.471	.000	.018
H(unb)	.069	.272	.000	.000	.053	.427	.475	.000	.018
H(D.C.)	.071	.286	.000	.000	.054	.393	.446	.000	.018

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	56	56	56	56	56	56	5	5	5
Allele									
a	1.000	.768	1.000	1.000	1.000	.580	1.000	1.000	1.000
b	.000	.232	.000	.000	.000	.420	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.357	.000	.000	.000	.487	.000	.000	.000
H(unb)	.000	.360	.000	.000	.000	.491	.000	.000	.000
H(D.C.)	.000	.357	.000	.000	.000	.446	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.113 (S.E. 0.042)

Mean heterozygosity per locus (unbiased estimate) = 0.114 (S.E. 0.042)

Mean heterozygosity per locus (direct-count estimate) = 0.109 (S.E. 0.040)

Mean number of alleles per locus = 1.47 (S.E. 0.14)

Percentage of loci polymorphic (0.95 criterion) = 26.32

Percentage of loci polymorphic (0.99 criterion) = 36.84

Percentage of loci polymorphic (no criterion) = 42.11

Table 58: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Upper St. Croix Lake, Wisconsin. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	34	34	34	34	34	34	34	34	34
Allele									
a	1.000	.956	1.000	.985	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.044	.000	.015	.000	.000	.000	.000	.000
H	.000	.084	.000	.029	.000	.000	.000	.000	.000
H(unb)	.000	.086	.000	.029	.000	.000	.000	.000	.000
H(D.C.)	.000	.088	.000	.029	.000	.000	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	34	34	34	34	34	34	34	34	34
Allele									
a	1.000	1.000	1.000	1.000	1.000	.471	1.000	1.000	.529
b	.000	.000	.000	.000	.000	.529	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.471
H	.000	.000	.000	.000	.000	.498	.000	.000	.498
H(unb)	.000	.000	.000	.000	.000	.506	.000	.000	.506
H(D.C.)	.000	.000	.000	.000	.000	.529	.000	.000	.529

Mean heterozygosity per locus (biased estimate) = 0.058 (S.E. 0.036)

Mean heterozygosity per locus (unbiased estimate) = 0.059 (S.E. 0.036)

Mean heterozygosity per locus (direct-count estimate) = 0.062 (S.E. 0.038)

Mean number of alleles per locus = 1.21 (S.E. 0.10)

Percentage of loci polymorphic (0.95 criterion) = 10.53

Percentage of loci polymorphic (0.99 criterion) = 21.05

Percentage of loci polymorphic (no criterion) = 21.05

Table 59: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Big Arbor Vitae Lake, Wisconsin. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	59	59	59	59	59	59	59	59	59
Allele									
a	1.000	1.000	1.000	.983	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.017	.000	.000	.000	.000	.000
H	.000	.000	.000	.033	.000	.000	.000	.000	.000
H(unb)	.000	.000	.000	.034	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.034	.000	.000	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	59	59	59	59	59	59	59	59	59
Allele									
a	1.000	1.000	1.000	.907	1.000	.508	1.000	1.000	1.000
b	.000	.000	.000	.093	.000	.492	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.169	.000	.500	.000	.000	.000
H(unb)	.000	.000	.000	.171	.000	.504	.000	.000	.000
H(D.C.)	.000	.000	.000	.186	.000	.508	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.037 (S.E. 0.027)

Mean heterozygosity per locus (unbiased estimate) = 0.037 (S.E. 0.027)

Mean heterozygosity per locus (direct-count estimate) = 0.038 (S.E. 0.028)

Mean number of alleles per locus = 1.16 (S.E. 0.09)

Percentage of loci polymorphic (0.95 criterion) = 10.53

Percentage of loci polymorphic (0.99 criterion) = 15.79

Percentage of loci polymorphic (no criterion) = 15.79

Table 60: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Little Dog Lake, Ontario. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	68	68	68	68	68	68	68	68	68
Allele									
a	1.000	1.000	1.000	.978	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.022	.000	.000	.000	.000	.000
H	.000	.000	.000	.043	.000	.000	.000	.000	.000
H(unb)	.000	.000	.000	.043	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.044	.000	.000	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	68	68	68	68	68	68	68	68	68
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.002 (S.E. 0.002)

Mean heterozygosity per locus (unbiased estimate) = 0.002 (S.E. 0.002)

Mean heterozygosity per locus (direct-count estimate) = 0.002 (S.E. 0.002)

Mean number of alleles per locus = 1.05 (S.E. 0.05)

Percentage of loci polymorphic (0.95 criterion) = 0.00

Percentage of loci polymorphic (0.99 criterion) = 5.26

Percentage of loci polymorphic (no criterion) = 5.26

Table 61: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from the Rock River, Wisconsin. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	15	15	15	15	15	15	15	15	15
Allele									
a	1.000	1.000	.967	1.000	1.000	.000	.067	1.000	.833
b	.000	.000	.033	.000	.000	1.000	.467	.000	.167
c	.000	.000	.000	.000	.000	.000	.467	.000	.000
H	.000	.000	.064	.000	.000	.000	.560	.000	.278
H(unb)	.000	.000	.067	.000	.000	.000	.579	.000	.287
H(D.C.)	.000	.000	.067	.000	.000	.000	.733	.000	.333

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	15	15	15	15	15	15	15	15	15
Allele									
a	1.000	1.000	1.000	1.000	.967	.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.033	1.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.064	.000	.000	.000	.000
H(unb)	.000	.000	.000	.000	.067	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.000	.067	.000	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.051 (S.E. 0.032)

Mean heterozygosity per locus (unbiased estimate) = 0.053 (S.E. 0.033)

Mean heterozygosity per locus (direct-count estimate) = 0.063 (S.E. 0.041)

Mean number of alleles per locus = 1.26 (S.E. 0.13)

Percentage of loci polymorphic (0.95 criterion) = 10.53

Percentage of loci polymorphic (0.99 criterion) = 21.05

Percentage of loci polymorphic (no criterion) = 21.05

Table 62: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from the Nipigon River, Ontario. The most to least common alleles are designated "a"-"b", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	66	66	66	66	66	66	66	66	66
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	66	66	66	66	66	66	66	66	66
Allele									
a	1.000	1.000	.985	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.015	.000	.000	.000	.000	.000	.000
H	.000	.000	.030	.000	.000	.000	.000	.000	.000
H(unb)	.000	.000	.030	.000	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.030	.000	.000	.000	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.002 (S.E. 0.002)

Mean heterozygosity per locus (unbiased estimate) = 0.002 (S.E. 0.002)

Mean heterozygosity per locus (direct-count estimate) = 0.002 (S.E. 0.002)

Mean number of alleles per locus = 1.05 (S.E. 0.05)

Percentage of loci polymorphic (0.95 criterion) = 0.00

Percentage of loci polymorphic (0.99 criterion) = 5.26

Percentage of loci polymorphic (no criterion) = 5.26

Table 63: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Puckaway Lake, Wisconsin. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	15	15	15	15	15	15	15	15	15
Allele									
a	1.000	1.000	.933	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.067	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.124	.000	.000	.000	.000	.000	.000
H(unb)	.000	.000	.129	.000	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.133	.000	.000	.000	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	15	15	15	15	15	15	15	15	15
Allele									
a	1.000	1.000	.967	1.000	1.000	.567	1.000	1.000	.867
b	.000	.000	.000	.000	.000	.433	.000	.000	.000
c	.000	.000	.033	.000	.000	.000	.000	.000	.133
H	.000	.000	.064	.000	.000	.491	.000	.000	.231
H(unb)	.000	.000	.067	.000	.000	.508	.000	.000	.239
H(D.C.)	.000	.000	.067	.000	.000	.467	.000	.000	.267

Mean heterozygosity per locus (biased estimate) = 0.048 (S.E. 0.028)

Mean heterozygosity per locus (unbiased estimate) = 0.050 (S.E. 0.029)

Mean heterozygosity per locus (direct-count estimate) = 0.049 (S.E. 0.028)

Mean number of alleles per locus = 1.21 (S.E. 0.10)

Percentage of loci polymorphic (0.95 criterion) = 15.79

Percentage of loci polymorphic (0.99 criterion) = 21.05

Percentage of loci polymorphic (no criterion) = 21.05

Table 64: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Silver Falls Creek, Ontario. The most to least common alleles are designated "a"-"b", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	9	9	9	9	9	9	9	9	9
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.944
b	.000	.000	.000	.000	.000	.000	.000	.000	.056
H	.000	.000	.000	.000	.000	.000	.000	.000	.105
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.111
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.111

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	9	9	9	9	9	9	9	9	9
Allele									
a	1.000	1.000	1.000	1.000	1.000	.611	1.000	1.000	.556
b	.000	.000	.000	.000	.000	.389	.000	.000	.444
H	.000	.000	.000	.000	.000	.475	.000	.000	.494
H(unb)	.000	.000	.000	.000	.000	.503	.000	.000	.523
H(D.C.)	.000	.000	.000	.000	.000	.556	.000	.000	.889

Mean heterozygosity per locus (biased estimate) = 0.057 (S.E. 0.035)

Mean heterozygosity per locus (unbiased estimate) = 0.060 (S.E. 0.037)

Mean heterozygosity per locus (direct-count estimate) = 0.082 (S.E. 0.054)

Mean number of alleles per locus = 1.16 (S.E. 0.09)

Percentage of loci polymorphic (0.95 criterion) = 15.79

Percentage of loci polymorphic (0.99 criterion) = 15.79

Percentage of loci polymorphic (no criterion) = 15.79

Table 65: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Prelate Lake, Ontario. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	52	52	52	52	52	52	52	52	52
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	52	52	52	52	52	52	52	35	35
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.857
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.143
H	.000	.000	.000	.000	.000	.000	.000	.000	.245
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.248
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.229

Mean heterozygosity per locus (biased estimate) = 0.013 (S.E. 0.013)

Mean heterozygosity per locus (unbiased estimate) = 0.013 (S.E. 0.013)

Mean heterozygosity per locus (direct-count estimate) = 0.012 (S.E. 0.012)

Mean number of alleles per locus = 1.05 (S.E. 0.05)

Percentage of loci polymorphic (0.95 criterion) = 5.26

Percentage of loci polymorphic (0.99 criterion) = 5.26

Percentage of loci polymorphic (no criterion) = 5.26

Table 66: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Sandstone Lake, Ontario. The most to least common alleles are designated "a"-"b", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	92	92	92	92	92	92	92	92	92
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.995
b	.000	.000	.000	.000	.000	.000	.000	.000	.005
H	.000	.000	.000	.000	.000	.000	.000	.000	.011
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.011
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.011

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	92	92	92	92	92	92	92	92	92
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(unb)	.000	.000	.000	.000	.000	.000	.000	.000	.000
H(D.C.)	.000	.000	.000	.000	.000	.000	.000	.000	.000

Mean heterozygosity per locus (biased estimate) = 0.001 (S.E. 0.001)

Mean heterozygosity per locus (unbiased estimate) = 0.001 (S.E. 0.001)

Mean heterozygosity per locus (direct-count estimate) = 0.001 (S.E. 0.001)

Mean number of alleles per locus = 1.05 (S.E. 0.05)

Percentage of loci polymorphic (0.95 criterion) = 0.00

Percentage of loci polymorphic (0.99 criterion) = 0.00

Percentage of loci polymorphic (no criterion) = 5.26

Table 67: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from the Flambeau River, Wisconsin. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	16	16	16	16	16	16	16	16	16
Allele									
a	1.000	1.000	1.000	1.000	1.000	.469	.938	.938	1.000
b	.000	.000	.000	.000	.000	.531	.063	.063	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.498	.117	.117	.000
H(unb)	.000	.000	.000	.000	.000	.514	.121	.121	.000
H(D.C.)	.000	.000	.000	.000	.000	.438	.125	.125	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	16	16	16	16	16	16	16	16	16
Allele									
a	1.000	1.000	1.000	1.000	1.000	1.000	.969	1.000	.500
b	.000	.000	.000	.000	.000	.000	.031	.000	.031
c	.000	.000	.000	.000	.000	.000	.000	.000	.469
H	.000	.000	.000	.000	.000	.000	.061	.000	.529
H(unb)	.000	.000	.000	.000	.000	.000	.063	.000	.546
H(D.C.)	.000	.000	.000	.000	.000	.000	.063	.000	.625

Mean heterozygosity per locus (biased estimate) = 0.070 (S.E. 0.037)

Mean heterozygosity per locus (unbiased estimate) = 0.072 (S.E. 0.038)

Mean heterozygosity per locus (direct-count estimate) = 0.072 (S.E. 0.039)

Mean number of alleles per locus = 1.32 (S.E. 0.13)

Percentage of loci polymorphic (0.95 criterion) = 21.05

Percentage of loci polymorphic (0.99 criterion) = 26.32

Percentage of loci polymorphic (no criterion) = 26.32

Table 68: Locus, sample size, mean heterozygosity per locus (biased (H), unbiased (H(unb)) and direct count (H(D.C.)), mean number of alleles per locus and percentage of loci polymorphic for *Etheostoma nigrum* from Whitefish Lake, Wisconsin. The most to least common alleles are designated "a"-"c", respectively. Liver and white muscle gels stained for *IDH** are indicated by *IDH-L** and *IDH-W**, respectively.

	*PGM-1**	*PGM-2**	*LDH-B**	*LDH-A**	*G3PDH**	*PGDH**	*GPI-A**	*GPI-B**	*EST-1**
N	7	7	7	7	7	7	7	7	7
Allele									
a	1.000	1.000	1.000	1.000	1.000	.929	1.000	1.000	1.000
b	.000	.000	.000	.000	.000	.071	.000	.000	.000
c	.000	.000	.000	.000	.000	.000	.000	.000	.000
H	.000	.000	.000	.000	.000	.133	.000	.000	.000
H(unb)	.000	.000	.000	.000	.000	.143	.000	.000	.000
H(D.C.)	.000	.000	.000	.000	.000	.143	.000	.000	.000

	*EST-2**	*EST-3**	*IDH-L**	*IDH-W**	*ME-1**	*ME-2**	*AAT-1,2**	*AAT-3**	*AAT-4**
N	7	7	7	7	7	7	7	7	7
Allele									
a	1.000	1.000	1.000	1.000	1.000	.786	1.000	1.000	.500
b	.000	.000	.000	.000	.000	.214	.000	.000	.071
c	.000	.000	.000	.000	.000	.000	.000	.000	.429
H	.000	.000	.000	.000	.000	.337	.000	.000	.561
H(unb)	.000	.000	.000	.000	.000	.363	.000	.000	.604
H(D.C.)	.000	.000	.000	.000	.000	.429	.000	.000	.429

Mean heterozygosity per locus (biased estimate) = 0.054 (S.E. 0.034)

Mean heterozygosity per locus (unbiased estimate) = 0.058 (S.E. 0.036)

Mean heterozygosity per locus (direct-count estimate) = 0.053 (S.E. 0.031)

Mean number of alleles per locus = 1.21 (S.E. 0.12)

Percentage of loci polymorphic (0.95 criterion) = 15.79

Percentage of loci polymorphic (0.99 criterion) = 15.79

Percentage of loci polymorphic (no criterion) = 15.79

McIntyre River, Ontario, sample. However, the Neebing-McIntyre River sample does not exhibit and significant difference with other locations possessing the *ME-2*125* allele. Many significant differences exist between larger samples and those possessing the *ME-2*125* allele (*e.g.*, Boulevard Lake and the Nipigon River, Ontario, vary significantly from the Neebing-McIntyre River, Ontario, and Upper St. Croix Lake, Wisconsin).

Highly significant differences exist between the Rock River, WI, and all locations but McVicars Creek, ON, at the *PGDH** locus. The McVicars Creek sample does not exhibit any significant differences when compared to other locations that possess the *PGDH*150* allele (McIntyre River, ON, Whitefish Lake and Flambeau River, WI) but does exhibit significant differences with all other areas. These three areas do not exhibit any significant differences in the frequency of the *PGDH*150* allele when compared to each other and they do not exhibit significant differences when compared to other areas. All other areas do not exhibit any significant differences when compared on a pairwise basis.

The Rock River, Wisconsin, and McVicars Creek, Ontario, are most similar in terms of the allele frequencies they possess. In turn, McVicars Creek is most similar to the nearby Neebing-McIntyre River. Specimens from the Rock River are almost all classified as scaled while fewer specimens from McVicars Creek meet the definition. Additionally, there is little similarity in habitats between these two locations. The Rock River is warm, turbid and weedy and harbours several species of sunfish (centrarchids) whereas McVicars Creek is clear with little vegetation and

harbours spawning runs of rainbow trout (*Oncorhynchus mykiss*). The McVicars Creek sample also exhibits several significant differences with most other locations with the exception of the nearby (< 5 km) Neebing-McIntyre River. Specimens from the Neebing-McIntyre River exhibit slightly more squamation than do those from McVicars Creek. Habitat within the Neebing-McIntyre River is similar to that of the Rock River and centrarchids, although less diverse, are present in the Neebing-McIntyre.

The dendrogram produced using Nei's (1978) unbiased genetic distance (Fig. **61**) is very similar to the one produced using Nei's (1972) genetic distance. All enzymes were utilized in the creation of these dendrograms except for *XDH** and *AAT** due to the reasons given earlier. Both dendrograms show the Rock River to be very distinct, separated from other sampled areas at D = 0.149. McVicars Creek and the Neebing-McIntyre River form a distinct cluster, joining other samples at D = 0.037. The Flambeau River is also distinct at D = 0.023. Six of the seven remaining Ontario samples cluster together with the exception of Silver Falls Creek which clusters with three Wisconsin samples (Fig. **61**). The Whitefish Lake, Wisconsin, sample clusters with the majority of Ontario samples. The cophenetic correlation coefficient for this dendrogram is 0.940. Most lacustrine samples are genetically similar while riverine samples exhibit the majority of variation. The matrices of distance and similarity in Table **69**, created with the same 15 loci as the dendrogram, show that several samples exhibit virtually no differences even if from different drainages and harbouring either the scaled or unscaled form (*e.g.*, Sandstone (scaled)

Figure 61: UPGMA dendrogram constructed using Nei's (1978) unbiased genetic distance. The cophenetic correlation coefficient for this dendrogram is 0.940. Drainage abbreviations after sample location are; LS=Lake Superior, MI=Lake Michigan, MS=Mississippi River and HB=Hudson Bay.

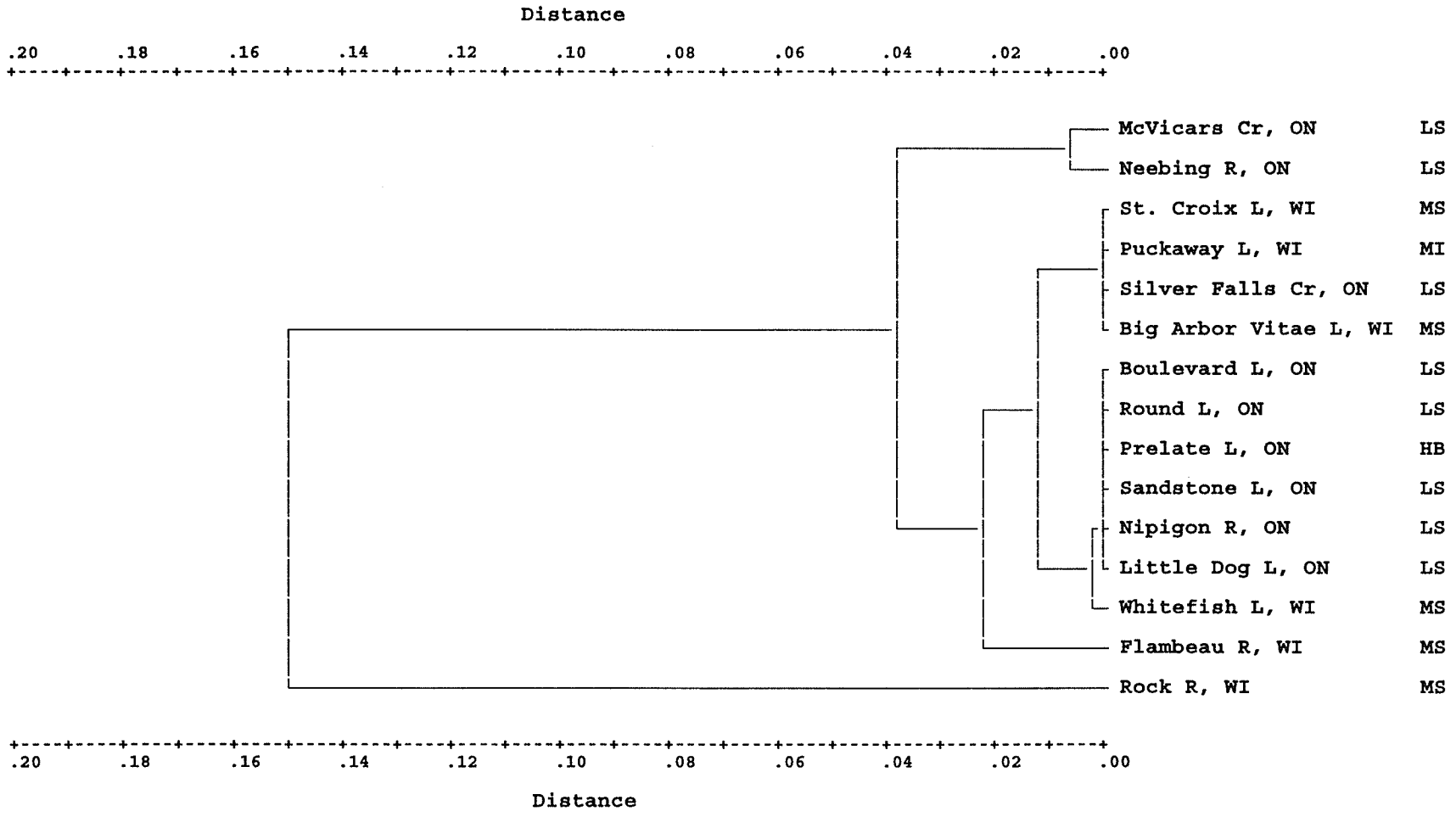

Distance
.20 .18 .16 .14 .12 .10 .08 .06 .04 .02 .00
McVicars Cr, ON LS
Neebing R, ON LS
St. Croix L, WI MS
Puckaway L, WI MI
Silver Falls Cr, ON LS
Big Arbor Vitae L, WI MS
Boulevard L, ON LS
Round L, ON LS
Prelate L, ON HB
Sandstone L, ON LS
Nipigon R, ON LS
Little Dog L, ON LS
Whitefish L, WI MS
Flambeau R, WI MS
Rock R, WI MS
.20 .18 .16 .14 .12 .10 .08 .06 .04 .02 .00
Distance

Table 69: Matrix of genetic similarity and distance coefficients among 15 samples of *Etheostoma nigrum* from Ontario and Wisconsin. Below diagonal: Nei (1972) genetic identity. Above diagonal: Nei (1978) unbiased genetic distance.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 McVicars Cr, ON	****	.007	.043	.043	.058	.059	.052	.059	.058	.044	.043	.058	.058	.041	.043
2 Neebing-McIntyre R, ON	.992	****	.018	.019	.028	.028	.085	.028	.028	.018	.017	.028	.028	.026	.016
3 Upper St. Croix L, WI	.956	.981	****	.000	.018	.018	.132	.018	.018	.000	.000	.018	.018	.037	.005
4 Big Arbor Vitae L, WI	.957	.981	.999	****	.016	.016	.136	.016	.016	.000	.000	.016	.016	.035	.005
5 Boulevard L, ON	.942	.972	.982	.984	****	.000	.188	.000	.000	.011	.009	.000	.000	.018	.002
6 Little Dog L, ON	.942	.971	.982	.984	1.00	****	.189	.000	.000	.011	.009	.000	.000	.018	.002
7 Rock R, WI	.948	.917	.875	.871	.827	.827	****	.189	.188	.140	.142	.188	.188	.126	.150
8 Nipigon R, ON	.942	.971	.982	.984	1.00	1.00	.827	****	.000	.011	.009	.000	.000	.018	.002
9 Roundtable L, ON	.942	.972	.982	.984	1.00	1.00	.827	1.00	****	.011	.009	.000	.000	.018	.002
10 Puckaway L, WI	.956	.981	.999	.999	.988	.988	.868	.988	.988	****	.000	.011	.011	.031	.002
11 Silver Falls Cr, ON	.956	.982	.998	.999	.990	.990	.866	.990	.990	.999	****	.009	.009	.027	.000
12 Prelate L, ON	.942	.972	.982	.984	1.00	1.00	.827	1.00	1.00	.988	.990	****	.000	.018	.002
13 Sandstone L, ON	.942	.971	.982	.984	1.00	1.00	.828	1.00	1.00	.988	.990	1.00	****	.018	.002
14 Flambeau R, WI	.958	.973	.963	.965	.982	.982	.880	.982	.982	.968	.971	.982	.982	****	.015
15 Whitefish L, WI	.956	.982	.993	.994	.997	.997	.859	.997	.997	.996	.998	.997	.997	.983	****

and Prelate (unscaled) lakes (approximately 0.000 distance; 1.00 identity)).

Canonical Correlation Analysis

After biochemical analyses, thirteen alleles were included in canonical correlation analysis. These alleles (*GPI-A*100, 91, 82*; *PGM-1*109*; *PGM-2*100, 130, 60*; *PGDH*100, 150*; *EST-1*100, 105*; *EST-3*100, 90*) were those which exhibited some of the greatest variation within and between samples, were consistently scorable within all samples, and were found at rates greater than 5.0% within samples which exhibited variation for these alleles. Other potentially informative alleles were either too rare or could not be consistently scored for all samples. Canonical correlation analysis was performed with biochemical data and environmental variables, biochemical data and meristic variables, and environmental data and meristic variables.

The correlation matrix comprised of biochemical, meristic and environmental variables shows that highly significant correlations between meristic characters for this sample are mainly those identified in Table **27**. Many highly significant correlations exist between biochemical characters and squamation or meristic characters which suggests a possible means to separate scaled and unscaled specimens. Highly significant positive correlations ($P < 0.001$) exist between the *GPI-A*91* allele and the number of nape, cheek and opercle scales and the estimated percent of the breast covered by scales. Highly significant positive correlations also exist between this allele and the number of scales above the lateral line (both LL1 and LL2), the number of total left and right POM pores and the number of right and left

posterior infraorbital pores. Highly significant negative correlations exist between the *GPI-A*100* allele and the number of cheek and opercle scales suggesting that the most heavily scaled specimens lack this allele. Highly significant positive correlations also exist between the *GPI-A*82* allele and the number of scales on the cheek, those above (LL2) and below the lateral line and the total number of left and right POM pores. Highly significant positive correlations exist between the *EST-1*105* allele and the number of cheek and opercle scales and the total number of left and right POM pores. This allele is also significantly correlated ($P < 0.01$) with the number of nape scales. Highly significant negative correlations exist between the *PGDH*100* allele and the number of nape, cheek and opercle scales. These enzymes may thus provide some information on the biogeographic history of *E. nigrum*.

Many highly significant ($P < 0.001$) positive correlations exist between the biochemical data and environmental variables (Table **70**). Some correlations are difficult to interpret in a geographic manner. For example, although the samples from the Thunder Bay area could be expected to possess fewer frost-free and growing-degree days due to their latitude, the moderating influence of Lake Superior generally results in a similar number of these days as experienced by most Wisconsin sample locations (Phillips and McCulloch 1972). Other correlations are easier to interpret such as the occurrence of the *PGDH*150*, *GPI-A*91* or *GPI-A*82* alleles in rivers of relatively low stream order.

A plot of the first canonical axes from the biochemical and environmental data sets produced a plot which did not result in the specimens lying near the diagonal and

Table 70: Correlation matrix of environmental and biochemical variables. Correlations in bold are highly significant ($P < 0.001$).

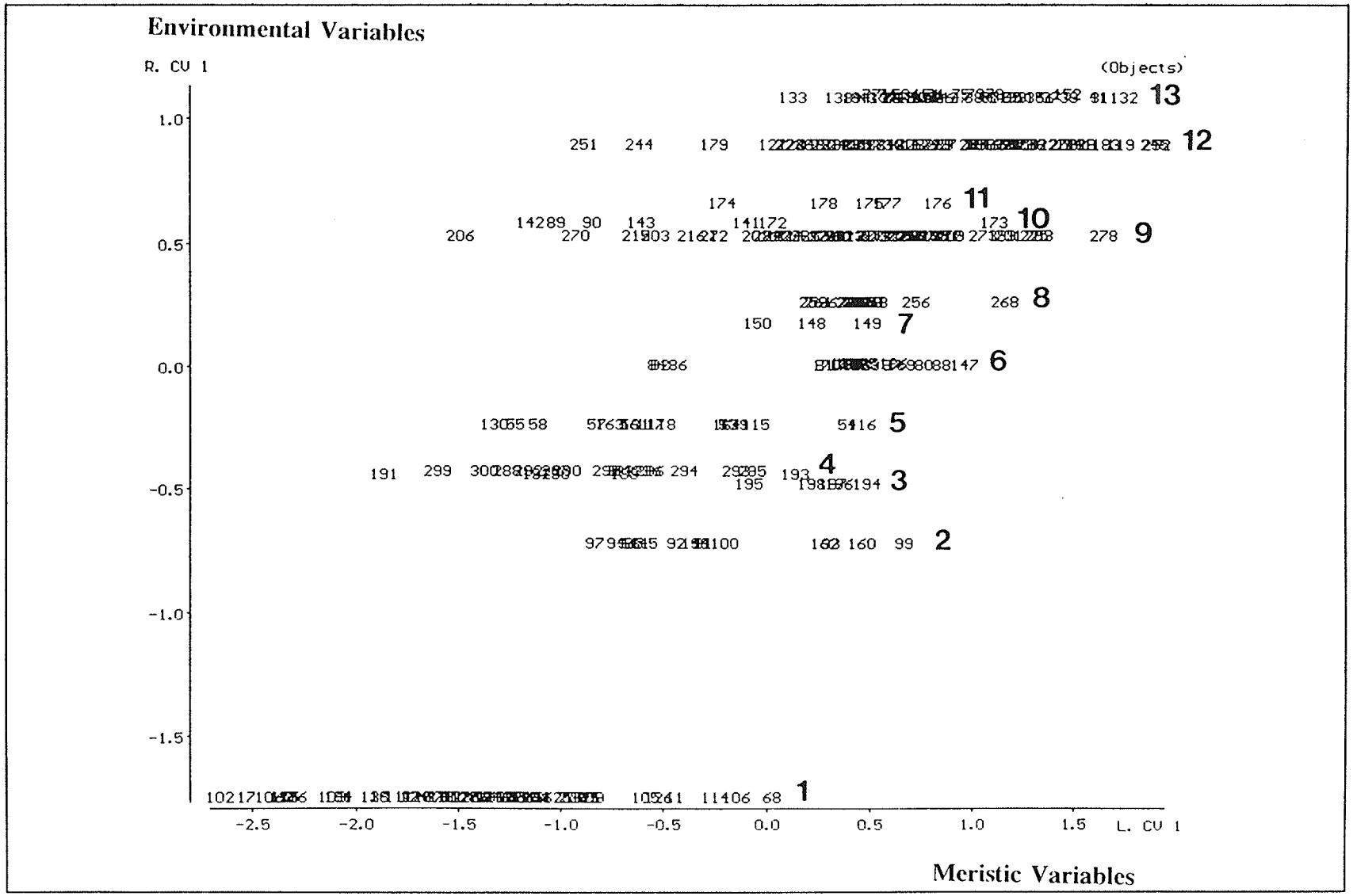
	LAT	LON	FFD	GDD	HABT	ORDER	ELEV
*PGDH*100*	**0.2346**	**0.2892**	**-0.3534**	**-0.2795**	**0.4001**	**-0.2531**	**0.2924**
*PGDH*150*	0.0528	**-0.3286**	0.1198	-0.0223	**-0.6664**	**0.3798**	**-0.5000**
*PGM-1*109*	0.1132	-0.0759	-0.0715	-0.1207	-0.1411	0.1122	-0.1406
*PGM-2*100*	-0.0893	0.0669	0.0552	0.0932	0.1089	0.0015	0.1089
*PGM-2*130*	**0.2645**	-0.1857	-0.1657	**-0.2795**	**-0.3267**	0.1542	**-0.3261**
*PGM-2*60*	-0.0339	**0.1971**	0.0538	0.0591	0.0928	-0.1116	-0.0113
*GPI-A*100*	0.0334	**0.3334**	-0.1719	-0.0526	**0.3938**	-0.1881	**0.3807**
*GPI-A*91*	**-0.2486**	**-0.2936**	**0.3586**	**0.2867**	**-0.3048**	**0.2200**	**-0.2649**
*GPI-A*82*	**0.2386**	**-0.4275**	-0.0375	**-0.2379**	**-0.6311**	**0.3022**	**-0.6074**
*EST-3*100*	-0.0893	0.0669	0.0552	0.0932	0.1089	0.0015	0.1089
*EST-3*90*	**0.3634**	**-0.2521**	**-0.2278**	**-0.3807**	**-0.4492**	**0.1901**	**-0.4363**
*EST-1*100*	-0.0266	0.1089	0.0187	0.0195	-0.0140	0.0205	0.0438
*EST-1*105*	**-0.2337**	**-0.1989**	**0.3130**	**0.2620**	-0.1378	0.1336	-0.1599

LAT = latitude
LON = longitude
FFD = average number of frost-free days
GDD = number of growing degree-days above 5.6°C
HABT: 1 = lake, 0 = river
ORDER: 1 = first order stream/headwater lake, 2 = second order stream, 3 = third order or higher stream, large rivers and lakes between streams
ELEV = elevation above sea level in metres

showed many overlapping values. The plot seemed to be arranged largely by latitude with the most northern specimens placed at the top of the scattergram. Although the first canonical correlation was high ($r^2 = 0.81$), the second was low ($r^2 = 0.69$). Part of this was due to a large number of specimens with similar alleles and the number of frost-free and growing-degree days varying in a similar manner with latitude. This plot was not considered useful. Redundancy, or the explained variance, was relatively high in this analysis suggesting that variation in some biochemical data is closely correlated with some environmental data. Analysis of biochemical and environmental data does suggest a trend for specimens possessing the *GPI-A*100* allele to be found in high elevation lakes. The *GPI-A*91* and *GPI-A*82* alleles are more common in low stream order rivers at somewhat lower elevations such as the Rock River, Wisconsin, (approximately 650 m above sea level) and both McVicars Creek and the Neebing-McIntyre River, Ontario (approximately 602 m).

Canonical correlation analysis of meristic and environmental variables suggests that the most heavily scaled *E. nigrum* are found in lower order rivers or lakes at relatively high elevations. This corresponds with both Big Arbor Vitae Lake, Wisconsin, and Sandstone Lake, Ontario, which both possess heavily scaled specimens and are at the highest sample elevations. Scaled specimens are not necessarily associated with either warmer or cooler temperatures but do tend to be correlated with the lower latitudes and longitudes from these samples. A plot of the first axis from the environmental and meristic variable set produces a high correlation ($r^2 = 0.82$) between the first axes of the two variable sets (Fig. **62**). Most specimens

Figure 62: Plot of the first two canonical axes of meristic and environmental variables on 300 specimens of *Etheostoma nigrum* from 15 locations. The horizontal axis (L CV 1) represents meristic variables while the vertical (R CV 1) represents environmental variables. Sample sites are identified as; 1=Big Arbor Vitae Lake, WI, 2=Puckaway Lake, WI, 3=Roundtable Lake, ON, 4=Sandstone Lake, ON, 5=Rock River, WI, 6=Flambeau River, WI and Little Dog Lake, ON, 7=Boulevard Lake, ON, 8=Prelate Lake, ON, 9=Neebing-McIntyre River, ON, 10=Whitefish Lake, WI, 11=Nipigon River, ON, 12=McVicars Creek, ON, 13=Upper St. Croix Lake, WI and Silver Falls Creek, ON.


Environmental Variables
R. CV 1
(Objects)
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
L. CV 1
Meristic Variables

are plotted along the diagonal with all samples forming distinctive groupings (Fig. **62**). Several Ontario and Wisconsin samples can be found together and some lake and river samples overlap. The overlap is due to a large number of specimens possessing similar meristic traits. This analysis also suggests an increase in dorsal rays with latitude, a correlation between the number of POM pores and riverine habitat and a greater number of infraorbital pores in areas with a warmer climate.

Analysis of the meristic and biochemical data sets produced a plot of the two variables sets which places most specimens in an overlapping manner near the top right of the diagonal due to similar genetic makeup. The correlation for this plot was not high on the first ($r^2 = 0.72$) or second axis ($r^2 = 0.54$). However, almost all specimens from the Rock River, Wisconsin, and McVicars Creek and the Neebing-McIntyre River, Ontario, samples are in the lower left portion of the scattergram suggesting that these samples are meristically and biochemically distinct from all others.

This analysis suggests that increasing POM pore counts are correlated with the presence of the *GPI-A*82* and the *PGDH*150* alleles, that squamation increases with an increase in the presence of the *GPI-A*91* and *EST-1*105* alleles, that an interrupted POM pore canal is correlated with the *GPI-A*100* and *PGDH*100* alleles, and that the number of dorsal rays is correlated with the presence of the *EST-3*90* allele. Canonical correlations and the chi-square values for each analysis are shown in Table **71**. All correlations on the first axes for all analyses are statistically significant.

Table 71: Canonical correlations, percent redundancy, chi-square values, degrees of freedom (df) and significance for the first two axes in canonical correlation analysis of meristic vs. biochemical variables, meristic vs. environmental variables, and biochemical vs. environmental variables for specimens of *Etheostoma nigrum* collected from 15 locations in Wisconsin and Ontario. The left set refers to that variable set listed first.

	Canonical Correlation	Redundancy Left set	Redundancy Right set	Chi-square	df	Sig
Meristic vs. Biochemical						
Axis I	0.718	4.305	10.072	547.59	299	< 0.05
Axis II	0.543	3.901	3.950	344.22	264	< 0.05
Biochemical vs. Environmental						
Axis I	0.815	13.870	22.094	591.46	91	< 0.05
Axis II	0.690	6.175	12.427	276.60	72	< 0.05
Meristic vs. Environmental						
Axis I	0.820	7.191	11.522	983.51	161	< 0.05
Axis II	0.778	7.900	20.686	667.53	132	< 0.05

DISCUSSION

SEPARATION OF *Etheostoma nigrum* FROM *E. olmstedi*

Both Stone (1947) and Cole (1965) examined *E. nigrum* and *E. olmstedi* in areas of sympatry and noted that the two species could be separated by, among other characters, the number of dorsal rays. Stone (1947) also relied heavily on squamation characters (nape, cheek and breast scaling) and the number of scales in and below the lateral line to separate the two species. However, because the number of lateral line scales are influenced by environmental conditions during development (Gosline 1947; Bailey and Gosline 1955; Barlow 1961) and because these same conditions may also affect the number of scales below the lateral line, squamation characters may not be suitable for discrimination. Cole (1965) thought that squamation among *E. olmstedi* might be as variable as it is among some populations of *E. nigrum* and also noted that populations of *E. nigrum eulepis* could occur in the area in which *E. olmstedi* and *E. nigrum* overlap. Cole (1965) suggested that the best separation of the two species could be accomplished using the number of rays in the second dorsal, number of pectoral rays and the number of pores in the infraorbital canal. Chapleau and Pageau (1985) did not consider pectoral rays a reliable discriminatory character for separating specimens of *E. nigrum* from *E. olmstedi*. Chapleau and Pageau (1985) utilized the number of POM and infraorbital pores, dorsal rays and the amount of cheek and nape squamation for their discriminant analysis.

Cole (1965) stated that most *E. olmstedi* possessed 13 dorsal rays, 13 pectoral rays and eight infraorbital pores on an uninterrupted canal. The same variables

enumerated on *E. nigrum* always resulted in lower counts and the infraorbital canal was almost always interrupted. Cole (1965) calculated a discriminant function using the number of dorsal rays, pectoral rays and infraorbital pores, which showed slightly more separation between the two species than did mine. His function gave a Y value of 89.5162 for *E. nigrum* and 104.0080 for *E. olmstedi* in comparison to 26.0287 for *E. nigrum* and 29.4201 for *E. olmstedi* by the function derived herein.

The difference between the discriminant functions prepared by myself and Cole (1965) may be accounted for by both the technique utilized and the mean values of the two species examined. All means for *E. nigrum* were higher in Cole's (1965) study than in this one. While my calculated mean for the number of pores in the infraorbital canal for *E. olmstedi* was the same as Cole's (1965), my dorsal ray count was lower and my pectoral ray count was higher. However, if both discriminant functions are placed on a similar scale, there is only a slightly lesser range than Cole's (1965) for the second function (utilizing the same characters) and a much greater range in my data for the first discriminant function (utilizing the same four variables).

The character index and the first discriminant function utilize the same four variables but produce conflicting results with the second function which excludes the condition of the infraorbital canal. The discriminant function which uses the most characters should result in the best discrimination due to the increased information content. Because many samples of both species, within and removed from the area of suspected interbreeding, possess similar pectoral or dorsal ray counts, the inclusion of

the condition of the infraorbital pore canal seems a necessity to provide good discrimination. Although all but four *E. olmstedi* I examined possess an uninterrupted infraorbital pore canal and almost all *E. nigrum* possess an interrupted canal, either the discriminating power of this character is reduced in areas where possible interbreeding has occurred or it shows that much interbreeding has occurred. It is impossible to determine which possibility may be true.

Excluding the specimens and samples used for discriminating the two species discussed above, out of 1959 specimens of *E. nigrum* examined, there are only three from the Rock River, Wisconsin which possess a complete infraorbital canal on both the left and right sides. A specimen from the Don River, Ontario, (ROM 11693) has a complete right infraorbital canal. Therefore the inclusion of the condition of the infraorbital canal utilized in the first discriminant function is an important discriminator between the two species.

Specimens of *E. nigrum* with 13 or more pectoral or dorsal rays can be found everywhere within portions of the species' range which I examined. Increased numbers of pectoral or dorsal rays are found more frequently than an uninterrupted infraorbital canal. Therefore, a reduction in the number of infraorbital pores or interruption of the infraorbital canal may be a more reliable indicator of hybridization between *E. nigrum* and *E. olmstedi* than is a reduction in the number of soft rays. This interruption of the infraorbital canal strongly suggests a large zone of mixing of the two species within the Lake Ontario-St. Lawrence River-New York state area. All samples in these areas possess at least some specimens with an interrupted

infraorbital canal. The uninterrupted canal strongly suggests close *E. olmstedi* affinities due to the near absence of specimens with an uninterrupted infraorbital canal throughout the rest of the examined range of *E. nigrum*.

Both *E. nigrum* and *E. olmstedi* occur near the site of Quebec sample 1 (Yamaska River tributary) although the presence of the interrupted infraorbital canal strongly suggests *E. nigrum*. The high frequency of specimens from the Lake Ontario-St. Lawrence area which possess a mixture of characters of the two species, including the condition of the infraorbital canal, strongly suggests there has been recent mixing of the two species. There is no evidence from this or other studies to suggest that northern *E. olmstedi* formerly or presently possess an interrupted infraorbital canal. Cole (1967) did note that populations of *E. olmstedi* (called *E. o. vexillare*) from the Cape Fear River, North Carolina, typically possess an interrupted infraorbital canal. However, these populations are far removed from the area of possible intergradation under consideration. These considerations support the hypothesis that Quebec sample 1 has been affected by interbreeding and it was removed from further analyses.

New York sample 3 (Oneida Lake) possesses an interrupted infraorbital pore canal and a pore count similar to that of typical *E. nigrum*. However, this sample is identified as *E. olmstedi* using the second discriminant function and the CI. Because of discrepancies between methods, this sample was removed from further analyses.

New York sample 1 from Lake Champlain, resembles *E. nigrum* except that some specimens are identified as *E. olmstedi* when using the second discriminant

function. Cole (1965) and Smith (1985) suggest that this location is inhabited solely by *E. olmstedi*. Smith (1985), however, notes that the construction of a number of canals within New York during the 1800's may have allowed for migration and consequent interbreeding between the two species long before extensive collecting of specimens began. Therefore, some traits may have appeared within the affected populations only recently and there is now no means to determine what the original population was like. The Lake Champlain sample (NY-01) could be considered to represent an intergrade population at best and was excluded from further analyses.

All the New York specimens I examined are distinct because their dorsal and pectoral ray counts generally exceed the means, not only of the baseline *E. nigrum* samples from the United States mid-west, but also of the *E. olmstedi* I examined from the Atlantic Slope. The possibility of an area of recent mixing by the two species resulting in intergrade populations seems unlikely as this would be expected to result in ray counts which were intermediate rather than higher than either parental form. Hubbs (1955) noted that it is an almost universal rule that natural hybrids are intermediate for almost all characters which differ between the parental forms. However, I did not examine a large number of *E. olmstedi* and some populations of this species may possess similar high or higher dorsal and pectoral ray counts than those of the specimens I examined. For example, Cole (1965) reported that almost 10.0% of his sample (from New York, Pennsylvannia, Connecticut and southern Ontario) possessed 15 or more dorsal rays as compared to approximately 1.0% with similar counts in this study. Because the increased ray counts among many New

York state specimens may be a result of *E. olmstedi* influences, I eliminated all New York samples from further analyses.

As dorsal and pectoral rays are generally higher among New York specimens than those in any other area, and yet the infraorbital canal is most often interrupted, it is difficult to view these specimens as resulting from recent hybridization. Environmental variables may be affecting the number of dorsal rays within this area as several samples examined from nearby areas (*e.g.*, Ontario sample 3 (*E. olmstedi*), Quebec sample 4 (*E. nigrum*)) also possess higher than average dorsal and pectoral ray counts for both of these species. An alternative is that these two samples, and perhaps several from nearby areas, may be genetically close to the New York specimens.

Examination of squamation characters shows great variability among samples of both species. Samples of *E. olmstedi* from the Atlantic Slope show evidence of a south to north clinal increase in the number of lateral line scales. This cline appears to continue to hold true within many, but not all, of the unclassified samples in which some specimens possess *E. olmstedi* characteristics. In general, the utility of squamation characters for separating the two species is questioned because my results do not suggest any squamation variables which consistently distinguish the species. However, *E. nigrum* is the least scaled of the two species where they occur in sympatry. This finding agrees with those of McAllister *et al.* (1972) who studied both species within the Ottawa River, Ontario. In areas further removed from the primary zone of contact (northern New York, St. Lawrence River), squamation

characters alone are not suitable for discriminatory purposes.

Based on the above analyses, there is evidence that *E. olmstedi* and *E. nigrum* are distinct species. Removal of these samples and individuals was required prior to any analysis of *E. nigrum* due to some overlapping characters. In most cases, both species can be readily distinguished from each other through the use of only a few characters. However, the large number of known or suspected hybrid specimens and samples suggest that additional analysis of the distribution of both species within this area would be beneficial and could help explain the reinvasion of that area following Wisconsinan glaciation.

Several specimen lots from the UMMZ should be relabelled to reflect the current status of *E. olmstedi*, which is recognized by the American Fisheries Society (Robins *et al.* 1991). Additionally, suspected specimens of both *E. nigrum* and *E. olmstedi* in Canadian museums should be re-examined for the possibility of misidentifications, especially in the St. Lawrence River area.

MERISTICS AND MORPHOMETRICS OF *Etheostoma nigrum*

With samples of pure *E. olmstedi* or hybrids now removed, analysis of meristic and morphometric variation in *E. nigrum* could continue. The purpose of these analyses was to identify samples of *E. nigrum* which possessed distinctive traits which might suggest adaptation to these areas or historic differentiation.

Morphometric Data

The morphometric variables examined explain only minor variation among samples of *E. nigrum* and are not considered useful for geographical analysis.

However, a possibility I did not consider at the onset of this study is that the specimens I examined for the initial selection of morphometric variables may have been exhibiting clinal variation in certain characters which did not vary to a great extent within the Thunder Bay area. There is a possibility that some morphometric characters of *E. nigrum* may show clinal trends and that utilizing specimens from a wider area for the initial selection of characters might reveal these clines. Due to differences noted between scaled and unscaled specimens for the number of scales below the lateral line, body depth is one character which should be examined in a future study.

Means of snout, pectoral and predorsal length are within the ranges given by Kott and Humphreys (1978) for southern Ontario and eastern Lake Superior samples and by Page (1981) for Illinois specimens. The greater pectoral fin lengths of males have been noted in earlier studies of *E. nigrum* (Becker 1983).

Statistically significant differences noted in snout length between the sexes are not due to examining an unequal number of specimens of either sex. Because significant dimorphism in snout-type was noted only in the transitional snout-type scores (*i.e.*, types 2 and 4), it is possible that some misclassifications in snout-type occurred. However, excluding the possibility of errors made during the assignment of snout-type, presumably only on one sex, there is real sexual dimorphism in snout lengths which is not related to snout-type. Because the snout-type of Lake Michigan specimens is more subconical than intermediate, snout-type may be affected by habitat or primary forage within the area or these specimens may be genetically different

from those in other areas.

Meristic Variables

Pearson correlation analysis of meristic variables with latitude and longitude using either the individual, sample mean or one-degree grid matrices often produced different results. The one-degree grids, while providing information on latitudinal and longitudinal trends due to environmental influences, sometimes included samples from different watersheds within drainages and thus may have included specimens with different evolutionary histories. Therefore, correlations produced with the one-degree grids are considered the least informative of these three methods for the purposes of this study. Because the sample means average the meristics of many specimens, these correlations are considered more valuable in determining latitudinal and longitudinal trends than the individual specimen matrix.

Although several meristic variables are geographically informative when examined individually, there are few which exhibit similar patterns of geographic variation and provide information on the biogeographic history of populations of *E. nigrum*. The upper and middle Ohio Basin samples of *E. nigrum* exhibit several meristic variables which vary in a distinct and geographically informative manner. Scaled darters possess squamation and several additional meristic characters which vary in a similar manner. Those variables which may provide information on the biogeographic history of *E. nigrum* are discussed in detail.

Branchiostegal Rays

The most common branchiostegal ray number for *E. nigrum* is six. Samples

possessing specimens with five branchiostegal rays are confined primarily to the middle and upper Ohio Basin. Although some scattered samples, mainly near or within the Mississippi River drainage also possess some specimens with five branchiostegal rays, their numbers are far less than those in the upper reaches of the Ohio River. The tendency for five branchiostegal rays to develop may be a genetic trait because it is less frequent outside of the upper or middle Ohio Basin area. Specimens with five branchiostegal rays in samples from the Lake Erie drainage likely attest to both recent connections between the Ohio and Erie basins (Underhill 1986) and a probable lack of immigration by individuals with six branchiostegal rays into these areas. Hence, populations which may have been influenced by recent immigration exhibit the typical count of six branchiostegal rays in some specimens.

The majority of Wabash River specimens were expected to possess five branchiostegal rays due to connections with the Lower Ohio River. However, recent connections between the Wabash and Maumee rivers via the Wabash-Erie Canal (Hubbs and Lagler 1983) may have allowed individuals with different genotypes to enter this area. Thus, samples of *E. nigrum* from this area possess the trait of five branchiostegal rays with varying frequencies. Similarly, canals joining the Upper Ohio River and Lake Erie may have led to a reduction in the frequency of this trait within other areas. Lack of isolation may have allowed the trait for six branchiostegal rays to spread to areas in which it was formerly absent.

The lower number of branchiostegal rays may have developed during a period of separation from other populations of *E. nigrum*. While it is possible that an initial

founder effect from a small population was responsible for this trait, the size of the area currently inhabited by individuals which possess five branchiostegal rays suggests that the character was well established in a larger population prior to their dispersal throughout the presently inhabited area. Although the total distribution of populations with five branchiostegal rays is unknown, the lack of specimens with six branchiostegal rays in many widely separated samples suggests that it may be present within much of the intervening area. Similarly, while any hypothesis explaining the origin of this trait is speculative, it may have evolved during impoundment of the Teays River (pre-Illinoian) (Teller 1973) or may have developed in areas further to the south (Kanawha-New River area, West Virginia) at a later date. Movement facilitated by stream capture and dispersal into the middle Ohio Basin by individuals possessing this trait would have occurred after these events. The area near or above the Kanawha River falls (*i.e.*, New River) may have provided the necessary isolation for this trait to develop and spread to surrounding areas. Hocutt *et al.* (1986) and Burr and Page (1986) cite many locations in the Cumberland and Tennessee river systems at which more southern populations could have moved north into rivers presently draining into the middle Ohio Basin. Therefore, although the trait may have spread either from the north or the south, at present, because there is no evidence that *E. nigrum* was in the Teays River area prior to the Illinoian and because *E. nigrum* examined from West Virginia, Kentucky and Tennessee do possess five branchiostegal rays, it is most likely that the trait evolved near or within these areas and spread northward. Thus, populations of *E. nigrum* in the middle and upper Ohio Basin

which possess five branchiostegal rays likely have a long and unique genetic history, separate from that of other, nearby unscaled *E. nigrum* populations.

Seven branchiostegal rays are considered a plesiomorphic trait among *Etheostoma* (Bailey and Etnier 1988). However, the location of specimens which possess seven branchiostegal rays on one or both sides do not follow any interpretable geographic pattern. Because scaled specimens are not the only ones which exhibit this trait, the occurrence of seven branchiostegal rays cannot be correlated with other plesiomorphic traits such as increased squamation or a complete lateral line. The occurrence of this trait on a few specimens within a single location appears to be due to chance.

Lateral Line Scale Counts

The lateral line scale counts for *E. nigrum* exhibit a prominent southeast to northwest clinal increase. Lateral line scale counts are known to increase with latitude, mainly due to slower development because of decreased temperature (Bailey and Gosline 1955; Barlow 1961). The two areas where lateral line scale counts do not closely follow this trend, the Sheyenne River of North Dakota and the Driftless Area in southwestern Wisconsin, are perhaps due to warmer than average temperatures for the latitude during the spawning and developmental period.

The single Michigan specimen with a lateral line count of 61 (Muskallonge Lake: UMMZ 68829) represents an extreme high count for the number of lateral line scales in *E. nigrum* among the samples I examined. Other specimens from this lake, as in several other lakes on the Michigan Upper Peninsula or northern Wisconsin,

also possess a higher than average number of lateral line scales for their latitude. Bailey and Gosline (1955) noted that percid populations which possessed high vertebral counts which did not fit the general trend for south to north increases in vertebral number were from waters which experienced cooler than normal temperatures during the spawning or developmental period. Cooler temperatures were ascribed to increasing elevation, lake depth or lake size. Schultz (1927) arrived at a similar conclusion when studying anal fin ray variation among populations of *Notemigonus crysoleucas*. The highest anal fin ray counts were associated with populations in mountain regions while lower counts predominated in populations inhabiting warmer coastal regions or in the warm, shallow bays of large lakes. The northern Michigan and northern Wisconsin samples which exhibit higher lateral line scale counts than other *Etheostoma nigrum* in the area are likely examples of the same phenomena as the majority of these samples are from upland lakes similar to those examples given by Bailey and Gosline (1955).

A south-north clinal increase in lateral line scale counts was previously suggested by Kott and Humphreys (1978) for Canadian samples. Overall, the lateral line scale counts I observed are similar to those reported in other studies (*e.g.*, Cole 1971; Kott and Humphreys 1978; Becker 1983; Page 1983). Because they are subject to phenotypically based clinal variation, lateral line scale counts do not provide any information about the biogeographic history of *E. nigrum* populations.

Completeness of the Lateral Line

Page (1977, 1983), Becker (1983), Trautman (1957) and Smith (1985) state

that *E. nigrum* typically possesses a complete lateral line. Robinson and Buchanan (1988) state that the lateral line on Arkansas specimens is usually complete or nearly complete. The lateral line is often complete in samples of *E. nigrum* around the southern fringe of its range that I examined. There is a trend for samples to possess a more incomplete lateral line as one moves further north. A very incomplete lateral line, with more than three unpored scales or an interrupted lateral line, is found on the majority of specimens from Lake Superior tributaries. However, there are enough specimens with incomplete or interrupted lateral lines in samples within and between the northern- and southern-most examined areas so as to question whether a trend exists in any area. The complete lateral line in 63.3% of the scaled specimens and within the majority of the samples comprising these specimens might suggest retention of a plesiomorphic trait. If the scaled form is, in fact, plesiomorphic, a complete lateral line could be expected to occur within these samples and the complete condition of the lateral line would correlate with increased squamation.

Page and Swofford (1984) have noted a strong tendency among darter species which inhabit slow, deep water (*i.e.*, lakes) to have a lower percentage of pored lateral line scales and fewer POM and infraorbital pores. A well-developed system of canals to protect sensory neuromasts from fatigue is apparently needed in fast-moving water, but is not required in slow or standing water (Page and Swofford 1984). Because the scaled form is more common in rivers than lakes, a more complete lateral line is expected on these specimens. However, because the complete lateral line is always twice as common on the scaled form as it is on the unscaled form, regardless

of habitat (Table **24**), the complete lateral line suggests retention of a plesiomorphic trait. Except for the frequent occurrence of a complete lateral line on scaled specimens, however, the condition of the lateral line exhibits a largely uninterpretable geographic pattern.

A complete lateral line in the southwestern portion of the range (*e.g.*, Colorado, Wyoming) seems to at least partly invalidate the "progression rule" (Wiley 1981) which states that the most derived traits (*e.g.*, very incomplete or interrupted lateral lines) are found near the limit of the range. However, there is no reason to believe that all populations at the limit of the range of a species will develop any or all of what are considered derived traits. Divergence of peripheral populations may be random relative to that of the ancestral form (Wright's rule) (Wiley 1981). Additionally, there is still disagreement as to whether the more derived forms displace the more plesiomorphic forms or whether it is the derived forms that radiate outwards. Displacement of either form depends largely on how the two forms interact with their biotic and abiotic environments within the original area of habitation. Excluding the possibility of a founder effect or genetic drift within the area, additional possible explanations for the complete lateral line in the southwest area include the following: (**1**) The southwestern samples possess the more ancestral state of the lateral line condition in *E. nigrum* because they represent descendants of populations which survived glaciation in this or a nearby area. (**2**) This area has always been far enough removed from distant populations so as to avoid immigration of individuals carrying a gene (or genes) for an incomplete or interrupted lateral line.

(3) These populations may have only recently (late Pleistocene) crossed the Mississippi from the southeastern portion of the range and carried the plesiomorphic trait into the area. (4) There is some tendency towards or selection for an incomplete lateral line among populations which inhabit slower waters (Page and Swofford 1984).

Possibility (3) seems doubtful based on populations of several species of *Etheostoma* on either side of the Mississippi that are considered racially distinct or sister species (*i.e.*, Tsai and Raney 1974; Mayden 1988). Thus there is some evidence that few *Etheostoma* have crossed the Mississippi since the Wisconsinan. Possibility (4), while seeming to hold true in some areas, such as northern lakes, does not hold true in all areas and perhaps can be discounted. Although the river conditions from which the Colorado sample was collected are unknown, a complete lateral line is predicted in riverine environments. Possibilities (1) and (2) are somewhat similar and are the most probable explanations. Therefore, the complete lateral line in the southwestern area is interpreted as due to retention of a trait carried from the southeast which has remained intact due to a lack of immigration by individuals with incomplete lateral lines.

Scales above the Lateral Line

The number of scales above the lateral line are largely similar to the counts reported in regional studies of the species by McAllister *et al.* (1972) and Kott and Humphreys (1978) for Canadian samples.

The number of scales above the lateral line (LL1) closely follows the trend in nape scaling; those fish with scaled napes have the highest counts while those without

scaled napes possess the lowest. Wisconsin thus possesses both the majority of scaled specimens and the majority of specimens with a high number of scales above the lateral line. However, other areas which have scaled specimens do not possess similar counts suggesting no simple correlation between increased squamation and a high number of scales above the lateral line. While lateral line scale counts exhibit a clinal increase to the northwest, the number of scales above the lateral line decreases. There is thus a clinal decrease in the number of scales above the lateral line radiating to the southeast and northwest with Wisconsin and Minnesota samples, predominantly those with scaled specimens, possessing the highest counts. When considered with body squamation, the number of scales above the lateral line exhibits a meaningful trend and further helps to describe the distribution of the scaled form of *E. nigrum*.

Pectoral Rays

The number of pectoral rays is lowest among samples from south of and near the Lower Great Lakes and within the middle and upper Ohio Basin. In this area, a reduced number of pectoral rays is often found in conjunction with a reduced number of branchiostegal rays. A reduced number of pectoral rays is thus another character which defines upper and middle Ohio Basin *E. nigrum*. This character may have arisen during the same period of suspected isolation from other populations of *E. nigrum* during which the trait for five branchiostegal rays developed.

Throughout the rest of the examined range, pectoral ray counts are generally similar so they do not provide any information on the biogeographic history of *E. nigrum*. The number of samples from lake or river locations, and yet possess similar

pectoral ray counts, suggests that variation in the number of pectoral rays is likely not just a response to habitat. However, a highly significant correlation with latitude and longitude suggests a graded increase in pectoral rays in the northwest. Pectoral ray counts from southern Ontario and Lake Superior are similar to those given by McAllister *et al.* (1972) and Kott and Humphreys (1978).

Preoperculomandibular Pores: Eastern and Western Forms

Chapleau and Cooper (1992) suggest that the current variation seen in POM pore counts between eastern and western *E. nigrum* populations in Canada is due to two postglacial dispersal routes: (1) via the Mississippi into Glacial Lake Agassiz and north and eastward into Glacial Lake Barlow-Ojibway and, (2) via the Ohio River and the Lower Great Lakes into the St. Lawrence River area and northeastward. My data basically concurs with their distributional findings. Higher counts predominate in the east and southeast (*e.g.*, southern Ontario, Pennsylvannia, Ohio, Tennessee, Virginia) while the lower counts predominate in much of the west (*e.g.*, Saskatchewan, Manitoba, North Dakota) and the northern portion of Ontario. However, due to a large number of samples from Wisconsin, Minnesota and Michigan, I have detected a much larger "mixing zone" for the eastern and western forms than is suggested by their (Chapleau and Cooper 1992) data.

Chapleau and Cooper (1992) stated that a cline for POM pores would be suggested if a large number of samples from the geographic area between the high and low POM pore count morphs were detected which possessed an average pore value of between seven and eight. I have noted 51 samples with such an average

number of pores with 28 of these sites occurring in Michigan (12), Minnesota (10) and Wisconsin (6) alone. The POM counts decrease from the southeast to the northwest and a large number of specimens from the central portion of the examined range possess an intermediate POM pore number (7 or 8).

The results of the Mann-Whitney test suggest that there may be an association between habitat and the total number of left POM pores. However, because the test detects small differences, the biological significance of the test is somewhat questionable. Additionally, although the results are highly significant, great differences occur between the mean number of POM pores for lacustrine populations of scaled and unscaled darters from Wisconsin (Table **23**). There may be an environmental effect, perhaps similar to that affecting some lacustrine populations of *Gasterosteus aculeatus* and *Culaea inconstans* which exhibit pelvic spine reduction (Bell and Ortí 1994; Nelson and Atton 1971). Local, but unequal, adaptation to these types of environments may be causing selective shifts for this trait among populations of both scaled and unscaled *Etheostoma nigrum.*.

The tendency for lacustrine darter species to possess fewer POM pores than riverine populations (Page and Swofford 1984) casts some doubt on the hypothesis of Chapleau and Cooper (1992). Although they cite the work of Starnes and Starnes (1979) and Cole (1971) as further support for homogeneity of the high POM pore eastern morph, all of the population statistics provided by Starnes and Starnes (1979) and Cole (1971) are from riverine populations. The results of the Mann-Whitney test presented above and by Chapleau and Cooper (1992) suggest that riverine samples can

be predicted to possess a greater number of POM pores regardless of geographic location. Because most of Chapleau and Cooper's (1992) eastern samples were from rivers, their results are similarly predictable. However, because both Chapleau and Cooper's (1992) and my data suggest that this prediction does not hold true in all areas (*e.g.*, the Hudson Bay-Manitoba drainage) there is some evidence for a southeast to northwest clinal decrease in the number of POM pores.

There are, perhaps, only two explanations for the appearance of eastern and western forms with a higher and lower number of POM pores, respectively. First, populations of *E. nigrum* may have been separated, either on both sides of the Mississippi (or in southern and northern tributaries), at some point during the late Pleistocene and developed, or had already developed, the higher or lower POM pore counts (the separation hypothesis). Alternatively, clinal variation in POM pores developed after Wisconsinan glaciation and has unequally affected the scaled and unscaled form (the developing cline hypothesis). Each of these possibilities is considered below.

I/ <u>Separation Hypothesis</u>

While not specific about the area, Chapleau and Cooper (1992) suggested two Mississippi refugia for the present eastern and western forms; one in the upper and one in the lower Mississippi. These areas could be interpreted as being the present day Upper Mississippi Basin above the confluence with the Missouri River and an area south of the Missouri River junction, perhaps within the present day Lower Ohio Basin (Robison 1986). The Wisconsinan glaciation is the most likely period during

which this separation would have occurred because establishment of this distributional pattern of POM pores during earlier glacial or interglacial periods would have allowed the mixing of both forms during later periods and possibly created a larger area of intergradation.

A consideration of the separation hypothesis requires isolation of some populations of *E. nigrum* either east and west of the Mississippi or in ancestral Hudson Bay drainages (Robison 1986). Pre-Pleistocene drainage patterns in the present Hudson Bay drainage are thought to have included much of the present Upper Missouri drainage and perhaps the extreme upper portion of the present Mississippi River in Minnesota (Owen *et al.* 1981; Robison 1986; Mayden 1988). It is probable that somewhat similar drainage patterns occurred during the Sangamon interglacial approximately 100,000-70,000 years BP (before present) (Hocutt *et al.* 1986). There is a good possibility that a generalist species like *E. nigrum* occurred in these waters. Both the scaled and unscaled form may have inhabited the Hudson Bay drainages isolated from those farther to the south although it seems possible that only the scaled form inhabited the area (see below). The trait for a lower number of POM pores may have thus developed during a period of isolation in either of these areas with Wisconsinan glaciation forcing fish possessing the trait farther south, still mainly west of the Mississippi. A reduction in the number of POM pores is most likely as Atlantic Slope *Boleosoma*, considered the most plesiomorphic members of the subgenus, possess higher numbers of POM pores (Cole 1971; Page 1983). Northern populations forced south would have been confined by the Des Moines and Superior

ice lobes to the north and the Chippewa, Green Bay and Lake Michigan lobes to the east (Becker 1983; Farrand and Drexler 1985). Unscaled *Etheostoma nigrum* likely existed on both sides of the Mississippi River and had reduced contact during the Wisconsinan advances.

Assuming that habitat is not the complete cause of high or low POM pore counts, an eastern and western morph with higher and lower POM pore counts, respectively, would each also have to be subdivided into scaled and unscaled, if the pattern for POM pores is not clinal. As the development of squamation is not directly affected by environmental conditions, the only alternatives explaining variation in POM pore counts between scaled and unscaled samples are that: (**1**) there were eastern and western scaled and unscaled forms which have since re-invaded the same areas, (**2**) unscaled darters have managed to pass the reduced (or increased) POM pore count into scaled populations without those populations losing squamation, or (**3**) scaled darters possess a higher number of POM pores because they are exhibiting the more plesiomorphic trait. The cross breeding experiments of Lagler and Bailey (1947) suggest that possibility (**2**) is not likely because the resulting offspring would be classed as intergrading specimens. We would be left with either possibility (**1**), which seems even less likely, or assume that (**3**), the scaled form is exhibiting the more plesiomorphic trait. The results in Table **23** showing that unscaled, riverine *E. nigrum* do not possess the same high number of POM pores as do scaled populations further suggests that: (**1**) unscaled, western populations did not possess high POM pore counts during the Wisconsinan, or (**2**) a decreased number of POM pores has

developed independently within both scaled and unscaled lacustrine populations.

II/ Developing Cline Hypothesis

Based on the results of the Mann-Whitney test, it is possible that some populations of the scaled form may have developed a reduced number of pores while inhabiting lakes. Scaled populations with the higher counts might indicate those with a history of river habitation. If this hypothesis is correct, then a separate eastern and western scaled form is no longer required. However, if pore reduction occurs within populations during a prolonged period of lake habitation, then those samples from the east occurring in lakes should also possess a reduced number of pores. In many cases however, these eastern lacustrine samples do not exhibit a reduced number of POM pores. However, without detailed information on the river and stream sample sites examined, it is impossible to determine if "river" or "stream" necessarily means fast flowing water. Therefore, some river or stream collections might have very little current which could allow riverine specimens to mimic the meristics (*i.e.*, low POM pore counts) or morphology of lacustrine specimens. This could seriously disrupt the discovery of any trends and place the biological significance of the Mann-Whitney test in doubt.

The above considerations suggest that the variation in POM pore counts exhibited by *E. nigrum*, decreasing from high counts in the southeast to low counts in the northwest are partly clinal. The cline appears to have been established following the dispersal of both the scaled and unscaled forms. There is also some evidence for eastern and western unscaled forms, especially in the area near Wisconsin, and the

"cline" may be a result of mixing of an eastern and western unscaled form. Habitat appears to play some role in moderating the number of POM pores as the "cline" is greatly disrupted in lacustrine habitats in the north. The scaled form of *E. nigrum* is unequally affected by this "cline" and may be exhibiting signs of adaptation to individual habitats as suggested by the Mann-Whitney test. The scaled form may also simply be exhibiting the more plesiomorphic trait. There are, however, too few scaled samples from the Hudson Bay drainage to determine if those populations are affected to a similar extent as the unscaled form appears to be in this area. Further research should be performed to determine the possible causes of this variation in *E. nigrum*.

Discriminant and Character Index Analyses

I/ Regional Analysis: Fox River, Wisconsin

Failure to arrive at a means independent of squamation to separate consistently scaled and unscaled *E. nigrum* within the Fox River system of Wisconsin with greater than 69% accuracy suggests that both forms possess few distinct meristic differences other than squamation itself, at least within this river. Two of the characters selected, number of scales in the lateral line and the number of dorsal rays, were noted by Hubbs and Greene (1935) to be higher in the scaled form. Environmental conditions within the river itself may be causing selection for some of the meristic traits thus contributing to these similarities. Similarly, upstream and downstream habitats may be contributing to the differences exhibited by the two forms within the river system. However, there are some character differences between the two forms within this

river system which are consistent within other areas (*e.g.*, complete lateral line).

II/ Analysis of Examined Range

Discriminant analysis of the entire data set identified few additional characters besides those identified with the Fox River samples which could be used for discrimination throughout the entire range of *E. nigrum*. Excluding the number of nape, cheek and breast scales, the number of scales at the nape-parietal juncture and the number of opercle scales, are typically at their highest on scaled darters. The complete condition of the lateral line holds true throughout most of the range of the scaled form. The number of dorsal rays may have been selected due to the large number of Wisconsin specimens in which high counts are typical and thus may be but a regional character. Hubbs and Greene (1935) reported a slightly higher number of dorsal rays on the scaled form. The number of scales below the lateral line may be a result of the scaled form possessing a slightly deeper body. However, because body depth was not measured, I have no basis on which to assess its importance as a morphological discriminator of the two forms or body depth is responsible for an increased number of scales below the lateral line. Hubbs and Greene (1935) did state that the scaled form was the more robust of the two forms so a greater number of scales below the lateral line may be a "good" discriminating character. Overall, however, when squamation is removed from analyses, discrimination of the two forms is weak.

An additional 416 specimens from a wide area are classified as scaled when squamation is excluded. Because some of these specimens possess only minor

amounts of squamation, and also because some previously identified scaled specimens are excluded, classification without squamation variables is not a good method. As many of the "new" specimens were identified from previously unidentified samples, there is some support for the possibility that some meristic characters selected for discriminating specimens of scaled *E. nigrum* when squamation is excluded are the result of regional latitudinal or altitudinal environmental effects on the phenotype. However, intergrades between the two parental forms would also be expected to occasionally possess some of the characters which are unique to the scaled form. Additionally, because regional or localized character differences are part of the basis for declaring subspecific status, regional differences exhibited by the scaled form of *E. nigrum* are not unexpected.

TEMPORAL VARIATION ANALYSIS

Stability of meristic characters over time might suggest: (**1**) lack of immigration by individuals with different genotypes, (**2**) consistency of environmental influences upon phenotypes, (**3**) a strong genetic basis for that variable (little plasticity), or (**4**) a combination of these factors. Immigration can be ruled out in isolated areas. Changes in environmental influences, such as temperature, during the usual developmental period can often be ruled out by examining size classes presumably representing different age classes which may have experienced different temperatures during their developmental period (Chernoff 1982). If characters are similar within and between size classes for that sample, the probability is high (although it is difficult to rule out completely) that environmental influences have been

minimal or remained constant between years during the developmental period (Barlow 1961). A final possibility is that the examined characters themselves are not greatly affected by the environment.

Since the number of scales in the lateral line is known to be influenced by temperature during early development in most fish species (Hubbs 1926; Barlow 1961; Chernoff 1982), it is possible that the number of scales on other body areas such as the nape or breast is similarly affected. Barlow (1961) suggests that the characters which appear last during development are the most labile and Hubbs (1926) noted that the nape is the last area to be scaled in a number of fish species. However, the appearance of scales where they are normally absent has not been reported. Additionally, the extremes in squamation seen in *E. nigrum* are largely unknown among darters (Page 1983) although somewhat similar variation exists in *E. olmstedi* (Cole 1967) and, to a lesser extent, in *E. zonale* (Tsai and Raney 1974).

Because the populations examined have inhabited these areas for 10,000 years, adaptation to local environments is likely complete. If squamation were temperature controlled, temperature variation during time of hatching could express itself in that some size classes (*i.e.*, year classes) would be much more heavily scaled than other size classes within the same sample. However, significant differences in squamation between size classes were not observed. If squamation, like serial elements, increases with decreasing temperatures, then heavily scaled specimens could have been expected in most northern locations. Because scaled specimens were not found in the far north, this casts doubt on the influence of temperature as a causal agent for increased

squamation.

If increased squamation arose due to some environmental influence alone, it is likely that many more populations of scaled *E. nigrum* should have been noted since the original description of Hubbs and Greene (1935). The formulation of a tenable biogeographic hypothesis explaining this distribution would be difficult. I examined a large number of samples collected prior to and since the original description and only noted a few new scaled populations from areas which were not sampled prior to 1935 and, therefore, were not available to Hubbs and Greene (1935). Because the distribution of the scaled form has not increased appreciably since it was first noted, even though some former sites have been re-sampled, there is further evidence that the trait for increased squamation does not arise independently among populations.

Squamation variables did not vary to the same extent or in the same direction within each of the five samples. Individually, however, all samples exhibited changes in the same direction for all squamation characters. Changes to squamation were not always in the same direction as changes in characters related to the lateral line. This result suggests that scaled areas (lateral line associated and body squamation) are not influenced by the same factors and/or not to the same extent. Both lateral line scale counts and body squamation generally remained as constant between size classes as they did between the historic and recent collections. Because the number of lateral line scales can be influenced by temperature during development, constant lateral line scale counts strengthens the possibility of constant environmental influences during development within the examined samples. However, this does not mean that the

environment has maintained increased squamation.

The majority of the characters examined exhibited only minor change over the 60+ years separating the historic and recent samples. It would be difficult to ascribe these changes to any particular environmental cause without having long-term data on each site. Changes to variables which also might be under the same external influences as serial elements (*e.g.*, number of scales in, above and below the lateral line) increased in some samples but decreased in others. Since the number of dorsal, anal and pectoral fin rays also remained relatively constant within samples, it is unlikely that the observed changes to the numbers of scales in, above or below the lateral line are affected to the same extent, by factors such as temperature, which cause changes in serial elements.

The samples exhibiting the greatest variation in scales associated with the lateral line are river samples (Rock and Flambeau) and thus some of the variation may be due to immigration of individuals with different genotypes. In the case of the Flambeau River, information provided by UMMZ suggests that the original site was re-sampled in 1992. The single heavily scaled specimen within the recent sample seems to suggest immigration. Lindsey (1953) noted meristic differences in samples of *Richardsonius balteatus* collected from different localities within the same lake, so variation within river samples seems highly likely.

A distinct change was noted in snout-type variation over time. A code of "3" was given to those specimens with an intermediate type snout while "1" signified a blunt snout and "5" denoted a subconical (pointed) snout. A value of "3" was the

mean for 2111 specimens examined. There was a trend towards a more intermediate type snout from a blunt one among three of the recent samples. The shape of the snout may be indicative of where darters feed rather than on what they feed (Page and Swofford 1984). Because many of the recent collections were assumed or known to have come from the same or nearly the same location as the historic collections, the observed trend towards an intermediate snout-type may reflect a physical change to the habitat, such as in-filling of substrate by siltation, or it may indicate changing distribution or abundance of the invertebrate community. If snout-type is selected for based on the habitat of primary forage, then a change in the substrate, the invertebrate community, or some combination of the two, may have caused a shift in where feeding was occurring and selected for a different snout-type. A food habit study utilizing historic and recent collections would help resolve this question.

The number of scaled specimens decreased 22% in Big Arbor Vitae Lake since the historic sample was taken. However, because specimens without any cheek scales and a low number of nape scales are present in the historic sample, the occurrence of several specimens with the same characteristics in the 1992 sample is not unexpected. Lakeshore development has likely had some kind of impact on the lake (*e.g.*, eutrophication, increased summer water temperatures). While this development may have somehow altered conditions which selectively acted against the scaled phenotype, the development also appears to create the conditions under which the scaled form was formerly thought to be most common. If conditions brought on by this development has acted against the scaled form, then there could be some underlying

adaptive cause for increased squamation and these conditions are now selecting a less scaled form. However, evidence from other studies suggests that some characters do not have any adaptive significance but are simple plesiomorphic traits (Wiley 1981).

Sample sizes were small for all locations except Big Arbor Vitae Lake and therefore, the results are somewhat preliminary. The observed increase in squamation in the Rock River but a decrease in its tributary the Crawfish River, where most specimens are of the scaled type (Greene 1935), could be the result of failure to detect actual differences due to the sample size. Changes in squamation characters in the Crawfish River were no greater than those exhibited by other meristic characters (*e.g.*, dorsal rays, lateral line counts). Variation in squamation characters should not be considered to be any different than that exhibited by meristic characters.

Temporal variation analysis of samples of *E. nigrum* suggests that squamation remains relatively constant and varies within ranges similar to those of other meristic characters. These changes are similar to the variation observed in other studies of temporal variation in fish (*e.g.*, Underhill and Merrell 1959). Thus there is some evidence of long-term consistency of squamation characters in some populations of *E. nigrum*.

Distribution of Scaled Specimens

The results presented above show that the percent of scaled specimens is not constant within sampled populations. Some of these results may be in error as breast counts were not available from four of the recent collections and therefore some of the specimens identified as scaled may not actually meet the requirements. However,

because the historic and the recent Crawfish River specimens remain "scaled" even when the number of breast scales are excluded, I have assumed that the same would be true among the other four recent collections. If the number of breast scales is excluded from the qualifying definition of scaled, only 4.6% (11 of 240) of the darters identified as scaled in this study do not actually possess 47 or more breast scales. Therefore, the assumption that specimens from the recent collections would qualify as scaled if their breast scale counts were available seems reasonable.

Excluding the Flambeau River sample which may have included a migrant with increased squamation, two of the recent collections exhibit increases in the percent of scaled specimens and two exhibit a decrease. The changes between these four samples are all of approximately the same magnitude (16% average). Reist (1981) examined the pelvic phenotypes of *Culaea inconstans* and noted that in ten sampled creeks, approximately 22% of the specimens in any sample did not possess a pelvic skeleton. The percent frequencies of other phenotypes were also relatively stable between these same locations. Reist (1981) suggested that there might be a seasonal, selective environmental factor acting on some of the phenotypes producing their variation in frequency. However, I have collected heavily scaled specimens from early spring to the late fall in several areas near Thunder Bay, Ontario, which argues against seasonal selection for the scaled or unscaled phenotype.

Increases and decreases in the amount of squamation, similar to the changes in other meristic variables (*e.g.*, dorsal rays, dorsal spines), suggests that squamation fluctuates in a manner similar to other variables due to the effects of recombination.

Assortative mating between scaled individuals could maintain the number of scaled specimens whereas matings between scaled and unscaled specimens would reduce the amount of squamation. Lagler and Bailey (1947) found that some crossings of suspected "pure" scaled parents resulted in some offspring with less squamation than the parents in one or several characters (nape, breast or cheek). They attributed this to one or both of the parents being near "the lower genetic limit of normal variation in the character" resulting in "transmission of the atypical condition" (less squamation) in the offspring. Although assortative mating has not been shown by this or any other study, Lagler and Bailey (1947) cite a verbal report by M. Trautman stating that in Ohio waters where the distribution of the two forms overlapped, the unscaled form migrated upstream prior to spawning.

A possible 32-60+ generations between samples seems more than adequate to explain the variation seen in this study. Although Bailey and Etnier (1988) suggest that squamation may be either lost or gained in *E. nigrum* due to their plastic phenotype, the above results provide no support for this. Although there are no known conditions which induce increased squamation or could maintain it at such a consistent rate, the possibility that such condition(s?) exist and have remained relatively constant within these populations over the past 60 years cannot yet be completely discounted. However, when additional characters, which vary little within and between samples, are considered, there seems to be no explanation for the retention of extra squamation within some geographic areas other than it has a strong genetic basis and, therefore, remains relatively constant through time.

CONSISTENCY OF SQUAMATION AND MERISTIC CHARACTERS

I/ Squamation Characters

The purpose of examining the consistency of squamation was to determine if specimens classed as scaled exhibited similar amounts of squamation within and between geographic areas or if other trends existed for the amount of squamation in different areas of the range. Examination of samples collected from the same areas over 60 years apart showed evidence of long-term stability of these characters.

Because the number of nape scales is approximately similar from sample to sample, even if the distribution of these scales is sometimes different (*e.g.*, 90% or 100% coverage), the presence of nape scaling at a consistent level argues for the probable isolation of portions of a once larger population. There is evidence for a strong genetic influence in determining squamation characters because the examined samples were collected over a 30 year span and yet the number of scales on these body areas remained relatively constant between geographic locations. Independent development of this trait among many small populations does not seem tenable since, unlike the species pairs of *Gasterosteus* (McPhail 1992, 1993), there are no known sympatric populations of scaled and unscaled *Etheostoma nigrum* which might indicate divergence through partitioning of resources. Therefore, enumeration of a similar number of nape and breast scales on both large and small specimens within samples collected over time and between geographic areas, suggests that environmental influences likely have minimal effects on the number of scales.

Lesser numbers of breast scales on specimens, especially to the east (*e.g.*,

Ohio, southern Ontario) but also in Missouri and to the west of Wisconsin, may suggest the effects of intergradation with the unscaled form or regional differentiation of populations. Local or regional differentiation allows a reverse argument to be used; that scaled populations are exhibiting squamation due to the influence of some biotic or abiotic variable. However, because the number of nape scales is relatively consistent over a wide geographic area, local differentiation due to some external influence is not likely.

Although nape length was not measured on all specimens, the predorsal length provides a close approximation of its length. The lack of statistically significant correlations between the number of scales on the nape and both the predorsal length and the nape length, suggests that the number of scales is not dependent on the size of the area covered. Because a large number of specimens were not measured for nape length, morphological adaptation to discrete habitats affecting the length or width of the nape cannot be totally ruled out as a cause of more or fewer nape scales in some samples. However, as there is no evidence of this from my samples, I do not believe that further examination will show any correlation between nape length and number of scales.

Independent of crowding, there are times when a lesser number of scales can cover an area as or more completely than a larger number of scales. Underhill (1963) noted that the size of the scales on the nape, cheek and breast was variable and abandoned his counts of the number of scales within these areas. I have shown that size of nape scales varies considerably (up to 20%) between darters which possess a

100% scaled nape and come from the same sample. Because this variation is found mainly within samples and between specimens of the same size, this variation may be due to developmental rate and/or genetic influences. Admittedly an extreme, one specimen exhibited a difference of 10 lateral line scales from the sample mean of 49; 20.4% variation. Differences in lateral line scale counts of 10-12% are not uncommon within samples. It is possible that, like lateral line scaling, there is enough variation in the genotype that some individuals will develop more or fewer scales on the nape or breast than will others. Therefore, similar differences in the number of nape or breast scales among specimens from the same sample are not unexpected.

Because lacustrine scaled specimens possess more scales than those in rivers, it is possible that reduced squamation offers an adaptive advantage which plays a role in streamlining the fish or allowing it to better hold its position in current. Page and Swofford (1984) have found a strong correlation between a reduction in squamation, such as that exhibited by species of *Ammocrypta*, and sand, riffle habitats. However, from personal collecting experience, there is no evidence for a correlation between the presence or absence of squamation and substratum in *Etheostoma nigrum*. Additionally, scaled darters were found to be more common in rivers than lakes in this study and thus argues against the selective advantage of streamlining in this case.

Because the nape is one of the last areas to be scaled (Hubbs 1926), it appears unlikely that increased squamation near the head could serve as any kind of effective defence against predacious insects or parasites and could be selected for on young

individuals. However, once formed, increased squamation on older individuals, especially those inhabiting waters with large numbers of parasites or predacious insects, could perhaps hold some slight advantage in terms of resistance to these attacks.

Increased squamation on some specimens may serve as some kind of useful camouflage by breaking up the outline of the body enough so as to be unobserved by some predators. A darter with a scaled nape generally has a much darker nape than one without this squamation due to the extra skin and pigment associated with the ctenal margin of the scales. There may be a potential selective advantage for the scaled phenotype over certain colours or types of substrate. However, based on the wide variety of habitats in which it occurs, this possibility also seems doubtful.

II/ Variation in Nape Squamation

All but eight of the 240 darters classified as scaled in this study possess some scales at the nape-parietal juncture. There were many instances when the nape was very obviously scaled across its entire posterior. Most darters which possessed only a few scales were scaled posteriorly. I recorded only 34 partially scaled darters which exhibited prominent anteriorly scaled napes (*e.g.*, Fig. **19** "B" and "H"). Most anterior nape scaling was recorded for Minnesota, Wisconsin, northwestern Ontario (Thunder Bay area) and a few Saskatchewan samples.

Underhill (1963) and Kott and Humphreys (1978) noted that the majority of their specimens exhibiting partially scaled napes were scaled anteriorly. Their anterior nape scaling seems to correspond with my nape scales at the nape-parietal

juncture but extends further posteriorly. Part of the reason for their recording so much anterior scaling could be due to not having a precise definition for the nape with posterolateral limitations. Therefore, posterolaterally placed scales may not have been consistently counted by Underhill (1963) and Kott and Humphreys (1978). However, because I did note some specimens with anterior nape scaling, there is a strong indication that anterior nape squamation is a discrete pattern in some geographic areas. There may be some underlying reason for this pattern in certain areas (*e.g.*, common origin, stream captures) although I have not been able to determine any.

If the scales at the nape-parietal juncture had been included as actual nape scales, as they seem to have been by others, then the definition of the nape on a scaled darter could have likely been increased to one which possessed 34 or more scales. Including nape-parietal juncture scales as part of the nape scale count and leaving the qualifier as 30 nape scales increases the number of scaled darters by 11 (4.5%) a small difference. All of these additional specimens would come from locations already identified as possessing scaled specimens. The mean number of scales on the nape of a scaled darter would then increase from 44.7 to 48.9. This suggests that all but eight scaled darters are scaled both anteriorly and posteriorly. This further evidence suggests that Underhill (1963) and Kott and Humphreys (1978) did not have sufficient posterolateral limitations for the nape when they reported large numbers of *E. nigrum* scaled only anteriorly.

Collette (1962) noted that scales first appear along the lateral line on the caudal peduncle, grow forward along the lateral line and finally appear dorsally and

ventrally during ontogenetic development of *E. fusiforme*. The last areas to be scaled include the anterior portions of the nape, belly, breast and the head area (cheek and opercle). If this type of scale development holds true for *E. nigrum*, it is difficult to explain the observed patterns of anterior nape squamation (Fig. 19: "B" or "H"). If the scales at the nape-parietal juncture are not controlled by the same genes as those causing nape squamation, then some of the observed patterns of nape squamation are more easily explained. Additionally, separate genes for nape scales and those at the nape-parietal juncture would not require explaining why some otherwise unscaled *E. nigrum* possess a few scales at the nape-parietal juncture. Furthermore, while it does appear that the belly squamation patterns observed in *E. zonale* might be explained by ontogenetic shifts in scale development with specimens becoming increasingly scaled from the posterior to anterior (Tsai and Raney 1974), the patterns of nape and breast squamation in *E. nigrum* are not similar and appear to be much more complex than could be explained by simple developmental rates.

Probably only three possibilities exist as to why some fish with few scales appear in samples which possess otherwise heavily scaled specimens. The primary reason appears to be lack of isolation within some areas. Populations which are not subject to immigration (many lakes or areas above barrier falls) by unscaled individuals have the most consistent patterns of squamation. Those areas which may be more easily accessed occasionally produce specimens which possess high, low and intermediate squamation values. Therefore, migration with subsequent crossbreeding by scaled and unscaled individuals results in intergrades.

A second hypothesis, that incomplete dominance exists for darters which carry the trait for squamation, is a valid possibility and I agree with Underhill (1963) in this conclusion. Although there could be several genes controlling the distribution of nape scales with some populations having lost the genes which code for scales in the anterior, middle or posterior positions, I have no evidence to support this hypothesis. Because specimens with complete and partial nape scaling are sometimes found within the same sample, it does not seem plausible that a single gene is lost in some populations. Specimens which are of the intergrade type in isolated areas with scaled specimens may thus represent offspring with recessive or incompletely dominant genes for squamation. Examination of the samples in which some darters possess a 100% scaled nape shows that occasional wide variation exists both within the percentage of nape area covered by scales and the number of scales themselves. These patterns are probably best explained through incomplete dominance at several loci (*i.e.*, heterozygotes possess an intermediate phenotype) or multiple gene inheritance (*i.e.*, several genes affect the same character in an additive fashion allowing for a wide range of phenotypes). Complex patterns of inheritance may explain why the scaled form of *E. nigrum* exhibits so many patterns of squamation (*e.g.*, anterior or posterior nape squamation) and also why no one has yet been able to determine exactly how many genes may control this squamation (*e.g.*, Underhill 1963). My own attempts (not reported) to determine the basis for squamation patterns using contingency tables resulted in failure.

There is a third possible reason for the occurrence of some unscaled specimens

within mainly scaled samples. Some of the sampled areas, mainly streams or rivers, may hold small populations of unscaled specimens which occasionally migrate up or downstream. There may also be assortative mating within populations which could promote an increase in the number of unscaled or heavily scaled specimens when these two forms come into contact. Cole (1967) assumed at least some measure of assortative mating within the heavily scaled form of *E. olmstedi* when it was in contact with the lightly scaled form, although he provided no evidence of this.

The consistency of squamation characters suggests, even without the benefit of the desired breeding experiments, a strong genetic component for squamation. There are probably minimal influences from the environment except for the actual distribution of the scales on some body areas (*i.e.*, crowding). The increased squamation likely lacks any adaptive significance and is not selected for. Based on the varied habitats in which the scaled form can be collected, it seems to be a heritable, plesiomorphic trait. The geographic distribution of these darters suggests an interpretable pattern explainable through examination of geologic events and probable historic distribution rather than a random distribution.

III/ Meristic Characters

Unlike squamation characters, many meristic characters of the scaled form exhibit a wide range of variation. Meristic differences between lacustrine and riverine scaled *E. nigrum* are attributed to adaptation to these particular environments. There is, however, both regional and watershed consistency in meristic characters among scaled specimens. When compared on a regional or local basis, character

variation exhibited by the scaled form is often in a different direction (increases or decreases) than that exhibited by the unscaled form. Scaled and unscaled *E. nigrum* can generally be separated by the means or modes of several variables, not only within discrete watersheds (*e.g.*, Fox River), but also within a large portion of their ranges. These considerations again suggest that the scaled form is distinct and may qualify for subspecific status.

CLUSTER ANALYSES

Cluster analyses utilizing variables identified with heavy weights on the first and second PC axis based on either the PC of sample means or the PC of the 2111 specimens themselves, both produce somewhat similar results. However, because the main purpose of the analyses was to cluster samples, the PC scores derived from sample means appear to be more indicative of the samples and eliminates some of the weight given to characters which exhibit extreme variation (*e.g.*, high or low lateral line scale counts). Principal component analysis using sample means also explained more of the variation between samples and identified more variables than did the PC on individual specimens which thus improves the information content of the clusters. Therefore, I discuss only those dendrograms created using characters identified by the sample means PCA.

Clustering using five squamation characters and the number of scales above the lateral line (LL1 and LL2) did not cluster all of the original samples identified as possessing scaled specimens. This is due to several samples possessing only a small percentage of specimens which met the definition of scaled relative to the size of the

sample (*e.g.*, Echo Lake, Saskatchewan; Yellow River, Wisconsin). The Illinois sample not identified as possessing scaled specimens, but clustering with scaled samples, is due to the large amount of squamation possessed by the majority of specimens within this sample. This dendrogram thus provides little in the way of new information.

The inclusion of an additional ten characters showed squamation overrides many of the additional characters. Most of the samples clustered in the first dendrogram remain intact. The clustering of samples with similar squamation and meristic characters from adjacent areas is impossible to ascribe to either common origin or the effects of environmental influences. However, the suspected movement of several fish species from Manitoba into northern Ontario by way of connections between Glacial Lake Agassiz and Glacial Lake Barlow-Ojibway, following the period when Glacial Lake Agassiz discharged into Lake Superior (Stewart and Lindsey 1983; Crossman and McAllister 1986), suggests that similarities among *E. nigrum* samples from some areas are likely due to common origin. Overall, however, the information content of this dendrogram is low.

Clustering using eight characters excluding squamation did not construct many easily interpretable groupings although broad eastern and western clusters are formed. Although some clustering of scaled samples occurs, these samples are sometimes geographically distant. This could suggest similar environmental effects within areas inhabited by the scaled form causing similar phenotypic development. It could also indicate a suite of characters which vary sufficiently from the unscaled form to

identify it from the unscaled form when squamation is removed.

The final dendrogram created with 12 variables (Fig. 37) is informative as many geographically based clusters are created. Clusters are based on geographic location of the samples, mainly east and west of the Mississippi River suggesting eastern and western forms. Northwestern and central-northwestern samples, those from the Upper Ohio Basin, and additional eastern samples, remain very distinct. Scaled samples are usually lost within the larger clusters and again suggests the possibility of phenotypic response to habitat.

If some samples did not possess extra squamation and thus stand out as being unique, I believe that in many cases I would have attributed the clustering of eastern and western scaled samples to a similar phenotypic response to environment. Therefore, many meristic characters of *E. nigrum* may be influenced by the environment. There may not be enough information provided by these eight characters to cluster samples in a more geographically informative manner or *E. nigrum* from many sampled areas may have undergone extensive local adaptation to their environments which may mask evidence of historic relationship. However, because eastern and western samples which clustered were often from different habitats, a combination of these two possibilities seems most probable.

When squamation is removed as a clustering variable, contiguous scaled and unscaled samples do not cluster closely, suggesting that additional differences remain. Recent environmental influences may have lessened historic genetic differences which developed during periods of isolation. Lyons (1990) stated that clustered samples of

mid-west populations of *Cottus cognatus* did not correspond with any hypothesis (clustering due to traits developed in isolation, clinal variation in traits, or habitat effects on characters) and suggested that the examined populations had not been separated long enough to develop distinctiveness. Chernoff (1982), however, found relatively quick changes (< 300 years) in the phenotype of *Menidia beryllina* after habitat changes. Many additional instances have been cited of phenotypic change or adaptation based on local environments (*e.g.*, Gill *et al.* 1992; Shields and Underhill 1993; Hindar and Jonsson 1993) although none of these has dealt with darters.

Clustering the 152 one-degree grid samples by drainages using 12 variables suggests distinctive eastern and western forms. Samples with scaled specimens typically fall within a northwestern cluster although the samples are not distinct due to the grid method which sometimes mixed scaled and unscaled samples. The upper and middle Ohio Basin samples continue to be easily recognizable. Clustering by one-degree grids strongly supports eastern and western forms and an Ohio group.

Eastern samples appearing within groups which cluster samples from the west, or vice versa, may in some cases represent descendants of populations which dispersed. Alternatively, they may have taken on the phenotypes of distant populations due to local environmental effects (Bailey and Gosline 1955). Only very unique traits with a strong genetic basis, such as increased squamation or a reduced number of branchiostegal rays, are likely to have remained distinctive within discrete areas. Mitochondrial DNA testing or electrophoresis along known or probable dispersal corridors might disclose additional connections between areas.

Clustering samples thus suggests that many samples from the middle and upper Ohio Basin immediately south of Lake Erie are distinct. Differences exist between samples from north and west of Lake Michigan and the Mississippi River and those to the east. Therefore, unscaled Ohio Basin, and an unscaled eastern and western form are identified. Scaled darters are distinct only when squamation characters are considered. This method provides little information in explaining their biogeographic history.

LATERAL ASYMMETRY

The lack of any correlation for sample specimens to exhibit similar amounts of asymmetry in a number of characters suggests concordance with the findings of Van Valen (1962) who noted a similar lack of correlations among characters he examined in three animal species. The lack of large amounts of asymmetry in a number of characters may suggest a lack of environmental or genetic influences, a high buffering capacity to compensate for these developmental "stresses" or, a combination thereof.

Etheostoma nigrum exhibits only limited amounts of asymmetry although some asymmetry is present in most samples (218 of 240). There is slightly more asymmetry than average in some northwestern sample areas such as Manitoba and Minnesota. However, this area is also the transitional area where samples are more likely to come from lakes than rivers and these northwestern samples do not always possess high asymmetry values for all variables. Perhaps selection for, or adaptation to, lentic or lotic habitats through a reduction in the number of POM or infraorbital pores as suggested by Page and Swofford (1984) may be the cause of elevated levels

of asymmetry for these characters in those areas.

The hypothesis that gene flow between scaled and unscaled darters is the cause of increased asymmetry is rejected because scaled, unscaled and intergrading specimens possess a similar percentage of asymmetric individuals and no area exhibits a higher than average amount of asymmetry. The examination of fluctuating asymmetry does not provide any useful information for this study.

BIOCHEMISTRY

Electrophoresis has become an important tool in describing species and to construct biogeographical hypotheses (McKeown *et al.* 1984; Wood and Mayden 1992; Howard and Morgan 1993). However, only regional studies (*e.g.*, Falls 1982) or incidental inclusion of specimens of *E. nigrum* in larger works (*e.g.*, Page and Whitt 1973a,b; Martin and Richmond 1973; Dimmick and Page 1992) have examined it biochemically.

The few previously published accounts of electrophoresis utilizing *E. nigrum* limit direct comparisons. However, more variation in *LDH** was seen in this study than has previously been reported (Page and Whitt 1973a,b) for *E. nigrum*. More variation in *GPI** was seen in this study than in other darter studies (Buth *et al.* 1980). Phosphoglucomutase (*PGM**) was less variable in this study than in other darter studies which examined (*Microperca*) (Buth *et al.* 1980), (*Catonotus*) (Wolfe *et al.* 1979) and (*Etheostoma*) (McKeown *et al.* 1984). The one available study dealing largely with *E. nigrum* (Falls 1982) found much less variation in *LDH**, *GPI**, *PGM** and *EST* than was present in this study.

Previous examinations of *LDH** in darters, including *E. nigrum* (Page and Whitt 1973a,b), have used head extracts and results suggested the presence of two loci. Using liver tissue, two loci are plainly visible. However, these are not the same as those identified using white muscle tissue as patterns seen on liver gels were not consistently replicated on white muscle gels. The three banded pattern typically seen in this study is identical to that shown by Page and Whitt (1973a) and Falls (1982). Many studies of other percids including darters (*e.g.*, Buth *et al.* 1980; Falls 1982; Billington *et al.* 1990) suggest that *LDH** is controlled by only two loci within white muscle tissue, similar to the results of this study. The cathodal tetrameric bands on white muscle gels on some specimens seems to correspond to the *LDH*-C_4* bands usually seen in eye and other nervous tissues (Markert and Faulhaber 1965; Coppes 1992). However, because the cathodal movement was observed on specimens from three locations and care was taken to keep all tissues free of contamination by other tissues, this movement must represent a unique allele at this locus.

An alternative to the interpretation presented above for gels stained for the activity of *AAT** is that three separate alleles may exist at the *sAAT-3,4** locus. Although these would create almost similar dimeric and tetrameric patterns, some patterns would be different from those observed. However, because the two samples not meeting Hardy-Weinberg expectations were those which exhibited variation in *AAT-3,4**, there is support for the possibility that my initial interpretation is incorrect.

Excluding *AAT** (due to incomplete examination) the overall divergence exhibited by *E. nigrum* (F_{ST} = 0.433) is generally higher than that reported for other

fishes (*e.g.*, two species of *Notropis* from North Carolina (F_{ST} = 0.413) (Wood and Mayden 1992); *Cottus bairdi* in the central Appalachians ((F_{ST} = 0.602) (Howard and Morgan 1993); western North American samples of *Gasterosteus aculeatus* (F_{ST} = 0.163) (Haglund *et al.* 1992); *Stizostedion vitreum* from the Great Lakes, Ontario and Manitoba (F_{ST} = 0.108) (Ward *et al.* 1989). As in other studies, the majority of variation occurs between rather than within samples. This high level of genetic diversity, in itself, cannot be taken as evidence of a long period of separation between scaled and unscaled phenotypes.

There are no distinct biochemical differences between the scaled and unscaled forms although the *PGDH*150* allele and the *GPI-A*91* and *GPI-A*82* alleles are most common only in the scaled form from some areas. Lavin and McPhail (1993) noted a similar lack of genetic distinction yet great morphological differentiation in two parapatric forms of lake and stream dwelling *Gasterosteus*. Thus, phenotype may differ without any observable difference noted using electrophoresis. Wisconsin and Ontario samples are not biochemically distinct. There are no clines in allelic frequencies which might result from selection within the environment as has been noted by Merritt (1972) and Koehn (1970) on studies of other fish species.

River samples were typically more heterozygous than lake samples. The high heterozygosity in McVicars Creek and the Neebing-McIntyre River could be due to gene flow from darters inhabiting deeper portions of Lake Superior or from those migrating into these areas from nearby areas. However, the lack of a large amount of heterozygosity in the Pigeon River watershed samples (Roundtable and Sandstone

lakes) and those from the Nipigon River argue against genetic input from either of these areas. Additionally, *Etheostoma nigrum* is unknown in the tributary streams or littoral zone of Lake Superior between the Pigeon River to the south and the Sibley Peninsula to the east; a distance of over 40 km in either direction (Stephenson 1991). Therefore, gene flow originating from individuals with different genotypes inhabiting deep waters of Lake Superior areas, immigration from headwater areas or maintenance of heterozygosity within these rivers are the only possible causes for the heterozygosity seen in these samples. *Etheostoma nigrum* is essentially a littoral fish and only a few specimens have been collected in deepwater trawls conducted by the Ontario Ministry of Natural Resources in the western Lake Superior Basin (pers. observ.). Because individuals with unique genotypes entering McVicars Creek and the Neebing-McIntyre River from Lake Superior would also be expected to have done so in the Nipigon River and because samples from both the Neebing-McIntyre and Nipigon rivers were collected within one km of their mouths, below any barriers to immigration from Lake Superior, genetic variation within McVicars Creek and the Neebing-McIntyre River is not a result of immigration by individuals from Lake Superior. Immigration from headwater lakes is not possible within the Neebing-McIntyre River or McVicars Creek as *E. nigrum* is unknown from headwater areas (Stephenson 1991). Therefore, heterozygosity appears to be maintained with these populations themselves.

It is difficult to explain the allozyme similarity between the Rock River, Wisconsin, sample and that of McVicars Creek, Ontario. Habitats are not similar

between the two locations and some of the other sampled locations are much more similar to the Rock River than is McVicars Creek. Because the specimens from these samples do not possess similar amounts of squamation, it is also difficult to view these similarities as being due to phenotype. Additionally, because several samples possessed heavily scaled specimens and yet did not exhibit a large amount of heterozygosity, it appears that the presence of some alleles at certain loci are not well correlated with increased squamation. That two areas so far removed appear to be most similar genetically may be due to chance.

Specific allozyme genotypes have been shown to vary in their sensitivity to the toxic effects of contaminants in some fish species. Fish populations under environmental stress have been reported to exhibit great variation in *GPI** (Heagler *et al.* 1993; Gillespie and Guttman 1993). Populations of *Astyanax mexicanus* (Avise and Selander 1972) and *Rhinichthys cataractae* (Merritt *et al.* 1978) exhibited a high degree of *GPI** variation. The Neebing-McIntyre River and McVicars Creek, Ontario, samples as well as the Rock River, Wisconsin, sample exhibit great *GPI** variation. Falls (1982) also noted large variation for *GPI** in his samples of *Etheostoma nigrum* from the James River, Virginia. Riverine specimens heterozygous at the *GPI** loci may have a selective advantage over homozygotes. Heterozygotes may possess some advantage under riverine conditions although the question of neutrality or non-neutrality of screened alleles under electrophoresis is debatable (Selander and Johnson 1973; Gall 1987). It may be but chance that these three samples exhibit increased squamation.

Avise and Selander (1972) note that under a niche width-variation hypothesis, those species having more or broader niches should be more genetically polymorphic since different environmental factors will select for different phenotypes. One would suspect that the genotype of generalist species like *E. nigrum* should be highly variable. Todd and Hatcher (1993) have noted that *Perca flavescens*, a wide ranging percid found in a variety of habitats, exhibits low genetic variability over most of the area believed colonized from the Mississippi refugium. Todd (1981) also reports nearly monomorphic populations of several species of ciscoes (*Coregonus*) from Lake Superior. Lake trout (*Salvelinus namaycush*) have been reported to exhibit low levels of heterozygosity within Lake Superior (Dehring *et al.* 1981). Northern pike (*Esox lucius*) exhibit similar low levels of heterozygosity within that area (Seeb *et al.* 1987). These findings suggest that a number of species believed to have recolonized from a Mississippi refugium lack genetic variability (Todd and Hatcher 1993). Because many populations of *Etheostoma nigrum* exhibit a high degree of genetic diversity, this diversity may be explained by one or more of the following possibilities: (**1**) the observed variability exhibited by *E. nigrum* in some areas is the result of interbreeding of two or more forms which were separated for a sufficient period, probably during the late Pleistocene, which allowed for the development of alternate alleles in some populations, (**2**) because *E. nigrum* is a widespread, generalist species, it follows the general pattern suggested by the niche width-variation hypothesis, or (**3**) *E. nigrum* has lost the majority of its genetic variation secondarily as it entered habitats which have remained relatively stable and not required genetic variability.

It is impossible to accurately determine if possibility (**1**) is valid although the study of lateral asymmetry provided no evidence of this. Possibility (**2**) also appears to be a valid, but because other species (such as *Esox lucius* and *Perca flavescens*) are ecologically diverse and yet essentially genetically monomorphic, its probability may be low. Possibility (**3**) seems likely at least in some locations where small, founding populations resulting from long distance dispersal and/or colonization of stable habitats have resulted in low genetic diversity. It appears most likely, based on meristic and morphometric differences, that populations of *Etheostoma nigrum* were isolated during portions of the Pleistocene and that alternate alleles appeared at that time. Populations which have invaded new habitats often exhibit reduced variability (Gall 1987). Genetic drift in small populations of *E. nigrum* may have resulted in random fixation of a particular allele when neither was particularly advantageous in a given situation. A founder effect could also be responsible in some cases.

The dendrogram showing the Rock River to be distinct (Fig. **61**) is due largely to fixation of the *PGDH*150* and *ME-2*125* alleles. Similarly, the distinctiveness of the McVicars Creek and Neebing-McIntyre River samples as well as the Flambeau River sample is due to the presence of the *PGDH*150* and the *GPI-A** alternate alleles. The work of Gorman and Renzi (1979) suggest that even with small samples and samples of variable size, the dendrograms created accurately depicts the genetic distances of the populations. The presence of these two alleles alone may suggest evidence of a long, unique history for the ancestors of the Rock River sample or may be the effect of chance. Studies of *E. radiosum* in headwater areas show a great deal

of inter-basin variation within a small area suggesting only limited movement between areas (Echelle *et al.* 1975). However, some evidence interpreted as instream movement has been seen in this study. It seems likely that most, if not all, individuals within this section of the Rock River possess these alleles, otherwise other alleles would have been encountered. Tables presented by Simpson *et al.* (1960) suggest that with the given Rock River sample size (N = 15), any character would have to be present in less than 20% of the population for it not to be detected in this study. However, because some alleles were found at < 5.0% frequency, it does not discount the possibility that alternate alleles may be present at this locus in the Rock River. The lack of the *PGDH*150* allele in most other samples and its exclusion from all unscaled samples does, however, suggest a strong possibility of a unique history for the Rock River specimens. Additionally, Greene's (1935) collection of only three specimens of what he considered "unscaled" *E. nigrum* from the entire Rock River system, suggests that this, or both alleles, may be representative of scaled specimens.

Canonical Correlation Analysis

Canonical correlation analysis of meristic and environmental variables provides some evidence that the scaled form has not adapted to its habitat through increased squamation (*i.e.*, not found in overly warm or cold areas) and lives in a variety of habitats (some scaled and unscaled samples almost completely overlap in Fig. **62**) suggesting there is no preferred habitat. Likewise, there is some support for the hypothesis that the scaled form is present in some areas (*e.g.*, headwater areas) because these areas have generally remained free of immigration by the unscaled

form. Increased squamation in low stream order areas suggests either a long history within the area or exclusion of the unscaled form further downstream (Rock River, Wisconsin) or general isolation from the unscaled form (McVicars Creek, Ontario).

While there are high correlations between increased squamation and the presence of the *GPI-A*91*, *GPI-A*82* or the *PGDH*150* alleles and increased POM pore counts, correlation does not mean causation. Because some scaled specimens lack these alleles, there is no evidence that either of these alleles are responsible for the traits they are correlated with and they cannot be used as "markers" for scaled specimens. Because increased POM pore counts are typically associated with riverine samples (see Table **23**) and most river samples are heterozygous, these correlations seem more indicative of habitat than squamation.

Correlations between increasing latitude and decreasing squamation are due to the inclusion of genetically homozygous, unscaled samples more northerly than any scaled sample included in the analyses. Correlations between increasing latitude and increasing ray counts are predicted by a known increase in serial elements with decreasing temperatures (Barlow 1961). Therefore, while providing interesting trends in several areas, canonical correlation analysis provides no distinct evidence of differences between scaled and unscaled *E. nigrum*.

PATTERNS OF VARIATION

The variation exhibited by many meristic characters of *E. nigrum* does not produce many patterns which can be used to explain colonization movements or areas of possible origin. Most characters are similar over a wide area and there are few

which are variable within a small area. Part of this may be due to geologically recent stream captures and anthropogenic effects such as canal construction which has permitted some movement by individuals with different genotypes. For example, areas of historic flooding linking the Fox and Wisconsin rivers of Wisconsin (Lake Michigan and Mississippi River drainages, respectively) and the Maumee and Wabash rivers (Lake Erie and Ohio River drainages, respectively) were eventually turned into canals to facilitate shipping (Hubbs and Lagler 1983). Underhill (1957) cites a case of fish migration between the Mississippi and the Red River (Hudson Bay drainage) headwaters. Therefore, movement between drainages may have led to the loss of some distinct, yet highly plastic, characters or led to the establishment of traits in areas in which they were formerly absent.

Squamation and additional plesiomorphic characters are important in explaining colonization and/or origin within the Upper Great Lakes and the north-central United States region. Branchiostegal rays, and to a lesser extent, both dorsal and pectoral rays, are important characters within the upper and middle Ohio Basin. This presents the possibility of four rather distinct forms of *E. nigrum*: a widespread unscaled morph subdivided into (**1**) eastern and (**2**) western forms; (**3**) an unscaled morph with distinctive meristic characters which inhabits the upper and middle Ohio Basin, and (**4**) the scaled morph. The Ohio Basin and scaled morphs are discussed further.

I/ Upper and Middle Ohio Basin *Etheostoma nigrum*

Many samples of *E. nigrum* within the middle and upper Ohio Basin and

nearby areas possess several meristic characters which make them distinct and easily identifiable. These meristic variables include a reduced number of branchiostegal rays (modally five), and a reduced number of dorsal rays (modally 11). Pectoral rays among sampled populations often number 11 or less. Reductions in all of these characters are considered derived percid traits (Wiley 1992).

The occurrence of some *E. nigrum* with similar traits in Lake Erie tributaries suggests both their relatively recent movement into the area and/or a lack of genetic exchange between these and other nearby populations of *E. nigrum* with different traits. Underhill (1986) estimated it has been approximately 10,000 years since the severing of natural connections between Lake Erie and the Upper Ohio River. Retention of these traits in the Lake Erie Basin may be due to a lack of immigration by individuals with the higher counts for these variables. Lack of movement, both in and out of the currently inhabited areas, may be due to inhospitable habitats between preferred habitats or possibly, predation by, and competition with other fish species which have invaded nearby areas since deglaciation. Ohio waters presently harbour at least 22 darter species (including species of *Percina* and *Ammocrypta*) (Page 1983). Competition and microhabitat partitioning between many darter species is known to be high (Page and Schemske 1978; Paine 1990; Chipps *et al.* 1994) which might limit movement by large numbers of individuals of one species. Recent crossings of drainage divides through man-made canals or during natural floods could explain the distribution of specimens with five branchiostegal rays in some areas. However, the locations of these specimens are generally far removed from canals and known

crossover areas, however (Trautman 1957; Hubbs and Lagler 1983).

Due to the apparent consistency of the number of dorsal and branchiostegal rays among samples of *E. nigrum* from the upper and some middle Ohio River tributaries, they may qualify for subspecific status. There are no environmental variables which are so widespread and constant within the area that these traits are a response to the environment. These darters are likely descendants of populations which were long separated from the other, large populations of *E. nigrum*. Thus there is an implied genetic and evolutionary basis to these differences which correlate well with geographic subdivision and, possibly, dispersal history.

II/ Scaled *Etheostoma nigrum*

Many samples from Wisconsin, Minnesota and nearby areas exhibit increased squamation which readily identifies them as distinct from the unscaled form. Some additional characters, such as the complete lateral line, also help to make them distinctive. The scaled form likely maintains these traits and its identity through lack of contact and interbreeding with the unscaled form.

Lagler and Bailey (1947) demonstrated through their cross-breeding experiments of scaled and unscaled *E. nigrum*, that squamation has a genetic basis. The possibility that extant populations of the scaled form are those that are free from immigration by the unscaled form, thus maintaining their genetic identity, seems a reasonable hypothesis. For example, populations of the scaled form in lakes and streams at the Ontario-Minnesota border (Pigeon River watershed) are isolated due to the presence of a series of impassable barrier falls near Lake Superior. Scaled

populations, such as those on the Bass Islands of Lake Erie, may similarly retain this trait due to isolation by distance.

Hubbs and Greene (1935) stated that the scaled form was more common in lakes than streams or rivers. Greene (1935) stated that the scaled form was most common in lakes or headwater areas, often above barrier falls. Lagler and Bailey (1947) suggested that the scaled form of *E. nigrum* was most common in slow moving, base-level waters with an abundance of aquatic growth and bottoms often composed of mud. However, many of the scaled specimens examined in this study have been in samples collected from various sections of watersheds. From personal collecting experience at waterbodies from which the scaled form has been collected in Ontario, Minnesota and Wisconsin, I have noted that the scaled form is present in reaches of small streams, in medium and large rivers, and lakes inhabited by cool, cold and warm water fish species. I have collected the scaled form in weeded areas and areas free of vegetation, over sand and gravel-rubble substrates, in clear and turbid water, in swiftly flowing small streams, and in the calm water areas of rivers, small streams and lakes. Assigning the scaled form a preferred habitat is as difficult as assigning one to the unscaled form. This study does concur with most earlier findings suggesting that the most heavily scaled specimens are more likely to be found in lakes and large rivers than smaller streams. This does not imply, however, that any preferred habitat exists.

Chi-square tests reveal a highly significant difference between the frequency of occurrence of the scaled form in lakes and rivers or streams. As well, several

significant differences in meristic characters occur between habitats. It may be, as noted by Underhill (1963), that in some areas the scaled form is more common in one environment than the other. While the most heavily scaled specimens are from lakes, the occurrence of scaled *E. nigrum* predominantly in rivers may be an artifact of sampler preference, accessability to one waterbody type over another or due to the number of riverine collections I examined. Fago (1992) notes that in Wisconsin sampling from 1974-86, *E. nigrum* has occurred in only 15% of the surveyed lakes. Fago (1992) also notes, however, that many more rivers and streams have been sampled than lakes.

The scaled form of *E. nigrum* thus appears to have a unique genetic history, inhabits a relatively discrete range although surrounded by the unscaled form, and is identifiable through increased squamation. It exhibits meristic differences from the unscaled form in areas in which both the scaled and unscaled form inhabit the same watershed. Therefore, similar to upper and middle Ohio Basin *E. nigrum*, the scaled form may also qualify as a subspecies. The possibility of subspecific status for the scaled form is considered in greater detail below.

ORIGIN OF THE SCALED JOHNNY DARTER

Lagler and Bailey's (1947) breeding experiments and the tendency for increased squamation to exhibit discrete patterns consistent over time in distant geographic locations, supports the argument that squamation is genetically controlled. A completely scaled body as well as a complete lateral line are frequently associated characters on the scaled morph and both are thought to be plesiomorphic (Bailey and

Etnier 1988; Simons 1992). This, in combination with the geographic distribution of the scaled morph as previously outlined, suggests that it is a plesiomorphic relict, surrounded by more apomorphic forms, which exhibit reduced squamation. The apomorphic forms are the upper and middle Ohio Basin form, a widespread, eastern unscaled morph, and a northern and western unscaled form occupying the Hudson Bay, Upper Mississippi and Missouri drainages.

Pre-Wisconsinan Distribution

The presence of scaled *E. nigrum* mainly near, but south of the Great Lakes, and generally only slightly north of the maximum known extent of Wisconsinan ice strongly suggests that it survived glaciation near those areas, especially in the mid-western United States. Forced south by Wisconsinan ice, but already being cold tolerant due to its presence in the north, the scaled form likely survived in the northern-most inhabitable areas south of the maximum ice advance. Greene (1935) suggested the Driftless Area of Wisconsin was perhaps one area in which the scaled forms survived during the Wisconsinan. Greene (1935) based his hypothesis primarily on the apparent centre of distribution of the scaled form within that area. This study has not found the scaled form to be more abundant within any other area which supports this hypothesis. The biochemical uniqueness of specimens from the Rock River seen in this study also suggests that northern portions of Illinois may have provided suitable habitat. The scaled form may have also been present in a pre-Wisconsinan Laurentian system which is believed to have included all or most of the present Great Lakes (Radforth 1944; Robison 1986). However, *E. nigrum* from the

upper and middle Ohio Basin typically exhibit reduced belly squamation and few samples possessing specimens with increased squamation exist anywhere in the Ohio River system. If scaled *E. nigrum* occurred in the Lower Laurentian system prior to the Wisconsinan, it is likely that the trait for increased squamation would have been passed on to their descendants within that area. Because the scaled form is completely absent from the Ohio Basin (although some specimens with some squamation are found in that area), and its present distribution north of that area (*e.g.*, Lake Erie) can be explained using known corridors of dispersal from the Wisconsin area (Bailey and Smith 1981; Underhill 1986), it is probable that scaled *E. nigrum* did not occur within the Laurentian system prior to the Wisconsinan. The scaled form is thus assumed to have arisen during or survived the period prior to the Wisconsinan glaciation either within a western Hudson Bay drainage and/or in the extreme northern portions of an ancestral Mississippi River.

Scaled specimens from the Spring River, Missouri (Neosho-Arkansas system), suggest that the scaled form was forced south by the Wisconsinan glaciation as were other species (Cross *et al.* 1986) and has since become extirpated in all but the most favourable locations or areas that are free from immigration by the unscaled form or other competitive species. Disjunct populations of many typical northern species are present in suitable southern areas (*e.g.*, *Hybognathus hankinsoni*, *Notropis heterolepis*, *Etheostoma microperca* (Lee *et al.* 1980)). This demonstrates that not all populations were as adept at following the retreating ice sheets as they were in staying ahead of advancing ones. However, the Missouri specimens raise the possibility that

either the scaled form was widespread prior to the Wisconsinan or that it survived in areas other than near or within the Driftless Area.

Cross *et al.* (1986) suggest that, based on geological evidence and faunal similarities, there have been connections between the Missouri (Osage River system) and Upper Arkansas rivers. Scaled *E. nigrum* in the Missouri River may have entered the Upper Arkansas via such connections and been unable to exit in the same way. If scaled *E. nigrum* repopulated more northern areas from Missouri, there should probably be some agreement between their distribution and those of other species suspected of surviving in or near this area. Miller (1968) suggested that *E. blennioides* likely populated the Ohio Basin from a refugium in Missouri, perhaps dating to Illinoisan time. However, *E. blennioides* is absent from the Driftless Area. Burr and Smith (1976) suggested that *Campostoma oligolepis* did not survive the Wisconsinan in the Driftless Area as postulated by Greene (1935) because there are few records for the species in that area. The paucity of *C. oligolepis* populations in the Driftless Area suggested a later arrival to the area and Burr and Smith (1976) further suggested that the Ozark Highlands was the most probable and only refugium for *C. oligolepis* during the Wisconsinan. A larger, more mobile species such as *C. oligolepis* would be expected to have a distribution somewhat similar to *Etheostoma nigrum*, at least within Wisconsin, if both species repopulated northern regions from contiguous areas.

Lower Ohio Basin *E. nigrum* are not only almost completely unscaled but also fail to cluster closely with scaled or Upper Mississippi samples during cluster analyses

(Fig. 37) and instead cluster with more eastern samples. Many Ohio Basin *E. nigrum* possess five branchiostegal rays except in those areas which have likely been influenced recently by canals, where most specimens possess six. Thus, if scaled *E. nigrum* entered the Ohio Basin from an Upper Arkansas or Lower Missouri river refugium, the complete swamping of scaled individuals, the failure of many populations to develop six branchiostegal rays and the lack of concordance in the distribution of scaled *E. nigrum* with other species known or suspected of surviving near or in these areas, would all have to be explained.

Due to the above considerations, the present distribution of scaled *E. nigrum* is interpreted as being due to survival mainly within or very near the Driftless Area during the Wisconsinan glaciation. The Spring River, Missouri, scaled populations are interpreted as true disjunct populations forced south during the Wisconsinan which did not contribute to the repopulation of more northern areas.

Post-Wisconsinan Dispersal

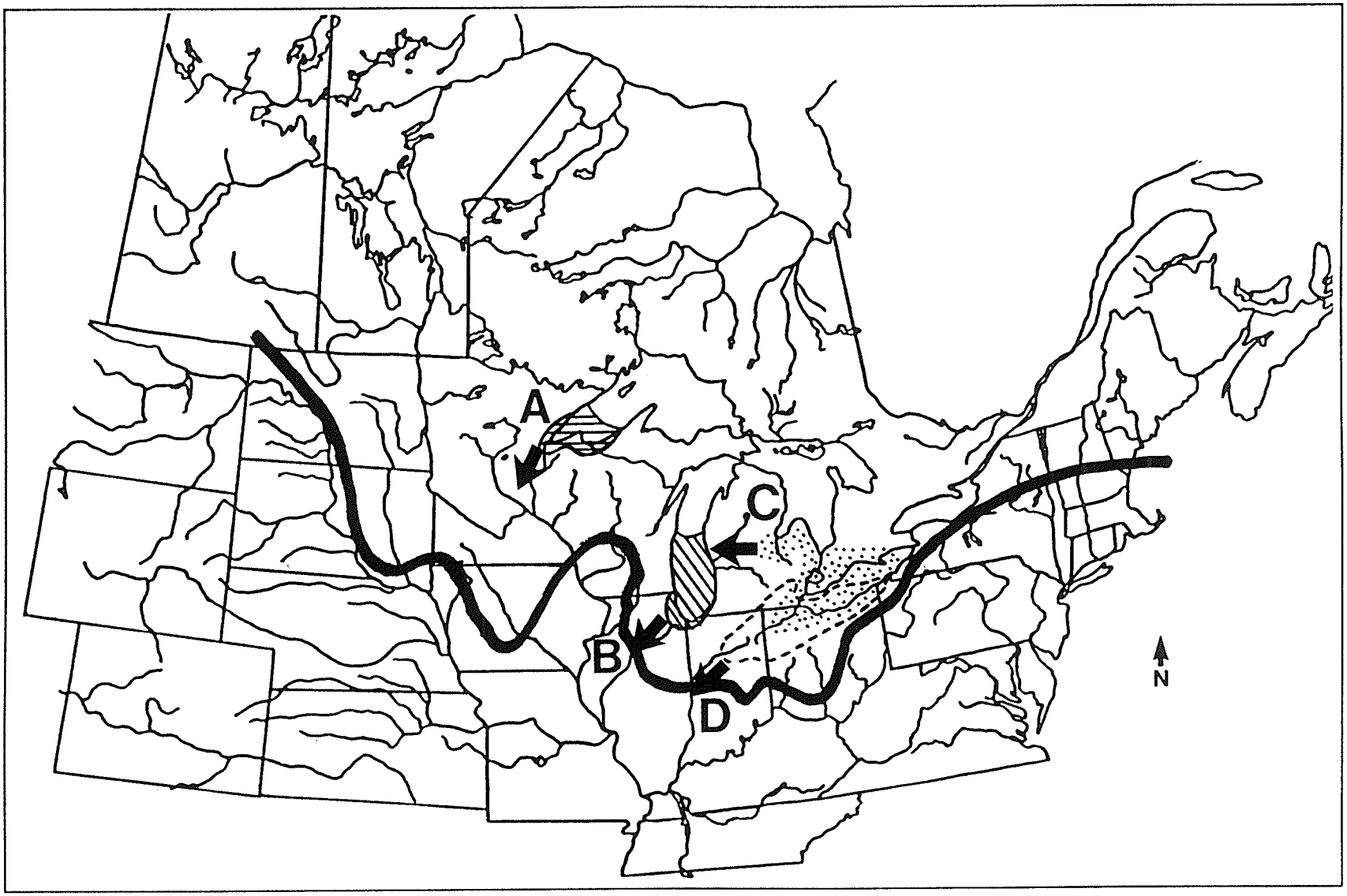
Survival of the scaled form in either the Driftless Area or the northern-most inhabitable areas of the mid-west United States would have permitted movement into all of its present areas of habitation within Wisconsin and allowed it to follow receding ice fronts into Minnesota and the Lake Superior Basin. Greene (1935) could not explain the apparent absence of the scaled form from the Superior Basin if his hypothesis of survival in the Upper Mississippi Basin was correct. Lack of surveys in many tributaries of western Lake Superior until recently (*e.g.*, Stephenson 1991) explain why the scaled form was unknown from the Lake Superior Basin at that time.

Greene (1935) further stated that in considering the present amount of intergradation between the scaled and unscaled forms, it was difficult to imagine how both forms could be both independent and likely contiguous, and yet the scaled form remained relatively distinct throughout the Wisconsinan. Survival of the scaled form in close contact with the unscaled form did not seem possible. However, the continued presence of both forms in many adjacent areas or within portions of the same watershed suggests that, in some instances, this did occur.

Habitation of the scaled form in the area south of, or within, Wisconsin would have permitted access to Lake Chicago (pre-Lake Michigan). Movement into the early Lake Huron and Lake Erie basins was possible utilizing the numerous outlets which ran across the middle of the Michigan Lower Peninsula between 12,500-12,000 years BP (Bailey and Smith 1981; Underhill 1986) (Fig. **63**). The existence of highly scaled and scaled specimens in southeast Lake Michigan tributaries support the hypothesis of entry into Lake Chicago. Use of Lower Michigan outlets from Lake Saginaw (early Lake Huron) or Lake Warren (early Lake Erie) would place the scaled form in an area from which it could access other areas in which it now occurs, including Lake Ontario. Although the conditions in these areas would have been harsh, the scaled form perhaps existed within similar or harsher conditions in or near the Driftless Area.

Trautman (1957) suggested that the scaled form may have utilized the Maumee outlet (draining into an early Wabash River) to access an early Lake Erie (Lake Maumee) approximately 13,000 years BP. Lake Maumee existed long enough to

Figure 63: Approximate location of furthest Wisconsinan advance (wide line), limits of glacial lakes and important outlets discussed in text. **A**=St.Croix outlet, **B**=Lake Chicago outlet, **C**= Grand River outlet and **D**=Lake Maumee (Ft. Wayne) outlet. Limit of Lake Duluth in horizontal hatching, limit of Lake Chicago in diagonal hatching, limit of Lake Maumee shown by dashed line and limit of Lake Warren shown within stippled area.


A
B
C
D
N

form a well-defined beach ridge (Trautman 1957). Lake Warren, formed perhaps 2000 years after Lake Maumee (Underhill 1986), also left a prominent beach ridge lower than that of Lake Maumee (Trautman 1957) (Fig. **63**).

If the scaled form accessed the Lake Erie area via a Maumee outlet, it could be expected to be present in areas above the Warren beach ridge due to its earlier arrival. Trautman (1957) stated that only unscaled or intergrade *E. nigrum* were present above the Warren ridge. Thus if the scaled form accessed the area when water levels were higher (Lake Maumee), it is difficult to explain why they did not become the dominant form in some headwater areas and why increased squamation is observed only infrequently on Wabash River (Lake Maumee outlet) specimens. Even with man-made canals linking the Wabash River to Lake Erie, it is still difficult to explain the lack of heavily scaled specimens in the area. However, specimens with increased squamation are relatively common from samples across Lower Michigan (*e.g.*, Thornapple River trib.: UMMZ 80676, Huron River: UMMZ 138004). If the scaled form accessed the Lake Erie Basin during the lower Lake Warren stage via connections across the Michigan Lower Peninsula (Underhill 1986), then their distribution mainly in lower gradient waters in Ohio (limit of Lake Warren beach ridge), the almost complete lack of specimens with increased squamation from the Wabash River and their presence across Lower Michigan are more easily explained. Thus the scaled form is believed to have accessed the Erie Basin during the Lake Warren stage approximately 12,000 years BP via channels across Lower Michigan.

Stephenson and Momot (1994) suggest the scaled form may have accessed the

Minnesota-Ontario border area shortly after the retreat of the Marquette lobe from the Superior Basin. Entry into the western Superior Basin during the high water Lake Duluth stages near the end of the Wisconsinan (9900 years BP) would have allowed the scaled form access to areas that developed barrier falls as water levels dropped to the lower Minong levels. From the beginning of the Marquette retreat to its final withdrawal from the Superior Basin (9700 years BP) leading to the Minong water levels would have provided almost 200 years during which the scaled form could have entered the basin. Entry into the basin likely occurred through the Portage or Brule outlets from the St. Croix River, possibly only during periods of low flow.

Movement of the scaled form into Minnesota could have occurred via the St. Croix River into glacial lakes Aitken or Upham (Underhill 1989) or directly up the Minnesota River into early Glacial Lake Agassiz. Movement through the Sheyenne spillways into Glacial Lake Souris (Owen *et al.* 1981) could have led to the establishment of scaled traits in southern Saskatchewan. Arrival to the Agassiz Basin during this time (11,500 years BP) (Underhill 1989) would have permitted entry of the scaled form into all of the presently inhabited northern Minnesota locations. Movement into Spirit Lake and other northern headwater areas in Iowa may have occurred via stream capture from Minnesota River tributaries or the area may have been part of a refugium during the Wisconsinan (Bailey and Allum 1962).

Possibility of Further Scaled Populations

The failure to note any increased squamation on *E. nigrum* north of the Thunder Bay, Ontario, area (Stephenson 1991; this study) strongly suggests that the

scaled morph never dispersed that far to the northeast following deglaciation. The trend north of this area is for a further reduction in squamation exhibited by decreasing belly squamation and a decreasing number of scales above the lateral line. The presence of more scaled populations within the Superior Basin is unlikely, even in the poorly sampled tributaries of the Canadian north shore. Waters (1977) notes that most north shore Minnesota tributaries to Lake Superior are short and steep. Many Ontario tributaries of Lake Superior are similar. Because ice from the Marquette re-advance almost completely filled the Superior Basin and due to the short length of most western Lake Superior tributaries, scaled darters which may have colonized shorter northern or western tributaries prior to the re-advance would have been extirpated by the advancing Marquette ice as it covered these watersheds. Only those western tributaries which extend a great distance inland would have provided the refugia needed to survive this re-advance. With the exception of the Pigeon at the international border, and the St. Louis near Duluth, Minnesota, few rivers extend far enough inland from western Superior to have provided such a refuge. My earlier work (Stephenson 1991), which covered all Lake Superior streams and rivers from the international border to east of the town of Nipigon and examined Minnesota collections not examined by Underhill (1963), revealed only one Minnesota location where specimens exhibit increased squamation (Sucker River). Samples with increased squamation were also noted from the Neebing-McIntyre River and McVicars Creek, Ontario. However, for the reasons outlined above, additional scaled populations will not likely be detected in future surveys of Lake Superior tributaries.

Excluding the Lake Superior Basin, it seems possible that small populations of scaled *E. nigrum* may yet be undetected. The first *E. nigrum* from the Pigeon River watershed (international border, Lake Superior drainage) were not collected until 1989 even though the area had been previously sampled (Stephenson 1991). Page (1983) has noted that darters exhibit clumped distribution and strong microhabitat selection has been noted for many darter species (Chipps *et al.* 1994). *Etheostoma nigrum* apparently does not utilize all available habitats even in the absence of other darter species (Leidy 1992) and populations can be very localized. Thus there are portions of watersheds which have not yet been adequately sampled which may reveal the presence of scaled *E. nigrum*.

The majority of scaled populations will likely occur within the area encompassing the known range of the scaled form or along known dispersal routes between areas currently inhabited by the scaled form. This hypothesis is based on results from this study and previous work (Stephenson 1991) which suggests: (**1**) That the scaled form has left "tracks" in the form of some heavily scaled specimens or populations within areas in which it formerly occurred or dispersed through (*e.g.*, the Minnesota shore of Lake Superior). (**2**) Some populations harbouring the scaled form have only recently been documented suggesting that there may be more. Possible areas for their presence include smaller streams across the Lower Peninsula of Michigan near the site of the Grand Valley and other outlets, southwestern Lake Michigan tributaries (due to their proximity to the Lake Chicago outlet), tributary streams of the Big Sioux River, Iowa, and, a wide area between northwestern

Minnesota and the Qu'Appelle Valley, Saskatchewan, but likely, due to its numerous tributaries, centred on the upper Souris River. (3) Although this study has not greatly increased the range of the scaled form since the initial description by Hubbs and Greene (1935), there are enough new locations to suggest that some areas may be located through examination of museum and university collections or increased field sampling. The presence of undetected scaled populations may explain why specimens with high squamation are found in peripheral areas like the Qu'Appelle Valley.

Staying near the margin of wasting ice led some fish into upland areas which became isolated as the ice retreated over a high point of land. This is the most parsimonious explanation for the occurrence of scattered populations of scaled darters in upland pockets. If they remained in these areas, their genetic integrity remained intact. Moving downstream, however, results in intergradation and likely, reduction or complete loss of squamation either due to sheer numbers or possible competition with the unscaled form. Aspinwall *et al.* (1993) suggest that "swamping" is likely never complete between hybridizing species and thus remnants of squamation should be expected to be present anywhere the two forms met.

Ontario populations near Fort Severn (55°59'9" N 87°38'9" W) exhibit a reduction in squamation as do Kentucky and Virginia specimens. The Ontario populations are as free of immigration as are some upland Virginia populations, due to both their remote location in the Hudson Bay drainage and the continuing isostatic rebound throughout the entire drainage (Teller and Thorleifson 1983) which helps prevent stream capture. Additionally, because members of *Etheostoma* are seldom, if

ever, used as bait fish, introduction of exotic genotypes into either of these areas by man does not seem likely. Samples with specimens exhibiting one to many scales on the nape, cheek and/or breast, suggests that squamation is a trait still present in populations which formerly had contact with individuals which possessed a high degree of squamation. Because extra squamation is not found in the Fort Severn or Upper Cumberland River areas, it is highly unlikely that these, and other areas with exclusively unscaled *E. nigrum*, had any contact with a scaled form. All possible alternative explanations are not suitable.

Distribution in Western Canada

The Qu'Appelle Valley, Saskatchewan, and some Lake Manitoba (including Delta Marsh), Manitoba, specimens are distinct because their increased cheek, breast and nape squamation suggests that the scaled form moved into that area well before it was genetically swamped or out-competed by the unscaled form within the rest of those provinces. Approximately 4500-2200 years BP there were connections between the Assiniboine River and Lake Manitoba (Stewart *et al.* 1985). Some scaled *E. nigrum*, with a postulated origin within the Qu'Appelle Valley area, may have reached Lake Manitoba during that time. A history of habitation by the scaled form in the Lake Manitoba area since deglaciation could be expected to have led to a more widespread distribution of the scaled form or more specimens possessing increased squamation. However, the scarcity of heavily scaled specimens in Lake Manitoba suggests either later arrival via these temporary conditions or small numbers of early migrants that have been largely extirpated or crossbred with the unscaled form.

Stewart *et al.* (1986) note the occasional operation of the Assiniboine River Floodway which diverts water into Lake Manitoba near Delta from the Assiniboine River. Very recent entry into the Delta Marsh area is an additional means to explain the few heavily scaled individuals in the area without requiring that the trait be widespread within Lake Manitoba. Examination of specimens from the Assiniboine River (all unscaled) does not suggest that the scaled traits have reached Lake Manitoba in recent times, however. The entry of *E. nigrum* with scaled traits into Lake Manitoba is more likely to have occurred prior to 2200 years BP. This earlier date of entry, allowing a greater amount of time for crossbreeding, probably explains the low, but yet increased, amount of squamation on these specimens.

Large collections are not available from the Qu'Appelle Valley. Available samples contain only a few specimens which meet the definition of this study as scaled or are partially scaled. However, the presence of even a few scaled specimens implies that the scaled form did enter this area. The scaled form, with some mating with the unscaled form, could produce the partially scaled Qu'Appelle Valley specimens examined in this study. Partially scaled specimens from this area are thus interpreted as a result of intergradation while those meeting the definition of scaled may be due to (1) recombination, (2) assortative mating, or (3) migration from undetected scaled populations. In any event, there must have been an early arrival of scaled darters to the area. However, there seems to be no natural barriers that would have excluded further immigration of unscaled *E. nigrum* into that area and the reason for continued identity of the scaled form in the area is problematic. Perhaps later

arriving individuals had difficulty moving into the area. There is the possibility of predators removing individuals (of either morph) that move too far from a certain area or small areas acting as "islands" with the area between these islands being too inhospitable to cross.

The hypsithermal period (ca. 5000-6000 years BP) (Pielou 1991) was a warming period which allowed some fish species to increase their ranges while others were reduced (Hubbs and Lagler 1949). Some streams dried up or became too warm for continued habitation by the species present at the time. Increased water temperatures may have increased the incidence of disease. Populations of the scaled (and unscaled) form may have been affected by disease and loss of habitat during this period. The probability of habitat loss or increased disease seems higher within prairie areas than in most others (Trautman 1957; Pielou 1991). Thus some populations of the scaled form may have been extirpated during the hypsithermal or may have been forced from isolated pockets leading to intergradation with the unscaled form. The relict population of scaled *E. nigrum* in the Qu'Appelle Valley may be the result of the area being a hypsithermal refugium due to the number of lakes within the area. At least 30 fish species currently inhabit the Qu'Appelle River and at least 44 species are found in the Qu'Appelle-Assiniboine rivers (Crossman and McAllister 1986). These numbers are almost equal to those of the Manitoba Great Lakes (Crossman and McAllister 1986) suggesting that the lakes in the Qu'Appelle-Assiniboine system may have provided the necessary refuge during this warming period. Similarly, the specimens exhibiting increased squamation from Lake

Manitoba may be due to this area acting as a refuge during the warming period.

Squamation as an Apomorphic or Plesiomorphic Character

Bailey and Etnier (1988) and Simons (1992) suggest that reduced squamation is the more derived state in darters. However, Bailey and Etnier (1988) also suggest that the redevelopment of scales is a possibility, especially in taxa which once possessed scales and have since invaded quiet water (*e.g.*, through the switching on/off of regulatory genes). Tsai and Raney (1974) suggested that the highly scaled form of *E. zonale* was closest to the hypothetical ancestor of that species and that the naked form was a better adaptation to riffle habitats. Page and Swofford (1984) noted the correlation between sand habitat and a reduction of scales in both *Ammocrypta* and a sand inhabiting species of *Percina*. The adaptive significance of reduced squamation for sand or riffle dwelling darters is increased streamlining. Because the scaled form of *Etheostoma nigrum* is found in a wide variety of habitats and has presumably lived within these habitats for many years, the hypothesis that increased squamation is a response to habitat is not supported. If this hypothesis were correct, scaled populations of *E. nigrum* could be expected to be found in areas in which they are currently absent (*e.g.*, Ohio Basin, Hudson Bay-Ontario drainage). Therefore, the scaled form of *E. nigrum* is most likely plesiomorphic.

The possibility that increased squamation may arise as a result of the plastic nature of *E. nigrum* as a response to environmental influences (Bailey and Etnier 1988) is not supported by this study. Because (**1**) squamation does not exhibit a highly discontinuous distribution (the distribution of most scaled populations are

within an area only slightly south of the Great Lakes and wholly contained within the range of the unscaled form), (**2**) the number of breast and nape scales is relatively constant between samples collected at widespread locations, (**3**) this study has not greatly increased the known distribution of the scaled form, (**4**) several plesiomorphic meristic characters are commonly associated with the scaled form, (**5**) squamation is relatively stable between samples collected over 60 years apart, and (**6**) the scaled form is found in a variety of habitats, there is a strong suggestion that squamation does not develop as a response to environmental cues. Differences in water chemistry eventually causing increased squamation does also not seem to be a reasonable explanation due to the number of nearby, but yet morphologically distinct (*i.e.*, scaled or unscaled) populations in adjacent watersheds or within upstream and downstream areas of the same watershed. These considerations, coupled with the information from Lagler and Baileys' (1947) breeding experiments, constitute a strong argument for a genetic basis for increased squamation. For these reasons, I suggest that scaled populations represent the remnants of a formerly more widespread phenotype of *E. nigrum*.

THE SUBSPECIES CONCEPT

Although there has always been little consensus as to what the definition of a subspecies should be, Mayr's (1969) definition of a subspecies as "an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of the species" seems a suitable starting point for any further discussion.

Because *Etheostoma* is the second most speciose of the North American

freshwater fish genera, it is not surprising that a genus which exhibits such radiation should also possess many suspected subspecies. Mayden *et al.* (1992) list 119 species of *Etheostoma* and suggest that 29 of these, including *E. nigrum*, are in need of additional study which might result in subspecific or specific status for some populations. Presently these species are simply called polytypic which resolves little.

The taxonomic history of many fishes has included the naming of subspecies based on slight differences in only one or a few traits. Changes to colouration, the number of lateral line scales and head or body shape modification are commonly seen adaptations to local environments (*e.g.*, Hubbs 1934, 1940; Hubbs and Raney 1946; Hubbs and Lagler 1949) and many early subspecies were described based on such characters. Most of these early "subspecies" were later recognized as resulting from local environmental adaptation and their subspecific status has been abandoned. However, the recognition and naming of subspecies remains an important part of systematic ichthyology and new subspecies continue to be recognized (*e.g.*, Hanssens *et al.* 1995).

Critics of the validity of the scaled and unscaled subspecies of *E. nigrum* and *E. olmstedi* (Underhill 1963; Collette and Knapp 1967; Zorach 1971) have centred their arguments on the doubtful genetic basis of distinguishing characters in both species, the wide distribution of intergrading specimens and the scattered distribution of the scaled and unscaled forms. Zorach (1971) suggested that the wide disjunctions in the distribution of the scaled form of *E. olmstedi*, *E. o. atromaculatum*, could imply the independent origin of some populations and suggested that *E. o.*

atromaculatum was a polytopic subspecies. Polytopic subspecies may be morphologically indistinguishable but, their populations are not necessarily more closely related to each other than to other less phenotypically similar populations (Mayr *et al.* 1953). Zorach (1971) suggested that *E. o. atromaculatum* was an ecological form which developed increased squamation as an adaptation to its environment. Similar arguments have been used against the recognition of *E. nigrum eulepis*, the scaled form of the johnny darter.

I have agreed with Greene (1935) that scaled and unscaled *E. nigrum* likely occupied distinct ranges prior to the Wisconsinan. This is basically the case argued by Cole (1967, 1971) in his analysis of subspecies of *E. olmstedi*. Although Zorach (1971) suggested that the scaled form of both species may have evolved independently several times, he provided no support for this hypothesis besides the possibility alone. Mayr (1969) suggests that habitat selection by phenotypes, or ecological polymorphism, could explain the distribution of polymorphic species such as *E. nigrum*. However, the wide variation in the physical habitats in which the scaled form of *E. nigrum* exists, suggests that ecological polymorphism is not a satisfactory explanation. Underhill (1963) could not determine ecological specializations for the scaled form, further suggesting that ecological polymorphism does not explain their occurrence. Similar character states among populations in different habitats in distant geographic areas is a strong argument against ecological polymorphism and refutes the hypothesis of possible repeated evolution of similar traits much more convincingly than the hypothesis is supported by the statement that such evolution may have

occurred. The possibility of genetic drift acting in a similar fashion among populations in different habitats and subjected to different environmental pressures does not seem plausible. Additionally, evidence presented above shows that since the scaled was first recognized in 1935 (Hubbs and Greene), its range has not been noted to have increased greatly. Thus, even though collections of *E. nigrum* continue to be made and placed in institutions, new collections of the scaled form are not made in previously sampled areas in which they were absent because the scaled form does not arise as a response to environment.

The separation of subspecies in different habitats is not the cause of subspeciation but rather a condition of it (Borgmeier 1957). Geographic isolation at some stage is usually required for speciation as non-allopatric speciation is rare (Futuyma and Mayer 1980). However the habitats in which speciation occurs is not the cause of the event itself. Thus, ecological specializations are not required between subspecies as separation of populations in similar habitats may still result in their divergence. Subspecies are present in a given habitat or area because this is where they originated or because they were forced or dispersed into the habitat following differentiation. Scaled *E. nigrum* are found in habitats into which they dispersed that have not been invaded by the unscaled form.

The decision as to when to decide to assign subspecific status appears to rest largely on when the forms are considered distinct enough to be separated with a large degree of accuracy. Some statistical methods, such as the coefficient of difference established by Mayr *et al.* (1953), have been utilized to help make the decision.

These statistical methods have been designed to make the final decision less subjective but, not necessarily less arbitrary, on the part of the individual. There are, however, a few requirements that most researchers in *Etheostoma* systematics seem to agree on or have utilized to present their cases for subspecific status.

Most generally recognized subspecies of *Etheostoma* are those which exhibit nearly or completely allopatric distributions (e.g., Miller (1968), *E. blennioides*; Tsai and Raney (1974), *E. zonale*; Buth (1979), *E. blennius*; Page and Cordes (1983), *E. euzonum*; Retzer *et al.* (1986), *E. whipplei*). Thus, most researchers agree that subspecies of *Etheostoma* should be largely or completely allopatric.

The degree of morphological divergence in generally recognized subspecies of *Etheostoma* ranges from minor variation to differences so great that the subspecies may yet qualify for specific status (*e.g.*, *E. blennius sequatchiense* (Burr 1979)). Burr (1979) presents tables showing some overlap in all meristic and morphometric characters between *E. blennius blennius* and *E. b. sequatchiense*. Complete separation of the subspecies is possible only through examination of the amount of squamation on the upper portion of the opercle. Moore and Rigney (1952) present tables showing overlap in all characters among three subspecies of *E. radiosum*. Overlap in meristic characters also occurs between subspecies of *E. euzonum* (Page and Cordes 1983), *E. blennioides* (Miller 1968), *E. zonale* (Tsai and Raney 1974), *E. whipplei* (Retzer *et al.* 1986) and subspecies of *Percina caprodes* (Morris and Page 1981). Squamation is the single character in which most subspecies of *Etheostoma* rarely overlap. Many subspecies of *Etheostoma* are thus defined based on mean or

modal differences in meristic or morphometric characters and differences in squamation. Thus a second condition is that subspecies of *Etheostoma* should exhibit one or several distinctive traits which, although they may overlap on some specimens, should vary significantly between the subspecies and enable the identification of intergrade specimens. All agree that the trait(s) should not be clinal and/or otherwise correlated with some environmental variable. Additionally, the specimens making up such populations are in the majority and are not individual variants or the result of seasonal variation in characters, such as those associated with sexual maturation.

The majority of described and generally recognized subspecies of *Etheostoma* also exhibit only limited amounts of intergradation. However, most of these subspecies are found in areas which have not been directly impacted by Wisconsinan glaciation. Although all areas of North America were influenced by the Wisconsinan glaciation through increased precipitation and cooler temperatures, few of the above subspecies currently inhabit an area which was ice covered. Presumably, their distribution was not directly affected as ice advanced and retreated whereas reinvasion complicates understanding the former distribution of the scaled form of *E. nigrum*. Additionally, few of these subspecies, with the exception of *E. blennioides*, has a range close to the size of that encompassed by *E. nigrum*, suggesting that most subspeciation has been the result of local, and sometimes, geologically recent, events. This may be a reason why most subspecies of *Etheostoma* do not exhibit the patchy distribution exhibited by suspected subspecies of *E. nigrum* and *E. olmstedi*. Additional evidence for the impact that glaciation has on speciation comes from

McPhail (1993) who notes that sympatric but disjunct pairs of sibling fish species are typically associated with glaciated areas.

To summarize, the criteria for recognizing subspecies in *Etheostoma* are: (**1**) allopatry, (**2**) distinguishing characters which may vary only in means or modes but do not vary in a clinal or seasonal fashion, and (**3**) typically, only limited zones of intergradation. Using these criteria, it is possible to determine whether the scaled or other forms of *E. nigrum* meet the requirements for subspecies. Scaled populations of *E. nigrum* are not comprised of seasonal or individual variants as shown through: (**1**) examination of specimens collected during different time periods at different geographic locations, and (**2**) examination of the number of nape and breast scales, which vary little between geographic areas, showing that the scaled phenotype is not a clinal variant or an eco-phenotype. The scaled form is easily distinguished from the unscaled or intergrade form by its increased squamation and by additional characters when the mean or mode of these characters are considered. The area of intergradation between the two forms is often small. Scaled populations are found as complete, allopatric populations, sometimes over a large area (*e.g.*, Fox and Rock rivers, Wisconsin), where immigration is unknown. Therefore, by current standards used to define subspecies of *Etheostoma*, *E. nigrum nigrum* and *E. n. eulepis* meet the requirements for subspecific status.

IMPLICATION OF SUBSPECIES OF *Etheostoma nigrum* TO DARTER SYSTEMATICS

Starnes and Starnes (1979) suggest that *E. nigrum susanae* (described as

Boleosoma susanae by Jordan and Swain (1883)), found above the fall line in the Upper Cumberland River of Kentucky, be recognized as subspecific. Cole (1967) recognized *Etheostoma nigrum susanae* as a valid subspecies while Jenkins *et al.* (1971) did not. Although this form is free of genetic influence from populations down river, the reduced squamation exhibited by this form (compared to the "typical" unscaled form of this study) is also present in northern samples. Additional specimens I examined from Kentucky and Virginia also exhibit reduced squamation on the belly, cheek and opercles. Etnier and Starnes (1993) report similar reduced squamation on specimens they examined from the Red River, Oklahoma. Additional distinguishing traits of this form (always interrupted POM canal, preorbital spot) may be adaptations to the environment. These additional traits are variable characters and are occasionally present in other samples I examined.

The small number of specimens examined (12) with which earlier workers hypothesized subspecific status for *E. n. susanae* is too small to make an accurate appraisal. Starnes and Starnes (1979) were able to examine an additional 30 specimens, however the total number of specimens examined is still very small. Additionally, there is no evidence to suggest that the traits exhibited by this form are heritable and not brought on by some environmental factor. Thus, due to confinement within a restricted area, the small number of available specimens examined, the possession of traits which also occur in several other areas (without a biogeographic hypothesis as to how those traits developed there), and the difficulty associated with distinguishing intergrading specimens (Starnes and Starnes 1979), I do

not believe this form should be granted subspecific status. With declines reported in the abundance of this form (Starnes and Starnes 1979; Etnier and Starnes 1993), it may be impossible to give the form the examination that would be required to make a detailed, reliable assessment of its possible subspecific status in the future. Therefore, I conclude that subspecific status of *E. n. susanae* is not warranted at this time.

For somewhat similar reasons, I suggest that the upper and middle Ohio Basin populations cannot be designated as subspecific at this time. Like the Upper Cumberland River *E. nigrum*, these populations require much more study to determine their total distribution, which could assist in determining under what conditions and where they may have evolved and whether their distinguishing traits have a genetic basis. Although they are distinctive over a wide area, several of the traits possessed by the Ohio Basin populations are at least occasionally found on specimens from other areas. These traits usually do not occur together on the same specimen when found outside of the upper or middle Ohio Basin. However, until the distribution of these traits over the entire range of *E. nigrum* is better understood, it is better to simply note their presence rather than naming an invalid subspecies.

Other recognizable forms of *Etheostoma* must thus meet certain criteria, currently utilized by those doing research on the genus, to be recognized as subspecific. The variation in the distinguishing characters should be consistent among populations and the characters should be consistent over the size range of the specimens indicating a strong genetic basis for those characters. Samples taken from

within the range of the subspecies should exhibit consistency of the characters. The distinguishing characters should not be found in scattered areas over the entire range of the species, which would suggest environmental influence or local adaptation. If populations possessing the trait are disjunct, there must be a reasonable explanation as to how they arrived in the separate areas. When possible, disjunctions should be correlated with the distribution of other fish species producing a "generalized" track, which can be used to substantiate dispersal routes using the vicariant biogeographic method (Wiley 1981). Subspecies may exhibit intergradation of distinguishing traits where the two subspecies meet. Finally, a sufficiently large number of specimens should be examined to determine the extent of intergradation and to ensure that the trait does not vary clinally. Although some subspecies may possess an extremely limited range, sample sets of greater than 50 specimens from a number of habitats (*e.g.*, riffles, shoreline locations) should be examined.

Others working with the subspecies problem in fishes may disagree with my decision to call *E. nigrum eulepis* a subspecies. While there are few strict rules for deciding subspecific status, I have attempted to expand on the methods and philosophy used by others working with species of *Etheostoma* by analyzing many aspects of variation over a wide geographic area, and have tried to present an argument based on the information available from this and other studies. Additionally, I have attempted to reconstruct the pre-Wisconsinan distribution and post-glacial dispersal of the scaled morph, and showed convincing evidence that it has acted as a genetically discrete unit during dispersal, rather than being merely an adaptation to local conditions.

Two subspecies, *Etheostoma nigrum nigrum* Rafinesque and *Etheostoma nigrum eulepis* Hubbs and Greene, are therefore recognized. Their separating characteristics and distribution are given in the following key.

1a Nape, cheek and breast usually completely scaled with an excess of 30 nape, ten cheek and 47 breast scales. Belly completely scaled. Opercle usually heavily scaled with mean of 21 scales. Mean number of lateral line scales 48. Lateral line usually complete. Widely distributed in Wisconsin and northern Minnesota. Present in the Lake Erie and Lake Ontario basins. Present near international border of Minnesota-Ontario. Relict populations in northern Illinois and Iowa, the Qu'Appelle Valley of Saskatchewan and the Spring River and tributaries of Missouri-Kansas.

Etheostoma nigrum eulepis - scaly johnny darter.

1b Nape, cheek and breast uniformly naked. Belly variously scaled with reduction in northern-most range and portions of the Ohio Basin. Opercle variously scaled with mean of six scales. Mean number of lateral line scales 47. Lateral line usually slightly incomplete (1-3 unpored scales) or very incomplete (more than three unpored scales). Widely distributed from Quebec to Saskatchewan and Hudson Bay to Alabama. Middle and upper Ohio Basin populations often with five branchiostegal rays with modally 11 dorsal rays and 11 pectoral rays may qualify for subspecific status.

Etheostoma nigrum nigrum - central johnny darter.

I further suggest that the information included below be added to the original description made by Hubbs and Greene (1935).

Etheostoma nigrum eulepis Hubbs and Greene

Boleosoma nigrum eulepis Hubbs and Greene, 1935, tributary of west branch of the Rock River, Wisconsin.

Holotype - A 45.0 mm (standard length) specimen collected in a tributary of the West Branch of the Rock River, 2 miles (3.2 km) north of Atwater, Dodge County, Wisconsin, catalogued at the Museum of Zoology, University of Michigan (UMMZ 77225).

Paratypes - A large number of paratypes from Wisconsin, Minnesota and Iowa, ranging in standard length from 25.0 to 59.0 mm are in collections of the universities of Michigan, Wisconsin and the United States National Museum. Collette and Knapp (1967) presented a long list of paratypes selected by Hubbs and Greene (some of which are given below) from Wisconsin, Iowa and Minnesota which must be checked against the definition developed in this study to determine if they should all remain as paratypes of *E. n. eulepis*. Collections examined which meet the definition of *E. n. eulepis* developed for this study are indicated with an asterisk (*). Presently extant UMMZ collections from Wisconsin entered on their database as paratypes include (number of specimens in parentheses);

> **72370** (13) Polk Co., Apple R., 2 mi. W. of turtle L. on rt. 14; P. Okkelberg; Aug. 26, 1928. **72382** (13) same data. **72414** (60) Vilas Co., SE. side of Big Arbor vitae L.; Metzelaar and Langlois; Sept. 19, 1925. **72447*** (9) Oneida Co., Kawaguesaga L., W. of Minoigua: Metzelaar and Langlois; Sept. 20, 1925. **72467** (2) Vilas Co., Trout L.; Metzelaar and Langlois; Sept. 20, 1925. **72495*** (14) Vilas Co., Little Arbor Vitae L.; Metzelaar and Langlois; Sept. 21, 1925. **72502*** (18) Vilas Co., St. Germain L.; Metzelaar and Langlois; Sept. 21, 1925. **73442** (15) Columbia Co.; Fox R., 10 mi. E. of Portage on rt. 33: Greene and Jones; Aug. 18, 1925. **73458** (1) Columbia Co.; French Cr., below dam; Greene and Jones; Aug. 19, 1925. **73464*** (35) Columbia Co.; French Cr., 1 mi. above mill pond; Greene and Jones; Aug. 19, 1925. **73472** (4) Columbia Co.; Spring Branch Cr., above co. rd.; Greene and Jones; Aug. 19, 1925. **73475** (3) same data. **73563*** (11) Marquette Co.; trib. of Neenah Cr., 1.5 mi. from Neenah Cr.; Greene and Jones; Aug. 21, 1925. **73571*** (5) Columbia Co.; outlet of Spring L., Pardeeville; Greene and Jones; Aug. 22, 1925. **73579*** (28) Columbia Co., old channel, below Park L. Dam; Greene and Jones; Aug. 22, 1925. **73586** (16) Columbia Co.; Park L.; Greene

and Jones; Aug. 22, 1925. **73627** (89) Marquette Co.; Neenah Cr., 2 mi. NE. of Briggsville on rt. 23; Greene and Jones; Aug. 24, 1925. **73640** (2) Columbia Co., trib. of Neenah Cr., 6 mi. E. of Briggsville; Greene and Jones; Aug. 24, 1925. **73670** (66) Marquette Co., Belle Fountaine Cr., 4 mi. N. of Dalton; Greene and Jones; Aug. 25, 1925. **73683** (11) Columbia Co.; Neenah Cr., near mouth; Greene and Jones; Aug. 26, 1925. **73693** (3) Columbia Co.; French Cr., at mouth; Greene and Jones; Aug. 26, 1925. **73706** (7) Columbia Co.; Fox R., below mouth of French Cr.; Greene and Jones; Aug. 26, 1925. **73728** (4) Columbia Co., Fox R., opposite Lock 25; Greene and Jones; Aug. 26, 1925. **73735** (6) Columbia Co., Fox R., at Portage; Greene and Jones; Aug. 26, 1925. **73744** (47) Fond du Lac Co., Grand R., above Fairwater Millpond; Greene and Jones; Aug. 5, 1925. **73810*** (14) Green Lake Co., Grand R., below Kingston Dam; Greene and Jones; Aug. 29, 1925. **73821** (4) Green Lake Co., Grand R. at Kingston; Greene and Jones; Aug. 29, 1925. **73831** (5) Green Lake Co., trib. of Grand R., 4 mi. SW. of Uthey; Greene and Jones; Aug. 31, 1925. **73835** (85) Fond du Lac Co., trib. of Grand R., 2 mi. S. of Fairwater; Greene and Jones; Sept. 1, 1925. **73862** (30) Green Lake Co., Grand R., 3 mi S. of Marquette; Greene and Jones; Sept. 1, 1925. **73874** (26) Marquette Co., trib. of Grand R., 2 mi. W. of Kingston; Greene and Jones; Sept. 1, 1925. **73877** (8) Marquette Co., trib. of Grand R., 1.5 mi. S. of Welsh Cr.; Greene and Jones; Sept. 1, 1925. **73888** (57) Marquette Co, trib. of Grand R., Welsh Cr.; Greene and Jones; Sept. 1, 1925. **73955** (34) Fond du Lac Co., Rush Cr., near Waupun; Greene and Cuchio; July 4, 1926. **95933*** (3) Sawyer Co., Grindstone L., 10 mi. SE of Haywood; Creaser and Jones; Sept. 2, 1928. **96085** (1) Washburn Co., outlet of Spooner L. below dam; Creaser and Jones; Sept 7, 1928. **96111** (9) Washburn Co., Long L., 14 mi. SE. of Spooner on rt. 53; Creaser and Jones; Sept 7, 1928. **100771** (79) out of UMMZ **77225**.

Material examined - Specimens meeting the definition of *E. n. eulepis* examined during this study but not all designated as paratypes by Hubbs and Greene (1935) are listed by drainage and river system. Specimens identified as paratypes in Collette and Knapp (1967) are denoted with an asterisk (*). Abbreviations of institutions from which material was borrowed follow Leviton *et al.* (1985) with the exception that **LU** = Lakehead University, Ichthyology Collection, Thunder Bay, Ontario and **PC** represents donated specimens from the JFBM, University of Minnesota, now in my personal collections.

Missouri River Basin - **UMMZ 114083**, IA. Meadow Creek, 2 miles E of Spencer (5); **UMMZ 101528***, IA. Spirit Lake (Dickinson Co.) (8).

Mississippi River Basin - **LU uncatalogued**. WI. Crawfish River, at Hwy "G" (Dodge Co.) (7); **UMMZ 167071**, SD. Big Stone Lake (Roberts Co.) (8); **UMMZ 166905**, SD. Whetstone Creek, near Big Stone City (Grant Co.) (23);

UMMZ 151843, MO. Coon Creek, 1 mile N of Jasper, tributary of N Fork Spring River (2); **UMMZ 151794**, MO. Muddy Fork, N Fork of Spring River, tributary to Neosho River (6); **UMMZ 94832***, MN. Mississippi River, between Cass Lake and Bemidji (Beltrami Co.) (10); **PC**, MN. Deer Creek (Itasca Co.) (4);

Lake Winnipeg-Hudson Bay Basin - **UMMZ 118445**, MN. Mud River, near Redby (Beltrami Co.) (5); **PC**, MN. Red Lake River, 25 miles E of Thief River Falls (Pennington Co.) (11); **PC**, MN. Otter Tail River (Becker Co.) (11); **ROM 17830**, SK. Echo Lake, Qu'Appelle Fishing lakes (10); **ROM 16769**, SK. Echo Lake, Qu'Appelle River (4); **NMC 58-188**, SK. Qu'Appelle Valley, E of Regina (1).

Lake Superior Basin - **LU uncatalogued**, ON. McIntyre River, at Thunder Bay (10); **LU uncatalogued**, ON. Roundtable Lake (14).

Lake Michigan Basin - **UMMZ 114925**, IN. Coffee Creek, near Chesterton (48).

Lake Huron Basin - **ROM 4447**, ON. McGregor Bay (6).

Lake Erie - Lake St. Clair Basins - **UMMZ 55923**, MI. Lake St. Clair, at Fair Haven (McComb Co.) (5); **UMMZ 159810**, OH. South Bass Island, Squaw Harbor, Lake Erie (11); **UMMZ 55027**, PA. Lake Erie, Erie Harbor (Erie Co.) (4).

Lake Ontario Basin/St. Lawrence River Drainages - **ROM 11138**, ON. Rideau Creek, near Andrewsville (11); **ROM 40132**, ON. Niagara River (5).

Etymology - *nigrum* means black; *eulepis* means well scaled.
Diagnosis - Member of subgenus *Boleosoma* as diagnosed by Page (1981). The only species of *Boleosoma* which is not limited solely to the Atlantic Slope or tributaries of the St. Lawrence River and Lake Ontario. Infraorbital canal interrupted. Preoperculomandibular pores usually numbering nine or less.

Description - Lateral line scales 40-58 (mode 48); lateral line complete (63% of specimens) or only slightly incomplete (3 or fewer unpored scales) (18% of specimens); scales from first dorsal origin to lateral line 3-6 (mode 4); from posterior origin of first dorsal to lateral line 4-7 (mode 5); from lateral line to anal origin 5-10 (mode 7); dorsal VIII-X, 8-14 (modes IX, 12); anal I, 6-10 (mode I,8); left pectoral fin rays 11-13 (mode 12); branchiostegal rays 6-7 (mode 6); preoperculomandibular pores 6-12 (mode 6); infraorbital canal incomplete, anterior infraorbital pores 2-5 (mode 4); posterior infraorbital pores 1-4 (mode 2); belly 100% scaled; nape fully to almost fully scaled (90% of specimens) 30-62 scales (mode 48)); breast fully to

almost fully scaled (50% of specimens) 47-156 scales (mode 88)); cheek fully to almost fully scaled (80% of specimens) 10-66 scales (mode 20)); opercle fully to almost fully scaled (88% of specimens) 7-36 scales (mode 22)); number of scales at nape-parietal juncture 0-7 (mode 4).

Comparison of *E. n. eulepis* to *E. n. nigrum* - *Etheostoma nigrum eulepis* is similar to *E. n. nigrum* except that the cheeks, nape and breast are usually completely or almost completely scaled. The belly is always completely scaled compared to variably scaled in *E. n. nigrum*. Napes scaled only partially and in the anterior position most common in northern Minnesota. Scales in lateral line average 48 compared to average of 47 in *E. n. nigrum*. Number of opercle scales average 21 as compared to 8 in *E. n. nigrum*. Dorsal spines average 9 with 10 common in some areas. Scales above lateral line generally 4 or greater and seldom 3. Number of scales at the nape-parietal juncture average 4 with higher counts in samples from northwestern Minnesota. Lateral line most often complete (63%) of specimens compared to only 31% complete in *E. n. nigrum*. Colouration similar to *E. n. nigrum* although many specimens of *E. n. eulepis* appear darker due to squamation on the nape, breast and cheek.

Distribution - Common in Wisconsin in the Rock, Fox and portions of the St. Croix river systems. Probably extends into northern Illinois in the Rock River system. Common in the lake area of the north-central portion of Wisconsin (Wisconsin River drainage). Common in the Otter Tail River of Minnesota and some nearby Red River tributaries. Present in Big Stone Lake, South Dakota, and adjacent tributaries. Common in the Pigeon River system at the international border of Minnesota-Ontario. Present in the Niagara River at the international border. Also present in areas nearby both these Ontario locations. Common at the Bass Islands and other locations in western Lake Erie and in Lake St. Clair. Relict populations in the Spring River, Missouri and Kansas, the Qu'Appelle Valley, Saskatchewan, and Spirit Lake and northern streams in Iowa. A small population occurs in a south shore tributary of Lake Michigan in Indiana.

Habitat and Ecology - *Etheostoma n. eulepis* is often found over barren sand or silt type substrates. Vegetation may or may not be present. Also abundant in some silted areas with vegetation and over gravel or rubble substrates. Co-exists with a variety of cold and warmwater fish species. More common in lakes in some areas (Minnesota) but less so in others (Wisconsin). Present in both turbid and clear waters. The heaviest scaled specimens normally occur in lakes. Although no one has studied the behaviour or spawning habits of this form exclusively, it is believed that it behaves very much the same as *E. n. nigrum*.