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Nest-Specific DNA Fingerprints of Smallmouth Bass in Lake Opeongo, Ontario

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Abstract.—Methods of DNA fingerprinting were employed to search for nest-specific markers in the population of smallmouth bass *Micropterus dolomieu* in Lake Opeongo, Ontario. Banding patterns produced from combinations of six restriction endonucleases and seven DNA fingerprint probes were evaluated. Each enzyme and probe combination detected very few polymorphic loci among fry from the same nest. However, comparisons of fingerprints between fry from different nests revealed significant differences in banding patterns, indicating that each nest had a unique fingerprint. Fry from 15 nests in the Jones Bay area of the lake were fingerprinted by using the restriction enzyme *Hae* III and the probe $(GACA)_4$. A double-blind test was conducted to determine the ability to assign an unknown fry correctly to its nest of origin and demonstrate that nest-specific fingerprints exist. All fry tested were assigned correctly to their nest of origin by visual comparison of their fingerprints with each of the nest fingerprints, thus demonstrating that nest-specific DNA fingerprints can be generated.

Recent advances in molecular genetics have led to the development of a powerful tool, DNA fingerprinting, that produces highly variable, genetically distinct marks for individuals (e.g., Fields et al. 1989). These individual-specific marks or "fingerprints" have provided a new source of genetic markers that have a variety of applications including studies involving forensics (Bär and Hummel 1991), parentage (Westneat 1990; Rico et al. 1991), linkage analysis (Jeffreys et al. 1986), and genetic diversity (Reeve et al. 1990; Schartl et al. 1991; Turner et al. 1991). In addition to producing individual-specific marks, researchers have begun exploring the use of this technique to generate family and population-specific fingerprints (Gilbert et al. 1990; Wirgin et al. 1991). Family-specific DNA fingerprints would permit monitoring of families in situations where physical tagging or identification is not feasible, a common problem in studies involving fish due to small size of the fish or to logistical problems posed by the behavior of individual fish.

Because of their spawning behavior, smallmouth bass *Micropterus dolomieu* provide an excellent opportunity to search for family (nest)-specific DNA fingerprints in a fish species. The males build conspicuous nests and defend their offspring, who initially remain together in a cluster

after hatching (Coble 1975). This behavior allows for easy collection of nest-specific tissue samples for DNA analysis.

Nest-specific DNA fingerprints would provide a new source of markers that could greatly assist research conducted on smallmouth bass. One area of research that may be advanced by application of this technology is determination of factors affecting individual reproductive success. A major obstacle to the estimation of individual reproductive success for smallmouth bass has been the inability to identify offspring from a particular mating due to dispersal and mixing of fry shortly after hatching. For this reason, past studies on individual reproductive success of smallmouth bass have focused only on estimates to the swim-up stage, prior to fry dispersal (Neves 1975; Goff 1986; Raffetto et al. 1990; Reynolds 1990). Because a large proportion of individuals survive to these early stages, factors influencing their success to a later life stage, after which the majority of them have died, may not be apparent. Generation of nest-specific DNA fingerprints of smallmouth bass would allow fisheries biologists to monitor the reproductive success of families to any life stage in a natural environment and determine which factors are important in regulating success. In addition, changes in reproductive success could be monitored in response to environmental changes and managerial modifications.

This paper describes the application of DNA fingerprinting methods to the smallmouth bass population in Lake Opeongo, Ontario. Individual as well as nest- (family)-specific DNA fingerprints were generated. How nest-specific DNA fingerprints are generated and their potential utility in studying various aspects of smallmouth bass biology are discussed.

Methods

Study population and sample collection.—Lake Opeongo (45°42'N, 78°22'W) is a 5,860-ha oligotrophic lake in Algonquin Park, Ontario. Smallmouth bass were introduced into the lake in the 1920s and have established a prominent, self-sustaining population (Martin and Fry 1973). Spawning sites of smallmouth bass are distributed throughout the lake but several areas, such as Jones Bay, have notably large concentrations of nest sites. Sample collection was confined to the 6 km of shoreline in Jones Bay, where approximately 40% of all smallmouth bass spawning in the lake occurs (M. Ridgway, Ontario Ministry of Natural Resources, personal communication).

Nest sites were located by snorkeling the shoreline of Jones Bay every 3 d during the spawning season. Once a nest was located, it was marked with a numbered brick. The guardian male (father) was angled from each nest and tagged with a Floy anchor tag (Floy Tag and Manufacturing, Inc.), and a tissue sample was obtained by clipping a portion of his pelvic fin. Upon reaching the swim-up stage, 10 offspring from each nest were collected, nonselectively with an aquarium net and placed separately in labeled vials. All samples collected in the field were kept on dry ice and stored later at −70°C until the DNA could be extracted.

DNA fingerprinting.—Genomic DNA was extracted from the guardian male fin tissue or whole fry by dicing the tissue and suspending it in a buffer solution (50 mM tris-HCl, pH 7.5; 100 mM EDTA, pH 8.0; and 2% sodium dodecyl sulfate, SDS). Proteinase K (200 μg) was added to the suspension, which was incubated for 2 h at 55°C. Two extractions were performed, one with an equal volume of phenol : chloroform (50:50) and another with an equal volume of chloroform : isoamyl alcohol (24:1). The DNA was precipitated by addition of an equal volume of isopropyl alcohol, chilled for at least 1 h at −20°C, centrifuged, and washed with 70% ethanol. The DNA was resuspended in TE buffer (10 mM tris-HCl, pH 7.5; 1 mM EDTA, pH 8.0) for subsequent storage at 4°C.

Approximately 6 μg of DNA was digested with a 10× excess of a restriction endonuclease (*Hae* III, *Hin*f I, *Alu* I, *Taq* I, *Msp* I, or *Pst* I) and incubated for 3 h according to the manufacturer's instructions. Samples were processed by electrophoresis through a 1% agarose gel (25 cm; 10-tooth–1-mm comb) at 60 V for 44 h (time at which the 1.6-kilobase [kb] marker fragment was at the bottom of the gel) in 1× TBE (tris–borate–EDTA) buffer. At approximately the midpoint through electrophoresis, the buffer was exchanged to prevent it from being exhausted.

After electrophoresis, the DNA in the gel was depurinated in 250 mM HCl for 5 min, denatured in 1.5 M NaCl and 500 mM NaOH for 30 min, and neutralized in 3 M NaCl with 500 mM tris-HCl (pH 7.5) for 30 min. The DNA was then transferred from the gel onto a nylon membrane (Hybond-Nfp, Amersham International) by overnight capillary Southern blotting (Sambrook et al. 1989) in 20× standard sodium citrate (3 M NaCl, 300 mM Na_3 citrate, pH 7.0). The DNA was fixed to the membrane by baking for 3 h at 80°C.

Prior to hybridization (the pairing of complementary, single strands of DNA), membranes were wet-

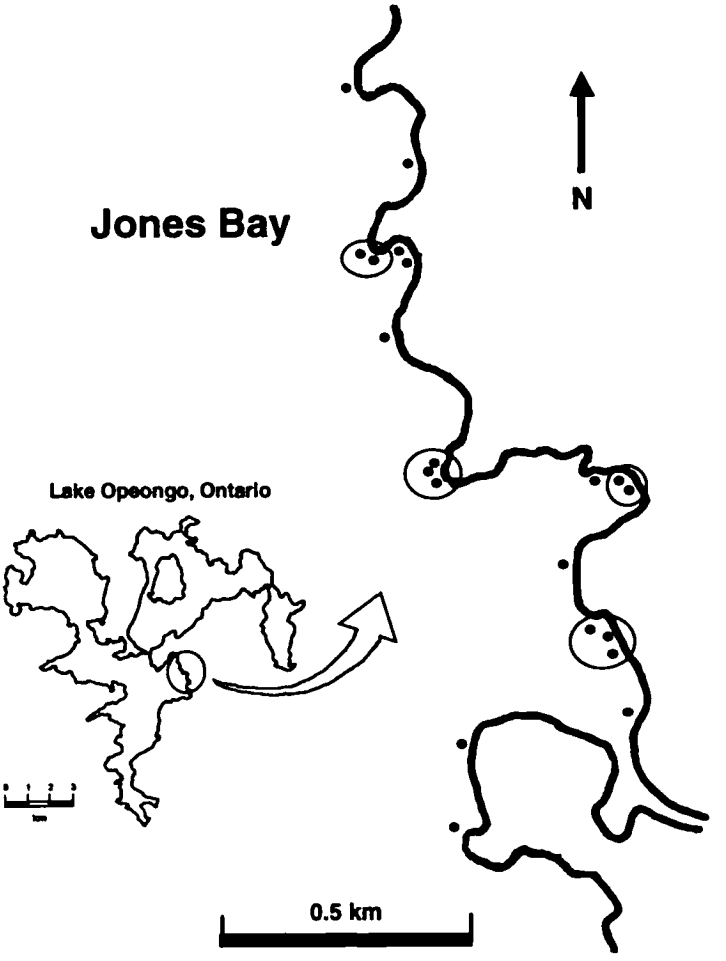

FIGURE 1.—Map of Lake Opeongo with the shoreline of Jones Bay highlighted to show the location of nests (●) from which fry were collected for DNA fingerprinting. Circles denote nests spaced closely together in clusters, for whose fry fingerprints were run on the same gel.

ted in a solution of 200 mM Na_2HPO_4 (pH 7.2) and then prehybridized for 1 h in a solution of 500 mM Na_2HPO_4 (pH 7.2) and 10% SDS. The membranes were hybridized overnight in a solution containing 200 mM Na_2HPO_4 (pH 7.2), 1% SDS, 1% bovine serum albumin (fraction V), 6% polyethylene glycol 8000, and ^{32}P-labeled probe (Amersham protocol). Probes and hybridization temperatures tested with each enzyme listed above were human minisatellites 33.15 and 33.6 (Zeneca Ltd.) at 62°C; oligonucleotides $(CAC)_5$ and $(GACGCTGGAGGTTCT)_4$ at 37°C; and $(GACA)_4$, $(GATA)_4$, and $(GGAT)_4$ at 42°C. Excess probe was removed by washing the membranes twice for 20 min at their hybridization temperatures in 200 mM Na_2HPO_4 (pH 7.2) and 0.1% SDS. Membranes were exposed for approximately 3 d to Kodak XAR-5 film at −70°C with intensifying screens.

Fingerprint scheme and evaluation.—A test gel containing DNA from several smallmouth bass was run for each of the six restriction enzymes. Test gel membranes for each enzyme were hybridized with the different probes to compare the fingerprint patterns that were generated. The combination of enzyme and probe that produced the best overall banding pattern for an individual, as well as having a high level of polymorphisms between individuals, was selected to test for nest-specific fingerprints.

Fish from 15 randomly distributed nests were fingerprinted with the same enzyme–probe combination (Figure 1). Nest fingerprint gels consisted

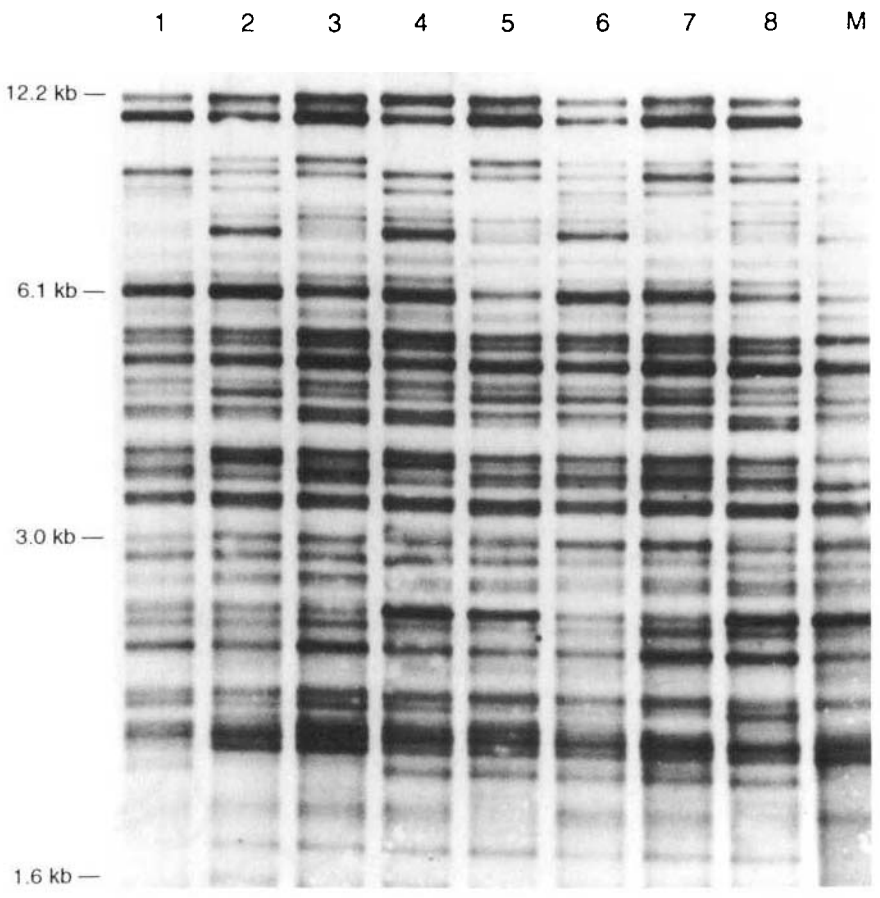

FIGURE 2.—The DNA fingerprints of eight smallmouth bass fry (lanes 1–8) and the guardian male (M) collected from nest C31 in Lake Opeongo (probe $[GACA]_4$ and restriction enzyme *Hae* III). Each numbered lane contains the DNA from an individual fry; the DNA has been cut into fragments with the enzyme and electrophoretically separated out by size through an agarose gel. Each band, ranging in size from approximately 1.5 to 13 kilobases (kb), is the result of a radioactively labeled probe pairing with the complementary sequence in a particular DNA fragment, which then has been visualized by exposure to X-ray film.

of one lane of 1-kb marker (Gibco BRL), eight lanes each containing the DNA of an individual fry from the nest, and one lane containing the DNA of the guardian male of the nest.

Again, with the same combination of enzyme and probe as above, two gels were run to examine the differences in fingerprints between nests in Jones Bay. One gel consisted of one fry randomly chosen from each of 10 nests and the other consisted of 10 guardian males. In addition, several gels were run to compare fingerprints of fry from nests that were spaced closely together in clusters (Figure 1).

A similarity index (SI) was computed to measure genetic variability. The SI between two individuals was calculated by the formula $100 \cdot 2N_{XY}/(N_X + N_Y)$; N_{XY} is the number of bands shared between individuals X and Y, and N_X and N_Y are the total number of bands scored for X and Y, respectively (Lynch 1990). Comparisons of bands were made between individuals in adjacent lanes down to approximately the 1.6-kb fragment. Although bands were clearly distinguishable below 1.6 kb, they were not included in the analysis because they might upwardly bias the SI estimates due to a higher probability that these bands are shared (Jeffreys et al. 1985). For all gels, the same investigator scored bands as identical if they had similar mobility and intensity based on visual estimation.

A double-blind test was conducted to determine the ability to correctly assign an unknown indi-

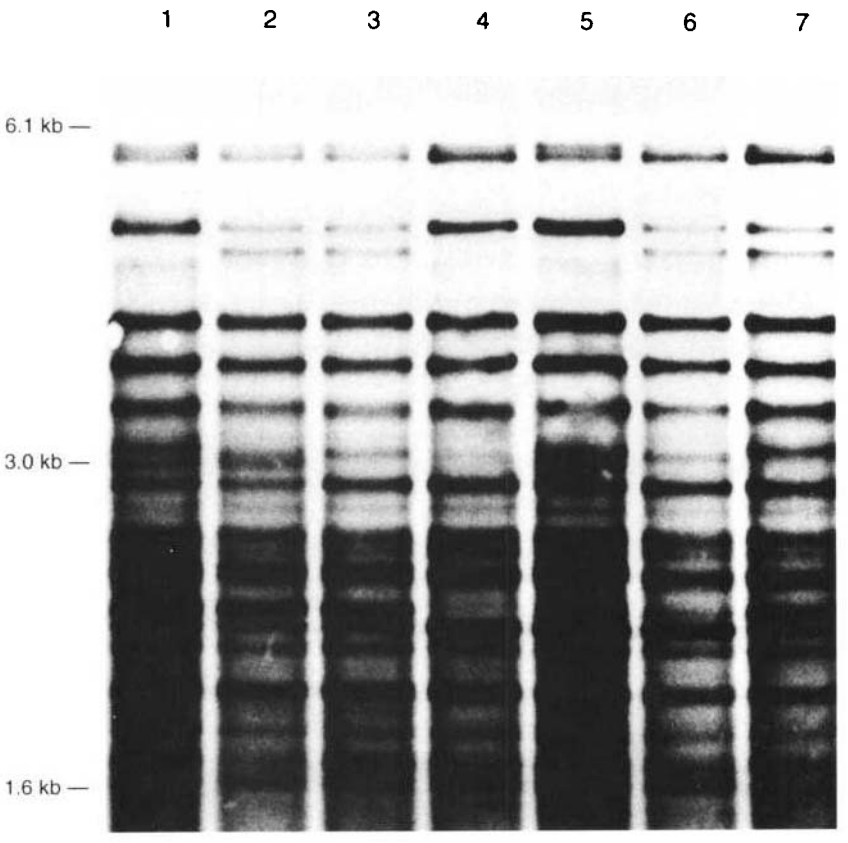

FIGURE 3.—The DNA fingerprints of seven smallmouth bass fry collected from nest C32 in Lake Opeongo (probe $[GGAT]_4$ and enzyme *Hae* III).

vidual to its nest of origin and assess if nest-specific fingerprints exist. Eight fry that had not been fingerprinted previously were chosen by a second party and their vials were arbitrarily relabeled; the second party kept a hidden list of the nest of origin of each vial. Fingerprints were generated for all fry and their banding patterns (distribution and intensity of bands) were compared visually to each of the 15 nest fingerprints to assign a nest of origin. A match was determined when the banding pattern of a fry concurred with the overall banding pattern of a particular nest fingerprint. Assignments were compared with the list of actual nest origins for an assessment of the ability of this technique to correctly identify nest origin.

Results

The best DNA fingerprint patterns were obtained with the restriction enzyme *Hae* III and the oligonucleotide probe $(GACA)_4$. Compared with the other combinations of enzymes and probes, the banding pattern generated by *Hae* III and $(GACA)_4$ consisted of a moderate number of bands per individual (36 ± 4, mean ± SD) that migrated throughout the 1.6–14-kb range (Figure 2). This combination of enzyme and probe produced a low level of polymorphic loci across members within a nest, as was evident from the average SI of 87.2 ± 4.4 (Table 1). Similar levels of polymorphism

TABLE 1.—Average similarity indices of DNA fingerprints and numbers of comparisons between individual smallmouth bass for three categories of fry or guardian males. Spatial relationships of individual nests and clusters of nests are shown in Figure 1.

Comparisons between	Number of comparisons	Similarity index (mean ± SD)
Fry within a nest	62	87.2 ± 4.4
Fry from several nests closely spaced together in clusters	13	67.2 ± 6.0
Fry or guardian males from different nests within Jones Bay	15	52.5 ± 5.8

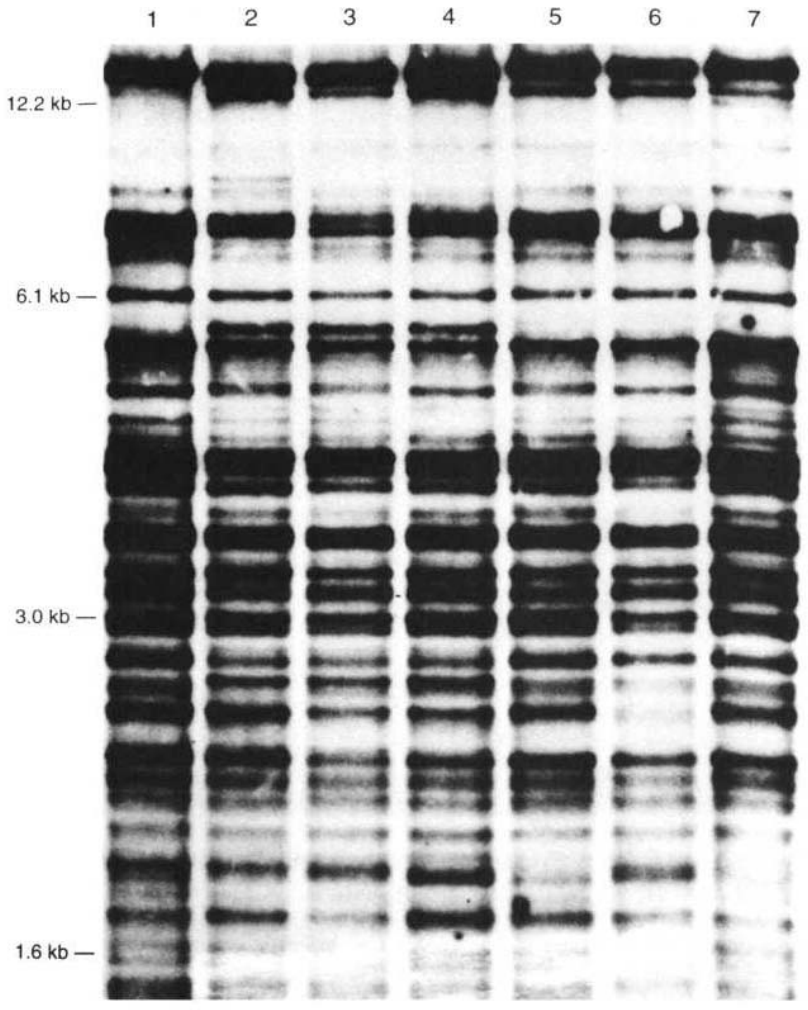

FIGURE 4.—The DNA fingerprints of seven smallmouth bass fry collected from nest 41 in Lake Opeongo (human minisatellite probe 33.15 and enzyme *Alu* I).

were obtained with other combinations, such as those shown in Figures 3 and 4.

Hybridizations of oligonucleotide probes $(CAC)_5$, $(GATA)_4$, and $(GACGCTGGAGGTTCT)_4$ were very weak under various conditions, suggesting low sequence homology in smallmouth bass. Oligonucleotide probe $(GGAT)_4$ generated only bands that were approximately 6 kb or smaller (Figure 3) and was therefore judged to not be useful for this study. Human minisatellite probes 33.15 (Figure 4) and 33.6 generated fingerprint patterns comparable to those of $(GACA)_4$, but they were not used because of the relative ease of use of and access to the oligonucleotide probe $(GACA)_4$. Other enzymes tested were excluded because they either produced too few (*Alu* I) or too many (*Taq* I) bands or were judged to be too expensive compared with *Hae* III.

The DNA fingerprints generated with *Hae* III and $(GACA)_4$ for smallmouth bass fry from different nests within Jones Bay showed that the bands are polymorphic across individuals from different nests (Figure 5). This observation is supported by the average SI of 52.5 ± 5.5 computed for the comparison of bands between fry or guardian males of different nests (Table 1). The average SI for comparisons between fry from nests that were spaced closely together in clusters was 67.2 ± 6.0 (Table 1).

The double-blind test confirmed our ability to

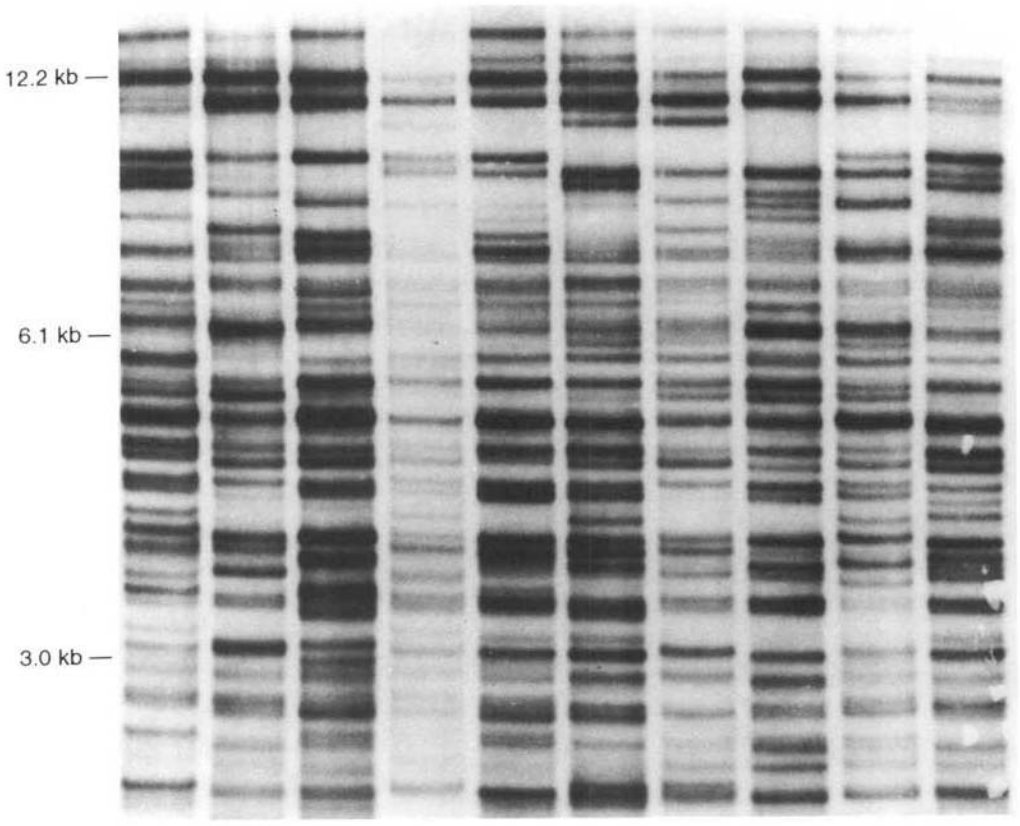

FIGURE 5.—The DNA fingerprints of 10 smallmouth bass fry, each chosen randomly from a different nest in Jones Bay, Lake Opeongo (probe $[GACA]_4$ and enzyme *Hae* III).

assign a unique fingerprint to each nest, as exemplified in Figure 6. All eight anonymous fry tested were correctly assigned to their nest of origin by visual comparison of their banding patterns (fingerprints) with the previously generated banding patterns (fingerprints) representing all prospective nests.

Discussion

Nest (family)-specific DNA fingerprints of smallmouth bass in Lake Opeongo are the result of a high percentage of bands being shared between and across family members (average SI, 87.2), but not shared across fish in the population (average SI, 52.5). Thus, for a given nest, each fry has a banding pattern very similar to that of its siblings but quite different from that of fry from another nest. This results in each family member having a mark (fingerprint pattern) that uniquely identifies it as belonging to a particular nest.

The DNA fingerprints are inherited in Mendelian fashion such that each band in an offspring's fingerprint can be matched with a corresponding band in either parent's fingerprint. Approximately fifty percent of the bands observed in the offspring's fingerprint are of maternal origin and fifty percent are of paternal origin. For a band to be similar in all offspring within a nest, at least one of the parents must be homozygous at that locus. This is exemplified by comparing the banding pattern of the male parent to those of his offspring in Figure 2. Many of the bands observed in the male are common to all the offspring, indicating that the male or both parents were homozygous for these loci. The remaining bands in the offspring, not observed in the male, must be contributed by the female (except for new bands resulting from mutations). In most cases, these remaining bands were also common to all offspring, indicating that these loci were homozygous in the female.

One possible explanation for the highly similar banding patterns observed among individuals within nests is that they are the consequence of philopatry and thus of inbreeding in this popula-

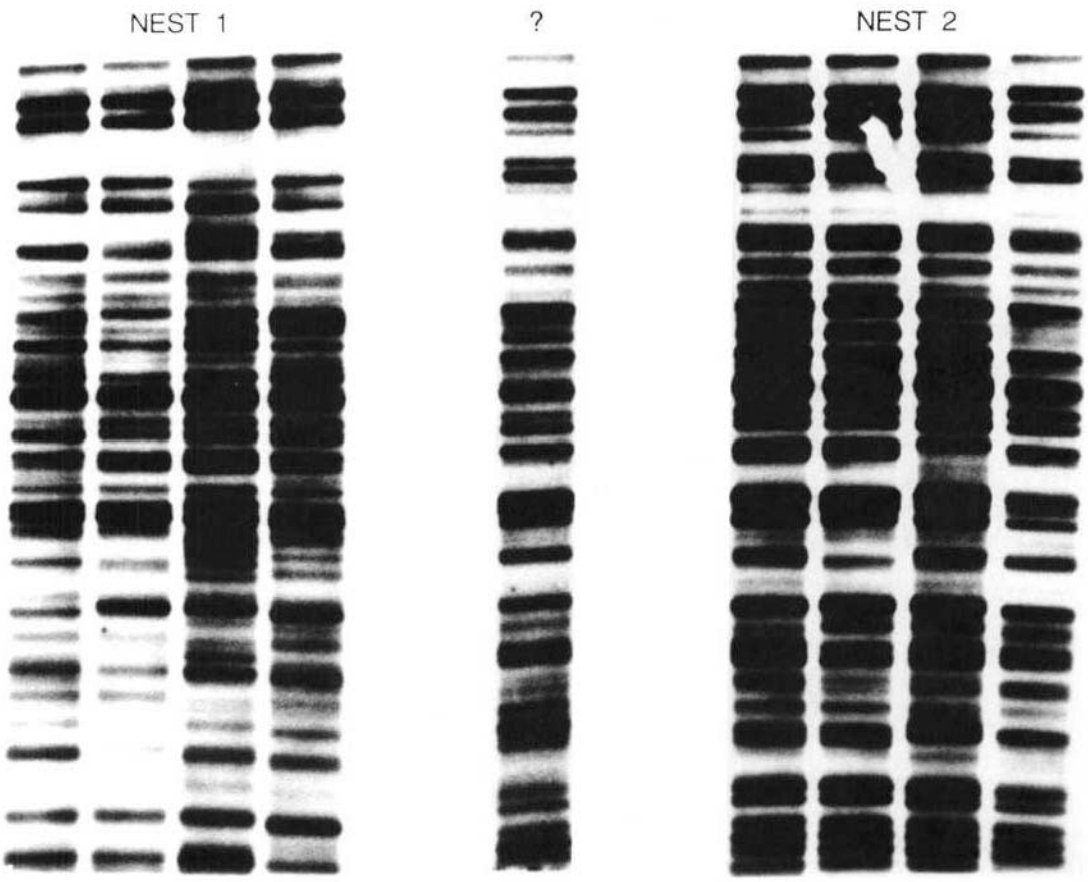

FIGURE 6.—An example of the decision process used in the double-blind test to determine a fry's nest of origin (unknown fry). Comparisons of banding patterns were made between the unknown fry (?) and four fry from two nests (nest 1 and nest 2), with each nest run on a separate gel and the unknown fry on a third gel (size markers on each gel are not shown). Solid brackets and upper-case letters denote some of the complementary regions where banding patterns match between the unknown fry and the fry from nest 2. Dotted brackets and lower-case letters denote some of the complementary regions where banding patterns do not match between the unknown fry and the fry from nest 1. In region A–a, the unknown fry and nest 2 fry both show to intense, higher-molecular-weight bands followed by a faint, lower-molecular-weight band; nest 1 fry show an inverted pattern of a faint band followed by two intense bands. In region B–b, the unknown fry and nest 2 fry show two intense bands followed by a faint band; nest 1 fry show only two intense bands with a greater relative distance between them than exists between the two intense bands from nest 2 fry. In region C–c, a band is present in nest 1 fry but is absent in the unknown fry and nest 2 fry. In region D–d, two close bands occur in the unknown fry and nest 2 fry, whereas a single band occurs in nest 1 fry. Comparisons among these four highlighted regions led to the correct conclusion that the unknown fry originated from nest 2.

tion. Inbreeding is the mating of relatives that share greater common ancestry than if they had been drawn at random from a large deme (Shields 1993). To locate and inbreed with relatives in a large system like Lake Opeongo during the short spawning season, smallmouth bass would have to be philopatric. Philopatry is defined as relatively localized dispersal of propagules or the return of mobile propagules to their birthplace for reproduction (Shields 1982). Species that are considered philopatric either remain near their birthplace throughout life, as is the case with some populations of the house mouse *Mus musculus* (Selander and Yang 1969), or they disperse widely as juveniles and return to mate at their natal site of origin, as is the case with the Laysan albatross *Diomedea immutabilis* (Fisher 1971). Philopatry tends to concentrate relatives during reproduc-

tion, thus increasing the probability of matings between close relatives. It is not clear whether inbreeding results from a species being philopatric or philopatry evolved to facilitate inbreeding (Shields 1982).

Our DNA fingerprinting data provide support for the hypothesis that smallmouth bass in Lake Opeongo are philopatric, resulting in some level of inbreeding. Reeve et al. (1990) found that full siblings of inbred colonies of naked mole-rats *Heterocephalus glaber* had an average SI of 95.0, and Schartl et al. (1991) calculated a similar value for a clonal fish, the amazon molly *Poecilia formosa*. Lehman et al. (1992), working with gray wolves *Canis lupus*, found that full siblings with inbred parents had an average SI of 78.7. On the other hand, average SI values calculated for outbred full siblings of largemouth bass *Micropterus salmoides* and threespine stickleback *Gasterosteus aculeatus* were 66.0 (Whitmore et al. 1990) and 56.0 (Rico et al. 1991), respectively. Therefore, the SI of 87.2 for full-sibling smallmouth bass in Lake Opeongo probably represents an inbred population.

Limited evidence for inbreeding also comes from comparisons of banding patterns between the male and female mates in two pairs of smallmouth bass captured while spawning, which had an average SI value of 69.5 (fingerprint data not shown). This value is higher than the population SI value of 52.5, suggesting that these two spawning pairs were inbreeding (rather than randomly mating) because they are more closely related to each other than to the population in general (the SI value increases with increasing relatedness; Lynch 1988). More extensive sampling of spawning pairs is needed to confirm the generality of this finding for the Lake Opeongo population.

Evidence of philopatry for the Lake Opeongo smallmouth bass is supported by the higher average SI value for fry from closely spaced nests (67.2) than for fry from nests distributed throughout Jones Bay (52.5). This results suggests that fry from nests clustered together are more closely related to each other than are Jones Bay fry in general. If nest site selection were random (no occurrence of philopatry), an SI value of approximately 52 would be expected for fry from closely spaced nests.

Although philopatry has not been explicitly demonstrated for smallmouth bass, the species is capable of homing (Larimore 1952) and adults have high nest site fidelity (Ridgway et al. 1991). Preliminary telemetry data for Lake Opeongo smallmouth bass indicate that the fish set up summer home ranges that can be very distant (several kilometers) from where they spawn (Mark Ridgway, Ontario Ministry of Natural Resources, personal communication). These observations suggest that adult fish move throughout the lake and are aware of other spawning sites, but still return to their previous spawning area.

An additional explanation for the observed high levels of homozygosity within nests, which form the basis of the observed nest-specific DNA fingerprints, may be that they are the result of a population bottleneck, a phenomenon that may have occurred when smallmouth bass were initially stocked into Lake Opeongo in the 1920s. A population bottleneck is defined as a severe temporary reduction in population size (Hartl and Clark 1989). Population bottlenecks increase the homozygosity of a population because a dramatic reduction in population size increases the likelihood of inbreeding. In our opinion, such a bottleneck is an unlikely cause of the relatively high homozygosities within nest cohorts. If a bottleneck had occurred, one would expect the population as a whole to have increased homozygosity and very similar DNA fingerprints among nest cohorts, thus precluding nest-specific fingerprints.

In conclusion, we have observed an unexpected pattern of nest-specific DNA fingerprints in one lake population of smallmouth bass. The generality of this phenomenon for lake and river populations of this species is currently unknown and requires additional research. These results raise intriguing questions about the roles of philopatry and inbreeding in the life history strategy of this Lake Opeongo population. Nest-specific DNA fingerprints could become a powerful tool for management of fisheries populations in which they occur because they would allow monitoring of individual reproductive success to any life stage. This would make it possible to determine patterns of temporal and spatial variability in individual reproductive success and to elucidate roles of myriad factors in control of reproductive success. Understanding these patterns and the factors that drive them will help managers to improve harvest regulation and direct habitat management so as to prevent diminution of individual reproductive success.

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References

Bär, W., and K. Hummel. 1991. DNA fingerprinting: its applications in forensic case work. Pages 349–355 *in* T. Burke et al., editors. DNA fingerprinting: approaches and applications. Birkhäuser Verlag, Basel, Switzerland.

Coble, D. W. 1975. Smallmouth bass. Pages 21–33 *in* H. Clepper, editor. Black bass biology and management. Sport Fishing Institute, Washington, D.C.

Fields, R. D., K. R. Johnson, and G. H. Thorgaard. 1989. DNA fingerprints in rainbow trout detected by hybridization with DNA of bacteriophage M13. Transactions of the American Fisheries Society 118: 78–81.

Fisher, H. I. 1971. Experiments on homing in Laysan albatrosses *Diomedea immutabilis*. Condor 73:389–400.

Gilbert, D. A., N. Lehman, S. J. O'Brien, and R. K. Wayne. 1990. Genetic fingerprinting reflects population differentiation in the California Channel Island fox. Nature (London) 344:764–766.

Goff, G. P. 1986. Reproductive success of male smallmouth bass in Long Point Bay, Lake Erie. Transactions of the American Fisheries Society 115:415–423.

Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics, 2nd edition. Sinauer, Sunderland, Massachusetts.

Jeffreys, A. J., V. Wilson, and S. L. Thein. 1985. Individual-specific 'fingerprints' of human DNA. Nature (London) 316:76–79.

Jeffreys, A. J., V. Wilson, S. L. Thein, D. J. Weatherall, and B. A. J. Ponder. 1986. DNA 'fingerprints' and segregation analysis of multiple markers in human pedigrees. American Journal of Human Genetics 39:11–24.

Larimore, R. W. 1952. Home pools and homing behavior of smallmouth black bass in Jordan Creek. Illinois Natural History Survey Biological Notes 28.

Lehman, N., P. Clarkson, L. D. Mech, T. J. Meier, and R. K. Wayne. 1992. A study of the genetic relationships within and among wolf packs using DNA fingerprinting and mitochondrial DNA. Behavioral Ecology and Sociobiology 30:83–94.

Lynch, M. 1988. Estimation of relatedness by DNA fingerprinting. Molecular Biology and Evolution 5:584–599.

Lynch, M. 1990. The similarity index and DNA fingerprinting. Molecular Biology and Evolution 7:478–484.

Martin, N. V., and F. E. J. Fry. 1973. Lake Opeongo: the ecology of the fish community and of man's effects on it. Great Lakes Fishery Commission Technical Report 24.

Neves, R. J. 1975. Factors affecting fry production of smallmouth bass (*Micropterus dolomieui*) in South Branch Lake, Maine. Transactions of the American Fisheries Society 104:83–87.

Raffetto, N. S., J. R. Baylis, and S. L. Serns. 1990. Complete estimates of reproductive success in a closed population of smallmouth bass (*Micropterus dolomieui*). Ecology 71:1523–1535.

Reeve, H. K., D. F. Westneat, W. A. Noon, P. W. Sherman, and C. F. Aquadro. 1990. DNA "fingerprinting" reveals high levels of inbreeding in colonies of the eusocial naked model-rat. Proceedings of the National Academy of Sciences of the USA 87:2496–2500.

Reynolds, C. R. 1990. Spawning habitat utilization and nest success of smallmouth bass (*Micropterus dolomieui*) in two Tennessee streams. Master's thesis. Tennessee Technological University, Cookeville.

Rico, C., U. Kuhnlein, and G. J. Fitzgerald. 1991. Spawning patterns in the three-spined stickleback (*Gasterosteus aculeatus* L.): an evaluation by DNA fingerprinting. Journal of Fish Biology 39:151–158.

Ridgway, M. S., J. A. MacLean, and J. C. MacLeod. 1991. Nest-site fidelity in a centrarchid fish, the smallmouth bass (*Micropterus dolomieui*). Canadian Journal of Zoology 69:3103–3105.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Schartl, M., and seven co-authors. 1991. On the stability of dispensable constituents of the eukaryotic genome: stability of coding sequences versus truly hypervariable sequences in a clonal vertebrate, the amazon molly, *Poecilia formosa*. Proceedings of the National Academy of Science of the USA 88:8759–8763.

Selander, R. K., and S. Y. Yang. 1969. Protein polymorphism and genic heterozygosity in a wild population of the house mouse (*Mus musculus*). Genetics 63:653–667.

Shields, W. M. 1982. Philopatry, inbreeding, and the evolution of sex. State University of New York Press, Albany.

Shields, W. M. 1993. The natural and unnatural history of inbreeding and outbreeding. Pages 143–172 *in* N. W. Thornhill, editor. The natural history of inbreeding and outbreeding. University of Chicago Press, Chicago.

Turner, B. J., J. F. Elder, and T. F. Laughlin. 1991. Repetitive DNA sequences and the divergence of fish populations: some hopeful beginnings. Journal of Fish Biology 39:131–142.

Westneat, D. F. 1990. Genetic parentage in the indigo bunting: a study using DNA fingerprinting. Behavioral Ecology and Sociobiology 27:67–76.

Whitmore, D. H., R. Cotton, and K. Sheridan. 1990. DNA fingerprinting. Pages 132–141 *in* D. H. Whitmore, editor. Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Ann Arbor, Michigan.

Wirgin, I. I., C. Grunwald, and S. J. Garte. 1991. Use of DNA fingerprinting in the identification and management of a striped bass population in the southeastern United States. Transactions of the American Fisheries Society 120:273–282.

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