

Genetic evidence for cuckoldry in bluegill *Lepomis macrochirus*

D. P. PHILIPP and M. R. GROSS*

Center for Aquatic Ecology, Illinois Natural History Survey, 607 E. Peabody Drive, Champaign, Illinois 61820, USA and

*Department of Zoology, University of Toronto, 25 Harbord Street, Toronto, Ontario M5S 1A1, Canada

Abstract

In colonies of bluegill *Lepomis macrochirus*, some males provide parental care for broods in nests, whereas other males steal fertilizations and do not provide parental care. Using experimental pond populations of bluegill of known genotype (determined through protein electrophoresis), we demonstrate that cuckolder males successfully fertilize eggs in parental male nests. Using electrophoretic techniques to assess the fertilization success of nesting parental male bluegill in Lake Opinicon, Ontario, we demonstrate that paternity of these males ranges from 41% to 100% among four colonies studied. This difference among colonies is related to the density of cuckolder males.

Keywords: cuckoldry, bluegill, alternative strategies, reproductive success, protein electrophoresis

Received 8 December 1992; revision received 29 April 1994; accepted 4 May 1994

Introduction

Understanding the extent of cuckoldry in nature is important to the study of animal breeding systems (Birkhead & Møller 1992; Westneat & Sherman 1993). Cuckoldry involves some individuals, either deceptively or forcibly, placing their genetic progeny in the care of unrelated individuals, and therefore, parasitizing the parental investment of those individuals. The advent of genetic marker techniques has made it possible to quantify natural rates of cuckoldry. Molecular techniques have been applied to birds (e.g. Burke *et al.* 1989; Gibbs *et al.* 1990; Smith *et al.* 1990; Gowaty & Bridges 1991) and mammals (McCracken & Bradbury 1977; Packer *et al.* 1991) and have revealed surprisingly high levels of cuckoldry. Although several studies in fish have shown that more than one male may fertilize eggs from the same female (Gandolfi 1971; Schroeder 1982; Maekawa & Onozato 1986; Hutchings & Myers 1988), to date, these techniques have not been applied to quantify natural cuckoldry levels. Researchers have relied on indirect measures such as changes in testes weight (Gross 1979) or behaviour in the nest (Dominey 1980; Gross 1982) as evi-

dence for cuckolder fertilization success. This is unfortunate because fish possess several attributes that make them a particularly interesting group in which to study cuckoldry. First, in contrast to the biparental care common in birds and mammals, parental care in fish is usually provided by the male alone (Blumer 1979; Gross & Sargent 1985). Secondly, also in contrast to birds and mammals, fish with male parental care typically have external fertilization (Gross & Shine 1981). Finally, many fish species have precocious males that pursue a specialized alternative mating behaviour as cuckolders specializing on reproductively parasitizing the life history of parental males (e.g. Gross & Charnov 1980; Gross 1984).

The bluegill *Lepomis macrochirus* is a species of freshwater teleost found in lakes and ponds throughout east-central North America (Scott & Crossman 1973). In Lake Opinicon, Ontario breeding occurs in colonies from late May through mid-July (Gross 1982). Males (aged 7–11 years) construct bowl-shaped depressions in the lake substrate as nests to which they attract females. At the onset of breeding, females (aged 4–8 years) congregate near a colony, and individuals enter nests to spawn with the resident males. During spawning the resident male and his female partner circle within the nest with the male on the outside to chase away any other males that attempt to intrude to fertilize or eat eggs. During this process females periodically dip over onto their side and extrude

eggs; males then release sperm to fertilize them. The eggs fall to the bottom of the nest where they immediately adhere to the substrate. When spawning ceases, the females leave the area, and the nesting males remain alone at the colony to provide parental care for the offspring. These males expend considerable energy fanning the eggs and guarding both eggs and larvae from predators (Coleman *et al.* 1985). It takes 6–13 days, depending on water temperatures, for the eggs to hatch and the larvae to develop into free-swimming fry and leave the colony; the males then disband the colony. Because their behaviour involves considerable parental investment, the life history of these nesting males has been termed 'parental' (Gross 1979).

During spawning, two kinds of males attempt to steal fertilizations from the resident parental males. The most common are precociously mature males, called 'cuckolders' (Gross 1979), that use sneaking and/or satellite tactics (Gross 1982). At 2–3 years of age, cuckolder males usually employ a sneaking behaviour that involves darting into a parental male's nest as a female dips to extrude eggs, releasing sperm, and then retreating quickly. However, cuckolders that are 4–5 years of age usually employ a satellite behaviour that involves the assumption of coloration and behaviour that mimics those of females. For spawning, a satellite male enters the nest of a parental male already spawning with one or more true females and poses as an additional willing female. If deceived, the parental male continues to spawn, allowing the satellite male to remain in the nest to spawn as well. Like females, cuckolder males leave the colony once spawning has ceased and never show parental care. In Lake Opinicon these cuckolder males die before reaching the age and size of parental males and therefore represent an alternative male life history (Gross & Charnov 1980).

Besides cuckolders, certain parental males will on occasion also attempt to steal fertilizations. Neighbouring males that have their own nests but no female present with which to spawn sometimes dart across the nest boundary in an attempt to steal fertilizations from adjacent males that are spawning. In addition, non-nesting males with the parental phenotype have also been observed to frequent colony areas during spawning and to dart into the nests of spawning parental males, presumably in an attempt to fertilize eggs. Following one of these transgressions, neighbouring and non-nesting parental males are driven away by the resident parental male.

The level of cuckoldry, as well as the success rates for cuckolding males, most likely varies widely among bluegill colonies (Gross 1991). Until now, however, cuckoldry levels have not been assessed directly. In the present study we use protein electrophoretic markers to accomplish two specific objectives: to demonstrate that cuckolders do, in fact, successfully fertilize eggs, and to quantify

natural levels of cuckoldry among bluegill colonies in Lake Opinicon with different habitat characteristics.

Methods

Protein electrophoresis

Genotypes at the *EST-1** locus were determined for individual bluegill using vertical starch gel electrophoresis coupled with histochemical staining procedures detailed in Philipp *et al.* (1979). Two alleles, *EST-1*100* and *EST-1*90*, were detected in the Lake Opinicon population. *In vitro* crosses using bluegill of known *EST-1** genotype produced offspring with appropriate genotypes, confirming that the banding patterns on the gels represented a heritable polymorphism. Nomenclature used was that of Shaklee *et al.* (1990). Tissue samples were taken from the caudal fin for immediate homogenization and electrophoresis or frozen at -20°C for later analysis. The removal of the tissue had no apparent effect on subsequent breeding behaviours of the fish.

Experimental pond populations

Two experimental populations of adult bluegill of known *EST-1** genotype were established in research ponds at the Illinois Natural History Survey (INHS) Aquatic Research Field Laboratory in Champaign, Illinois (USA). Specifically, bluegill were hand-netted from colonies in Lake Opinicon by skin divers during early June, 1985 and transferred live to containment facilities at the nearby Queen's University Biological Station (QUBS). Parental males were large individuals (> 170 mm total length) in breeding coloration holding territories; cuckolders were small, cryptic, reproductively mature males (< 110 mm total length) hiding in weed cover adjacent to the nests. Samples of caudal fin tissue were removed and used to determine the genotype at the *EST-1** locus for each individual as described above. Fish with the desired genotypes, parental males that were homozygous 90/90 and cuckolder males and females that were homozygous 100/100, were transported to the INHS and introduced into the research ponds (Table 1). These research ponds were 0.04 ha in size with a maximum depth of 1.5 m, had earthen bottoms, contained natural vegetation, and had natural populations of invertebrates to serve as food for the adults and their subsequent progeny. The test pond was stocked with females, as well as both parental and cuckolder males at a 1:6 ratio, similar to that found in Lake Opinicon (Gross 1979). In contrast, the control pond contained females and cuckolder males, but no parental males. The function of the control population was to determine if cuckolder males would switch their behaviour to construct nests and spawn as small parental males.

Table 1 Genetically tagged bluegill and their production of progeny in the experimental populations in INHS ponds

Phenotypes	Pond population	
	Control	Test
Adults		
Female		
Genotype	EST-1*100/100	EST-1*100/100
Number Stocked	10	10
Total Length (mm) ± SD	151.9 ± 5.49	152.3 ± 6.22
Number Retrieved	9	8
Total Length (mm) ± SD	171.2 ± 10.80	168.4 ± 6.82
Parental male		
Genotype	-	EST-1*90/90
Number Stocked	-	6
Total Length (mm) ± SD	-	182 ± 8.22
Number Retrieved	-	6
Total Length (mm) ± SD	-	189 ± 8.19
Cuckolder male		
Genotype	EST-1*100/100	EST-1*100/100
Number Stocked	36	36
Total Length (mm) ± SD	81.8 ± 17.47	83.2 ± 16.85
Number Retrieved	31	28
Total Length (mm) ± SD	128.7 ± 18.10	132.7 ± 10.34
Progeny		
Genotype	0	210
EST-1*90/100	-	141 (67.1%)
EST-1*100/100	-	69 (32.9%)

Fish spawned freely in the ponds, and cuckoldry activity was confirmed through visual observation. Cuckolder and parental males were distinguishable in the test pond by the difference in their body sizes. Both ponds were drained on 18 October 1985, the adult fish being transferred live to other research ponds, and the progeny being frozen for subsequent electrophoretic analysis. There was no significant difference between ponds in the initial total length of cuckolders or between ponds in the initial total length of females (Table 1, *t*-test, *P* > 0.05 for females & cuckolders). During the experimental period, adult mortality was low, and significant growth was shown by all sexual types, suggesting healthy populations (Table 1).

Natural colonies

To assess natural variation in fertilization success through cuckoldry, four test bluegill colonies were selected in Lake Opinicon that varied in physical habitat characteristics and as a result, in the density of cuckolders present during spawning (Table 2). Habitat characteristics and cuckolder densities were assessed by underwater observation at the time of spawning; observations were conducted for 15 min every 2 h from initiation of spawning activity to its cessation. Colonies selected were moderate in size, ranging from 29 to 73 nesting parental males.

Spawning was monitored visually, but allowed to proceed normally at each of the four colonies. Just prior to female departure following cessation of spawning, random samples of females and cuckolders were collected by skin divers with hand nets. These samples were frozen and later electrophoretically analysed to determine the frequency of the two alleles at the *EST-1** locus. Once spawning in each of these four colonies was completed, each nest that received eggs was labelled using a numbered tile. Each parental male was hand-netted from his numbered nest, a small sample of its caudal fin was taken for electrophoretic analysis as described above, and the male then returned to his nest. This entire procedure took less than 1.5 minutes per male, and all males quickly resumed parental care behaviour at their nests. Electrophoretic analyses of these tissue samples revealed the *EST-1** genotype for each parental male in all four test colonies.

On the third day post-hatching at each of the four test colonies, swimmers siphoned a random sample of approximately 1000 larvae from each of ten nests occupied by parental males homozygous for the *EST-1* 100* allele. These larvae were transported to Illinois and stocked as free-swimming fry in 0.08 ha research ponds at the INHS. Each of the four rearing ponds was stocked with 5000 bluegill larvae, a random sample of 500 from each of the ten nests from which larvae were removed from one of the four test colonies; each pond received larvae from a different colony. The bluegill were allowed to grow

Table 2 Characteristics of the four test bluegill colonies in Lake Opinicon, Ontario

Colony site	Cuckolder density	Nesting parentals	Location	Cover	Water depth (range)
CBBB	absent	61	sandbar across mouth of large bay	absent	1.0-1.3 m
WBBB	low	29	bar/shoreline interface	low(some short plants & woody debris)	0.8-1.0 m
SB	moderate	73	centre of small & windy bay	average (some plant growth, rock rubble & woody debris)	1.1-1.3 m
WP	high	53	near-shore along sheltered island	dense (heavy plant growth & debris)	0.9-1.1 m

CBBB, Central Birch Bay Bar; WBBB, West Birch Bay Bar; SB, Sandy Bay; WP, Whispering Pines.

through the summer, after which time the ponds were drained and samples of up to 250 progeny from each pond were frozen for electrophoretic analysis.

Calculations of paternity

Paternity of any offspring produced in the experimental pond populations could be determined directly from electrophoretic determination of genotypes at the *EST-1** locus. Because in these populations all cuckoldler males were homozygous 100/100, all parental males were homozygous 90/90, and all females were homozygous 100/100, progeny with a 100/100 genotype would have to have been produced by cuckoldler fathers and those with a 90/100 genotype by parental fathers.

Paternity of offspring from the four natural colonies in Lake Opinicon, however, could not be determined directly from *EST-1** genotypes, but were estimated from calculations based upon the allele frequencies at that locus in the population. Because all fry were sampled from nests of parental males that were homozygous for the *EST-1*-100* allele, progeny that were *EST-1*90/90* necessarily would have to have been fathered by cuckoldling males and not the resident nesting parental male. From the frequency of this genotype among offspring and the allele frequencies within the population, the equations developed below were used to calculate cuckoldry levels.

The level of cuckoldry (estimated proportion of non-kin) in nests is calculated as:

$$\frac{\text{observed frequency of non-kin}}{\text{probability of detection.}} \quad (1)$$

The observed frequency of non-kin in nests is calculated as:

$$\frac{\text{number of } EST-1*90/90 \text{ progeny}}{\text{number of total progeny.}} \quad (2)$$

The probability of detection is calculated as:

$$E_p(F_f \times F_p) + E_c(F_f \times F_c), \quad (3)$$

where F_f is the frequency of the *EST-1*90* allele in females, F_p is the frequency of the *EST-1*90* allele in intruding parental males, and F_c is the frequency of the *EST-1*90* allele in intruding cuckoldler males; E is the proportion of fertilizations that involve parental (E_p) or cuckoldler (E_c) males ($E_p + E_c = 1$). Since the frequency of the *EST-1*-90* allele did not differ significantly between the two types of males, cuckoldlers and parentals, then equation 1 can be reduced to $F_f \times F_m$ where F_m is the frequency of the *EST-1*-90* allele in all males. A discussion of somewhat similar methods for calculating probabilities of exclusion can be found in Westneat *et al.* (1987) and Wrege & Emlen (1987).

Several assumptions exist in our methods for calculating paternity. First, we assume that the alternative alleles at the *EST-1** locus have no differential effect on the survivorship and breeding behaviour of individuals. Second, we assume that the *EST-1*90* allele frequency in non-nesting intruding parental males, which were uncaptured, is the same as the nesting parental males that were sampled. Third, we assume that in the INHS research ponds there is no differential survival of progeny fathered by intruders and nest owners. Because the cuckoldler life history is at least partly inherited, and cuckoldler sons have faster growth than sons that delay maturation to become parental males (Gross & Philipp, unpublished data), cuckoldlers may acquire greater mortality through risk-prone foraging. There were, however, no piscivorous predators in the research ponds. Fourth, we assume that the sample of 10 parental nests per colony will adequately describe the average paternity of nesting bluegill in that colony.

Results

Cuckoldry in the experimental pond populations

Daily visual observations of the control pond population revealed that no cuckoldler males built nests and that no spawning took place during the 1985 breeding season. This observation was supported by the absence of offspring when the pond was drained. Thus, these cuckoldler males did not employ parental male behaviours in an attempt to reproduce.

In contrast, visual observations of the test pond population revealed two distinct spawning bouts. During both of these bouts only parental males were observed to build nests and rear young in them. Successful reproduction by cuckoldler males could have occurred therefore only through the employment of sneaker and/or satellite tactics. Cuckoldler males were observed frequently to intrude into parental male nests during spawning. Successful reproduction in the test population was verified when the pond was drained and 210 offspring were collected (Table 1). Electrophoretic analysis of all 210 offspring revealed that 32.9% had the *EST-1*100/100* genotype, indicating that they were not sired by the resident parental male, but by a cuckoldler male.

Cuckoldry in the natural colonies in Lake Opinicon

The genetic structure of the four test colonies in Lake Opinicon is summarized in Table 3 and analysed in Table 4. Neither genotype frequencies nor allele frequencies were significantly different among the colonies for any one sexual type. In addition, these frequencies were not significantly different between parental and cuckoldler

Table 3 Genetic structure at the *EST-1** locus for the four test bluegill colonies in Lake Opinicon.

Colony site	No. of nesting males	N	Genotype numbers			*90 freq.
			90/90	90/100	100/100	
Parental Males						
CBBB	61	56	6	18	32	0.268
WBBB	29	27	1	12	14	0.259
SB	73	73	5	29	39	0.267
WP	53	50	2	12	36	0.160
overall	216	206	14	71	121	0.240
HWE			12	75	119	
Cuckolder Males						
CBBB		-	-	-	-	-
WBBB		29	3	12	14	0.310
SB		55	4	21	30	0.264
WP		42	4	13	25	0.250
overall		126	11	46	69	0.270
HWE			9	50	67	
All Males						
CBBB		56				0.268
WBBB		56				0.286
SB		128				0.266
WP		92				0.201
overall		332				0.252
Females						
CBBB		64	13	26	25	0.406
WBBB		49	5	28	16	0.388
SB		46	4	26	16	0.370
WP		31	4	15	12	0.371
overall		190	26	95	69	0.387
HWE			29	90	71	
Males and Females						
CBBB		120	19	44	57	0.342
WBBB		105	9	52	44	0.333
SB		174	13	76	85	0.293
WP		123	10	40	73	0.244
overall		522	51	212	259	0.301
HWE			47	220	255	

N = number of fish genotyped;
 HWE = Hardy-Weinberg Equilibrium
 (expected number of genotypes).

Table 4 Statistical analyses of data in Table 3

	N	χ^2	d.f.	P
Genotype frequencies among colonies				
Parentals	206	7.35	6	> 0.25
Cuckolders	126	1.23	4	> 0.85
Females	190	5.76	6	> 0.45
Males and females	522	17.07	6	< 0.01
Allele frequencies among colonies				
Parentals	412	4.68	3	> 0.15
Cuckolders	252	0.68	2	> 0.70
Females	380	0.40	3	> 0.90
Males and females	1044	7.02	3	< 0.06
Overall HWE prediction	522	0.69	2	> 0.70
Allele frequencies between types				
Males and females	1044	22.05	2	< 0.001
Parentals vs. females	792	19.87	1	< 0.001
Parentals vs. cuckolders	664	0.85	1	> 0.30
Cuckolders vs. females	632	9.52	1	< 0.01

males. However, among colonies genotypic frequencies of males differed significantly from those of females. This difference between the sexes is apparently due to an 'excess' of the *EST-1*90* allele in females, perhaps because of random year class differences, rather than population differentiation among the colonies.

We assessed the genotypes for 748 bluegill progeny that were taken from these four colonies and raised in Illinois. Homozygous *EST-1*90/90* progeny, those fathered by intruders, were found in three of the colonies. Cuckoldry levels were calculated for each of these colonies using the exclusion formulae and the allele frequencies in each colony and sexual type (Table 5). The paternity of nesting parental males ranged from 41 to 100% among the four colonies, the difference in cuckoldry levels being significantly correlated with the relative densities of cuckolders that frequented the colonies ($N=4$, $r_s = 1$, $P = 0.05$).

Colony site	Progeny			Paternity		
	N	Genotypes		Parental Males	Cuckolding Males	
		90/90	90/100			100/100
CBBB	132	0	31	101	100	0.0
WBBB	250	4	91	155	85.6	14.4
SB	116	3	29	84	73.5	26.5
WP	250	11	95	144	41.3	58.7

Table 5 Paternity of nesting parental males versus cuckoldling males among natural bluegill colonies in Lake Opinicon, Ontario. Paternity was calculated using the observed frequency of 90/90 genotypes (known to be non-parental male offspring) and the allele frequencies within the population (Table 4), using the equations presented in the methods

Discussion

Data from the experimental pond populations unequivocally demonstrate that the behaviour employed by cuckoldler males permits them to fertilize eggs successfully. Data from the natural populations show that in Lake Opinicon cuckoldry rates vary widely (0–58.7%) among different colonies, depending upon the density of cuckolders present during spawning. We cannot presently quantify how much of this cuckoldry may have been due to intruding parental phenotypes (non-nesters or nesting neighbours), however, we note that over 90% of intrusions to steal fertilizations are by the specialized precocious males of the alternative cuckoldler phenotype (Gross 1982). It seems quite apparent therefore that in nature cuckoldler males are also highly successful at fertilizing eggs.

The high level of cuckoldry observed in certain colonies might be expected to destabilize parental care (Trivers 1972; Werren *et al.* 1980; Westneat & Sherman 1993). Coleman *et al.* (1985) have shown that the level of bluegill parental investment is a function of brood-number; parental males therefore might be expected to evaluate cuckoldry rates and alter their parental investment accordingly. However, even where almost 60% of the progeny in the colony were estimated to be non-kin, parental males provided care for the full parental care period. This result suggests that at least under some situations parental male bluegill cannot determine cuckoldry rates or are willing to tolerate high levels without altering their parental care efforts.

Several authors have suggested that paternal care is more common in fish than in birds or mammals because external fertilization provides higher paternity (Ridley 1978; Baylis 1981). The high levels of cuckoldry observed among bluegill, however, suggest that this mode of fertilization may have little determination on average parentage – an hypothesis previously disputed on theoretical grounds alone (Werren *et al.* 1980; Gross & Shine 1981).

The variation in paternity among colonies can be predicted largely from cuckoldler density, which is thought to be due to ecological differences in the availability of refuges from predation (Gross 1982, 1991). Because of this relationship, parental males should prefer colony sites

with minimal cover, where cuckoldler pressure is low (e.g. CBBB). Competition for females at these preferred sites, however, may well force colony establishment at some less preferred site (e.g. the WP colony site). This apparent trade-off between number of cuckoldler males and number of females predicts an ESS (Maynard Smith 1982) distribution of males among colony sites. The ecologically-based variation in paternity reported here and the accumulating evidence of high rates of cuckoldry in natural populations of a number of different taxa suggest that efforts to measure simple ecological variables to explain breeding dispersion must now also consider cuckoldry levels.

Acknowledgements

For field and laboratory assistance we thank J. Claussen, J. Epifanio, J. Koppelman, J. Van Orman, M. Horner, J. Weber, and I. Fleming, and especially David B. F. Philipp for all the time he spent underwater. We thank the management of the Queen's University Biological Station at Lake Opinicon for laboratory and field facilities. Comments on the manuscript were kindly given by N. Gerrish, J. Reynolds, A. Toline, I. Fleming, L. Dueck, and R. Coleman. Financial support was provided by the National Science and Engineering Research Council of Canada to Gross, and the Illinois Natural History Survey to Philipp.

References

- Baylis JR (1981) The evolution of parental care in fishes, with references to Darwin's rule of male sexual selection. *Environmental Biology of Fishes*, **6**, 223–251.
- Birkhead TR, Møller AP (1992) *Sperm Competition In Birds: Evolutionary Causes and Consequences*. London: Academic Press.
- Blumer LS (1979) Male parental care in the bony fishes. *Quarterly Review of Biology*, **54**, 149–161.
- Burke T, Davies NB, Bruford MW, Hatchwell BJ (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature*, **338**, 249–251.
- Coleman RM, Gross MR, Sargent RC (1985) Parental investment decision rules: a test in bluegill sunfish. *Behavioral Ecology and Sociobiology*, **18**, 59–66.
- Dominey W (1980) Female mimicry in bluegill sunfish – a genetic

- polymorphism? *Nature*, **284**, 546–548.
- Gandolfi G (1971) Sexual selection in relation to the social status of males in *Poecilia reticulata* (Teleostei: Poeciliidae). *Bulletin of Zoology*, **38**, 35–48.
- Gibbs HL, Weatherhead PJ, Boag PT, White BN, Tabak LM, Hoysak DJ (1990) Realized reproductive success of polygynous red-winged blackbirds revealed by DNA markers. *Science*, **250**, 1394–1397.
- Gowaty PA, Bridges WC (1991) Behavioral, demographic, and environmental correlates of extra pair fertilizations in eastern bluebirds, *Sialia sialis*. *Behavioral Ecology*, **2**, 339–350.
- Gross MR (1979) Cuckoldry in sunfishes (*Lepomis*: Centrarchidae). *Canadian Journal of Zoology*, **57**, 1507–1509.
- Gross MR (1982) Sneakers, satellites and parentals: polymorphic mating strategies in North American sunfishes. *Zeitschrift für Tierpsychologie*, **60**, 1–26.
- Gross MR (1984) Sunfish, salmon, and the evolution of alternative reproductive strategies and tactics in fishes. In: Potts G, Wootton R, eds. *Fish Reproduction: Strategies and Tactics*, pp. 55–75. Academic Press, London.
- Gross MR (1991) Evolution of alternative reproductive strategies: frequency-dependent sexual selection in male bluegill sunfish. *Transactions of the Royal Society of London B*, **332**, 59–66.
- Gross MR, Charnov EL (1980) Alternative male life histories in bluegill sunfish. *Proceedings of the National Academy of Sciences of the USA*, **77**, 6937–6940.
- Gross MR, Sargent RC (1985) The evolution of male and female parental care in fishes. *American Zoologist*, **25**, 807–822.
- Gross MR, Shine R (1981) Parental care and mode of fertilization in ectothermic vertebrates. *Evolution*, **35**, 775–793.
- Hutchings JA, Myers RA (1988) Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia*, **75**, 169–174.
- Maekawa K, Onozato H (1986) Reproductive tactics and fertilization success of mature male Miyabe charr, *Salvelinus malma miyabei*. *Environmental Biology of Fishes*, **15**, 119–129.
- Maynard Smith J (1982) *Evolution and the Theory of Games*. Cambridge: Cambridge University Press.
- McCracken GF, Bradbury JW (1977) Paternity and genetic heterogeneity in the polygynous bat, *Phyllostomus hastatus*. *Science*, **198**, 303–306.
- Packer C, Gilbert DA, Pusey AE, O'Brien SJ (1991) Kinship and cooperation in African lions: a molecular genetic analysis. *Nature*, **351**, 562–565.
- Philipp DP, Childers WF, Whitt GS (1979) Evolution of differential patterns of gene expression: A comparison of the temporal and spatial patterns of isozyme locus expression in two closely related fish species (northern largemouth bass, *Micropterus salmoides salmoides* & smallmouth bass, *Micropterus dolomieu*). *Journal of Experimental Zoology*, **210**, 473–488.
- Ridley M (1978) Paternal care. *Animal Behaviour*, **26**, 904–932.
- Schroeder SL (1982) The influence of intrasexual competition on the distribution of chum salmon in an experimental stream. In: Brannon EL, Salo EO, eds. *Salmon and Trout Migratory Behaviour Symposium*, pp. 275–285. School of Fisheries, University of Washington, Seattle.
- Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin, **184**, 1–966.
- Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature for protein-coding loci in fish. *Transactions of the American Fisheries Society*, **119**, 2–15.
- Smith HG, Montgomerie R, Poldman T, White B, Boag P (1990) DNA fingerprinting reveals relation between tail ornaments and cuckoldry in barn swallows *Hirundo rustica*. *Behavioral Ecology*, **2**, 90–98.
- Trivers RL (1972) Parental investment and sexual selection. In: Campbell BG, ed. *Sexual Selection and the Descent of Man (1871–1971)*. Aldine-Atherton Press, Chicago.
- Werren JH, Gross MR, Shine R (1980) Paternity and the evolution of male parental care. *Journal of Theoretical Biology*, **82**, 619–631.
- Westneat DF, Frederick PC, Wiley HR (1987) The use of genetic markers to estimate the frequency of successful alternative reproductive tactics. *Behavioral Ecology and Sociobiology*, **21**, 35–45.
- Westneat DF, Sherman PW (1993) Parentage and the evolution of parental behaviour. *Behavioral Ecology*, **4**, 66–77.
- Westneat DF, Sherman PW, Morton ML (1990) The ecology and evolution of extra-pair copulations in birds. In: Power DM, ed. *Current Ornithology*, Vol. 7, pp. 331–369. New York, Plenum Press.
- Wrege PH, Emlen ST (1987) Biochemical determination of parental uncertainty in white-fronted bee-eaters. *Behavioral Ecology and Sociobiology*, **20**, 153–160.