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* Send correspondence to S.N.Z.

21 December 1981; revised 8 March 1982

Is Sperm Cheap? Limited Male Fertility and Female Choice in the Lemon Tetra (Pisces, Characidae)

Abstract. *In the laboratory, fertilization rates achieved by male lemon tetras decline with spawning frequency. Even when the number of females is not limited, males can produce only four times as many offspring as females. Females show a preference for males that have not recently spawned as opposed to those that have. The cost of producing sufficient sperm to maximize fertilization rates may therefore reduce the intensity of sexual selection in this polygamous fish species.*

The massive difference in the size of the egg and the sperm forms the basis of much contemporary theory concerning sexual dimorphism and social behavior. A case in point is the theory of sexual selection developed by Darwin (*1*) to explain the evolution of characters, seen especially in males, that seemed unlikely to have evolved by natural selection but could offer a reproductive advantage either in competing with the same sex or in courting the opposite sex. For polygamous species in which paternal care is absent, Bateman (*2*) argued that since males invest so much less in each zygote than do females, males are potentially capable of producing many more offspring than are females. The reproductive success of females is limited by their ability to produce eggs, and the reproductive success of males is limited by their ability to obtain mates. Competition between males and female choice cause some males to achieve more of their potential reproduction at the expense of others, resulting in a higher variance of reproductive success among males than among females. This results in a greater intensity of sexual selection on males (*2*, *3*). Trivers (*4*) generalized this argument to include investment in parental care, concluding that the sex that invests less in each offspring is limited by the availability of the sex that invests more and is subject to a greater intensity of sexual selection. This conclusion has been advanced in recent discussions of the evolution of social behavior (*5*).

These theoretical arguments do not take into account the number of sperm produced to fertilize each egg. The rate of external fertilization is maximized only at sperm concentrations of about 10^6 sperm per milliliter, and similar concentrations are required in the ejaculates of internal fertilizers (*6*), potentially reducing the upper limit to male fertility (*7*). A decline in sperm concentrations or fertilization rates with successive ejaculations in internal fertilizers (*8*) may indicate the high cost of producing adequate sperm concentrations (*7*). However, many investigators (*9*), in field studies, assume that males are not limited by sperm supply (*10*). Although laboratory observations of sperm depletion may be artifacts resulting from matings at frequencies that do not occur in nature, we noted indications of fertility limitation in a small, tropical fish at mating frequencies that could occur in nature. Our study documents male fertility limitation in an external fertilizer; we investigated the mating frequencies at which males would be limited by mates and by sperm supply and compared maximal offspring production of males and females. We found that mate selection by females supports the argument that sperm limitation may occur in nature. The data call into question the assumption that "sperm is cheap" and show that the reproductive success of the sex that invests less in each gamete is not necessarily limited exclusively by the availability of the opposite sex.

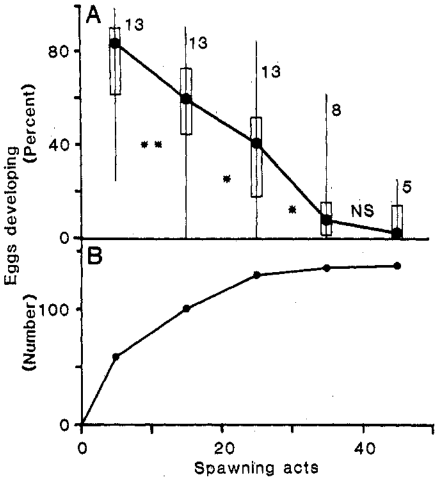

Fig. 1. The relation between total daily spawning acts and fertility (A) or estimated, total number of offspring (B) in male lemon tetras. The graph in (A) is based on 13 different tests carried out on six individual males. Eggs were removed after each ten spawning acts and incubated to determine fertility, but the total number of spawning acts achieved by each male varied as a function of female availability and fecundity on the test day. Dots show medians, open rectangles are upper and lower quartiles, and vertical lines represent ranges. Sample sizes are indicated, as is the statistical significance of the differences between adjacent means (Wilcoxon matched-pairs test; $**P < .01$; $*P < .05$; *NS*, not significant). The graph in (B) is based on the median fertility in (A) and an average production of seven eggs per spawning act.

The lemon tetra *Hyphessobrycon pulchripinnis* is a small (31 to 38 mm), characid fish native to the Amazon Basin. In our laboratory, spawning takes place during the first 2 hours of the morning. At this time males defend spawning sites while females school. A female ready to spawn enters a male's territory, is briefly courted, and approaches a spawning site such as a clump of plants. The male positions himself beside her. After a period of quivering, the pair leap forward releasing eggs and presumably sperm. On the average, ovulation occurs once every 4 days, and on this day each female spawns an average of 23 times, producing a total of 160 eggs. Mating is promiscuous, and males spawn every day that receptive females are available. Parental care is absent; both sexes school together after spawning (*11*). Male fertility as a function of the number of spawning acts was determined by placing one at a time females that had ovulated with an isolated male and observing the spawning frequency (*12*). Egg traps used as spawning sites (*13*) were replaced after each series of ten spawning acts. When a female stopped spawning she was replaced. Each egg collection was incubated separately for at least 4 hours, and the percentage of developing eggs was used as an index of the fertilization rate.

The percentage of eggs developing declines, nearly linearly, as a function of the number of spawning acts by the male (Fig. 1A). The decline is most easily explained by a reduction in the quantity or quality of sperm released at successive spawnings (*14*). A statistically significant difference in the rate of fertilization between the first and second set of ten spawning acts indicates that a male cannot fertilize all the eggs released in the 23 spawnings of a single female. After ten spawning acts male reproductive success is limited by both female availability and sperm supply. After 20 spawnings, male lemon tetras could enhance their reproductive success more by restoring their fertilization rate rather than by gaining additional spawnings. After 30 spawnings, male reproductive success is limited almost entirely by fertilization rate. If females are not limited, males could expect to fertilize about 136 eggs per day—the asymptote in Fig. 1B. This is almost identical to the number of developing eggs produced by females every 4 days (*15*).

Could fertilization rate limit reproductive success in nature? With a 1:1 sex ratio, all males participating equally, and female ovulations evenly distributed over days, each male would expect about six spawnings per day, and fertility limitation would be insignificant. However, random processes, intermale competition, female choice, and spawning site limitation could result in some males having much greater than average mating success (*16*). Since some males would be likely to mate more than 10 times per day, it is a reasonable possibility that fertility limitation occurs in the field. If so, females should choose males on the basis of previous spawning frequency (*7*). A second experiment was designed to determine whether females discriminate in this way.

Female-choice tests were carried out in 45-liter aquariums divided into three sections by clear Plexiglas partitions. Resident males were established in the two end sections. On the test day a female that had ovulated was transferred into the middle section. At the same time another ovulated female was placed with one of the resident males and a female that had not ovulated was placed with the other. After an average of 19.5 spawnings (standard error, 1.9) by pairs of resident males and ovulated females, the females were removed from the end compartments. At 15-second intervals for the next 25 to 40 minutes, we recorded the male to which the female in the center section was closer (*17*). On a subsequent day the test was repeated with the same resident males and different females, with the previously nonspawning male as the spawner. Then a different combination of males was established, and the test was repeated. In all, six pairs of tests were performed with 12 females and 6 males.

In 11 of 12 tests, the females spent more time near the unspawned male. The paired tests, a partial control for differences in attractiveness between the two males, show that all six males were more preferred if they had not spawned. On the average, females spent 62.5 percent of their time near the unspawned males (Table 1). Although not conclusive because there may be other advantages in avoiding sites of recent spawning, these observations support the suggestion that female mate preference is influenced by male spawning history, and that fertility limitation is a factor that influences the reproductive success of females in the field (*18*). Similar evidence comes from studies of the effect of prior mating on courtship success in *Drosophila* (*19*).

If male reproductive success is limited by sperm supply, then the intensity of sexual selection in males will be much less than expected in a polygamous species in which paternal care is absent. Furthermore, fertile males easily become a limited resource for females. Female avoidance of males with high recent spawning rates reduces the variation in male reproductive success. At the same time, competition for access to fertile males increases opportunities for sexual selection among females. Thus sexual differences in the intensity of sexual selection should be reduced as a function of the amount of sperm required to achieve fertilization.

Table 1. The effect of male spawning activity on female mate choice in the lemon tetra. The results are shown for six pairs of tests involving six males and 12 females in which females were scored for the percentage of time in which they were closer to the male that had not spawned. The mean preference controls for differential attractiveness of the two sides of the aquarium by averaging trials in which the same two males alternated as the spawners. Overall, the females preferred unspawned males 62.52 percent of the time (difference from 50 percent is statistically significant by *t*-test; $P < .01$).

Trial	Spawning acts (No.)	Time near nonspawned male (%)	Mean preference (%)
1a	18	74.5	70.45
1b	25	66.4	
2a	26	71.8	71.10
2b	23	70.4	
3a	13	53.0	62.00
3b	25	71.0	
4a	15	60.3	57.65
4b	6	55.0	
5a	15	50.9	57.55
5b	23	64.2	
6a	18	64.5	56.40
6b	27	48.3	

KEN NAKATSURU
DONALD L. KRAMER

Department of Biology,
McGill University,
1205 Avenue Docteur Penfield,
Montréal, Québec, Canada H3A 1B1

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10. The exceptions are mating systems in which males compete through sperm production [see, for example, G. A. Parker, *J. Insect Physiol.* **16**, 1301 (1970)].
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12. Males held under the same conditions as the females were isolated in their normal spawning territories about 4 hours before the lights went out on the day preceding a test.
13. Egg traps were 150-mm plastic petri dishes covered by nylon screening to protect the eggs from cannibalism, with bundles of thin, green plastic strips placed over the screen to attract the fish.
14. The decline was not due to decreased viability of eggs released later by a given female because the percentage of developing eggs continued to decline with subsequent females, and it was not due to a time-dependent change in viability of eggs because the same percentage of eggs was developing from females prevented from spawning for 2 hours as from females that started spawning at dawn.
15. If the median 83.7 percent development rate of eggs for the first ten spawning acts (Fig. 1A) represents the percentage of viable eggs, then females can produce an average of 134 developing eggs (160 × 0.837) every 4 days.
16. For examples in fishes see J. F. Downhower and L. Brown [*Anim. Behav.* **28**, 728 (1980)], R. R. Warner and S. G. Hoffman [*Evolution* **34**, 508

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17. Since we could not open the partitions to observe actual spawning rates because of high aggression in this confined space, we used proximity to indicate preference.
18. Females could have used morphological, chemical, or behavioral cues to discriminate between the two males, as well as the observed spawning frequency. However, there were no apparent differences in the appearance or behavior of the males that had spawned and those that had not spawned.
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28 August 1981; revised 7 December 1981

Interdigitation of Contralateral and Ipsilateral Columnar Projections to Frontal Association Cortex in Primates

Abstract. *The combined use of two anterograde axonal transport methods reveals that in the prefrontal association cortex of macaque monkeys, associational projections from the parietal lobe of one hemisphere interdigitate with callosal projections from the opposite frontal lobe, forming adjacent columns 300 to 750 micrometers wide. The finding of separate and alternating ipsilateral and contralateral inputs in the frontal association cortex opens up new possibilities for the functional analysis of this large but unexplored area of the primate brain.*

Over the past 25 years, one of the most important concepts for understanding the structure and function of the neocortex has been that of the vertical compartmentalization of its cells and connections. Such organization was suggested in electrophysiological analysis of somatosensory cortex by Mountcastle (*1*); later functional and morphological research extended this principle of organization to receptive field properties and connectivity of the primary visual and auditory as well as somatosensory areas of the cortex (*2*, *3*). In these systems, vertical "columns" or "bands" related to input serving one class of sensory receptor alternate with input from another group of receptors within the same modality. More recently, it has become apparent that vertical organization of inputs is not solely a property of sensory systems but applies to association cortex as well (*4*, *5*). Our previous studies using autoradiographic methods for tracing connections have shown that corticocortical projections to the prefrontal association cortex form well-defined bands or columns that in coronal section traverse the entire width of the cortex and alternate with vertical territories devoid of label. However, the input to the unlabeled spaces has so far remained unknown. By combining two anterograde tracers—one based on anterograde axonal transport of horseradish peroxidase (HRP) and the other on that of tritiated amino acids—one can label convergent projections in the same animal. Using this research strategy, we hereby provide what is, to our knowledge, the first evidence that callosal (contralateral) terminals alternate with associational (ipsilateral) terminals in selected cytoarchitectonic areas of primate association cortex. Such side-by-side registration of inputs from the two hemispheres may be relevant to the cerebral mechanisms underlying interhemispheric integration.

Our evidence is based on results obtained from four macaque monkeys (three rhesus and one fascicularis) killed 2 days after a mixture of [^{3}H]leucine and [^{3}H]proline (100 μCi/μl) was injected into the midregion of the dorsal bank of the principal sulcus (Brodmann's area 9) in the left hemisphere and acrylamide-bis gel HRP pellets (0.5 by 1.5 mm) were simultaneously implanted in the posterior rim of the intraparietal sulcus (Brodmann's area 7) of the parietal lobe in the right hemisphere (Fig. 1). In the monkey, these cortical areas project to the prefrontal cortex in the right hemisphere, via callosal and intrahemispheric routes, respectively (*4*, *6*). Since the terminals of these two inputs independently exhibit a columnar mode of termination in the prefrontal cortex (*4*, *7*), their arrangement within this zone could take one of several forms: (i) callosal and associational afferents may fully or partially overlap; (ii) they may interdigitate with one another, or (iii) they may have no discernible relationship to one another. To determine which of these possibilities obtained, alternate coronal sections through the prefrontal cortex of the right hemisphere were histochemically reacted with tetramethylbenzidene to reveal HRP reaction product (*8*). The intervening sections were processed by standard autoradiographic techniques. Through the use of blood vessels as fiducial marks, photographs and camera lucida drawings of the territories of terminal labeling in the adjacent 50-μm sections were overlaid to document the relationship of the labeled territories.

In accordance with previous results (*4*), analysis of the autoradiographic data revealed that labeled fibers originating from the opposite prefrontal cortex were distributed in 300- to 750-μm bands that extended across all layers of the cortex and alternated with spaces of variable width in which radioactivity was not above background (Fig. 2, A and B). For the most part, these callosal columns were located in the dorsal bank of the principal sulcus homotopic to the injection site, although some assumed a heterotopic position in the adjacent ventral bank of the same sulcus. The HRP-labeled fibers originating from the inferior parietal cortex were also distributed in roughly the same area of the prefrontal cortex; they also formed discontinuous bands alternating with spaces of variable width that contained little or no reaction product (Fig. 2, C and D). Like callosal columns, the ipsilateral associational columns spanned all cortical layers. Although the ipsilateral projections from the parietal lobe were distributed in various portions of the prefrontal cortex including the dorsal and lateral convexities, many were located in portions of the principal sulcus that contained radioactively labeled callosal terminals.

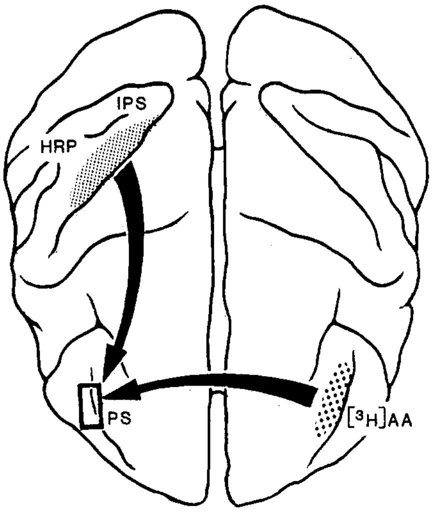

Fig. 1. Double-labeling strategy. Tritiated amino acids ([^{3}*H*]*AA*, coarse stipple) were injected into the principal sulcus (*PS*) of the left hemisphere to label callosal projections to the principal sulcus in the right hemisphere; HRP pellets (fine stipple) were implanted into the posterior bank of the intraparietal sulcus (*IPS*) in the right hemisphere to label associational projections to the principal sulcus in the right prefrontal cortex. The rectangle marks the areas of convergence of the ipsilateral and contralateral projections examined.