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ABSTRACT — In a population of immature leopard frogs (*Rana pipiens*) at Mud Lake, Washington County, Minnesota, a high frequency of unilateral deformity of the hind legs was observed in 1965. Metamorphosis from tadpole to frog took place during July, and samples in late July averaged 14.8% deformed frogs. By late September, the frequency of deformed frogs had decreased to 3.6%, presumably as the result of natural selection.

In 1953 Waddington, in reviewing the progress in the study of evolution over the previous 30 years, noted that two major lines of research had been followed, one the development of a mathematical theory of evolution and the other the experimental study of evolutionary phenomena in nature. Of the latter he said that the major contributions had been the revelation of the wealth of concealed genetic variation, both genic and chromosomal, in natural populations, and the discovery that the differences between races or related species were similar to those causing variation within a single breeding population. This finding permits the conclusion that evolution has been brought about by natural selection operating on this kind of genetic variability. Waddington went on to state, "Further, in a certain number of cases, the actual occurrence of natural selection can be demonstrated."

Two notable examples of natural selection have been reported since 1953, Allison's study (1955) of sickle cell polymorphism and Kettlewell's work (1961) on industrial melanism. However, the very frequency with which these two examples are cited in the literature indicates how few examples of natural selection are available.

This paper reports an apparent case of natural selection in a wild population of *Rana pipiens*. It seems to represent a case of stabilizing selection and, thus, is more comparable to the 1898 study by Bumpus with English sparrows than to either industrial melanism or sickle cell polymorphism.

Methods and Sampling

During 1965 and 1966 a number of leopard frog populations in the vicinity of Minneapolis and St. Paul, Minnesota, were sampled at monthly intervals during the spring, summer, and fall months. In one of these populations at Mud Lake, Washington County, Minnesota, an exceptionally high frequency of deformed individuals was found among the newly metamorphosed frogs at the end of July, 1965.

The frogs were captured with a net, scored in the field, toe clipped for individual identification, and released. For all samples, notes were routinely made on the unusual features of any frog. The samples were collected to get estimates of population size and the gene frequency of the burnsi gene (Merrell, 1965).

Some frogs showed signs of having been wounded. The wounds could have resulted from predators, from cars, or, rarely, from the net used to gather samples. The differences between wounded and deformed frogs were so clear cut that no hesitancy was felt in scoring one or the other condition. In Table I wounded frogs are included in the "normal" category.

In the deformed frogs the hind legs were affected, with the anomaly ranging from deformed toes through absence of foot, or lower leg, or even the entire leg in some cases. The deformity was unilateral in all instances.

A similar condition was found in *Rana pipiens* reared in the laboratory by L. W. Browder (personal communication.) However, this condition was bilateral and less severe. The underlying causative mechanism in that case was not determined, but since it occurred in several unrelated families, the rearing conditions were undoubtedly a major factor. The trait in the wild population probably also had a large environmental component, for it was asymmetrical and occurred among the progeny from many different matings developing in a common environment.

Leopard frogs in central Minnesota complete breeding in about a week in early spring. The egg masses are laid in shallow breeding ponds, usually in close proximity to one another. The tadpoles hatch out in about a week and then develop in the breeding ponds for about three months, usually completing metamorphosis by mid-July. The progeny from a single mating can be identified only so long as the egg mass is intact. As soon as the tadpoles start to swim about, the progeny of different matings start to mingle. By the time of metamorphosis it seems safe to conclude that tadpoles from all of the matings in a pond have been thoroughly and randomly mixed.

Mud lake is a large shallow pond split by a county road across its center and with a culvert connecting the two halves. The samples were taken along the 400-yard stretch of road through the pond. Even though the area sampled was only a small part of the available favorable habitat, the random mixing during the tadpole stage and the linear sampling technique both suggest that the samples were representative of the Mud Lake population.

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TABLE 1. Immature *Rana pipiens* in samples from Mud Lake, Washington County, Minnesota

1965 Date	Day	Pipiens Normal	Pipiens Deformed	Burnsi Normal	Burnsi Deformed	N	% Deformed
July Total		139	24	10	2	175	14.8%
July 28	0	56	17	6	1	80	22.5%
July 30	2	83	7	4	1	95	8.4%
Aug. 17	20	82	13	8	2	105	14.2%
Sept. 23	57	201	7	10	1	219	3.6%
Total		422	44	28	5	499	9.8%

$X^2_{1df} = 16.759^{**}$ $p < 0.01$ for difference between the July and August samples and the September sample.
$X^2_{1df} = 1.133$ No significant difference in frequency of deformity between pipiens and burnsi.

Results and Discussion

Table 1 gives the data on the frequency of deformed frogs among the immature frogs in the 1965 samples taken at Mud Lake. There was no significant difference between burnsi and pipiens frogs in the frequency of the deformity. However, the July 28 and July 30 samples were significantly different from one another at the 1% level ($X^2_{1df} = 6.81$; $p < 0.01$). There is no obvious explanation for the low frequency of the deformity in the July 30 sample. The only known difference is that the July 28 sample was taken in late afternoon and the July 30 sample in midmorning. Under the circumstances, it seems best to accept both samples as equally valid estimates for the July population and to combine them in order to estimate the frequency of the deformity in July. Even for the low July 30 sample there was less than one chance in ten that it was not significantly different from the September 23 sample ($X^2_{1df} = 3.117$; $0.10 > p > 0.05$).

In the large September sample the frequency of deformed frogs was only 3.6%, about one fourth the frequency in July and August. By that time the weather was very different from the typical midsummer conditions that prevailed for the July and August samples, when temperatures were in the low 80's, humidity was high, and the skies were partly cloudy. On September 23, the air temperature was 44° F. and a strong sharp wind was blowing under overcast skies. Consequently, the frogs were quite inactive, seemingly pinned down by the cold.

Collecting conditions were such that deformed frogs could not be identified in the tall grass prior to capture, and despite the deformities they seemed otherwise to be normal, vigorous individuals. They were as active as normal frogs but somewhat less agile. In other words, they seemed to jump as often as the undeformed frogs but not as far. However, this difference was unlikely to cause any significant sampling bias because both types of frogs were small and easy to capture. Movement of grass was the stimulus leading to capture.

A more serious problem is whether the September frogs were a part of the Mud Lake population or were moving through the area in the fall migration to deep water. This question arises because no marked frogs were recaptured in September although 281 had been released in the area. Futhermore, no frogs were found in the previous collecting area but they were located some 100 yards further away from Mud Lake. Yet there are several reasons to suppose that the September sample came from the Mud Lake population.

First, although the frequency of deformed frogs was only 3.6%, this frequency is still far greater than that found in other populations, and the type of deformity was identical to that seen earlier.

Second, the size distribution of the frogs in the September sample is consistent with the idea that they were drawn from the Mud Lake population, showing the normal amount of growth for the time period involved (Table 2). For simplicity, the sizes were grouped in 10 mm. classes for comparison with late September 1965 samples from two remote populations. The size distributions in those populations are strikingly different from those at Mud Lake and can be related to the degree of crowding of the tadpoles in the breeding ponds. Since different populations from ponds near Mud Lake would be expected to vary in a similar way, the size data are at least consistent with the idea that the September sample was in fact drawn from the Mud Lake population.

Finally the estimates of the size of the population of frogs at Mud Lake are so large that failure to recapture any marked frogs should occasion no surprise (Table 3).

TABLE 2. Snout to vent length in mm. of frogs studied

Date—1966	Day	30-39	40-49	50-59	60-69	70-79	80-89	90-99
July 28	0	15	50	15	0	2	1	2
July 30	2	8	58	29	0	4	2	1
Aug. 17	20	0	69	32	4	1	1	1
Sept. 23	57	1	94	92	32	0	0	0
Sept. 22 .... (Dakota Co.)	56	0	0	3	17	7	1	0
Sept. 25 .... (Isanti Co.)	59	13	20	4	0	0	0	0

TABLE 3. Estimated populations at Mud Lake.

1965 Date	Immature			Sexually Mature		
	N marked in area	Total in sample	N recaptures	N marked in area	Total in sample	N recaptures
July 30	80	95	5*	5	7	2
1. Estimated size Lincoln-Petersen index		1,520			18	
2. Adjusted estimate		30,400			360	
Aug. 16	169	105	5	10	3	1
1. Estimated size Lincoln-Petersen		3,549			30	
2. Adjusted estimate		70,980			600	

* One recaptured frog killed. Hence total marked in area after July 30 sample was 79 + 90 = 169.

(Toe clipping alone seems unlikely to lead to the rapid elimination of the marked frogs, for such frogs have been recaptured more than a year after marking, and mating success for either males or females is unaffected.) Separate estimates were made for the recently metamorphosd immature frogs and for the sexually mature frogs by the mark-recapture method and the Lincoln -Petersen index (Scattergood, 1954), with two estimates for each group.

Contrary to expectation, the second estimates indicate larger populations than the first. Of the various necessary assumptions relative to this index, the one most likely to have been violated is that the marked individuals had randomly mixed with the unmarked. In the second sample, taken two days after the first, 5 of the first 6 frogs collected were marked, yet only 7 marked frogs in all were collected in the sample of 102 frogs. Furthermore, the sampled area was roughly estimated to have covered only about 5% of the likely available habitat at Mud Lake. These points both suggest that the size of the population at Mud Lake was considerably underestimated. In the 18 day interval from the second to the third sample, more chance for mixing occurred, but mixing of marked frogs was probably still not entirely random throughout the area.

Attempts to correct for these two factors will inevitably introduce large errors. However, if it is assumed that random mixing occurred within the sampled area and there was no movement of frogs between the sampled and the unsampled parts of the area, then 20 times the Lincoln-Petersen estimate gives a very rough approximation of the total Mud Lake population. Both of these assumptions are undoubtedly in error, but they are opposite in effect so that they tend to cancel one another out.

When these adjustments are made, the maximum estimate for the population of adult frogs is 600 and for immature frogs, 70,980. The latter figure may seem unrealistic. However, the eggs produced by a single Rana pipiens female may exceed 5,000 so that only 20 females are needed to produce 100,000 young. Therefore, the large size of the breeding area at Mud Lake and the estimated size of the adult population make these estimates for the size of the immature population seem reasonable.

With estimates of such size, a 5-week sampling interval, and the fact that the frogs had moved from the earlier sampling area, it is no longer so surprising that none of the 281 marked frogs was recaptured in the final sample.

Therefore, the available evidence indicates that the last sample at Mud Lake was drawn from the same population as the earlier samples and that in the interval between the earlier samples and the last sample in September the frequency of deformed frogs had dropped from 14.2% to 3.6%.

Since it was postulated that the deformity had a large environmental component, it may be asked whether this case is an example of natural selection at all, or only in a trivial sense. However, selection is phenotypic. If selection operates within a homozygous population, it will be without effect in changing gene frequencies, but nonetheless selection will have occurred. Thus, whether gene frequency changes reflect death of the deformed frogs is irrelevant to the question of whether natural selection had taken place.

However, since the frogs formed an outcrossing population, some genetic differences between the deformed frogs and their normal cohorts undoubtedly existed. The form of selection then would seem of the sort first visualized independently by Plunkett (1932, 1933) and Muller (1932), and later referred to as "canalization" by Waddington (1957). They suggested that a system of modifiers is built up by selection that tends to stabilize the "wild-type" phenotype under a variety of environmental and genetic stresses. Such a theory of stabilizing selection seems applicable in this case.

Furthermore, one of the most frequent observations about wild populations is their phenotypic uniformity. Despite the wealth of genetic variability, the "wild type" seems uncommonly common. The rate of elimination of the deformed frogs suggests why this is so, for while the species ordinarily requires two years just to reach sexual maturity, most of the deformed individuals had been eliminated in a few weeks. As noted above, the deformed frogs were otherwise vigorous, normal individuals, and it is unlikely that they were dying as a direct result of their deformities. The most common predators of *Rana pipiens* in summer are the garter snakes of the genus *Thamnophis*, which often live in the midst of the frog populations. The Mud Lake population of frogs was supporting a sizable population of garter snakes, and much of the differential elimination of the less agile deformed frogs probably occurred during the more or less constant survival competition between the snakes and the frogs.

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I. T. Freshman Retention Improved by New Programs

An experimental program to help freshmen in the Institute of Technology at the University of Minnesota through the first year is showing promising results, according to the latest statistics.

Several approaches are being tried to reduce the high attrition rate among entering freshmen, including a three-hour mathematics short course during Freshman Week. Two hundred I. T. freshmen took this course voluntarily in 1968. Their performance during the subsequent Fall and Winter quarters was better than performance of first year students who had not taken the course, it was reported.

Another retention program was based on supervised study and tutorial sessions, available from 3:15 to 5 p.m., when many students were through with other classes.

A program of group housing for Institute of Technology freshmen who want to be in a residence hall, and in some cases "Institute of Technology Floors" in the residence halls, was started in the Fall of 1968 to further promote an I. T. identity.

The following table shows results to date:

End of	Percent of I. T. Freshmen Still in I. T. and doing "C" work or better					
	1965	1966	1967	1968	Dorm	I. T. Floor
First Quarter	54.47%	67.71%	69.7%	75%	80%	88%