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# History of Minnesota Frog Abnormalities: Do Recent Findings Represent a New Phenomenon?

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Two lines of investigation were used to determine whether recent Minnesota reports of frog abnormalities differ from historical reports: (1) museum collection studies paired with follow-up field surveys, and (2) comparison of recent and earlier field surveys in west-central Minnesota. For the museum study I examined 2433 northern leopard frogs (*Rana pipiens*) in the Bell Museum of Natural History collections of the University of Minnesota, collected during 1958–63. 0.7% of frogs were found to have abnormalities, consisting of 0.5% predator amputations and 0.2% malformations. Three types of malformation were found: missing hindlimbs, vestigial feet, and fused digits. Conspicuously absent from the museum collections were the malformation categories of extra limbs, split limbs, webbing of hindlimbs (cutaneous fusions), and bent/twisted limbs (anteversions). Postmetamorphic field surveys at some of the museum collection sites were attempted in 1997. One finding was that frogs were much more easily collected in 1958–63! During surveys in 1997 I found frogs at only 5 of 14 museum sites on record. Fifteen malformed frogs were recorded among 611 examined (2.5%), compared to one malformed frog among 276 (0.4%) in the corresponding museum collections. Field surveys of more museum sites are in progress. Frog surveys conducted in west-central Minnesota from 1976–97 were also analyzed for differences in abnormality frequencies and types. Nine sites were identified as having reasonable (>50) sample sizes in both pre-1993 and post-1995 surveys. Pre-1993 data included three abnormal frogs among 1772 examined (0.2%), compared to 59 abnormal frogs among 2548 (2.3%) in 1996–97. Only 2 categories of abnormality were found in the pre-1993 data, partial limbs and vestigial foot. I conclude that recent findings of anuran abnormalities in Minnesota do represent a new phenomenon. Frog abnormalities were more frequent, more varied, more severe, and more widely distributed in 1996 and 1997 than in 1958–93.

INDEX DESCRIPTORS: anurans, leopard frogs, *Rana pipiens*, abnormalities, malformations, Minnesota.

Reports of abnormal frogs can be found in both the scientific and popular literature dating back over two centuries. Most frequent have been reports of frogs with extra limbs (supernumerary limbs or polymelia), extensively reviewed by Van Valen (1974). Ouellet et al. (1997) reported high prevalences of hindlimb ectromelia and ectrodactyly in Canada, and further reviewed the scientific literature on amphibian abnormalities. The pre-1990 reports tend to be of sporadic occurrences of anomalies. The frequently-cited Van Valen (1974) review, for example, contains only one reference to a high number of abnormal North American anurans, the Volpe (1967) report of "in excess of 350 multilegged bullfrogs." That bullfrog example is clearly different from affected sites I am studying in Minnesota in that (1) other coexisting species were not affected, (2) affected frogs all showed the same anomaly of bilateral polymelia, and (3) no abnormalities recurred in subsequent years (Volpe 1967, Volpe, pers. comm. 1998). The other 21 Van Valen references to North American anurans that identify species, reviewing about 200 yrs of literature, report on only 62 individual abnormal frogs from six different species. By contrast, I have examined over 400 malformed frogs from seven different species at one site during 1997 alone.

A better way to compare the past with the present in a given region is to study museum collections from that region. Drawbacks in such a study include (1) individual museum collections often have low sample sizes, and (2) there may be bias in collectors either selecting the normal, typical specimens or being impressed by variants. The Bell Museum of Natural History of the University of Minnesota contains northern leopard frog (*Rana pipiens*) collections that counter those drawbacks. David Merrell and various assistants collected leopard frogs in the 1950s and 1960s largely for the purpose of mapping the distribution of burnsi and kandiyohi pattern variants (Merrell 1965). Some collections were examined and released in the field, others were preserved and placed in the Bell Museum. The museum collections total 3620 leopard frogs from 93 sites in 44 different Minnesota counties. In collecting at nearly 200 Minnesota sites, Merrell encountered substantial numbers of abnormal frogs only once, in Washington county (Merrell 1969). That finding also differs from my current Minnesota malformation observations in several respects: (1) only two types of malformation were noted, ectromelia and "deformed toes," whereas I now see an array of up to ten different malformations in individual populations, (2) the malformation frequency decreased later in the season, whereas now the malformation frequency is usually higher later in the season, and (3) there was apparently no recurrence, whereas my intensive sites have had significant malformations for 3–4 successive years.

Another method to assess differences across time is to examine field data on populations that have been surveyed over long periods of time. My own color polymorphism research has included surveys at some Minnesota sites dating as far back as 1975. Thus I report here data from two lines of investigation to determine whether recent Minnesota reports of frog abnormalities differ from historical reports: (1) museum collection studies paired with recent surveys at the same sites, and (2) comparisons of recent and earlier field surveys in west-central Minnesota.

## METHODS

Selected leopard frog collections in the University of Minnesota's Bell Museum of Natural History made by Merrell and assistants

Table 1. Types of *Rana pipiens* anomalies among abnormal museum frogs (including likely predator amputations and malformations). Four frogs had two malformations each.

| Category of anomaly | abnormal | malformed |
|---|---|---|
| hindlimb ectrodactyly | 7 | 3 |
| hindlimb syndactyly | 1 | 1 |
| hindlimb ectromelia | 8 | 3 |
| forelimb ectrodactyly | 3 | 0 |
| forelimb syndactyly | 1 | 1 |
| forelimb polydactyly | 1 | 1 |
| forelimb ectromelia | 1 | 1 |

during 1958–63 were examined. The frogs had been fixed in 10% formalin and preserved in 60% isopropanol. Collections were selected on the basis of adequate sample size and time of collection (i.e., midsummer collection dates so that new juveniles were well represented). The examined collections represent 43 sites in 26 Minnesota counties. Snout–vent length of individual specimens was measured using calipers. Length data among individual collections were used to categorize frogs as juveniles (the current summer's metamorphs), subadults (the previous year's metamorphs), and adults (>2 yrs). A total of 2433 museum frogs was examined, 2166 of which were juveniles. Presence of scar tissue was used to distinguish injury-caused ectromelia and ectrodactly ("amputations") from developmental anomalies ("malformations"). I consider amputations and malformations collectively to be "abnormalities."

Field collections were made by hand capture using insect sweep nets terrestrially and dip nets in the water. Frogs were stored temporarily in buckets with damp sphagnum moss, then examined, measured, and released on site. Malformed frogs were photographed before release.

Hypotheses of differences in frequencies between museum and recent collections, or between past years' vs. current years' collections were tested statistically using chi-square tests and related modifications. With the likelihood of different levels of risk across clustered samples such as these, chi-square tests may not always be valid. Homogeneity of association across sites was tested by the Breslow-Day procedure and differences in risk across time were also tested using the Cochran-Mantel-Haenszel procedure to combine evidence of association across sites (Agresti 1996).

## RESULTS

### Museum Comparisons

Among the 2433 preserved frogs examined, 18 abnormal frogs (0.7% abnormalities) were recorded, representing 12 sites in ten counties. All frogs from the remaining 33 sites in 16 counties were normal. Using scar tissue as a distinguishing criterion, the abnormalities consisted of 12 likely predator amputations (0.5%) and six malformations (0.2%). All malformed frogs were juveniles, hence the malformation frequency may be better expressed as 6 in 2166, or 0.3%. Seven specific types of abnormalities were found among museum specimens (Table 1), representing four general categories of abnormality (ectrodactyly, syndactyly, polydactyly, ectromelia), affecting the hindlimbs more often than the forelimbs.

Fourteen of the sites represented by the museum collections were surveyed in 1997; one site had also been surveyed in 1996 and those data are included. One finding was that frogs were much more abundant and/or easily collected in 1958–63, as the 1997 surveys found frogs at only five of the 14 sites. Survey data from 1996–97 were compared to respective sites in the 1958–63 museum collections (Table 2). Fifteen malformed leopard frogs were found in 1996–97 among 611 total juvenile frogs (2.5%), while only one of 276 juvenile frogs in the corresponding museum collections was malformed. The total malformation frequency was significantly higher in 1996–97 than in pre-1993 surveys (chi-square = 4.700, P = 0.03). Leopard frogs were at six times the risk of malformation in 1996–97 than in 1958–63 (relative risk = 6.776 with 95% confidence bounds of 1.200–38.246). Four of the five sites had at least one malformed frog in 1996–97, compared to one of the same five sites in the museum collections. Pearson chi-square tests of the total museum frequency of 0.3% malformed juveniles against 1.4% malformed juveniles among all random sites surveyed in Minnesota in 1996–97 also showed a significant difference ($P < 0.001$, relative risk = 5.2).

The Breslow-Day test for homogeneity of odds ratios was not significant for these sites (P = 0.83). Temporal increase in frequency of malformations is also strongly suggested, given the small number of sites (5) and sample sizes (35–85), by the Cochran-Mantel-Haenszel test (P = 0.104), but is not significant with 95% confidence given these data alone.

### Field Comparisons

Leopard frog surveys conducted in west-central Minnesota from 1975–97 were analyzed for differences in abnormality frequencies and types. The pre-1993 surveys were done specifically to study color and pattern polymorphism, not malformations. Hence, while physical abnormalities were noted on the data sheets, frogs were not examinined closely to attempt distinction between predator amputations and developmental malformations. Therefore I record and analyze total abnormalities in this section. Nine sites were identified as having reasonable ($n > 50$) sample sizes in both pre-1993 and post-1995 surveys (Table 3). Pre-1993 data included three abnormal frogs among 1772 examined (0.2%), compared to 59 abnormal frogs

Table 2. Malformed *Rana pipiens* from 1958–63 museum collections compared to 1996–97 field surveys of juvenile frogs at the same sites.

| | | Museum (1958–63) | | | Field (1996–97) | | |
|---|---|---|---|---|---|---|---|
| County | Site | n | malformed | % | n | malformed | % |
| Big Stone | 1 | 85 | 0 | 0 | 129 | 1 | 0.8 |
| Big Stone | 2 | 35 | 0 | 0 | 120 | 4 | 3.3 |
| Douglas | 3 | 40 | 0 | 0 | 85 | 1 | 1.2 |
| Grant | 4 | 50 | 0 | 0 | 24 | 0 | 0 |
| Traverse | 5 | 66 | 1 | 1.5 | 244 | 9 | 3.7 |
| Total | | 276 | 1 | 0.4 | 611 | 15 | 2.5 |

Table 3. Numbers of abnormal *Rana pipiens* comparing recent to earlier field findings at the same sites in west-central Minnesota.

| | | pre-1993 | | | 1996–97 | |
|---|---|---|---|---|---|---|
| County | Site | dates | n | abnormal | n | abnormal |
| Stevens | 1 | 1987 | 118 | 0 | 1250 | 20 |
| | 2 | 1987 | 152 | 0 | 173 | 8 |
| | 3 | 1975–80 | 482 | 2 | 102 | 2 |
| Traverse | 1 | 1988–90 | 80 | 0 | 220 | 7 |
| | 2 | 1990 | 146 | 0 | 73 | 1 |
| Douglas | 1 | 1991 | 123 | 0 | 180 | 17 |
| | 2 | 1991–92 | 441 | 1 | 117 | 1 |
| | 3 | 1990–91 | 135 | 0 | 53 | 3 |
| Lac Qui Parle | 1 | 1992 | 95 | 0 | 380 | 0 |
| Totals | | | 1772 | 3 | 2548 | 59 |

Table 4. Number of *Rana pipiens* showing various abnormalities, comparing pre-1993 west-central Minnesota surveys to post-1993 surveys at the same sites and statewide.

| | pre-1993 | 1996–97 | |
|---|---|---|---|
| Abnormality | west-central | statewide | west-central |
| n | 3637 | 6689 | 4722 |
| hindlimb webbing | 0 | 96 | 1 |
| hindlimb anteversion | 0 | 30 | 4 |
| hindlimb ectromelia: | | | |
| distal portion missing | 7 | 29 | 20 |
| underdev. portion | 1 | 17 | 15 |
| entire limb missing | 2 | 13 | 2 |
| hindlimb polymelia: | | | |
| extra complete limb(s) | 1 | 13 | 2 |
| limb distally split | 0 | 13 | 1 |
| ectrodactyly | 0 | 12 | 10 |
| missing eye | 0 | 5 | 5 |
| abnormal jaw | 0 | 2 | 2 |
| forelimb ectromelia | 0 | 2 | 1 |
| syndactyly | 0 | 2 | 1 |
| abnormal pigmentation | 0 | 2 | 1 |
| displaced hip | 0 | 1 | 1 |

among 2548 (2.3%) in 1996–97. The total abnormality frequency was significantly higher in 1996–97 than in pre-1993 surveys (chi-square = 34.034, $P < 0.001$). Leopard frogs were at 13 times the risk of malformation in 1996–97 than 1975–92 (relative risk = 13.677 with 95% confidence bounds of 4.294–43.560). The Breslow-Day test was not significant for these sites ($P = 0.104$). The Cochran-Mantel-Haenszel test confirms significant increase in west-central Minnesota ($P < 0.0001$). Eight of the nine sites had at least one abnormal frog in 1996–97, compared to two of the same nine sites in 1975–92.

The *types of abnormalities* found in all west-central Minnesota sites in pre-1993 surveys were compared to the types found in 1996–97 (1) at all Minnesota sites surveyed, and (2) at all west-central Minnesota sites (Table 4). Four types of abnormalities (three degrees of hindlimb ectromelia, hindlimb polymelia) were recorded in 1975–92 surveys, compared to 14 types in 1996–97.

## DISCUSSION

### Background Levels

An important question with all the media attention on malformed frogs in recent years, and given the history of malformations in the literature, is whether what we are seeing now is any different from what occurred in the past. Stated differently, are the frequencies and

types of malformation reported in recent years different from some sort of "background level" that might be expected in frog populations? Literature and anecdotal reports from the past rarely have frequency estimates, and mostly have focused on polymelia. Lesser malformations such as partial ectrodactyly would likely often go unnoticed in past surveys where malformations were not the main focus.

Read and Tyler (1994) reported "natural levels of abnormalities" in the trilling frog (*Neobatrachus centralis*) in one set of Australian sites to be about 1.6%, concluding that this apparently reflects the "background level" for that species. Most of the abnormalities they found were ectrodactyly, often involving loss of distal tarsal bones of a single digit. Ouellet et al. (1997) reported 0.7% malformations (all ectromelia) among 271 frogs of four ranid species in a set of pesticide-free sites in Canada in 1992 and 1993. Gray (1998) reported a frequency of 0.39% abnormalites among nearly 10,000 cricket frogs (*Acris crepitans*) in Illinois surveys conducted in 1968–71. Most of those (0.32%) were ectromelia. From reports like these and anecdotal information, a general "natural" level of frog abnormalities frequently used has been "about 1%." My museum findings and my past surveys in west-central Minnesota indicate the background frequency of both total abnormalities and malformations in Minnesota *Rana pipiens* to be less than 1%.

The 1958–63 Bell Museum data presented here represents perhaps the best documented estimate of a background frequency of abnormalities in *Rana pipiens.* Because the preserved frogs are there to be examined, the ectromelia and ectrodactyly estimates can be broken into predator amputations vs. malformations. From the 2433 frogs examined, the estimates of background frequencies are 0.7% total abnormalities consisting of 0.5% amputations and 0.2% malformations. The abnormality frequency is lower (0.2%) in the pre-1993 west-central Minnesota data. This is likely because ectrodactyly could often go unnoticed in studies where investigators were not specifically looking for abnormalities. Because I have frequently encountered leopard frog populations since 1995 having malformation frequencies of 1–22% (Tables 2–4, Hoppe 1996, Hoppe unpubl.), I conclude that malformations are occurring at higher frequencies now than in the past.

### Temporal Frequency Differences

Problems in finding good sample sizes of juvenile frogs in the same sites as were surveyed in 1958–63 hamper direct statistical testing of this hypothesis of increase. Data comparing 1996–97 surveys at sites originally surveyed in 1958–63 (Table 2) do, however, show an increase in malformation frequency from 0.4% to 2.5%. These sites were not selected for either low numbers of malformations in 1958–63 or high numbers in 1996–97, rather were the only five sites where reasonable sample sizes were captured in both time spans. Attempts to resurvey more of the 1958–63 sites are in progress.

A higher incidence of frog abnormalities is also shown by comparing pre-1993 to post-1995 west-central Minnesota surveys. I had better success in resurveying sites studied earlier, getting sufficient samples at nine of 12 sites attempted. The differences in recent vs. earlier surveys were greater than for the museum sites, with eight of nine sites having abnormal frogs in 1996–97 (freq. = 2.3%) compared to two of the same nine sites (freq. = 0.2%) in pre-1993 surveys. With more sites and higher sample sizes than the museum comparisons, this increase was significant using both chi-square and Cochran-Mantel-Haenszel tests.

### Abnormality Types

A striking difference between historical abnormality observations and current findings in Minnesota is the appearance of an array of anomalies not seen before. The most common malformation I see in all three frog species that I now survey intensively (*R. pipiens, R. septentrionalis, R. clamitans*) is a webbing of skin from femoral to tibiafibula regions (called "cutaneous fusion" by some investigators). This webbing frequently inhibits both the frogs' swimming and hopping capabilities. I have seen no such webbing in past collections, including the 2433 museum frogs examined. Similarly, the second most common and one of the most severe *R. pipiens* malformations in 1996–97, hindlimb anteversion, was seen neither among the 2433 museum frogs nor among 3637 west-central Minnesota frogs examined from 1975–92. Several malformations found at lower frequencies in 1996–97 were also absent in museum and pre-1993 collections, including split limb, missing eye, malformed jaw, forelimb ectromelia, and displaced hip. Hindlimb polymelia was ten times more frequent in 1996–97 than before 1993 in *R. pipiens.* These observations may be the strongest evidence that malformations are a different phenomenon now than in the past. An interesting aspect of the array of different malformations is finding differences between sites and/or geographic areas, for which my data need further analyses. For example, hindlimb webbing and anteversions are common in some central Minnesota sites, but uncommon in the west-central sites.

I conclude that recent findings of anuran abnormalities in Minnesota do represent a new phenomenon. Historical and current data reveal that frog abnormalities were more frequent, more varied, more severe, and more widely distributed in 1996 and 1997 than in 1958–93.

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## LITERATURE CITED

AGRESTI, A. 1996. An introduction to categorical data analysis. John Wiley and Sons, New York.

GRAY, R.H. 2000. Morphological abnormalities in Illinois cricket frogs, *Acris crepitans,* 1968–1971. Investigating amphibian declines. Proceedings of the 1998 midwest amphibian conference. H. Kaiser, and G.S. Casper, eds. Journal of the Iowa Academy of Science 107:92–95.

HOPPE, D.M. 1996. Historical observations and recent species diversity of deformed anurans in Minnesota. *In* Third Annual Meeting of the North American Amphibian Monitoring Program. http://www.im.nbs.gov/naamp3/naamp3.html.

MERRELL, D.J. 1965. The distribution of the dominant burnsi gene in the leopard frog, *Rana pipiens.* Evolution 19:69–85.

MERRELL, D.J. 1969. Natural selection in a leopard frog population. Journal of the Minnesota Academy of Science 35:86–89.

OUELLET, M., J. BONIN, J. RODRIQUE, J. DESGRANGES, and S. LAIR. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. Journal of Wildlife Disease 33: 95–104.

READ, J.L., and M.J. TYLER. 1994. Natural levels of abnormalities in the trilling frog (*Neobactrachus centralis*) at the Olympic Dam Mine. Bulletin of Environmental Contamination and Toxicology 53:25–31.

VAN VALEN, L. 1974. A natural model for the origin of some higher taxa. Journal of Herpetology 8:109–121.

VOLPE, E.P. 1967. Understanding evolution. W.C. Brown, Dubuque, Iowa.