

THE UNIVERSITY OF MANITOBA

AN ANALYSIS OF TOADS OF THE BUFO AMERICANUS GROUP
IN A CONTACT ZONE IN CENTRAL NORTHERN NORTH AMERICA

by

FRANCIS RUSSELL COOK

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ABSTRACT

Discriminant function analysis of 30 measured morphological characters was used to compare reference samples from Alberta to Prince Edward Island of 825 male Bufo americanus and 819 male B. hemiophrys and 59 female B. americanus and 118 female B. hemiophrys. Both analyses provided complete separation of the two taxa. When the resulting weights were applied to 2461 male and 225 female Bufo from southeastern Manitoba, an area excluded from reference samples, a relatively narrow zone of intermediate populations was defined. Separate analysis of three additional scored characters and of selected measured characters, alone or as ratios, followed the same pattern. Audiospectrograms of male breeding calls substantiated the presence of the intermediate populations and laboratory- and field-raised crosses between americanus, intermediate, and hemiophrys parents indicated some transformation success in all combinations.

Discriminant scores for B. woodhousei fowleri (19 males) and B. w. woodhousei (17 males) gave mean values different from the closest B. americanus or B. hemiophrys populations. Separate discriminant analysis comparing B. hemiophrys with B. cognatus (48 males) and B. boreas (60 males) showed complete separation of the taxa in areas of sympatry, and the morphological intermediacy of a single B. hemiophrys boreas field-identified hybrid was confirmed.

The taxa americanus and hemiophrys are conspecific and lack

apparent barriers to interbreeding. Because of the narrowness of the zone of intermediate populations, and the distinctiveness of these taxa outside of this zone, they can be considered megasubspecies: B. (americanus) americanus and B. (americanus) hemiophrys. Two previously recognized subspecies, B. a. copei (northern Ontario and Quebec) and B. h. baxteri (Wyoming) are not taxonomically distinct.

The intermediate zone between americanus and hemiophrys corresponds to the mid-point in transition between eastern and central herpetofaunas, elements of which are postulated to have been separated during a break in the trans-continental Boreal Forest during the Wisconsin, and perhaps earlier, glaciations.

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INTRODUCTION

Five species of toads of the 187 (Gorham, 1974) recognized in the nearly cosmopolitan genus Bufo (Amphibia: Anura: Bufonidae) occur in northern North America (Canada and Alaska). Their geographical relationships may be summarized from Conant (1975) and Stebbins (1966). Three are broadly distributed: the American Toad, Bufo americanus, the Canadian Toad, B. hemiophrys, and the Northwestern Toad, B. boreas. These are largely allopatric and replace each other from east to west (Figure 1). North-south boundaries lie through eastern Manitoba and western Alberta. All are widespread in the boreal or montane forests which cover much of this region, but none invade tundra or true alpine conditions. Also, they are abundant in the deciduous forests of the east (americanus), the parkland and northern fringes of the grassland of the central portion (hemiophrys) and the arid interior valleys and coastal rainforest of the west (boreas). Two additional species invade or approach the southern fringes of the region. In the east, Fowler's Toad, B. woodhousei fowleri, reaches its northern limit along the sandy north shore of Lake Erie in southern Ontario. Here and south to the Atlantic coastal plain it is geographically sympatric with, but largely ecologically separated from, Bufo americanus. In the central portion of the continent Woodhouse's Toad, B. woodhousei woodhousei, is allopatric to Bufo hemiophrys

Figure 1. The geographic distribution of toads in northern North America based on Conant (1975), Stebbins (1966) with modifications and additions from National Museum of Natural Sciences (Herpetology Section) collections and Cook (1977).

Top: Bufo americanus, B. hemiophrys and B. boreas replace each other across the continent from east to west respectively. Note the narrow zone of sympatry between B. hemiophrys and B. boreas in central Alberta, and the disjunct population of B. hemiophrys in Wyoming (bottom of map, west of center).

Middle: The Bufo woodhousei complex: B. w. woodhousei in the west (hatched) and B. w. fowleri in the east (solid). Compare with the top map for area of sympatry between B. w. fowleri and B. americanus in the east, for allopatry between B. woodhousei and B. hemiophrys in the central portion and sympatry between B. woodhousei and B. boreas in the west. Ecological separation occurs within the areas of geographic overlap.

Bottom: Bufo cognatus. Compare with top and middle for areas of sympatry with B. hemiophrys and B. w. woodhousei. There is some ecological separation between B. cognatus and the other species within their overlapping ranges.

along a line from northern Montana to northern South Dakota (Figure 1). The Great Plains Toad, B. cognatus, which is widely sympatric with B. woodhousei over the interior of the continent south to northern Mexico, is narrowly sympatric with B. hemiophrys along the grassland southern fringe of its range in southeastern Alberta and southwestern Saskatchewan (Figure 1). A disjunct population of B. hemiophrys occurs in the Laramie Valley of Wyoming. In western Alberta and in British Columbia, the Yukon and coastal Alaska, B. boreas is the only toad, but to the south its range interdigitates with other species (e.g. B. w. woodhousei in Washington and further south).

The literature on toads is voluminous (Blair 1972) and the evolutionary relationships, and many of the comparative aspects between species are extensively documented (osteology, chromosomes, electrophoretic patterns of proteins, venom constituents, and laboratory hybridization, among others). Blair (1972 and previous papers) has placed all the species considered here in his narrow-skulled (or thin-skulled) northern evolutionary line within the genus, and concluded that they represent three species-groups. B. americanus, B. hemiophrys and B. woodhousei are all included in the americanus group; B. cognatus and B. boreas each are placed in distinct groups. However, relatively little has been published on northern populations of these taxa and their geographic variation and interactions within this region have been poorly documented or are unknown.

This study attempts to clarify the relationship and interaction between B. americanus and B. hemiophrys. It has been possible to examine larger number of specimens than any previous authors had available to them from over the combined range of the two forms. This sample encompasses northern North America from Prince Edward Island to the eastern foothills of the Rocky Mountains in Alberta. The geographic variation of their populations over this vast area is compared to the variation in population samples obtained through transects of their contact zone in eastern Manitoba. Calls of individuals from intermediate populations are compared to those of both eastern (americanus) and western (hemiophrys) populations adjacent to the contact zone and the relationship of morphological variation and call variation is compared. The data on crossing success is less extensive and less satisfactory due to the hazards of the techniques, but it is the first attempt to field-rear tadpoles of crosses between these forms and compare their viability in different pond environments.

Also discussed are comparisons between the B. americanus-hemiophrys complex and samples of B. w. woodhousei, B. w. fowleri, B. cognatus and B. boreas.

Throughout the bulk of this paper I have used current terminology and referred to the taxa of primary concern as Bufo americanus and Bufo hemiophrys, or for brevity simply as americanus and hemiophrys. This usage is for convenience and not intended to confuse, contradict, or preempt the conclusion on their taxonomic

status advanced later in the discussion.

Metric units were used throughout the study for morphological features of toads, and celsius for temperatures. However the dimensions of tadpole rearing cages and, more importantly, the distances along roads which specify most localities, were taken in English units at the time of the study. These latter units are retained here because to convert them would either convey a false sense of additional precision if the equivalents were given exactly in metric decimals or else add further imprecision by rounding to whole metric units.

MATERIALS AND METHODS

Description of areas sampled

The primary vegetation zones of Canada have been discussed and mapped by Rowe (1959) and these are used on the base maps showing variation in reference samples of Bufo americanus and B. hemiophrys in eastern and central North America (Figure 2).

Forest region distributions depend on a variety of climatic, topographic, soil, and geologic variables and are, at least in part, a function of the combined effect of these on gross vegetation. Amphibian and reptile distributions are influenced by the same variables and often show some general agreement with vegetation zones (Savage 1960) though exact range limits for particular species vary as much as those of individual tree species. Forest regions are used here as a crude measure of climatic and other variables which affect toad distribution. The wide geographic area covered by the reference samples does not justify any attempt to correlate toad variability with more precise individual environmental measures at the present time.

Southeastern Manitoba was most intensively sampled because it includes the contact zone between toad taxa that is central to this study. Figure 3 shows the collecting localities and vegetation types in this area. The southern tongue of the Manitoba lowlands section of Rowe (1959) is important in the following analysis. This is an area of change from

Figure 2. Map of eastern and central North America showing the localities from which reference collections of toads were obtained. These localities are numbered separately within each province or state and are listed in Appendix I with catalogue numbers for each collection examined. The Forest regions shown are simplified from Rowe (1959) with southern extensions from Clarke (1973).

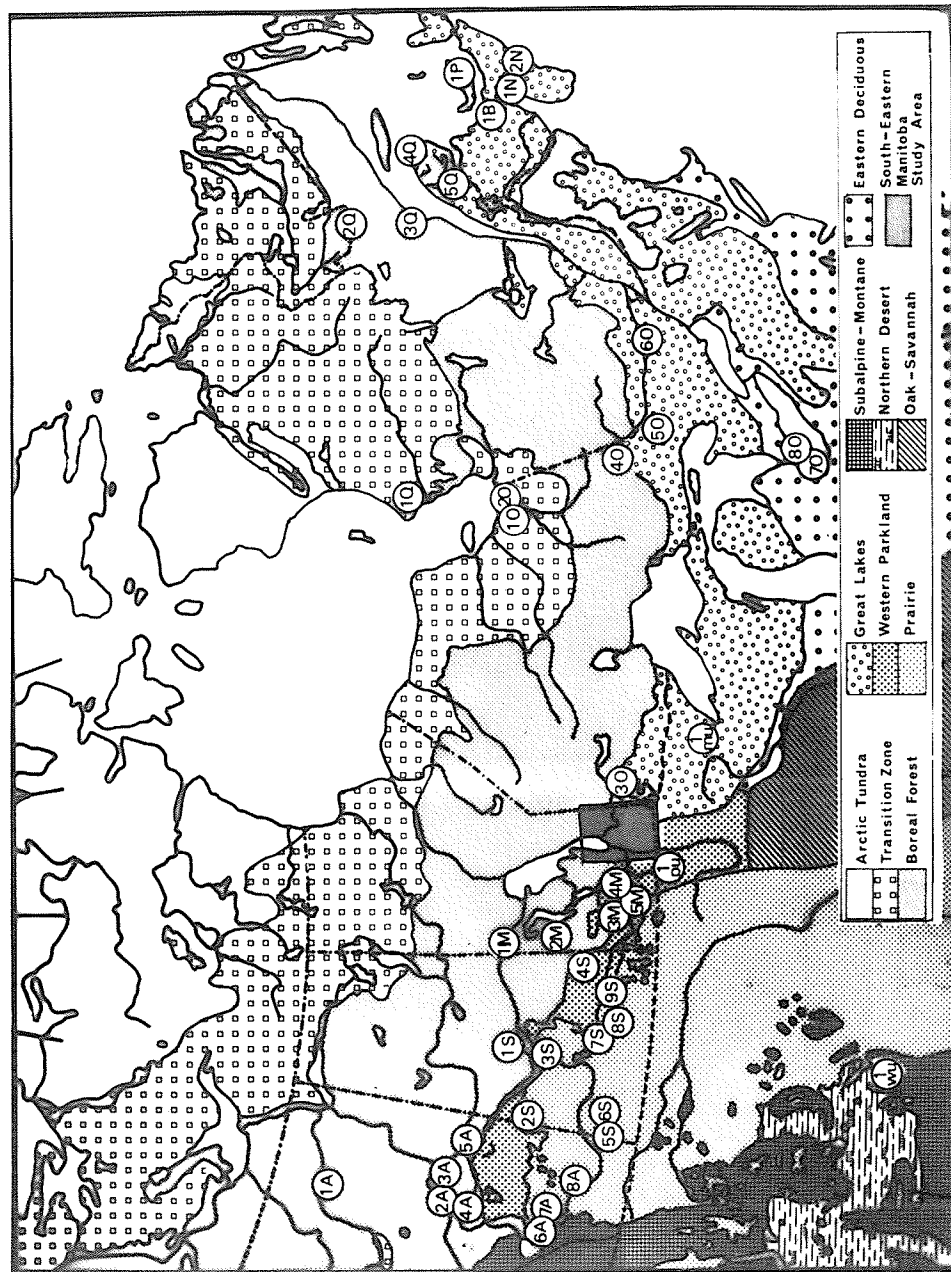
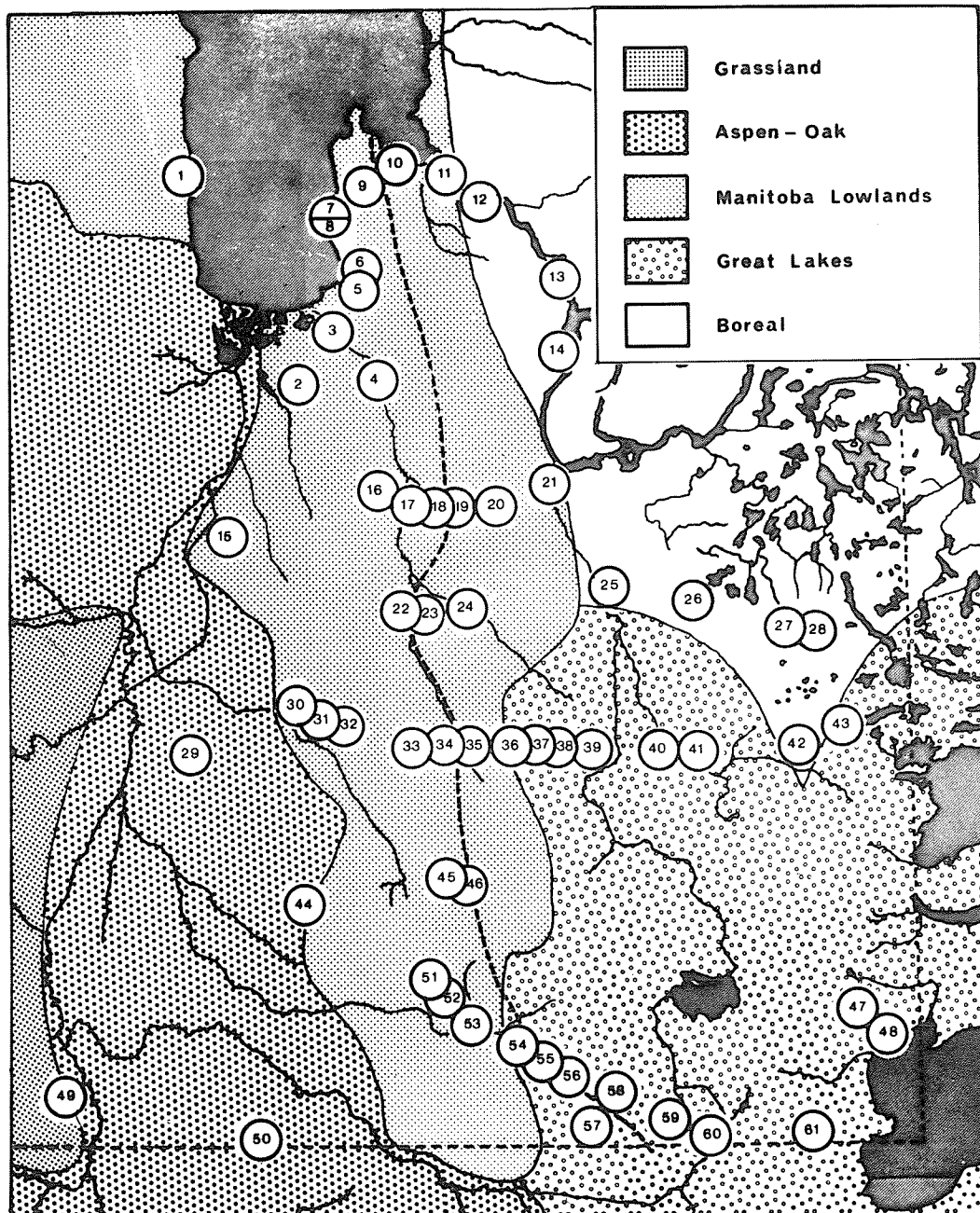


Figure 3. Map of southeastern Manitoba showing the localities from which collections of toads for analysis were obtained. Locality numbers are listed in Appendix I with catalogue numbers of specimens examined. The forest regions shown are simplified from Rowe (1959). The dashed line represents the mid-point of the contact zone between Bufo hemiophrys (west of line) and B. americanus (east of line) as discussed subsequently in the text.



predominantly coniferous trees of the Boreal and Great Lakes complex to aspen and grassland vegetation. At some localities (e.g. the Brokenhead River at Highway 1) the change is abrupt and can be readily appreciated in the field. However, various coniferous outliers exist to the west (e.g. at Birds Hill), and simple correlations of toad morphology and tree species cannot be readily made.

Southeastern Manitoba has been modified by clearing for tillage and drainage for agriculture, primarily in its western portion (Warkentin 1967). The vegetational transition and area of contact between toad taxa features abundant beach ridges of glacial Lake Agassiz and is bordered on the east by peat and swamp and to the west by clay soils (Figure 4 in Elson 1967).

Löve (1959) and Shay (1967) have presented discussions of the post-glacial vegetation in this area, demonstrating the changes that have occurred over the past 12,000 or more years.

Field Surveys

Field work was conducted largely by motor vehicle along highways and rural roads. For this reason roadside ditches, ponds, lakes, and rivers were the main areas sampled. Breeding toads were of prime concern. These are here assumed to provide the best population samples for geographic comparisons because the individuals are known to be mature adults. However, samples were also taken throughout the active season for amphibians and reptiles whenever possible, in order to obtain tadpoles of

various growth stages, metamorphosing individuals, and samples of juveniles and adults outside the reproductive season.

General survey collecting sites were chosen to provide a wide geographic sample. In Manitoba, transects were run across the southeastern corner of the province on the east-west network of public roads. Particular attention was given to intensive sampling where the putative zone of taxa contact seemed most obvious.

An eight-cell headlamp was used for illumination during after-dark collecting. At peak breeding, and usually at other times as well, this artificial light is apparently ignored by toads and they continue normal activity.

Attempts were made to obtain a minimum of 20 breeding males per locality, although this was not always possible. Much larger series (a hundred or more) were occasionally taken to provide a robust sample for statistical analysis. Many Trans-Canada Highway localities were resampled to allow analysis for possible within- and between-year variation.

Generally, a large proportion of males utilizing a particular site for breeding could be expected to be present on a peak breeding night, but females tended to arrive over a more extended period through the breeding season, breed shortly after joining the chorus, and leave after depositing their eggs. This results in few females relative to the numbers of males being available at any sampling period. In addition, females tend to come to the ponds in greatest numbers on a few peak nights of the breeding

season, whereas males, though in reduced numbers compared to peak intensity, continue to call and be present over much of the breeding season. Because of their call males are also more conspicuous. Juveniles were rarely present at the breeding site, but when present could be recognized as immature by size and lack of breeding condition.

Field notes on habitat, general topography and weather conditions, as well relative density and behaviour of toads, were made for each locality at the end of each sampling period.

Specimens for preservation were usually processed within 12 hours of capture, but occasionally they were retained in dampened cloth bags under refrigeration for longer periods.

All were killed with ether, were injected with 10% formalin and individually tagged, then immersed in formalin of the same strength (Cook 1965a). Permanent museum catalogue numbers were assigned to each collection in the field and individuals were given a subnumber within that catalogue number. For museum storage collections were rinsed and transferred to 45% isopropyl alcohol.

Morphological character set

The following character set was devised primarily to compare B. americanus and B. hemiophrys on the basis of characters which appeared to differ between the two taxa or which showed variation of interest within each taxon. It was later applied to one sample

of B. boreas and one of B. cognatus and to small samples of B. w. woodhousei and B. w. fowleri to compare their variation in these characters with B. hemiophrys and B. americanus. Additional characters could have been added which would have improved the separation in certain of these combinations.

Many of these characters, in the same or slightly different forms, have been used previously to differentiate americanus and hemiophrys. Blair (1957a), Wright and Wright (1949), Breckenridge (1944), Conant (1975) and others have presented comparative descriptions. Previous attempts at quantification have been made by Underhill (1961), to compare variation within populations of hemiophrys and between hemiophrys and woodhousei and americanus. Porter (1968) compared the Wyoming relict with samples from the continuous range of hemiophrys, and Henrich (1968) assessed populations from South Dakota which he showed to be intermediate between hemiophrys and americanus.

All measurements and coding states for the present analysis were made after several years preservation. Measurements were taken with dial calipers to the nearest 0.1 mm. The following were recorded for all specimens except for some Bufo boreas in which the cranial crests were not obvious, thus forcing the omission of all characters involving these structures. Figure 4 depicts the measured characters and Figure 5 the ventral pattern variation. Figures 6 and 7 compare dorsal views of toads studied here, and Figure 8 depicts ventral views of hind

Figure 4. Digramatic views of a toad showing underside of right hind foot, dorsal view of head and shoulders, and dorsal and lateral aspects of a whole animal.

CA: cranial crest: anterior width
 CL: cranial crest length
 CP: cranial crest: posterior width
 EL: eyelid length
 HW: head width
 IW: tubercle width (inter metatarsal tubercle)
 LW: diameter of largest wart on tibia
 NP: nostril to parotoid
 NS: nostril separation
 OW: "spade" width (outer metatarsal tubercle)
 PL: parotoid gland: length
 PS: parotoid gland separation
 PW: parotoid gland: width
 TL tibia length
 TH: tympanum height
 TL: tarsus length
 SL: spot length
 SV: snout-vent length
 SW: spot width
 WW: wart width

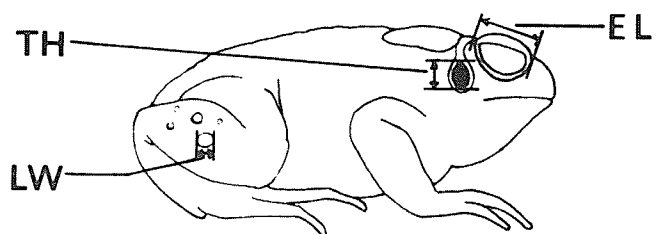
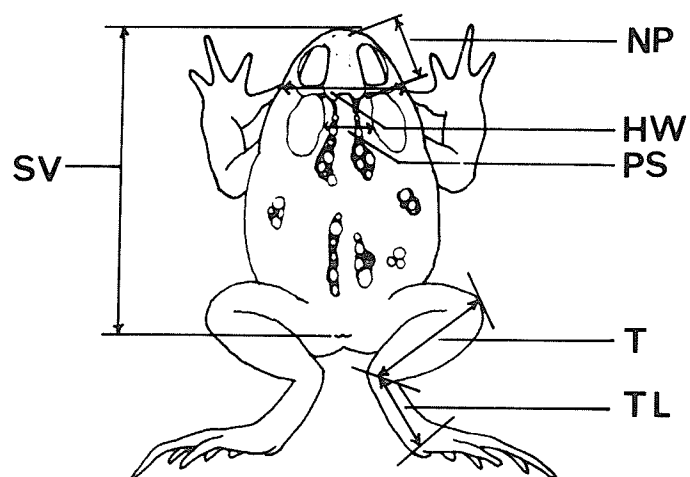
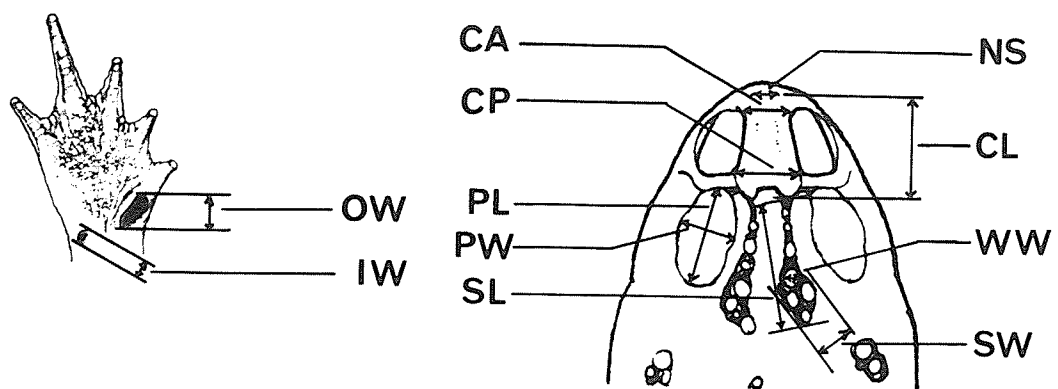
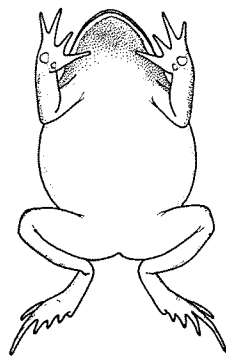
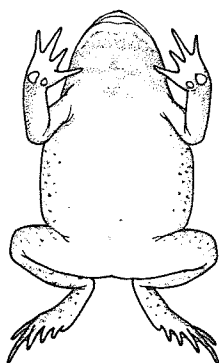


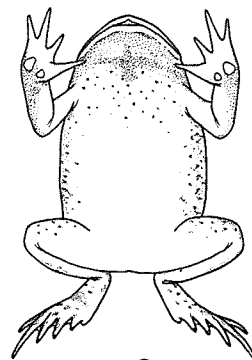
Figure 5. Diagrammatic venters of toads showing the range of ventral spotting and the scoring values (0-6) assigned (see text for definitions of scored conditions).



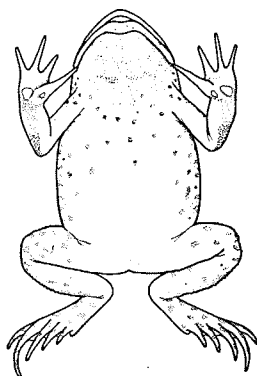
0



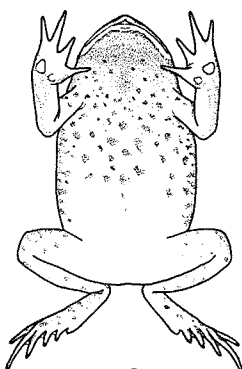
1



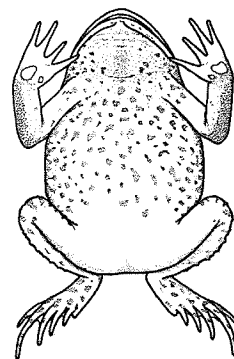
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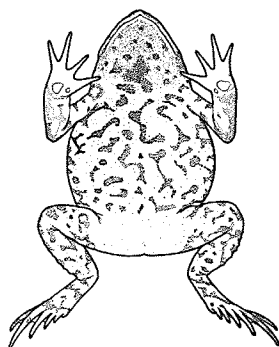
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4



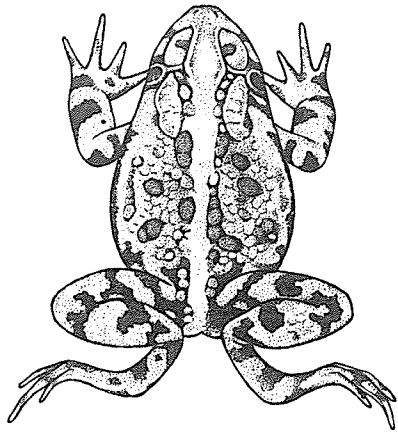
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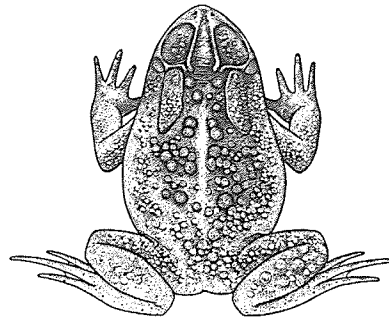
6

Figure 6. Dorsal views of selected male toads in the Bufo americanus-B. hemiophrys complex showing external structure and pattern.

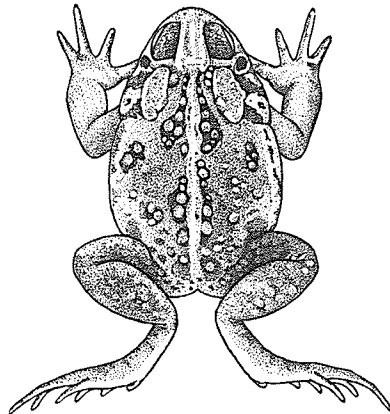
- A. B. americanus, NMNS(HS) 14797-4, Whitetop Creek, Moosonee, Ontario
- B. B. americanus, NMNS(HS) 11795-3, 3 miles west on Highway 1 of junction Highways 1&11, Manitoba
- C. B. americanus x hemiophrys, NMNS(HS) 8484-11, 17 miles west on Highway 1 of junction Highways 1&11, Manitoba
- D. B. hemiophrys, NMNS(HS) 12217-1, 30 miles west on Highway 1 of junction Highways 1&11, Manitoba
- E. B. hemiophrys, NMNS(HS) 8543-2, 10.5 miles west and north on Highway 28 of St. Paul, Alberta



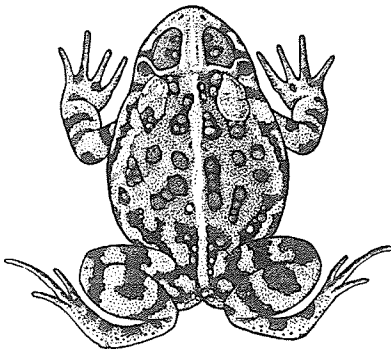
A



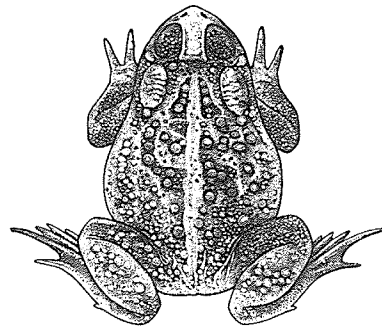
B



C



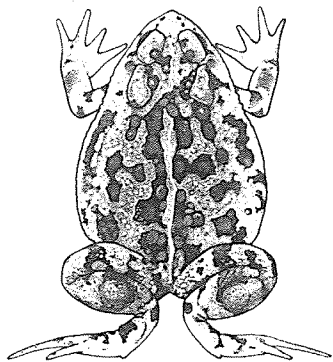
D



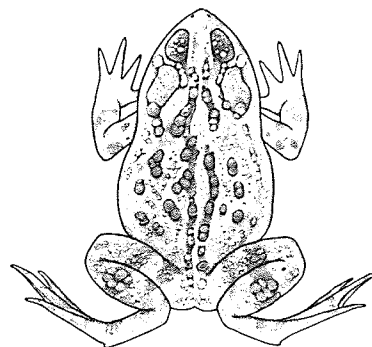
E

Figure 7. Dorsal views of other Bufo taxa and one hybrid.

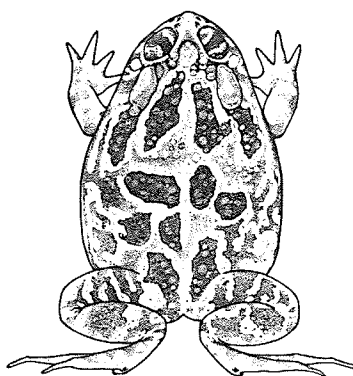
- A. B. boreas, NMNS(HS) 17014, Mile 1.9 on Kananaskis Lakes Road,
Alberta
- B. B. boreas x hemiophrys, NMNS(HS) 8523, 2.4 miles north on
Highway 44 of Westlock, Alberta
- C. B. cognatus, NMNS(HS) 4168, 3.1 miles east on Highway 1 of
junction Highways 1&21, Saskatchewan
- D. B. w. woodhousei, NMNS(HS) 8585-3, 3½ miles SSE of Stockton,
Rooks Co., Kansas
- E. B. w. fowleri, NMNS(HS) 3294-5, Turkey Point, Norfolk Co.,
Ontario



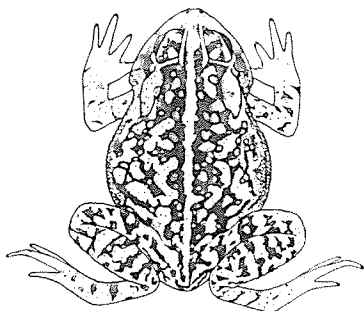
A



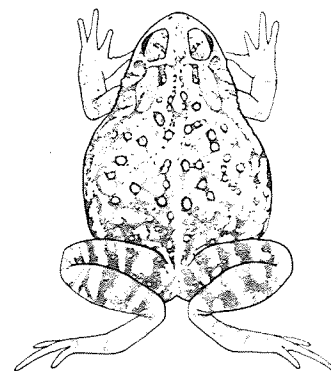
B



C



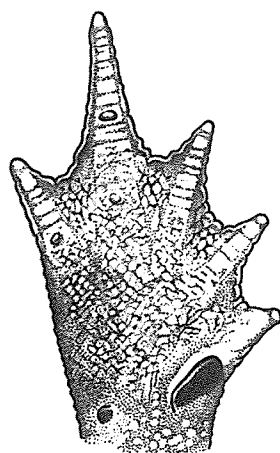
D



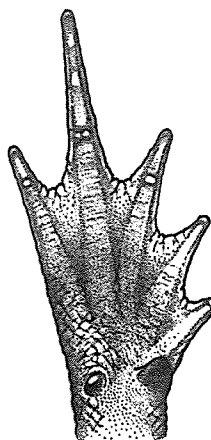
E

Figure 8. Ventral views of the right hind foot of selected toads.

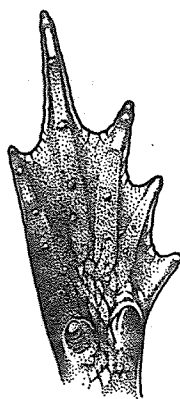
- A. B. hemiophrys, NMNS(HS) 8521-35, 2.4 miles north on Highway 44 of Westlock, Alberta
- B. B. americanus, NMNS(HS) 7064-33, 10 $\frac{1}{2}$ miles east on Highway 17 of North Bay, Ontario
- C. B. boreas, NMNS(HS) 8530-33, 2 miles west on Highway 2 of Athabaska, Alberta
- D. B. cognatus, NMNS(HS) 7110-5, 8.3 miles east on Highway 1 of Piapot (turnoff), Saskatchewan
- E. B. w. fowleri, NMNS(HS) 15783-55, Long Point, Norfolk Co., Ontario



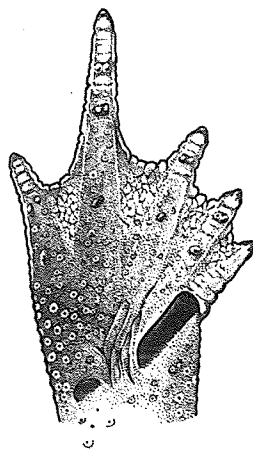
A



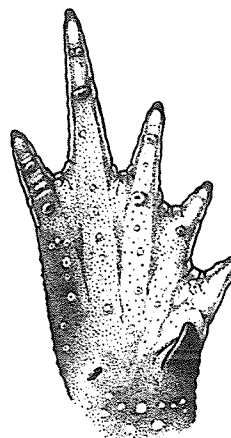
B



C



D



E

feet.

1. Sex: specimens were sexed by presence of nuptial pads and darkly pigmented vocal sac in males, both of which are absent in females. Since most collections analyzed were from breeding populations this was generally adequate. Specimens less than the breeding size of the smallest mature male for that locality were designated as juveniles.
2. Snout-vent: taken from the tip of the snout to the centre of vent, with the toad pressed as flat as conditions of preservation would allow. Differences in preservation affect snout-vent length more than other measurements.
3. Nostril separation: the distance separating the nostrils measured across the nose between their interior (medial) edges.
- 4,5. "Cranial crest" length, right and left sides: measured from the nostril to the posterior edge of the crest. The anterior edge of the cranial crest merges with the bony structure of the nose so variously that the nostril was the best fixed point for anterior reference although this necessitates including more than the crest itself.
6. Cranial Crest type: four arbitrary categories were based on the difference between extreme B. americanus and B. hemiophrys and the intermediate condition.

- 0)- a solid, filled-in boss caused by the cranial crests lacking a groove between them or having only a slight trace of a groove. The extreme B. hemiophrys condition.
- 1) - crests parallel or divergent anteriorly with a bridge joining them across the back, and a distinct, but shallow, groove between them in the boss. Many B. hemiophrys.
- 2) - crests variable, but always with either a trace of filling-in between them or a partial posterior bridge. Common in many individuals from hybrid populations.
- 3) - crests distinct, no trace of filling-in between them and usually convergent anteriorly and strongly divergent posteriorly, but often hooked in slightly toward each other at the extreme posterior. The typical B. americanus condition. Of the other toads analyzed, B. cognatus and B. woodhousei were also scored in this category. The anterior convergence and posterior divergence is extreme in cognatus, and the crests usually less divergent in B. woodhousei. B. boreas has low indistinct crests if they are visible at all, and all crest characters were omitted for this species.

7,8. Nostril to parotoid, right and left sides: the distance

from the middle of the nostril to the anterior edge of the parotoid gland on both the right and left sides. A problem sometimes arose in defining the anterior edge of the gland, which may be somewhat indistinct especially in cases where it was pressed against the post-orbital crest or where a small wart was present between its anterior edge and the post-orbital crest.

9. Cranial Crest, anterior width: cranial crests are raised bony structures that lie between the eyelids on the top of the head. They are more or less complete from the anterior edge of the eyelids to at least their posterior edges (and often beyond, especially in B. americanus). In americanus then tend to slightly converge anteriorly and flare widely posteriorly; in hemiohryys they may be more-or-less parallel (sometimes slightly bulging or contracting in the middle) but generally diverge anteriorly and slightly or strongly converge posteriorly.

The anterior width of the crests was measured across their exterior (lateral) edges at approximately the level of the anterior edge of the eyelids. Some difficulties in precision are inherent, as the crests, particularly in B. hemiohryys, merge with the bony crest of the nose, and the latter begins to spread just anterior to the reference point used. In B.

americanus the nose crest is less prominent and the anterior portion of crest is more distinct. Despite the potential for inconsistencies in measurement, the crest differences are sufficient to make this measurement one of the most apparent distinctions between taxa.

10. Cranial Crest, posterior width: taken across the posterior lateral cranial crest edges, just anterior to their junction with the post-orbital crest or (in many B. hemiophrys) just anterior to the post-orbital "wing", a short right-angled spur more-or-less in the plane of the post-orbital crest.

In B. hemiophrys the crests often do not extend past the post-orbitals and therefore a more posterior measurement, which would have accentuated the crest divergence in B. americanus, was not possible if consistency in measuring the two taxa was to be maintained.

11. Post-orbital crest: three arbitrary categories were based on the relative prominence of the bony crest that extends behind the eyelids, from the lateral edges of the cranial crests, at or near their posterior ends, to the tympanum.

0) - post-orbital crest absent or nearly so (not including in this evaluation the short spur on the lateral edge of the cranial crest present in some B. hemiophrys).

1) - an intermediate condition between 0 and 2, where the

crests are slightly evident, either low and relatively indistinct throughout or else broken on one or both sides.

2) - post-orbital crests elevated and generally distinct.

12. Eyelid length: the anteroposterior length of the protruding pad of skin above the eye.
 13. Head width: taken across the posterior portion of the head at the level of the tympani and between their upper edges. There are bony ridges along the upper edges giving a relatively solid structure to measure against.
 14. Tympanum diameter: the greatest diameter of the eardrum, usually taken on a vertical plane.
 - 15-18. Parotoid gland, right and left sides: the parotoid gland lies roughly over the shoulder of the toad and is generally an elongate, somewhat bean-shaped or oval gland. Generally, the margins are distinct in B. americanus but in B. hemiophrys they may be indistinct, especially at the lateral and posterior edges.
- Adjoining warts sometimes merge with the borders of the parotoid gland, particularly posteriorly, and make definition of gland boundaries difficult. The criterion applied for wart inclusion or exclusion in measurement of the parotoid was to omit them whenever possible, that is, whenever they had any appearance of being a

distinct wart, whether or not they did actually touch the parotoid gland. Often the left and right paratoids were unequal in size. Separate measurements were taken for left and right sides.

- 15, 16. Length: the longest axis of this gland.
- 17,18. Width: taken at right angles to length, at the widest point.
19. Parotoid separation: taken between the inside edges of the two parotoid glands at the point where the left and right glands approach nearest to each other.
- 20,21. Spot length, right and left sides: a measure of the size of markings on an individual toad and the various relations of this to wart size and number. The spot that is usually present on the back at the level of the posterior edges of the parotoid glands and adjacent to the midline on each side was chosen. In rare cases where this was absent the nearest spot to its position was used. There is considerable individual variation in spot size and shape in both species and in intermediates. The spot is most frequently round or oval but irregular shapes also occur and there is a variable degree of fusion of adjacent spots. Any complete separation, no matter how narrow, was used to define the boundary of this from adjacent spots, but generally constrictions of varying degrees were ignored. In

some cases, the spot would be quite regular but in others it would extend far beyond the usual position. Occasionally a spot was continuous far posteriorly on the back and sometimes anteriorly to the post-orbitals. Only rarely did spots cross the midline. Spots were usually aligned anteroposteriorly, parallel to the midline. In all cases this measurement was taken as the longest axis regardless of spot orientation.

22,23. Spot width: right and left sides: the widest "width", that is, the widest portion of the shorter of the two axes, subject to similar variation to that described in the above section.

23. Tibia: the length of the bone formed by the fusion of the right tibia and fibula in toads, measured from knee to heel with the leg flexed, as it is in most preserved specimens. The measurement taken will be slightly larger on preserved material than on the cleaned bone itself because of inclusion of adherent skin. Minor additional error may be introduced by difficulties in completely flexing the leg in preserved material and inadvertently including some of the adjoining articulation of other leg bones. With care this can be largely avoided. The right tibia was measured in all but a few specimens in which it was obviously deformed, broken, or missing. In such cases the left

was used instead.

- 24,25. Wart width, right and left sides: the width, or largest diameter, of the largest wart in the above spot. Generally warts are more or less circular, but in the rare instances of elongate warts the width was taken across the wart in the same axis as the width of the spot.
- 26,27. Number of warts per spot, right and left sides: the number of warts present in each spot defined above. Difficulties due to apparent merging or fusing of adjoining warts were resolved by counting as distinct warts each one defined by a separate peak or each portion separated by a distinct crease. Lack of complete separation of warts is most common in B. americanus.
- 29,30. Diameter of the largest wart of the tibia, right and left tibia: the longest dimension of the largest wart on the upper surface of the tibia. If two obvious peaks were present the wart was regarded as fused from two warts and the measurement taken across the wart so as to measure only the larger one. However, elongated warts which had no obvious peaks were considered one wart and the measurement taken on the long axis.
31. Tarsus: measured from a point on an imaginary line across the posterior (proximal) edges of the metatarsal tubercles (see below) to the "heel" - the junction of the

tarsus and tibia. There is a certain amount of measurement variation introduced because of difficulties in flexing the tibia clear of the end of the tarsus in some preserved specimens.

32. Spade width: the projection used for digging, largest of the two metatarsal tubercles on the outer edge of the tarsus, measured across its base from the inner (medial) corner to the outer (lateral) edge of the peak. This measurement is an attempt to express the effective digging edge width. It may not, as taken express the full width of spade but is chosen because of the clarity of its reference points.
33. Tubercle width: the smaller, medial, metatarsal tubercle on the tarsus measured across its base.
34. Venter: a scoring system based on Blair (1943) for the relative amount of the undersurface covered by dark markings (see Figure 5).
 - 0) - no markings on the ventral surface (exclusive of the throat)
 - 1) - spot in the pectoral region only
 - 2) - spots covering the chest region only
 - 3) - spotting over the anterior 1/3 of the venter
 - 4) - spotting over anterior 2/3 of venter
 - 5) - spotting over the entire venter
 - 6) - reticulated pattern over the venter

This system, unfortunately, excludes much additional variation in ventral markings. The spotting varies in intensity from dark to faint. However, because the variation due to fading in preservative could not be distinguished from natural variation no attempt was made to quantify intensity for geographic analysis. There are also differences in the size of the irregular ventral spots and in their relative density on the undersurface that were also ignored in this analysis.

Some additional characters, found useful by previous authors in comparing variation within or between these taxa, such as colouration (Gaige 1932, Ashton, Guttman and Buckley 1973, Conant 1975 to differentiate B. a. copei), width of mid-dorsal stripe, height of boss, ulna length (Porter 1968, to differentiate B. h. baxteri), parotoid distinctness (Breckenridge 1944, Henrich 1968), appearance of warts, snout length, head length, pattern of dorsal blotches (Henrich 1968), snout length and inter-orbital distance (Underhill 1961) were not included in the present analysis because of difficulties in objectively measuring or evaluating them, or because the variation they describe is accounted for by a different approach here. Of the omissions, colouration is the most serious because obvious differences are apparent between the two taxa. However, intermediates show such a complete gradation that an attempt at objective categories was abandoned. The character is useless with preserved material because of fading.

Call analysis

During the 1969 and 1970 field seasons, tape recordings of breeding calls of individual male toads were made on a Nagra III tape recorder. A hand-held microphone proved adequate because in virtually all cases toads could be approached and the microphone held within a few inches of the calling individual, effectively screening out adjacent members of the chorus. Occasionally adjacent toads were transferred to another portion of the breeding pond when they were too close to the individual being recorded.

Use of a parabolic reflector was thus avoided, together with any distortions such equipment might introduce.

An effort was made to obtain at least four complete, clear calls from each individual recorded. Most individuals were recorded at the ponds along the Trans-Canada Highway, the main transect along the interbreeding zone. In 1969 efforts were concentrated along the latter transect from about 3 miles west to about 31 miles west of the junction of Highway 1 and Highway 11 along Highway 1. Only the single transect was monitored for intermediate calls because of the time required to obtain individual calls. The assumption was made that it was potentially more informative to have many individuals along one complete transect than a few from each of several transects.

In 1970 call sampling was augmented on this transect and localities west in the range of hemiophrys to the Pas, Manitoba, and east in the range of americanus to Oxdrift, Ontario were also sampled.

The body temperature of the recorded toad, as well as adjacent water and air temperatures, was taken immediately after recording was completed. Initially, body temperatures were taken by inserting the probe into the mouth and into the vent but it soon became apparent that the difference between temperatures taken by the two methods was essentially negligible and the first method was abandoned. Temperatures were taken with a Yellow Springs single channel tele-thermometer (0° - 50° C) equipped

with thermistor probe of small flexible vinyl (YS1 Probe 402).

The latter was inserted through the vent into the body and the temperature read as insertion was completed. Every effort was made to insert and read the temperature as soon as the animal was captured in order to minimize the effect of struggling while being held (and thus transferring human body heat). In general this proved practicable.

Individual toads were tagged after recording and the tag number read with temperature, date and locality directly on to the taped call for that individual. The number read was subsequently used for the sub-number in the series in which that toad was preserved, thus providing a means of identifying the individual preserved toad with its call and allowing comparisons between morphological parameters and call parameters.

Tapes were analyzed in the laboratory on a "Sona-Graph" 6061A 85-8000 cps spectrum analyzer manufactured by Kay Electronics, Pine Brook, New Jersey. The Sona-graph produces audiospectrograms of a selected segment of a call. The segment used in the present analysis was chosen from the approximate mid-point of one uninterrupted call for each individual, after test samples of several calls each from selected individuals indicated no difference between calls for the variables considered. Zweifel (1968:271) also noted, in a study of B. americanus and B. w. fowleri, the same lack of difference between calls given by the same individual. The wideband (300 cps filter) generally was used for determining

pulse rate because with the narrow-band (45 cps filter) individual pulses were difficult to distinguish at warm temperatures. The number of pulses in a one second interval were counted directly from the audiospectrogram produced. The mid-point of the dominant frequency band was measured by means of a section to the nearest 100 cps. This is produced by the Sona-graph as a graphic representation of the relative amount of energy found at different frequency levels at a selected point in a recording. These sections were generally taken about half-way through a call.

All calls recorded by a given individual were timed by a stopwatch and the mean duration of the call over the sample determined.

Computer analysis of data

All measurements were transferred to computer punch-cards, with two cards required for the data from each specimen. Analysis was run on the IBM system 370 at the University of Toronto Computing Center.

The discriminant analysis program for two groups used for comparison of B. americanus and B. hemiophrys was programmed at the University of Toronto by D.M. Power, November 1967, revised December 1970 and December 1972. The procedure for this program had been taken from Anderson (1966). Discriminant function analysis requires the use of two groups chosen a priori. This was met by excluding all samples from southeastern Manitoba, well to either side of the area of peak interaction noted in field sampling. The reference

samples were all collections east and west of this area, chosen therefore on geographical origin without regard to morphology. The weights produced by the analysis were then used to produce scores for the unknowns from the excluded area. Scored characters were omitted from the discriminant analysis and males and females were run separately on the remaining 30 characters (all representing measured values except for two counts - the number of warts per spot on the left and right sides). Because the computer would accept only a maximum of 500 specimens in each reference sample with this many characters used, and over 800 were available in each of the reference samples (819 hemiphrys and 825 americanus) for males, the program was set up to run every other specimen in one run for each taxon and then re-run a second time with the alternate specimens. Each run compared approximately one-half the sample of one taxa with one-half the sample of the other. Group I included 412 B. americanus and 409 B. hemiphrys, Group II consisted of 413 B. americanus and 410 B. hemiphrys. The two sets of discriminant weights were then averaged and the resultant weight for each character was applied to the individual scores for all americanus and hemiphrys in the reference samples and to 2461 male Bufo from southeastern Manitoba. Means, variances, standard deviations, and standard errors were calculated for each collection. In addition, histograms of the individual scores for each collection were generated.

The same program was run for females. The smaller female samples (59 americanus and 118 hemiphrys) were included on one

computer run and the resulting weights applied to 225 females from southeastern Manitoba.

A sample of immature individuals proved too small to yield significant results, as although the taxa were distinctive when compared with the program the number of characters exceeded the number of specimens in one reference sample.

Programs for discriminant analysis comparisons of hemiophrys vs. boreas were also run in the University of Toronto program. Additional discriminant analyses involving morphological comparisons of hemiophrys vs. cognatus and the call comparison of americanus vs. hemiophrys were run on the National Museum of Natural Sciences, Ichthyology Section, Hewlett-Packard 9830 mini-computer "ICHTHOS" using Program 29: Hostellings T^2 and discriminant function for two groups from Davis (1971:284-291), as programmed by D.E. McAllister.

Analysis of call variables and a discriminant function involving these were also computed on "ICHTHOS", as well as the correlation of the discriminant function thus obtained for call and the one previously generated on morphological characters for americanus, hemiophrys, and intermediate populations.

Hybridization experiments

During 1969 calling males and gravid females were collected for mating experiments from choruses along the Trans-Canada Highway transect through the interbreeding zone. The localities used were:

31-29 mi. W. on Hwy 1 of Jct. of Hwys 1&11 "B. hemiophrys"

(see Figure 9)

17 mi. W. on Hwy 1 of Jct. of Hwys 1&11 intermediate
population (see Figure 10)

3 mi. W. on Hwy 1 of Jct. of Hwys 1&11 "B. americanus"
(see Figure 11)

A total of nine crosses with three replicates of each (27
sets) were attempted.

female		male
<u>americanus</u>	x	<u>americanus</u>
<u>americanus</u>	x	<u>intermediate</u>
<u>americanus</u>	x	<u>hemiphrys</u>
<u>intermediate</u>	x	<u>intermediate</u>
<u>intermediate</u>	x	<u>americanus</u>
<u>intermediate</u>	x	<u>hemiphrys</u>
<u>hemiphrys</u>	x	<u>hemiphrys</u>
<u>hemiphrys</u>	x	<u>intermediate</u>
<u>hemiphrys</u>	x	<u>americanus</u>

Males and females were collected the same night from all
three localities and paired in selected combinations in one or
two-and-a-half gallon jars in pond water from the locality where
the female had been collected. Mating and egg laying usually
took place overnight but occasionally was delayed until the next
day. If it did not occur within 48 hours the cross was attempted
with another male and female.

(TOP)

Figure 9. Ponds 31 miles West on Highway 1 of junction Highways 1 and 11, Manitoba (Locality 31). This site was a sample area for Bufo hemiophrys and aspen is the major tree cover. May 1969.

(BOTTOM)

Figure 10. Pond 17 miles west on Highway 1 of junction Highways 1 and 11, Manitoba (Locality 35). This site was a sample area for an intermediate Bufo population. The pond is just west of Brokenhead River and south of the Trans-Canada Highway.



(TOP)

Figure 11. Roadside ditches 8 miles West on Highway 1 of junction of Highways 1 and 11, Manitoba. Bufo americanus occurs at this site which is similar to the site 3 miles West on Highway 1 of junction Highways 1 and 11 (Locality 39). Note the dominance of coniferous trees. 23 June 1969.

(BOTTOM)

Figure 12. The margin aggregation behaviour of Bufo tadpoles. Locality and date as in Figure 11.



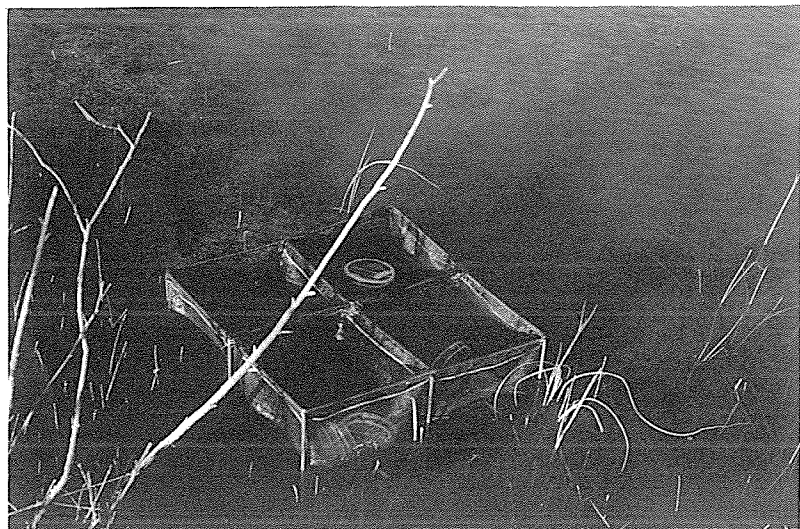
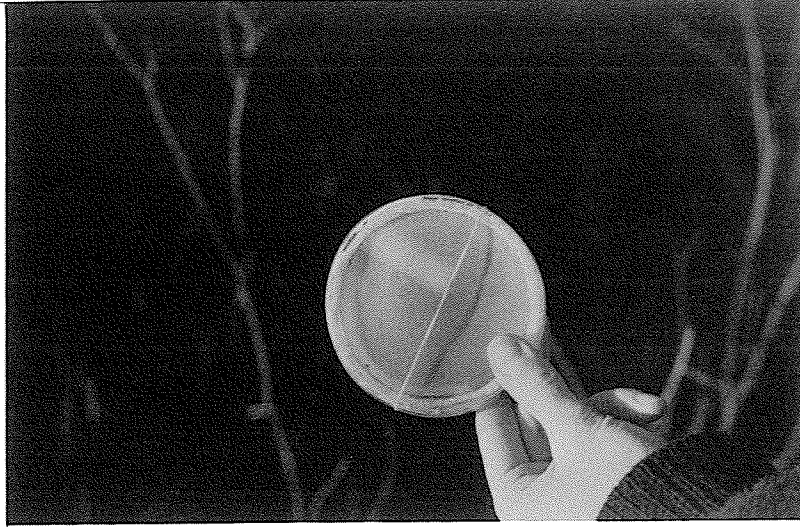
Two hundred eggs were counted out from the batch laid by each female of a successful cross, and divided into four lots of fifty eggs each. One set of 50 eggs from each cross was raised in the laboratory, one of each other lot was placed in a container in each of the three sites from which the adults had been collected. Laboratory crosses were raised in dechlorinated water in shallow plastic pans (Figure 16), field crosses were initially placed in circular petri dishes (Figure 13), each with a piece of their lid removed and replaced by plastic screening to permit water circulation. After hatching, the tadpoles were released into open plastic rearing cages made from plastic screening with a framework of plastic rods. Each unit was approximately 4 x 4 x 1 ft. and each included four 1 x 1 x 1 ft. compartments screened on the sides and bottom but open at the top (Figure 14). They were placed in the water at the edge of the ponds and submerged to approximately half their height (Figure 15). Receding pond levels due to evaporation during the period from hatching to transformation of the tadpoles and level rises after occasional heavy rains necessitated moving the units to compensate for these fluctuations in water levels. Initially, the eggs were checked at daily intervals, but later in tadpole development containers were checked at two-day intervals, until the last tadpole had metamorphosed. Tadpoles were fed Tetramin, a commercial "fish food" preparation that does not unduly contaminate

(TOP)

Figure 13. Plastic container with screening used to hold eggs from each Bufo cross until they hatched. May 1969.

(BOTTOM)

Figure 14. Four-compartment plastic screen rearing container for Bufo tadpoles raised in field sites. An egg container can be seen at the surface in the upper right compartment, and submerged containers are evident in other compartments. May 1969.



(TOP)

Figure 15. Rearing containers in position in pond 31 miles West on Highway 1 of junction Highways 1 and 11, Manitoba. Note sheet plastic lids to prevent predation. Each lid is weighted by stones. June 1969.

(BOTTOM)

Figure 16. Pans used for laboratory rearing of Bufo crosses. Tadpoles may be noted in each pan. June 1969.



water. The water in laboratory pans was changed whenever it appeared fouled but this was not necessary in field cages. Dead eggs and tadpoles were removed and noted at each inspection. Part way through the experimental period a major disaster occurred at the "americanus" site when all rearing cages were removed from the water and placed neatly at the roadside by person or persons unknown. The majority of tadpoles then desiccated due to exposure to air before the next regular inspection, thus terminating these crosses.

RESULTS

1. Field surveys

Field work in eastern Manitoba in the breeding seasons of 1968, 1969, and 1970 revealed no strong differences in breeding times, either in the initiation, peaks, or termination of calling between americanus, hemiphrys or intermediate populations. B. hemiphrys localities tended to be in more open sites, and the air and water temperatures dropped there more rapidly on cool evenings, resulting in hemiphrys choruses tapering off or stopping earlier than americanus choruses.

The general pattern evident in the 1968-70 field seasons in eastern Manitoba was an initial emergence from hibernation and arrival of an occasional individual at the breeding site as early as 19 April but as late as early May. The earliest calling was 24 April in 1969, with the earliest amplexed pairs and eggs 6 May the same year. The first good choruses were heard 13 May 1968, 14 May 1969, and 17 May 1970, indicating that an early or late spring does not alter the time of the initial peak of breeding by more than a few days. It was common in all years to find good choruses on warm nights from mid-May through to early June and for calling to gradually taper off with a few persistent individuals calling as late as the first week in July. A few small choruses were found in middle and late June in 1968 but the bulk of the series from breeding ponds were taken earlier (see list of

specimens examined in Appendix I for dates of strong choruses indicated by size of collections). A similar pattern emerges when the collection dates of breeding hemiophrys from throughout its range are examined. One large collection taken later in the year (60 specimens from the Spruce Woods Forest Reserve 20-21 July 1960) is a post-breeding sample from toads along a sandy road. All large breeding samples are from the latter half of May or early June.

It is notable that there is a much greater variation in the dates for americanus breeding series, especially the series (NMNS 6916) obtained adjacent to Rondeau Park in southern Ontario on 17 April 1963. In the milder climate of southwestern Ontario toads apparently may reach full chorus as much as a month earlier than is possible in eastern Manitoba or the Prairie Provinces in general. In eastern Manitoba, there was no consistent pattern during this study of either americanus or hemiophrys beginning calling appreciably before the other, or continuing later. The latest chorus sampled was of hemiophrys-like individuals (Grand Beach Provincial Park) 21 June 1968 but there were americanus choruses at N.W. Angle Forest Reserve a few days earlier, 16-17 June 1968. In both cases the proximity of the breeding localities to large lakes may have retarded the start of the breeding and prolonged it in comparison to other localities.

Bufo started to metamorphose in early July in eastern Manitoba. The earliest noted was 2 July 1969. This coincided with the



cessation of calling from the last few persistent males and represented roughly a month and a half for development after the initial peak breeding. The latest tadpoles were noted 5 August 1968, but most had transformed by the last week in July, representing a two-three week span of peak transformation that roughly corresponded with the span of most active breeding.

Breeding ponds for both americanus and hemiophrys in eastern Manitoba and elsewhere are variable. Artificial dugout ponds (see Figure 9 and 10) roadside ditches (Figure 11) and shallow quiet shores of rivers and lakes are common breeding sites. A fairly wide area of shallow water seems to be preferred. The breeding sites for hemiophrys, being mainly in aspen parkland and prairie, are in more open surroundings due to the general habitat, and no conclusions can be drawn on any difference in site preference. In general, the same types of breeding sites seem to be chosen by the two taxa.

In 1970 a simple mark-recapture program was carried out at the intermediate (contact zone) locality 17 miles west of junction Highways 1 and 11. Fifty-one male toads were marked and released between 17 May and 11 June. An additional 23 males were collected 18 May from this locality and preserved. Forty-nine recaptures of 28 individual toads were made. A total of 16 females was also taken during the same collecting period. Some males remain at the chorus through the peak breeding period although unmarked individuals may be arriving throughout. Four individuals marked and released 18

May were recaptured on each of 22, 23, and 24 May and three others were captured on two of those nights. There was no evidence of an uneven distribution of morphological types between early and later samples. A simple population estimate gives figures of 42, 47 and 64 male toads for 23 May, 24 May and 5 June, respectively.

2. Analysis of morphological variation for reference samples

a. Discriminant analysis of males

The two discriminations run on alternate specimens from the combined reference sample gave the weights or character coefficients shown in Table 1. A mean discriminant weight for each character was produced by averaging them (Table 1) and these mean weights used to compute discriminant scores for each individual.

The size of the weights reflect in part the relative importance of the characters considered in separating the taxa.

The relative effectiveness of the weights is dependant on the number of units an individual character varies over, and the separation between the taxa within this character. A method of expressing the general distance between taxa is the "Mahalanobis generalized distance" computed by finding the separation between the mean discriminant scores of the taxa. The contribution of each character weight to this value can be obtained by multiplying the weight by the difference between the means of each taxa for that character. This gives the weight contribution (to the Mahalanobis generalized distance) and the sum of these contributions is equal to the Mahalanobis generalized distance (D.E. McAllister,

Table I. Discriminant weights given each character in two analyses (Groups I and II) of male reference samples of Bufo americanus and B. hemiophrys, and the mean weight of the two groups used in computing discriminant scores for individual males, and the weight contribution to the Mahalanobis generalized distance between the combined groups.

	WEIGHT GROUP I	WEIGHT GROUP II	MEAN WEIGHT	MEAN CONTRIBUTION
Snout-vent length	+0.16522861	+0.18881679	+0.177023	+0.9242126
Nostril separation	-8.1142540	-8.586230	-8.350242	+2.5555703
Cranial crest length (right)	+0.76029348	+3.2909012	+2.025597	+3.5536756
Cranial Crest length (left)	+3.6722383	+0.50351000	+2.087874	+3.6579865
Nostril to parotoid (right)	+0.41164047	-0.78529143	-0.186825	-0.2177316
Nostril to parotoid (left)	-0.59715325	+1.0789080	+0.838030	+0.9472763
Cranial crest width (anterior)	-4.7215214	-4.5984650	-4.659993	+0.2671204
Cranial crest width (posterior)	+8.3745794	+8.6095295	+8.492055	+21.7769295
Eyelid length	-3.5560398	-3.2611437	-3.408592	-1.4677809
Head width	-2.0142698	-1.8766050	-1.945437	-1.3364795
Tympanum diameter	-0.65865707	-0.21684265	-0.437750	-0.3360502
Parotoid length (right)	+0.073451102	+0.31809098	+0.195771	+0.2395683
Parotoid length (left)	-0.48348296	-0.37021834	-0.426351	-0.4372118
Parotoid width (right)	+1.0284834	+0.13527966	+0.581882	+0.0483071
Parotoid width (left)	-0.89462119	+0.41438520	-0.240118	-0.0093115
Parotoid separation	-3.0141783	-2.6087561	-2.811467	+4.1812830

Table I Continued

	WEIGHT GROUP I	WEIGHT GROUP II	MEAN WEIGHT	MEAN CONTRIBUTION
Spot length (right)	-0.034990408	+0.036637843	-0.000824	-0.0008016
Spot length (left)	+0.05400848	+0.025961559	+0.040681	-1.0455871
Spot width (right)	-0.37663031	-0.18416786	-0.280399	+0.0474776
Spot width (left)	-0.76379973	-0.31844270	-0.541121	+0.0950692
Wart width (right)	+3.37714357	+1.0056877	+2.388562	+1.8619682
Wart width (left)	+3.5572758	+1.7886143	+2.672945	+2.1319337
No. warts per spot (right)	-0.090008795	-0.10848355	-0.099246	+0.1581416
No. warts per spot (left)	-0.098180711	-0.18084419	-0.139512	+0.2189280
Tibia length	-1.1040401	-0.79755020	-0.950795	-2.1168822
Diameter of largest wart on tibia (right)	+0.29262280	-0.93891734	+0.615770	+0.5780064
Diameter of largest wart on tibia (left)	+1.0001144	+1.13967094	+1.198411	+ 1.1220402
Tarsus length	+4.0203476	+4.3927183	+4.206533	+ 10.3077514
"Spade" width	-6.1017218	-6.1168079	-6.109264	+ 1.9970081
Inner metatarsal tubercle width	+3.0812159	+0.45624775	+1.768732	+ 0.6493347

Mahalonobis generalized distance (mean): 51.3517513

personal communication). This weight contribution to separation of the taxa is also given in Table 1.

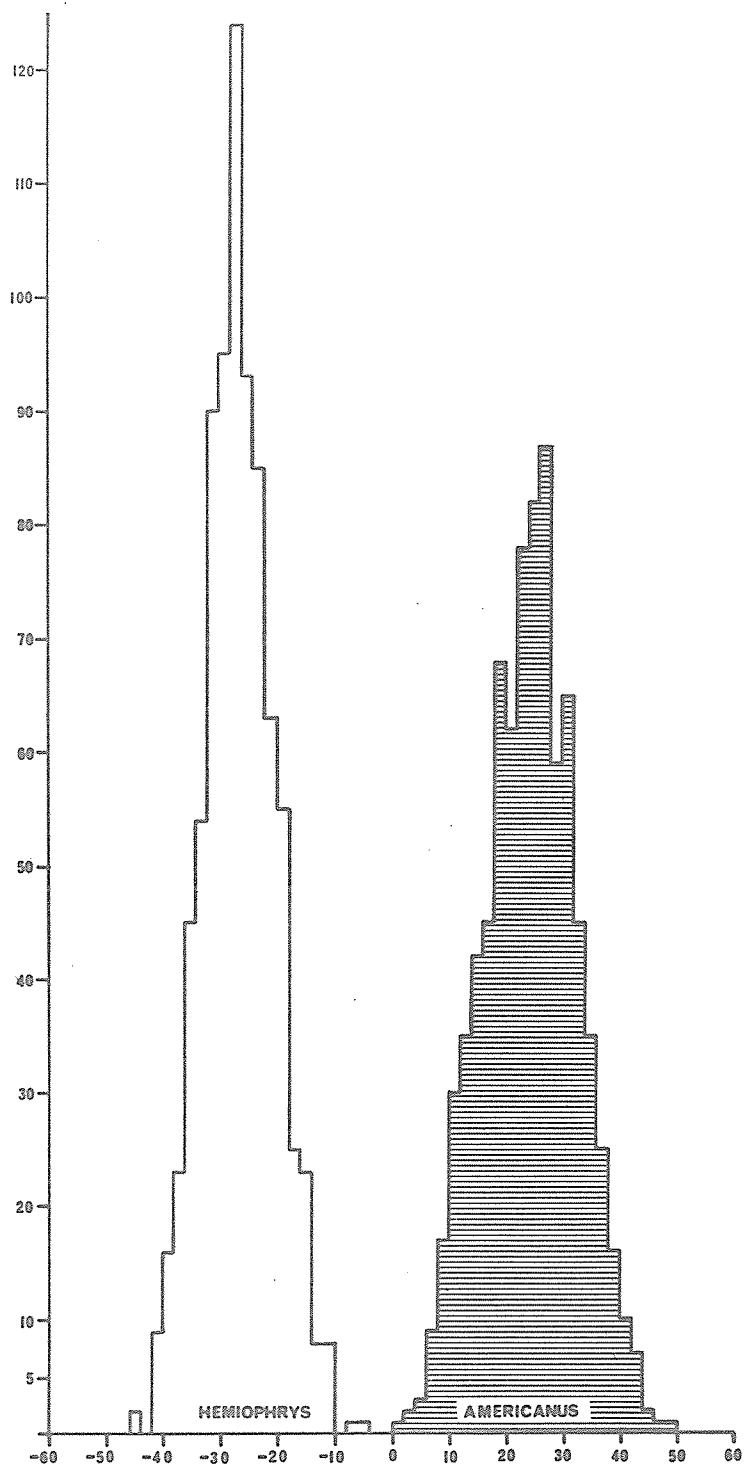
Inspection of these values indicates that posterior cranial crest width, tarsus length, parotoid separation, and cranial crest length are making relatively large contributions (in descending order) to the separation between taxa.

High values for nostril separation, anterior cranial crest width, head width, parotoid separation, eyelid length and width of the outer metatarsal tubercle ("spade") seem typical of B. hemiophrys, and high values of posterior cranial crest width, cranial crest length, width of largest wart in a blotch, tarsus length, and width of inner metatarsal tubercle seem typical of americanus.

When average discriminant weights were applied to the entire male reference sample of both species (1644 specimens) to obtain a discriminant value for each specimen, a single specimen, a hemiophrys from Delta, Manitoba, was misclassified, due to an error in measurement which was discovered on re-examination of the specimen and corrected. Recalculation with this measurement corrected gave a discriminant score of -10.2223, well within other hemiophrys scores.

The sample size for each population sample of males is given in Appendix I. Histograms for the pooled male reference samples are given in Figure 17. With six exceptions, sample sizes are over 20 males per locality.

Figure 17. Histograms of individual discriminant scores for male reference samples: 819 hemiophrys (open) and 825 americanus (hatched). Vertical scale represents number of specimens, horizontal scale the discriminant score.



The population mean for the discriminant score, ranging from -18.7 at Delta, Manitoba, to -30.8 at Qu'Appelle Valley in Saskatchewan for hemiophrys and -13.3 at Oxdrift, Ontario to +33.8 at Rondeau Provincial Park, Ontario, for americanus. The highest values for hemiophrys are in the Aspen Parkland and Boreal Forest of Manitoba and adjacent North Dakota with slightly lower values across middle Saskatchewan and Alberta and northern Alberta, largely in the Boreal Forest. The mean of the disjunct Wyoming population falls with these. Southern Saskatchewan and Alberta populations have the lowest values from shortgrass prairie and Aspen Parkland habitats. The lowest values for americanus are from northern Minnesota (Hibbing), northwestern Ontario (Oxdrift) and the southern end of James Bay (Whitetop Creek).

The variance, as an indication of the dispersion of discriminant values within the population samples, is given in Appendix II B. Variances of hemiophrys populations were generally lower than for americanus, although higher variances are seen in hemiophrys populations in areas adjacent to eastern Manitoba.

In addition, histograms of discriminant scores for each population sample, or pooled sample, are given in Appendix II C-H. In general, they confirm that the values are roughly normally distributed within each population and that individual values are well away from the mid-point value of the discrimination axis. The closest approach to the mid-point is made by an individual hemiophrys from Perryvale, Alberta, and an americanus from Oxdrift,

Ontario.

b. Univariate and ratio analysis of males

In addition to the discriminant function analysis, the geographic variation in three scored characters: cranial crest, post-orbital crest and venter (Appendix II, I-K) all excluded from the discriminant analysis, was examined. Snout-vent length and ratios of tibia/snout-vent, spot length/snout-vent, cranial crest posterior width/head width and outer metatarsal tubercle width/tarsus length (Appendix II, L-P), were also examined for patterns of variation. These measurements had been used individually, not as ratios, in the discriminant analysis.

Cranial crest score

Scores of 0 (boss) and 1 (grooved crest bridged posteriorly) were thought to be characteristic of hemiohryps. A score of 3 denoted a typical americanus crest. A score of 2 was assigned to any intermediate condition. Mean values for hemiohryps range from 0.1 to 1.0 and all mean values for americanus are 3.0. Samples for hemiohryps do not show a geographic pattern outside of Manitoba, although the Manitoba and North Dakota samples are consistently high, 1.0 to 0.8.

Post-orbital crest score

All americanus populations examined in this study had mean scores of 2.0 and 1.9 excepting 1.8 scores for the Lac Attila and Moosonee samples and 1.5 for Oxdrift. The means for hemiohryps ranged from 1.5 to 0.1 with high values (1.0 to 0.8)

in eastern Manitoba, but considerable variation exists between populations elsewhere in the range, with one central Alberta population (Perryvale) scoring 1.5, and one southern Saskatchewan population (Tompkins) scoring 1.3.

Venter score

This score ranged from 0 (immaculate venter) to 6 (reticulated venter) and as such covers the variation in americanus group toads. Means for hemiophrys populations varied from 4.3 to 5.0, with low values in central Manitoba (4.3 at Delta, and 4.6 at Spruce Woods). The highest values were in southern Alberta (5.2 at Brooks and at Sutherland) with northern values in Alberta, Saskatchewan and Manitoba all at 5.0. The range in americanus was greater, from 4.2 at Rondeau to 6.0 at Sept Iles. Northern populations of americanus all tended to have high mean scores (above 5.5) and southern populations had the lowest scores, (4.8 and lower) with those intermediate geographically tending toward intermediate scores (5.0 - 5.2). An anomaly is the Cap-des-Rosiers population at the tip of the Gaspé with a mean of 4.7. Less surprising is the 5.6 score for Roseneath, Prince Edward Island, putting it closer to northern populations in this character.

Snout-vent length

Although there is an obvious difference in means between species (Table II) for the whole reference sample there is a great deal of variation between population means within each species. For americanus

Table II. Mean values for each character measured in reference samples of male Bufo americanus and B. hemiophrys.

	<u>Bufo americanus</u>		<u>Bufo hemiophrys</u>	
	<u>Group I</u>	<u>Group II</u>	<u>Group I</u>	<u>Group II</u>
Snout-vent length	62.159164	62.004028	56.797302	56.924149
Nostril separation	3.9171495	3.9309130	4.2321920	4.2279654
Cranial crest length (right)	12.6222904	12.582693	10.848487	10.848342
Cranial crest length (left)	12.634803	12.616809	10.868276	10.879306
Nostril to parotoid (right)	12.759092	12.718702	11.580885	11.566053
Nostril to parotoid (left)	12.764392	12.719232	11.629278	11.593626
Cranial crest width (anterior)	4.9079227	4.9357510	4.9805956	4.9777222
Cranial crest width (posterior)	6.7011232	6.6863594	4.1363468	4.1223583
Eyelid length	7.5224867	7.4919243	7.0796156	7.0735712
Head width	17.055084	17.057892	16.364105	16.374908
Tympanum diameter	4.9554949	4.9359989	4.1847582	4.1713829
Parotoid length (right)	11.721546	11.619533	10.434548	10.459098
Parotoid length (left)	11.711373	11.650821	10.654340	10.659307
Parotoid width (right)	6.4105911	6.3711004	6.2896471	6.3260069
Parotoid width (left)	6.3710337	6.3008833	6.2554150	6.338944
Parotoid separation	7.7693291	7.8577881	9.3114061	9.2901602

Table II Continued

	<u>Bufo americanus</u>		<u>Bufo hemiophrys</u>	
	<u>Group I</u>	<u>Group II</u>	<u>Group I</u>	<u>Group II</u>
Spot length (right)	8.2746706	8.1989489	9.3226452	9.0972290
Spot length (left)	8.0421515	7.8604441	9.1226511	9.0211334
Spot width (right)	4.3023443	4.2669897	4.4395208	4.4684544
Spot width (left)	4.3076839	4.2541599	4.4869576	4.4262648
Wart width (right)	2.7535648	2.7376938	1.9527435	1.9794445
Wart width (left)	2.7809954	2.7781324	1.9698620	1.9940720
No. warts per spot (right)	2.7038832	2.9176750	4.4498777	4.3585358
No. warts per spot (left)	2.6941738	2.6973362	4.2933979	4.2365847
Tibia length	23.107529	23.026627	20.834091	20.847198
Largest wart on tibia (right)	3.0771122	3.0401163	2.1119099	2.1279736
Largest wart on tibia (left)	3.1288061	3.074981	2.1693630	2.1618786
Tarsus length	15.663939	15.685769	13.216190	13.232688
"Spade" width	4.7040462	4.6698914	5.0246153	5.0030861
Inner metatarsal tubercle width	2.4746885	2.4432659	2.0818386	2.1018782
Mean discriminant score				
(centroid):	24.037781	246.83716	-29.128174	-24.89318
Mahalanobis generalized distance:	between Group I centroids: 53.165955		between Group II centroids: 49.578034	

the range was from 71.8 mm (Rothierville, Quebec) to 53.9 mm (Moosonee, Ontario). Hemiophrys ranged from 70.8 mm (Calgary, Alberta) to 45.8 mm (Delta, Manitoba). For individual males, hemiophrys ranged from 79.1 mm (near Brooks, Alberta) to 41.3mm (Delta, Manitoba) and americanus from 88.4 mm (near Rondeau Provincial Park, Ontario) to 42.1 mm (Whitetop Creek, near Moosonee, Ontario). Commonly, there is about a 20 mm spread from the largest to the smallest mature male in any given sample, though it ranges from about 5 to 25 mm, with the smaller hemiophrys generally having less of a spread (often 10-15 mm) within one population sample. However, in the Rondeau americanus sample the range is 38.4 mm.

The largest hemiophrys sample means (61.2 mm and larger) are in the prairie region of Southern Alberta and Saskatchewan. There is no clear-cut geographic or vegetation region arrangement of the other groups, though there is a tendency for the sample means to be smaller in the east and larger in the west. Within americanus the smallest means are generally in the north (52.9 to 60.5 mm) but the large value of the sample from Mile 134, North of Sept Iles, is out of line at 64.9 mm and the small value from Moncton, N.B., at 57.3 mm is aligned with the smaller northern means.

Tibia/snout-vent length

The relative length of the tibia is variable in both americanus and hemiophrys. Within hemiophrys there is little apparent pattern, though southern Manitoba scores are among the lowest, and southern Alberta and Saskatchewan are among the

highest. In americanus there is a tendency for northern scores to be low and southern scores to be high.

Spot length/snout-vent length

The range in hemiphrys (0.148 to 0.175) is much narrower than that in americanus (0.090 to 0.216). Within hemiphrys there is no obvious geographic pattern to the sample means of this ratio from north to south or between vegetation areas. In americanus, however, the northern populations (Moosonee, Lac Attila, Mile 134, Sept Iles, Routhierville and Cap-des-Rosiers) have generally higher values (between 0.136 and 0.216) which encompass the hemiphrys means, while more southern populations (Oxdrift, Hibbing, Rondeau, Moncton, Roseneath and Musquodoboit) have low values (0.119 - 0.090). Kenogami, North Bay and Ottawa are intermediate (0.128 to 0.134) as is Wolfville (0.132). That the western americanus have smaller dorsal spots than hemiphrys heightens the contrast between the taxa in adjacent western Ontario and eastern Manitoba.

Posterior width of the cranial crest/head width

Hemiphrys population means of this character range from 0.240 to 0.281. There does not seem to be any obvious geographic pattern to the ratio within the taxa, as both high and low means occur generally throughout the range, except that the two highest values (0.280, 0.281) are in central Manitoba whereas all other values are 0.269 or below.

In americanus the values for means vary from 0.339 to 0.442.

The low value is from Oxdrift in western Ontario. The next lowest value is 0.370 from Whitetop Creek. All other values are 0.379 (Cap-des-Rosiers, Moosonee) or above. Values at 0.398 or above are predominant in the east (six out of eight means). In extreme southern Ontario values are 0.408 at Rondeau and 0.401 at Point Pelee. The other central values are 0.390 - 0.395 in the Kenogami to Ottawa area and 0.382 at Lac Attila. There is no clear north-south cline.

Spade/tarsus

The mean values for hemiophrys populations varied from 0.352 to 0.402. The lowest is from The Pas, Manitoba and the highest is from Stathmore in western Alberta. In americanus means varied from 0.267 at Lac Attila to 0.314 at Moncton, New Brunswick, and Sept Iles, Quebec. No clear geographic trends are evident in either taxon.

c. Discriminant function analysis of females

The discriminant weights and means for each character for females are given in Table III.

Posterior cranial crest width made the largest contribution as it did in the male analysis. Tarsus length and parotoid separation also make contributions but the largest wart on the right tibia, the cranial crest length (right side) and distance from nostril to parotoid on the left side seem to make disproportionate contributions to this separation compared to their contribution in the discriminant analysis of males. The Mahalanobis generalized

Table III. Discriminant weights and mean values for each character in discriminant analysis of female reference samples of Bufo americanus and B. hemiophrys, and the weight contribution to the Mahalanobis generalized distance.

	Discriminant Weights	Means Bufo americanus	Bufo hemiophrys	Contribution
Snout-vent length	+0.05570437	72.499863	59.72431	+0.711654
Nostril separation	-12.574883	4.5457535	4.3228445	-2.803055
Cranial crest length (right)	+8.7230959	14.881289	11.388057	+30.471798
Cranial crest length (left)	+0.99025917	14.857541	11.420262	+3.403797
Nostril to parotoid (right)	+2.4354296	15.081285	12.211794	+6.988443
Nostril to parotoid (left)	-0.99551696	15.127056	12.233808	-2.880277
Cranial crest width (anterior)	-5.6168242	5.8474321	5.2152014	-3.551129
Cranial crest width (posterior)	+11.437762	8.1440296	4.3813000	+ 43.037206
Eyelid length	-0.16800469	8.6168976	7.2346783	-0.232219
Head width	-0.66918558	20.243988	16.905833	-2.233842
Tympanum diameter	-0.59421539	5.7406559	4.3101177	-0.850048
Parotoid length (right)	+0.41642243	13.593149	10.921095	+ 1.112703
Parotoid length (left)	+1.6200924	13.721965	11.038900	+ 4.346813
Parotoid width (right)	-1.5974340	7.2253618	6.5355158	-1.101983
Parotoid width (left)	-3.1625957	7.1067219	6.5041580	-1.905666
Parotoid separation	-6.1137037	9.1507912	9.7041588	+ 3.383126

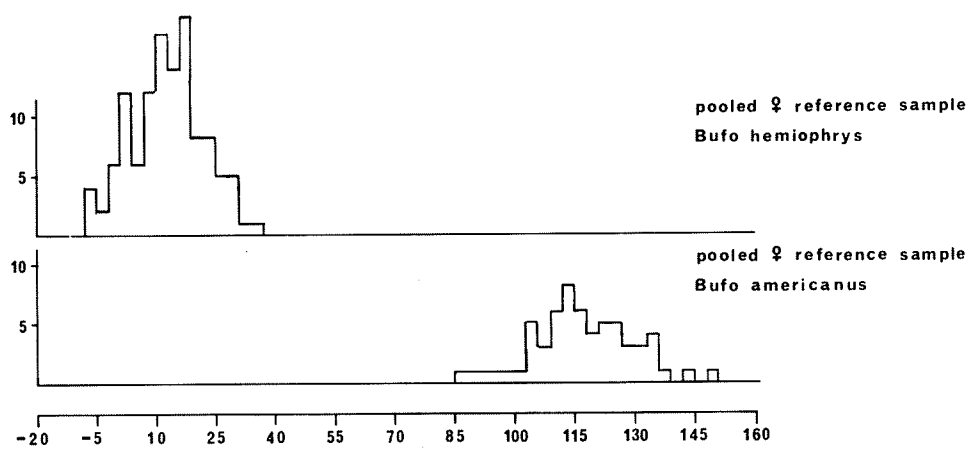
Table III Continued

	Discriminant Weights	Means Bufo americanus	Bufo hemiphrys	Contribution
Spot length (right)	+0.13028830	10.289782	9.6245070	+0.086678
Spot length (left)	+0.23475748	10.540627	9.6906052	+0.199549
Spot width (right)	+4.4119072	5.2016764	4.5041809	+3.077285
Spot width (left)	-1.2844067	5.1830263	4.4414635	-0.952468
Wart width (right)	+5.1356106	3.2084694	2.0423670	+5.988648
Wart width (left)	+3.3310843	3.1898260	2.0398245	+3.830752
No. warts per spot (right)	+0.66464460	2.6440678	4.4491520	-1.199739
No. warts per spot (left)	-1.7433395	2.6779652	4.3135586	+2.851395
Tibia length	-1.012804	26.891434	20.971939	-5.995288
Largest wart on tibia (right)	+6.8065100	3.7305031	2.1245699	+10.930800
Largest wart on tibia (left)	+0.53891832	4.4559221	2.1906691	+1.220786
Tarsus length	+2.0391092	18.149078	13.522799	+9.433488
"Spade" width	-10.362802	5.6287861	5.3338470	-3.056395
Inner metatarsal tubercle width	+0.42757177	3.0406723	2.2347364	+0.344595
Mean discriminant score (centroid):		117.73267		
Mahalanobis generalized distance: 104.6573390			13.075331	

distance is 104.6573390 for female reference samples compared to less than half that value (51.3517513; Table I) in the male analysis.

The histograms of the individual scores for the female reference sample are plotted in Figure 18, showing the separation obtained. Because of the generally higher weights in the discrimination and larger values of individual measurements the scores are higher than obtained in the male discrimination. As individual population samples were small, these have not been plotted separately.

Figure 18. Histograms of individual discriminant function scores for pooled reference samples of females of B. hemiophrys and B. americanus. Vertical axis shows number of individuals and the horizontal gives the score. List of localities, dates, catalogue numbers and number of specimens is given in Appendix I.



3. Morphological character comparison of southeastern Manitoba Bufo.

a. Discriminant scores of males

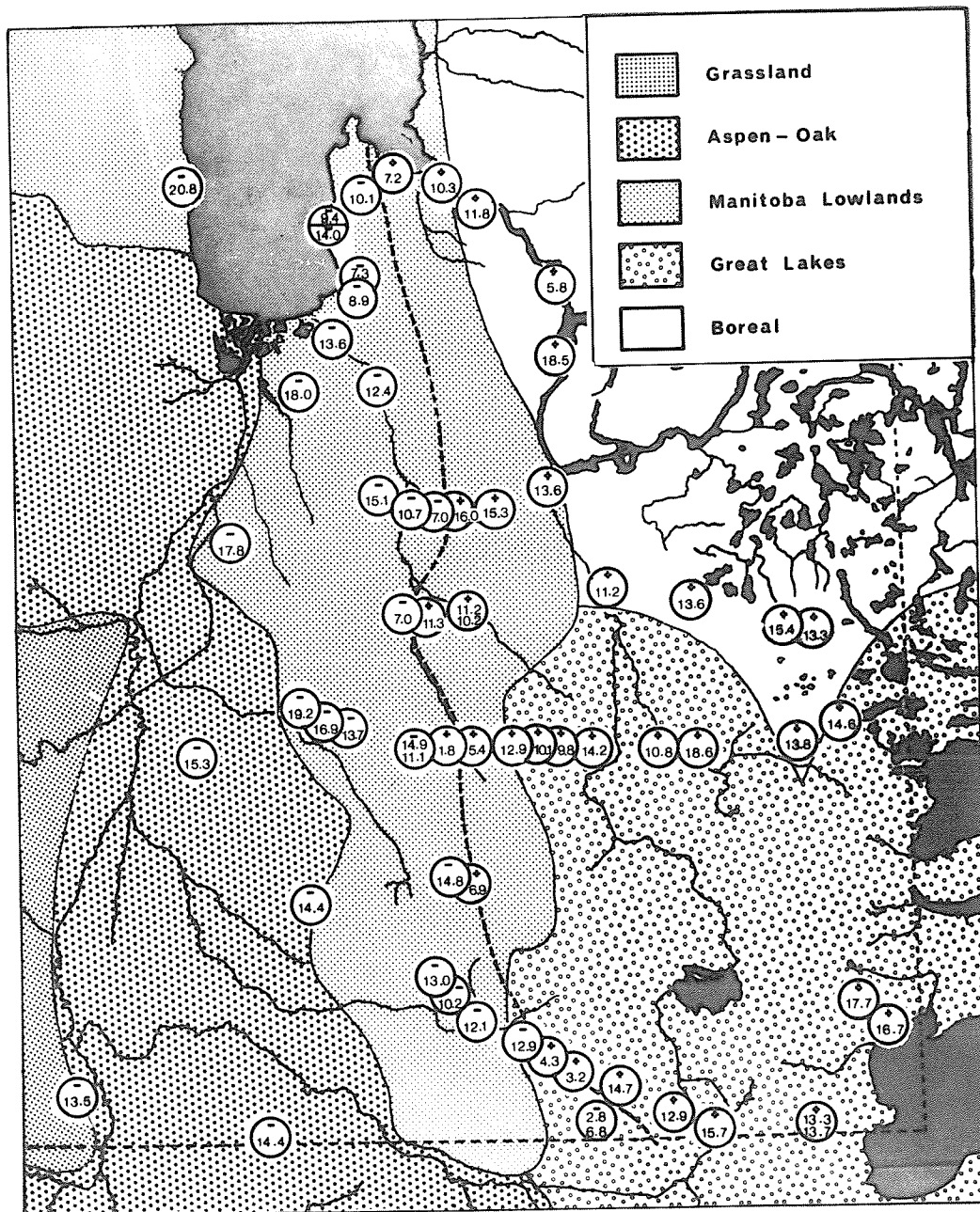
Figure 3 gives the distribution of samples in eastern Manitoba. None of these collections were included in the reference samples used for the discriminations between hemiophrys and americanus. These collections can be divided roughly into six west-east transects for discussion:

1. The northern transect: localities 1 to 14.
2. The Beausejour transect: localities 15 to 21.
3. The Vivian transect: localities 22-38.
4. The Trans-Canada Highway (highway 1): localities 29-43.
5. The Marchand transect: localities 44-48.
6. The southern transect: localities 49-61.

Sample sizes for breeding males are given in Appendix I. There is great disparity in number (2 - 268 individuals) largely because of local weather conditions during the breeding period. Some localities represent pooled adjacent samples, several samples from separate visits during the same breeding season and/or several collections taken in different years.

Figure 19 gives the mean discriminant score for each population sample. That western scores are low and eastern ones high is immediately apparent. The rapid transition from minus to plus (between -10 and +10) scores occurs largely in the eastern half of the Manitoba Lowlands Forest Region (of Rowe 1959), with

Figure 19. Map of southeastern Manitoba showing discriminant function mean scores for collections of male Bufo.



the southern transect transition in the western extreme of the Great Lakes Forest. Figure 20 gives the variance values for these samples. Generally a zone of very high variances (in the 80-258 range) clearly coincides with the intermediate means in five of the transects. In the northern transect the variances are not as large in the transition zone but values of 61 and 64 are larger than those in eastern and western samples in the region. Perhaps the exact middle of the transition zone has not been sampled as effectively in this transect as it was in the others. That high variances are correlated with intermediate scores is further brought out by Figure 21 where mean discriminant scores are plotted against the variance. Most very high variances are from the contact zone.

Figures 22 to 28 present histograms of the scores of individuals within populations sampled. In the northern transect (Figure 22) the transition occurs between localities 9 and 10. Various other samples show occasional individuals over the mid-point of the discrimination axis. In the Beausejour transect (Figure 23) the shift occurs between locality 18 and locality 19, but localities 17 and 20 show slight extensions over the mid-point of the discrimination. In the Vivian transect (Figure 24) the shift is between localities 22 and 23 but the samples from locality 24 show some extensions over the mid-point. This is also marginally evident in locality 25 and also in locality 28. The individual left of mid-point in the latter sample may be simply a chance extreme recombination from

Figure 20. Map of southeastern Manitoba showing variance of discriminant function scores for collections of male Bufo.

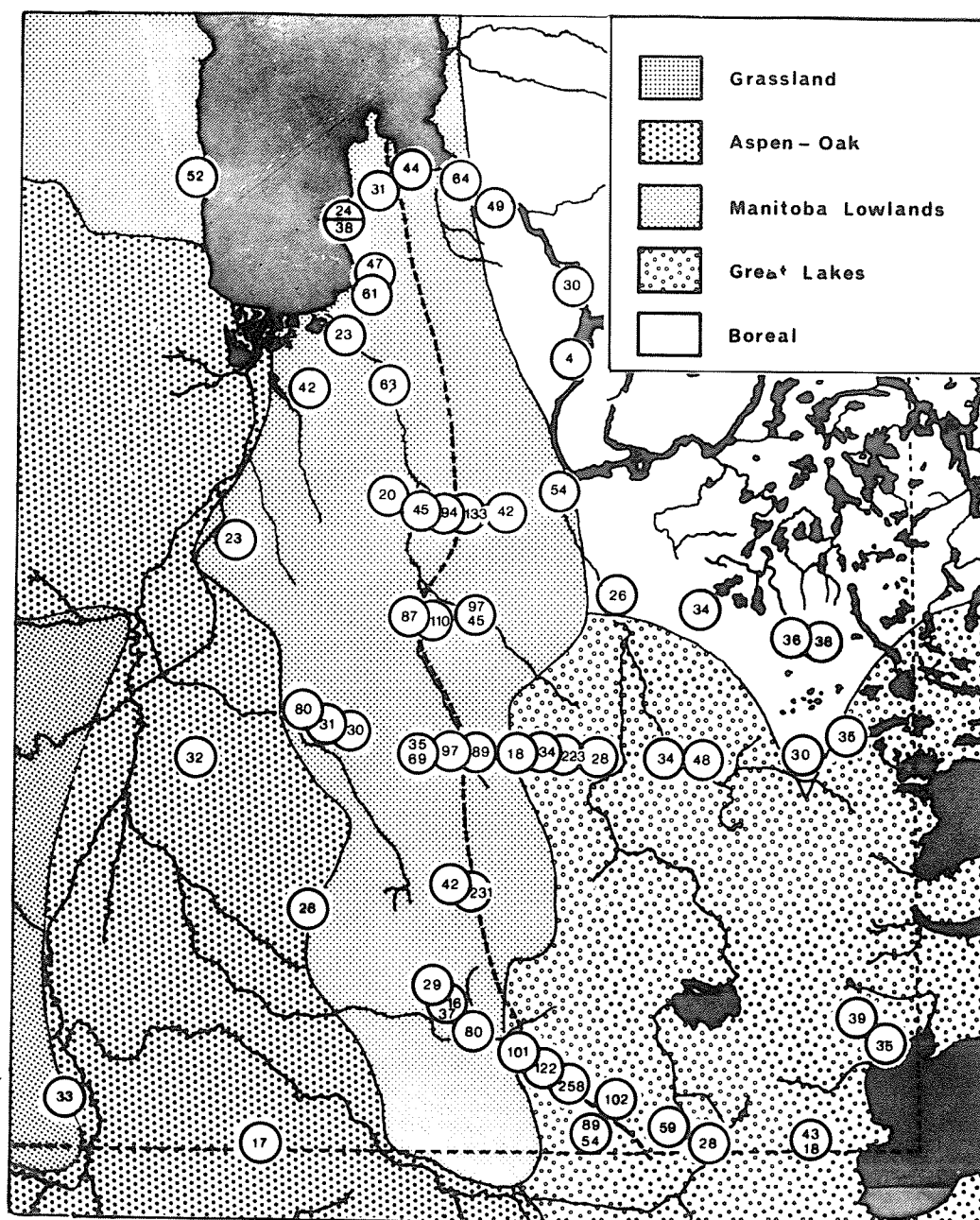


Figure 21. Relationship of mean discriminant score and variance for reference samples of B. americanus and B. hemiophrys and for Bufo samples from eastern Manitoba. The most intermediate scores have the largest variances.

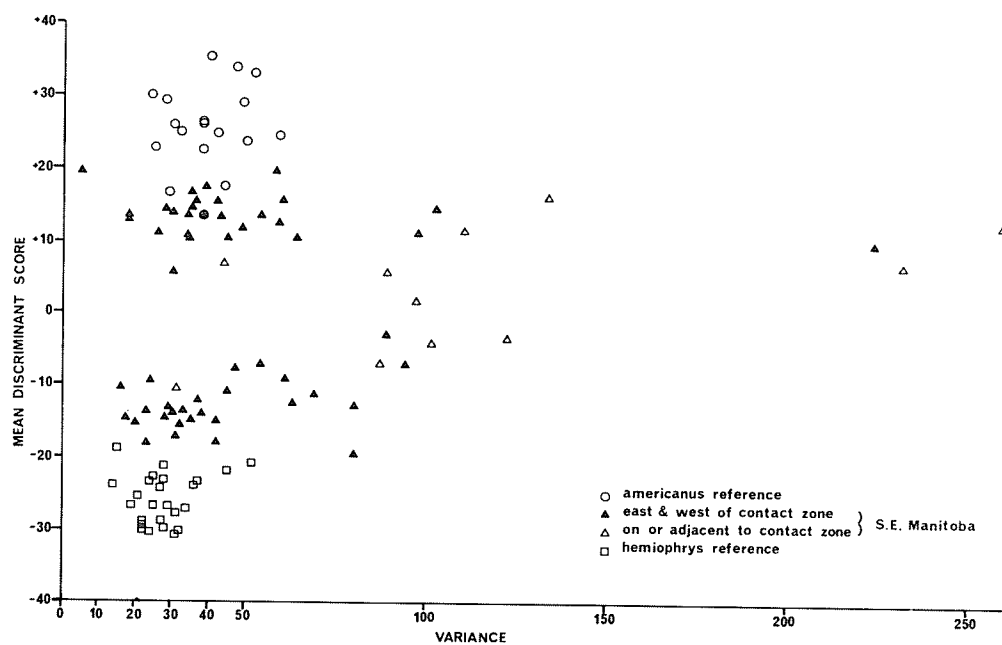


Figure 22. Histograms of individual discriminant function scores of collections of male Bufo for collecting stations 1-14, the northern transect through southeastern Manitoba. The two vertical lines indicate the mid-point of the discriminant analysis between americanus and hemiophrys reference samples. M indicates the position of the mean for the sample and D is the position of one standard deviation on either side of the mean (the unequal distances apparent in some histograms are due to rounding of values to fit the whole number scale units used). For each sample the locality designation is given, and to the right of it the catalogue number (National Museum of Natural Sciences, Herpetology Section, unless otherwise noted) followed by a dash and the number of specimens in that sample. The horizontal scale at the bottom gives the discriminant score values, the vertical one on the left gives the number of individuals.

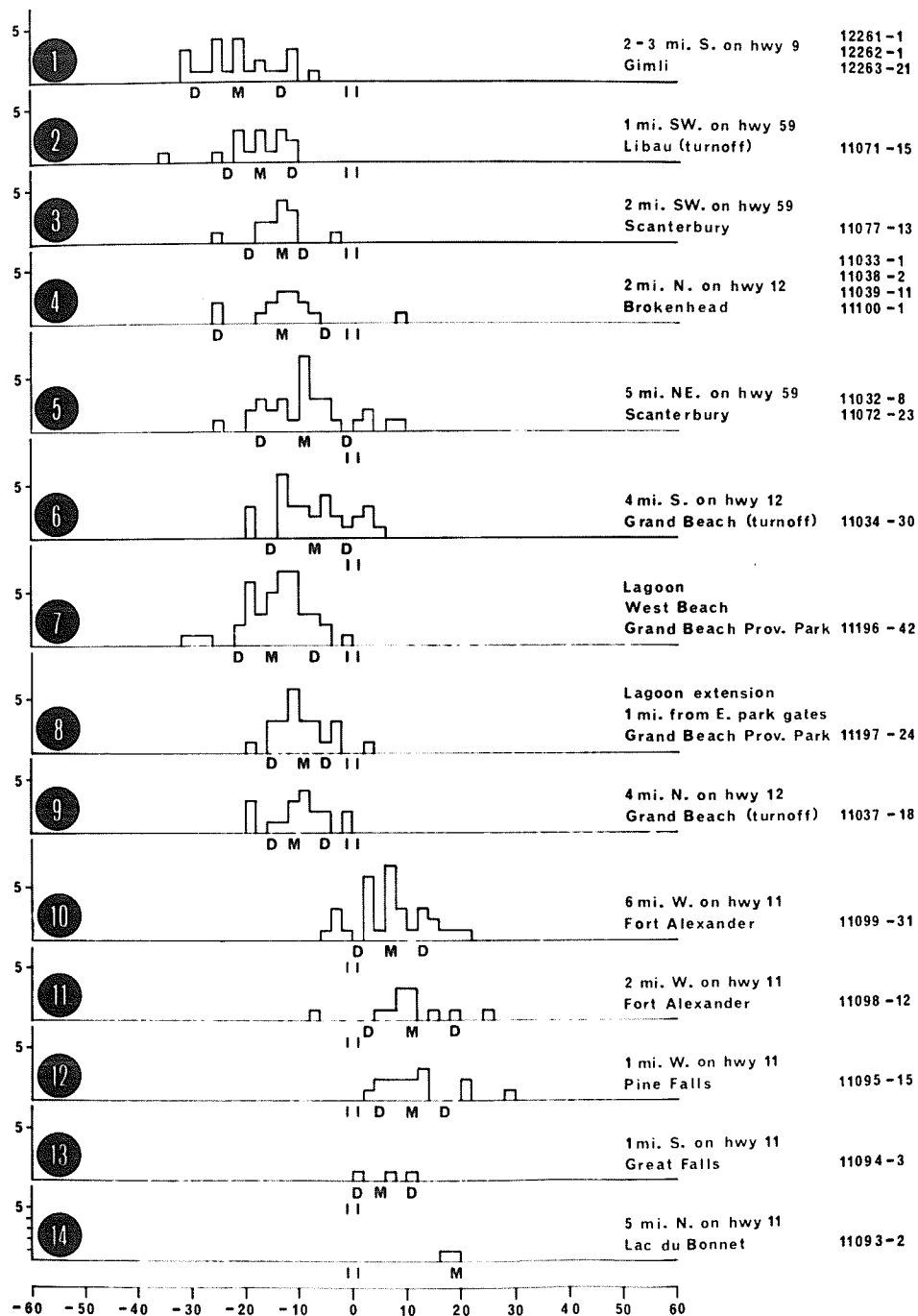


Figure 23. Histograms of individual discriminant function scores of collections of male Bufo for collecting stations 15 - 21, the Beausejour transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 22.

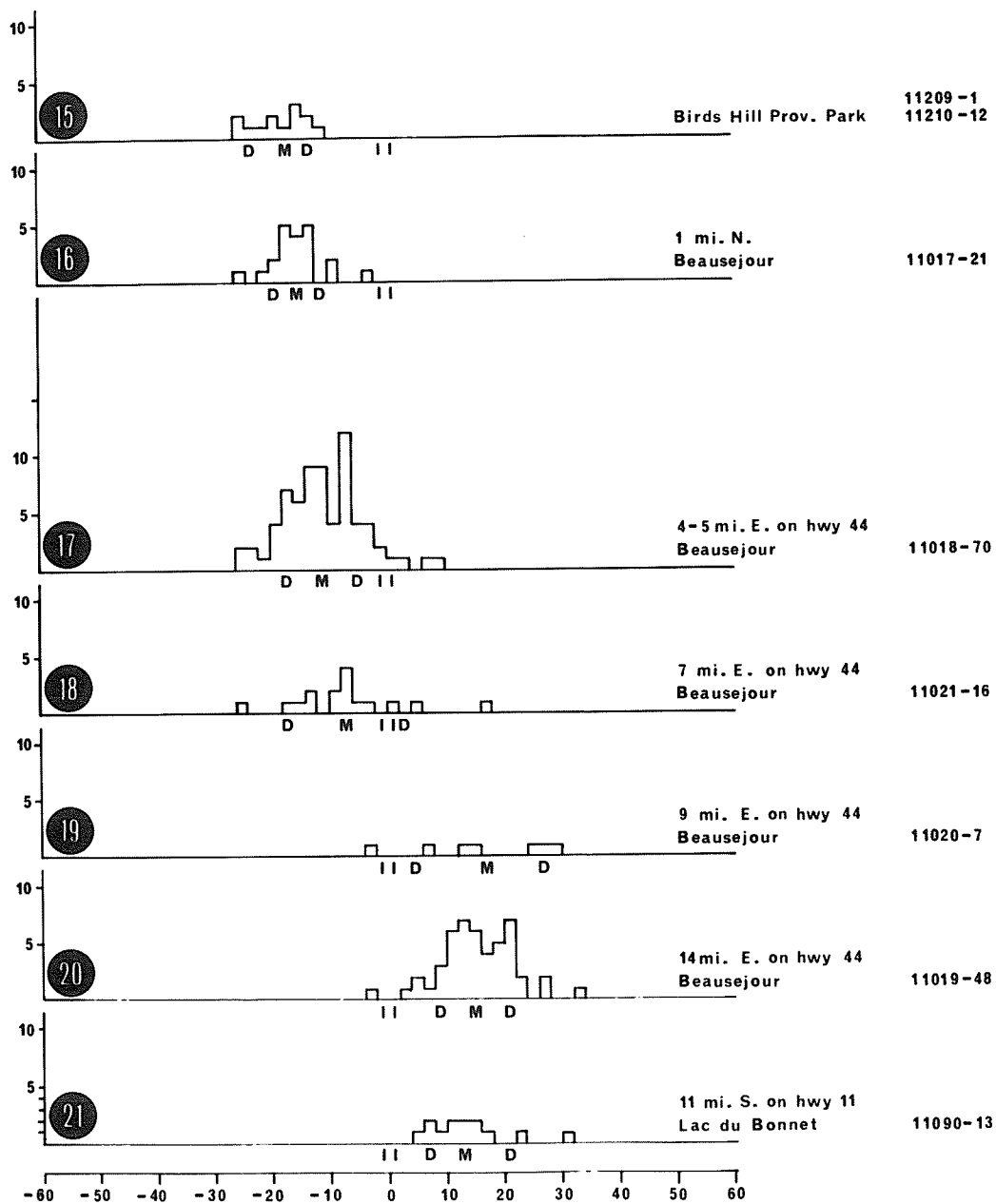


Figure 24. Histograms of individual discriminant function scores of collections of male Bufo for collecting stations 22 - 28, the Vivian transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 22.

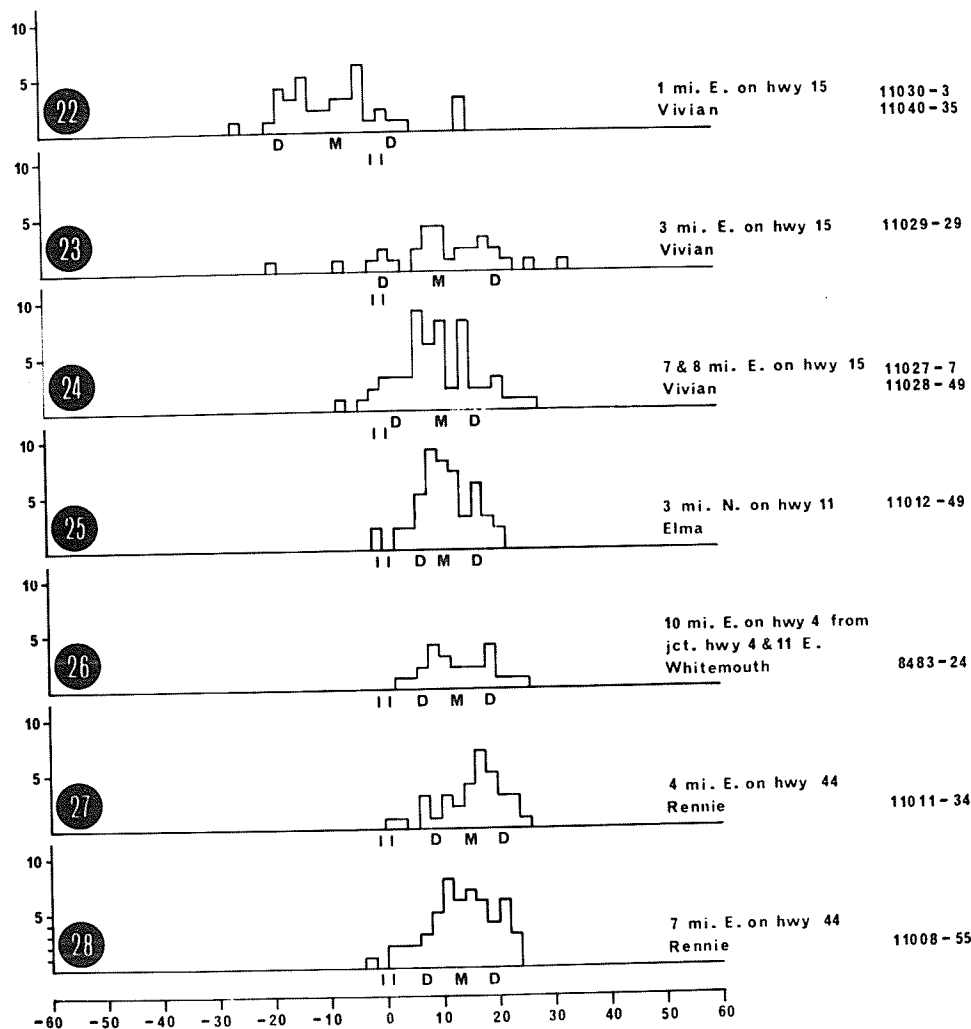


Figure 25. Histograms of individual discriminant scores of collections of male Bufo for collecting stations 29 - 34, the western portion of the Trans-Canada Highway transect through southeastern Manitoba. An explanation of the letters, scale, etc. is given in the legend of Figure 22.

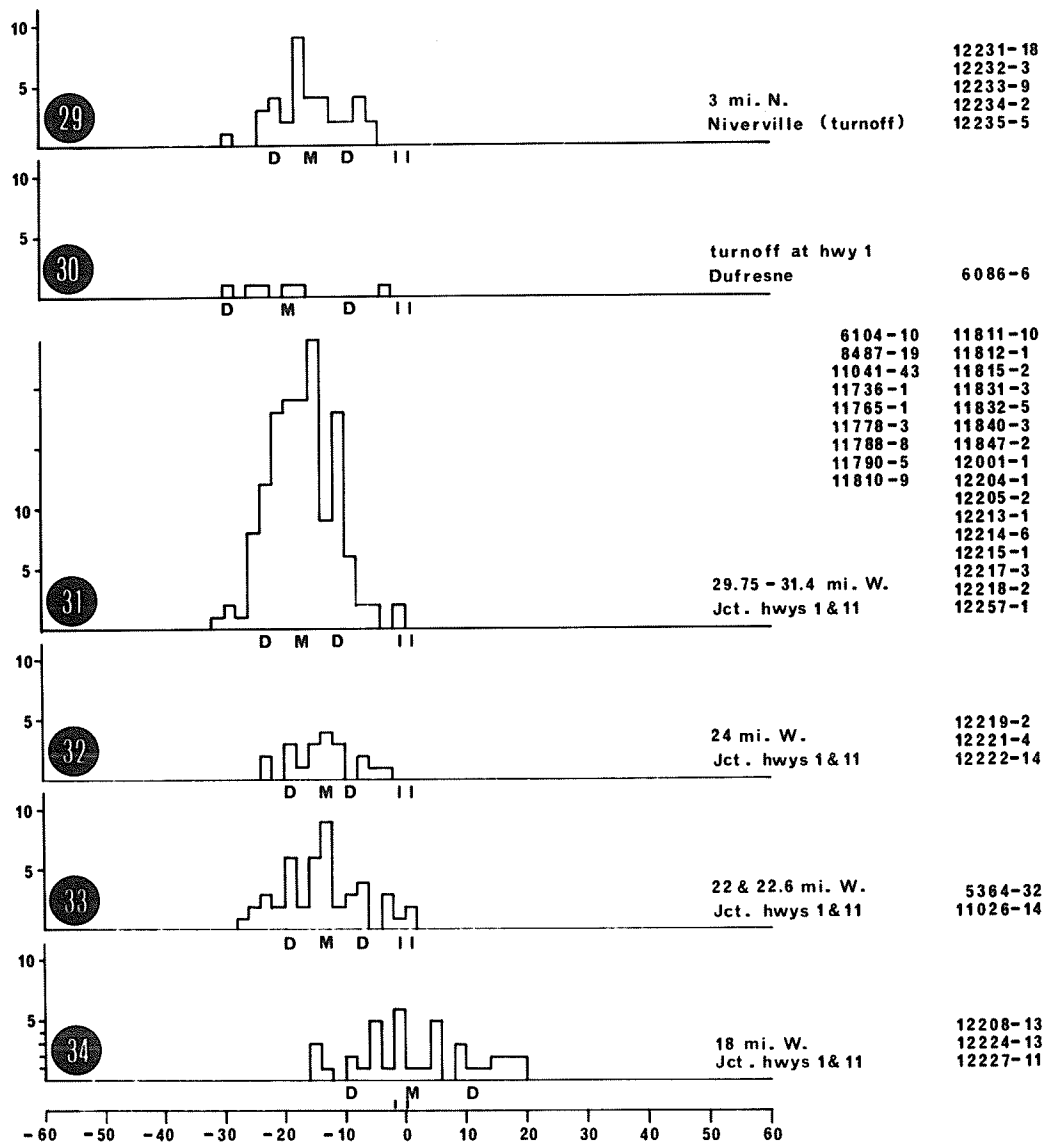


Figure 26. Histograms of individual discriminant function scores for collections of male Bufo for collecting stations 35 - 43, the eastern portion of the Trans-Canada Highway transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 22.

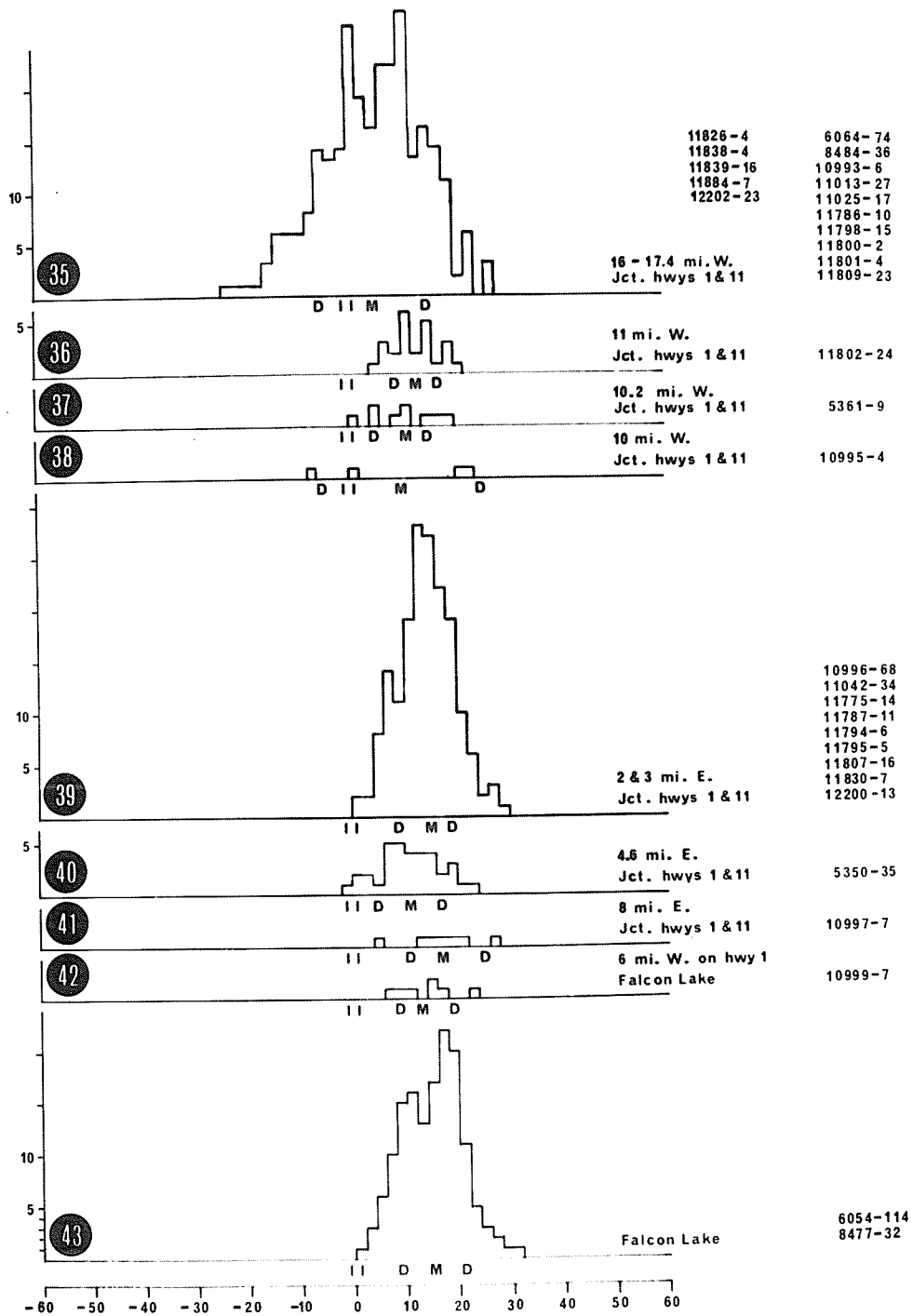


Figure 27. Histograms of individual discriminant function scores for collections of male Bufo for collecting stations 44 - 48, the Marchand transect, and stations 49 - 50, the western portion of the southern transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 22.

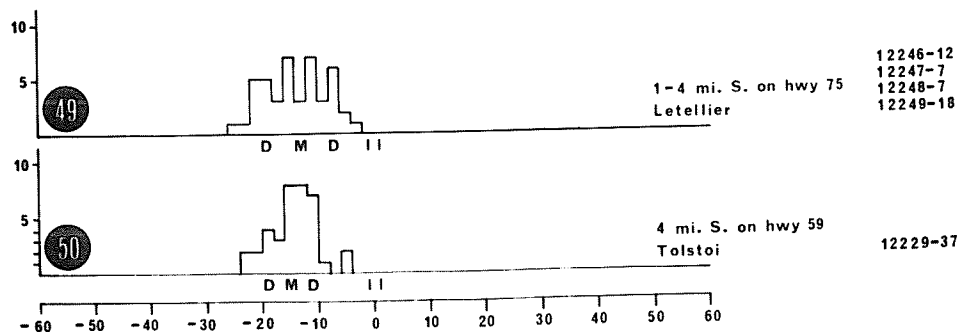
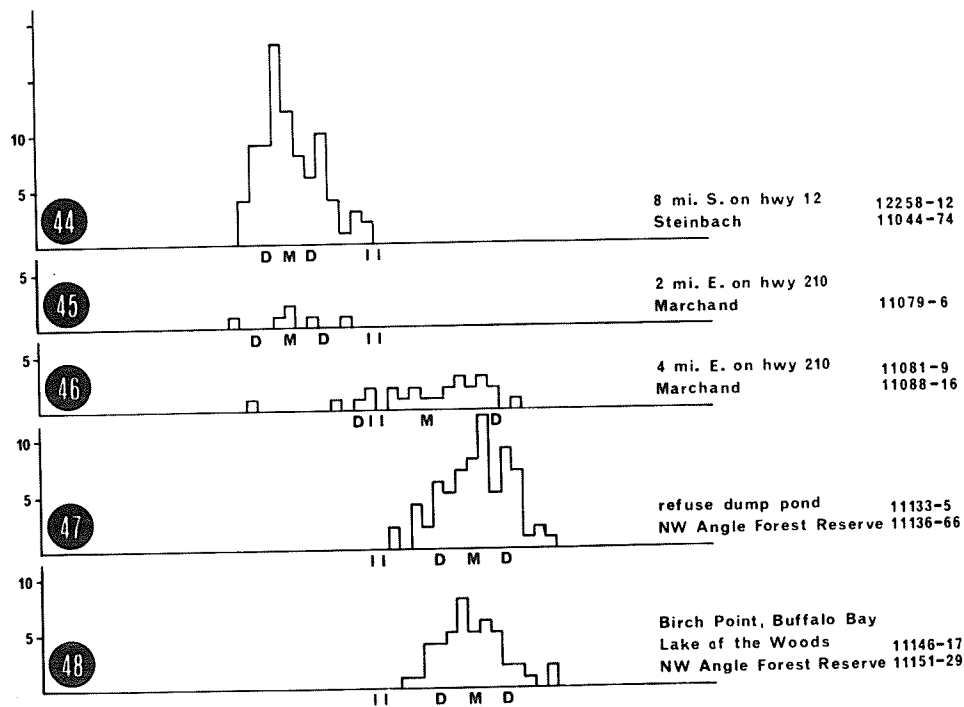
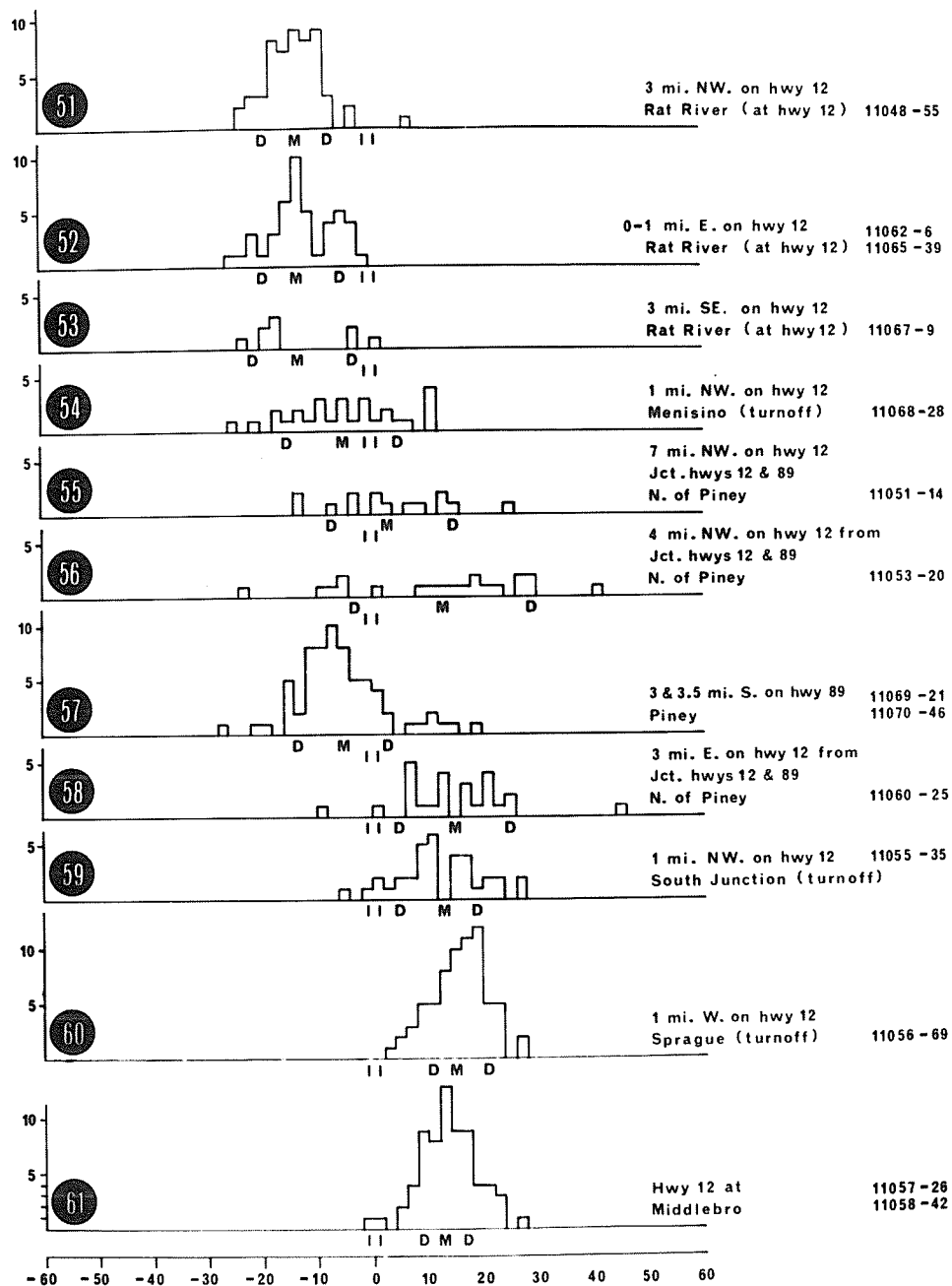


Figure 28. Histograms of individual discriminant function scores for collections of male Bufo for collecting stations 51 - 61, the eastern portion of the southern transect through southeastern Manitoba. An explanation of letters, scale, etc. is given in the legend of Figure 22.



introgression. In the Trans-Canada Highway transect, which is the most thoroughly covered of all the transects (Figures 25 and 26) the shift can be seen between locality 33 and 36. Localities 34 and 35 clearly are intermediate populations from the center of the zone of contact. Locality 38 shows one individual to the left side of mid-point and this is either a stray from a western locality or a chance extreme recombination. The Marchand Transect (Figure 27) is the most poorly sampled but clearly locality 46 has an intermediate population. The southern transect (bottom of Figure 28 and Figure 29) cuts a diagonal through the intermediate zone and one sample (locality 57) is geographically "out of place" in the arrangement of these samples as it is south of the general sample line (see Figure 3). The number of intermediate populations is greater because of the NW-SE axis of sampling instead of the general west-east line in other transects except the northern one. Clearly intermediate populations lie at locality 54, locality 55 and locality 56. Locality 57 is south of these and is largely on the hemiohryis side of the axis but has intermediate and americanus-like individuals. Other localities northwest and southeast show occasional individuals across the mid-point.

The mean discriminant scores for populations to the east and west of the narrow intermediate zone defined by the line connecting these points are consistently above 10 to the west with two exceptions, locality 13 mean +5.8 and locality 38

mean +9.8. In both these cases the sample size is very small, 5 and 4 specimens respectively, and only in the latter sample is an individual on the hemiophrys side of the discriminant axis present. However, the "hemiophrys" means in eastern Manitoba -10.2 to -19.2 are consistently higher than those for the reference sample (compare Appendix IIA and Figure 19). All reference sample means, except Delta at -18.7, are below -20.0. Similarly all "americanus" means in eastern Manitoba (with the exception of localities 13 and 38 mentioned above) are +10.1 to +18.6. In the reference sample of americanus, means are all above +20.0 with the exception of Oxdrift (+13.3), Hibbing (+17.5), and Whitetop Creek, NE of Moosonee (+16.2).

Figure 29, 30 and 31 show histograms of individual discriminant scores by date of collection for localities 31, 35, and 39 respectively. The pooled results of these scores were used in Figures 25 and 26 as population histograms and in Figures 19 and 20 for means and variances.

For locality 31, a hemiophrys site, several individual localities are designated in what is essentially a continuous roadside ditch and adjacent pond complex. Within-year samples were generally small and have therefore been pooled by year. There are no obvious shifts in distribution between years or adjacent localities.

Locality 35 is a transition site. Roadside pools of the Brokenhead River where it is crossed by Highway 1, adjacent

Figure 29. Histograms for individual discriminant function scores for collections of male Bufo from collecting station 31 in southeastern Manitoba to show variation in collections from adjacent sites and different years which were later pooled to form a combined sample for this station (see Figure 25). An explanation of letters, scale, etc. is given in the legend of Figure 22.

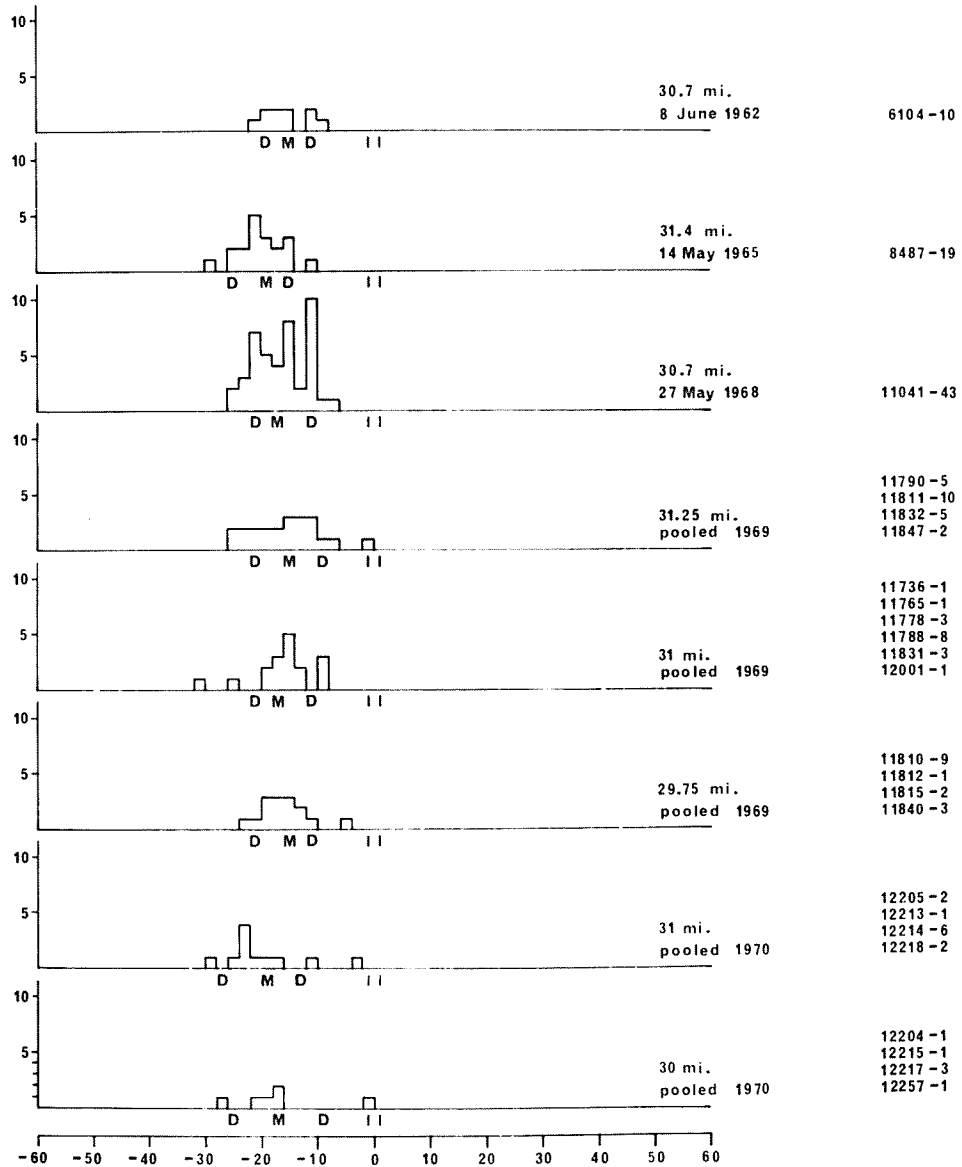


Figure 30. Histograms of individual discriminant function scores for collections of male Bufo from collecting station 35 in southeastern Manitoba to compare samples from different dates and years later pooled to form a combined sample for this station (see Figure 25). An explanation of letters, scale, etc. is given in the legend of Figure 22.



16-17.4 mi. W. on hwy 1 from jct. hwy 1&11

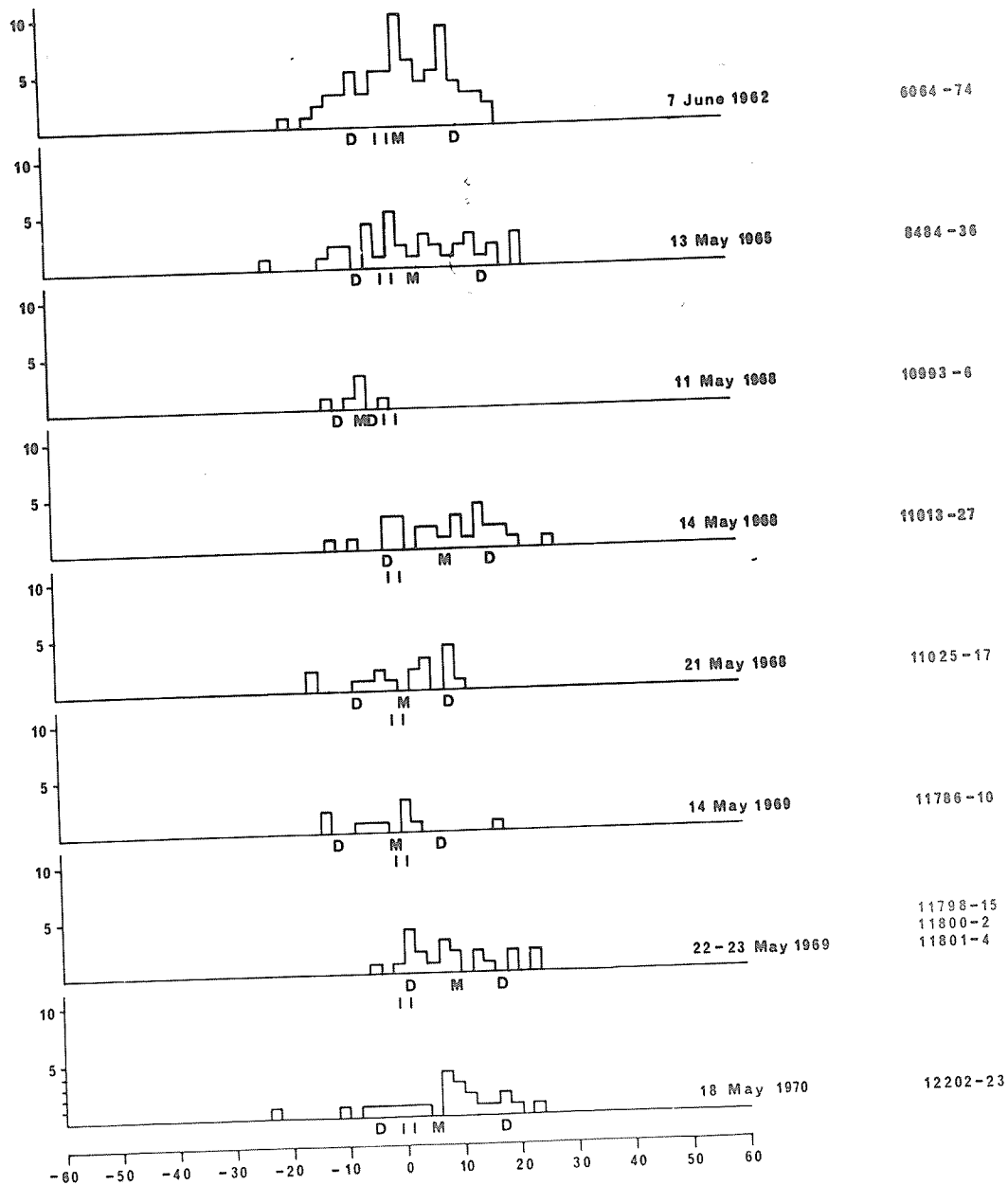
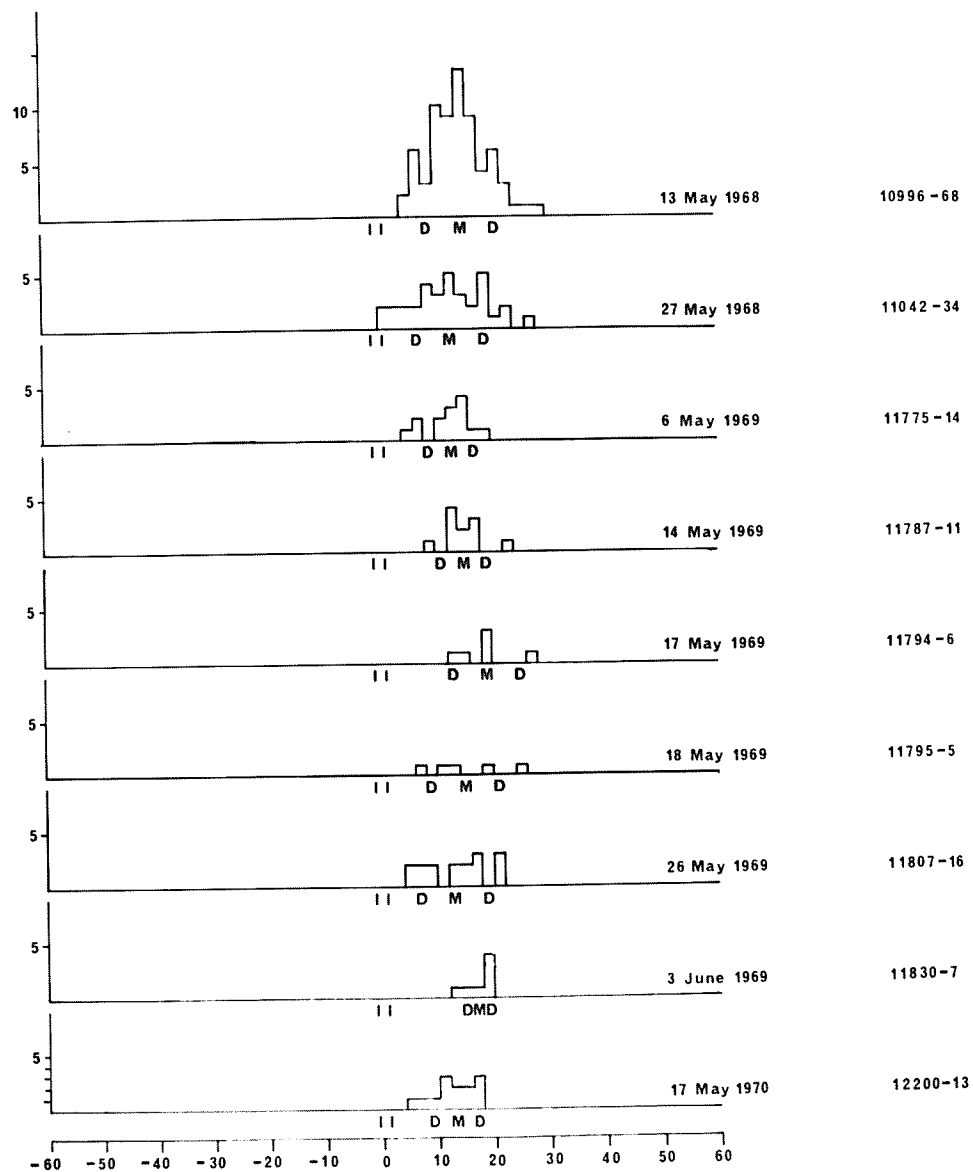


Figure 31. Histograms of individual discriminant function scores for collections of male Bufo from collecting station 39 in southeastern Manitoba from different dates and years later pooled to form a combined sample for this station (see Figure 25). An explanation of letters, scale, etc. is given in the legend of Figure 22.



roadside ditches, and one adjacent dugout pond back from the roadside are included in this data. These almost certainly represent one breeding unit (the 16 - 17.4 range in mileage represents odometer variability in different years rather than a true mile-and-a-half spread). There is some fluctuation between samples from either side of the mid-point particularly between the 11 May 1968 and the 22-23 May 1969 collections. The former only contains 6 males, however, and may not be representative. Comparison of the three 1968 samples (11 May, 14 May, 21 May) seems to rule out the possibility that hemiphrys-like individuals are breeding earlier and americanus-like ones later as the latest collection within the year (21 May) has the most intermediate sample. In the eight years spanned by the sampling (1962-1970) there seems to have been no shift toward either hemiphrys or americanus.

Locality 39 is an americanus site and represents roadside ditches along the Trans-Canada Highway spread along a distance of less than one mile. The samples show little variation within a year or between years.

b. Univariate and ratio analysis of males

The same characters examined individually or as ratios for geographic variation in the reference samples were computed for eastern Manitoba samples (Figures 32-39).

Figure 32. Map of southeastern Manitoba showing sample means for cranial crest scores for collections of male Bufo. In this and subsequent maps adjacent samples which were examined separately in discriminant mean and variance figures have been pooled to facilitate mapping.

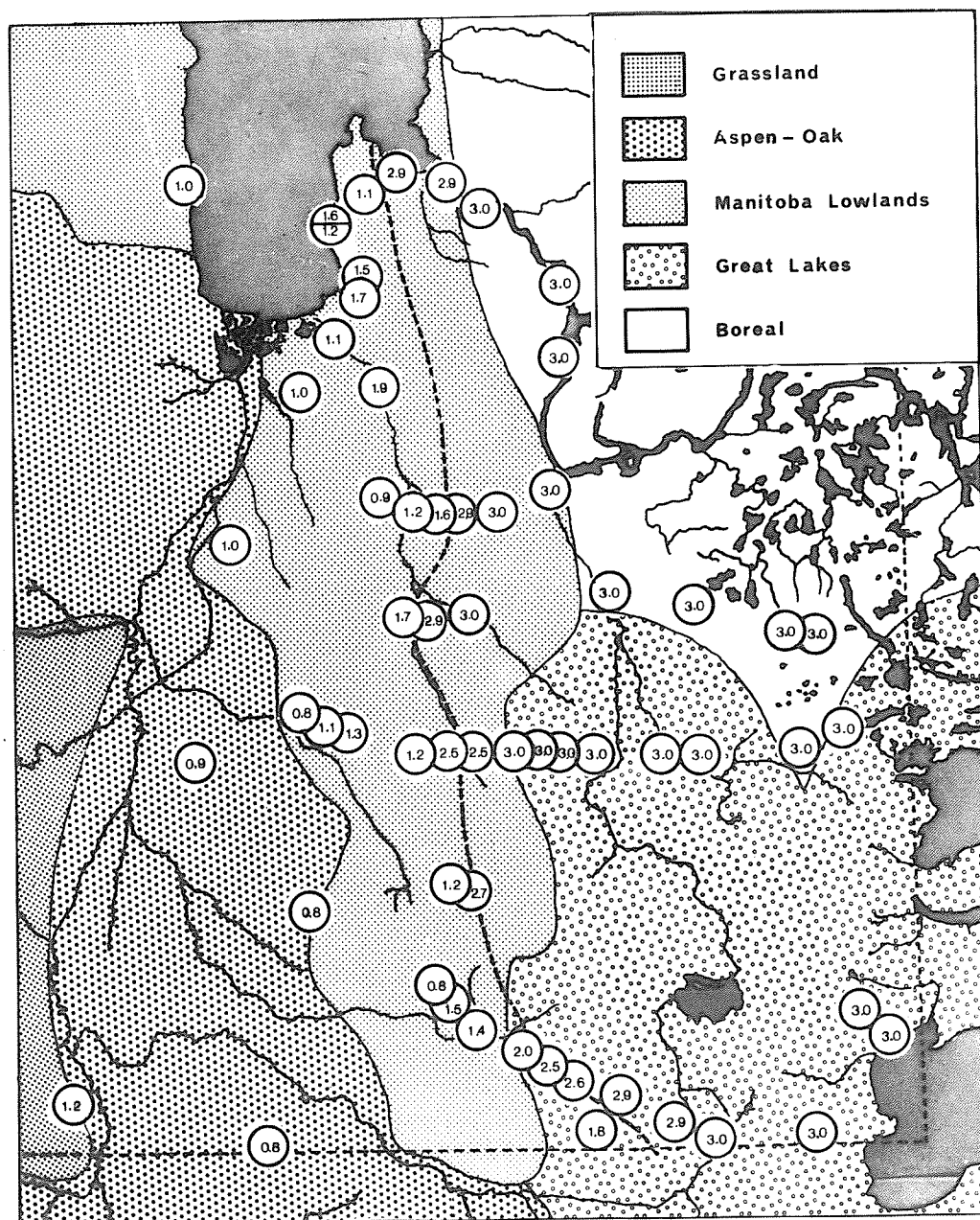


Figure 33. Map of southeastern Manitoba showing sample means for post-orbital scores for collections of male Bufo.

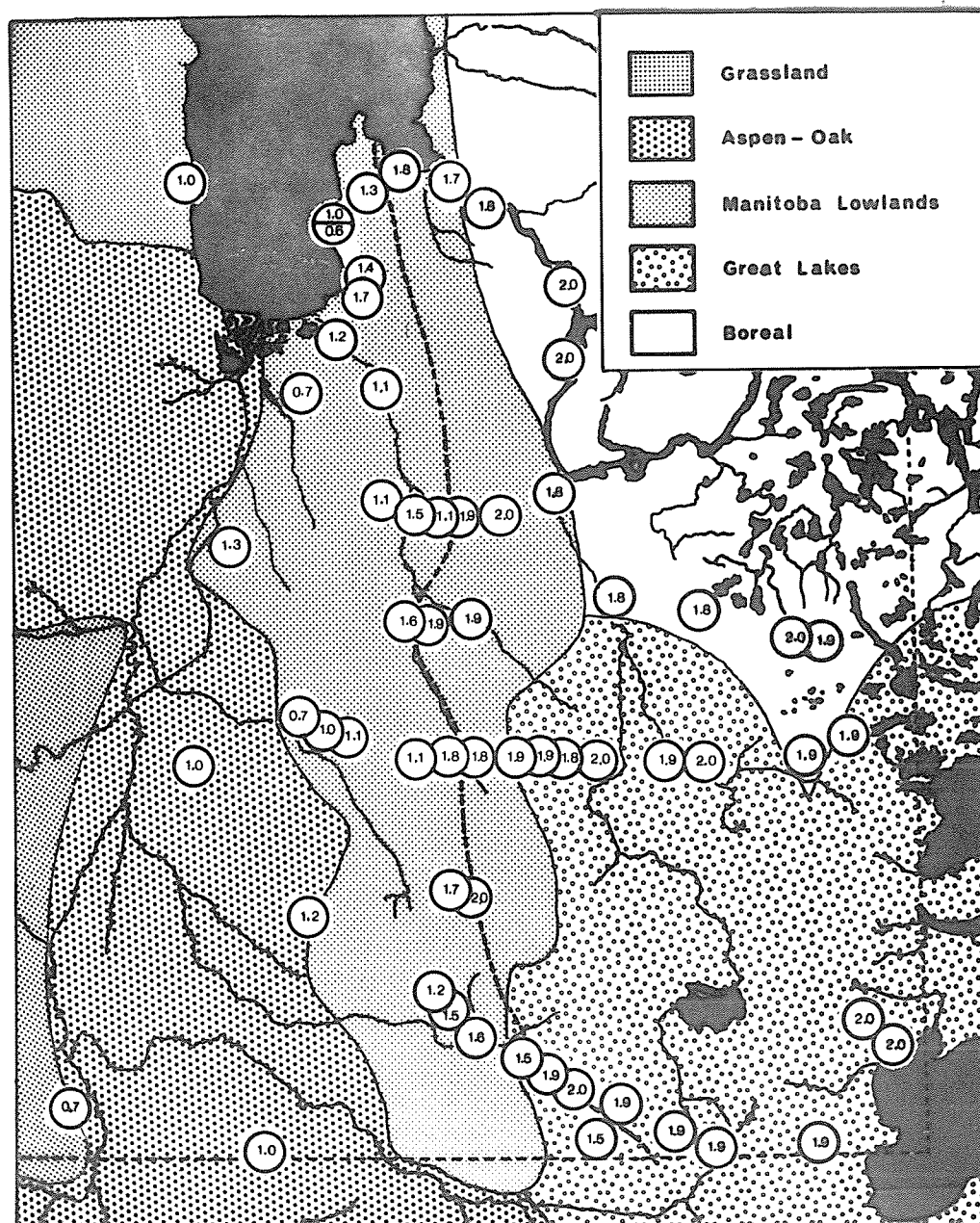


Figure 34. Map of southeastern Manitoba showing sample means for venter scores for collections of male Bufo.

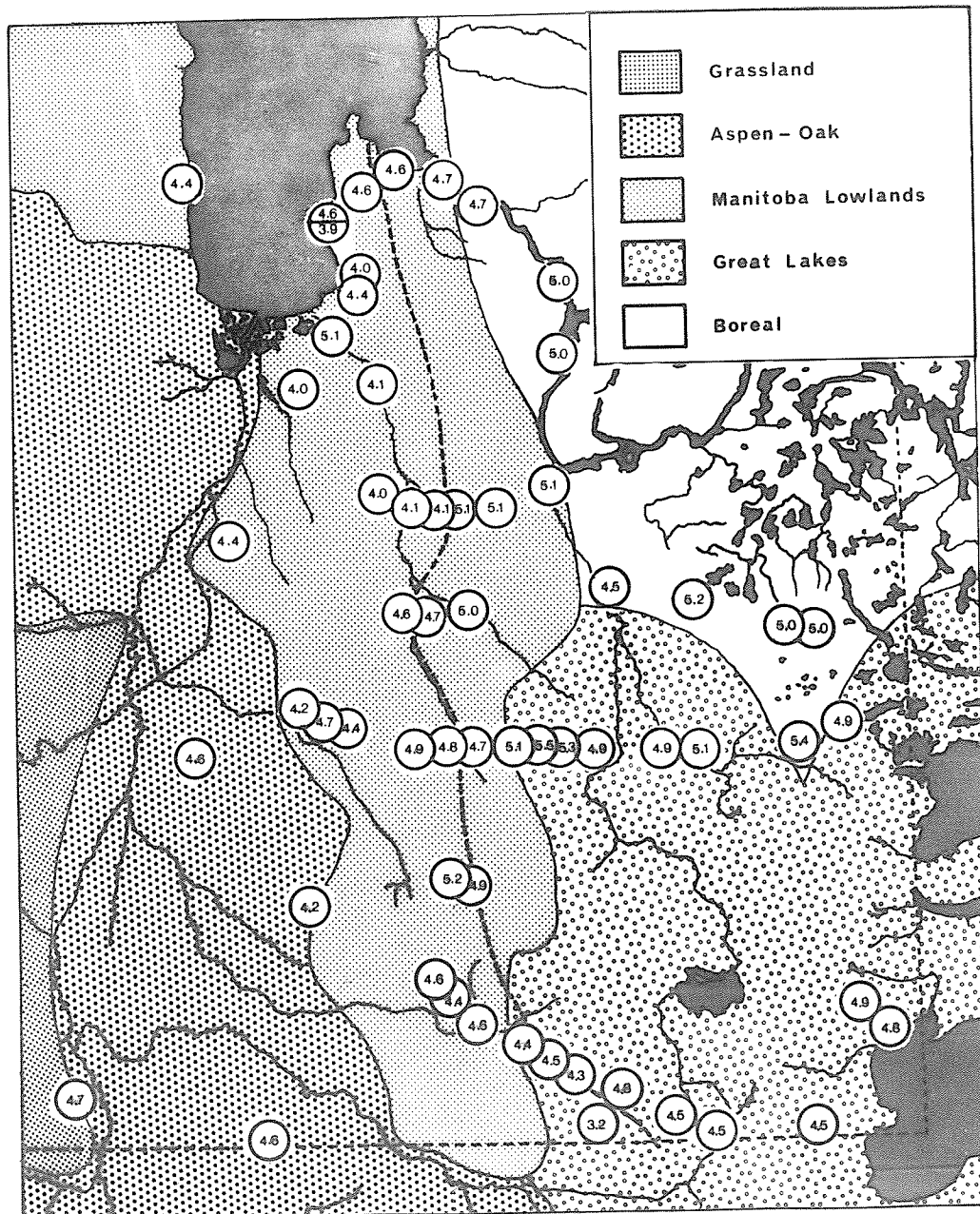


Figure 35. Map of southeastern Manitoba showing sample means for snout-vent length of collections of male Bufo.

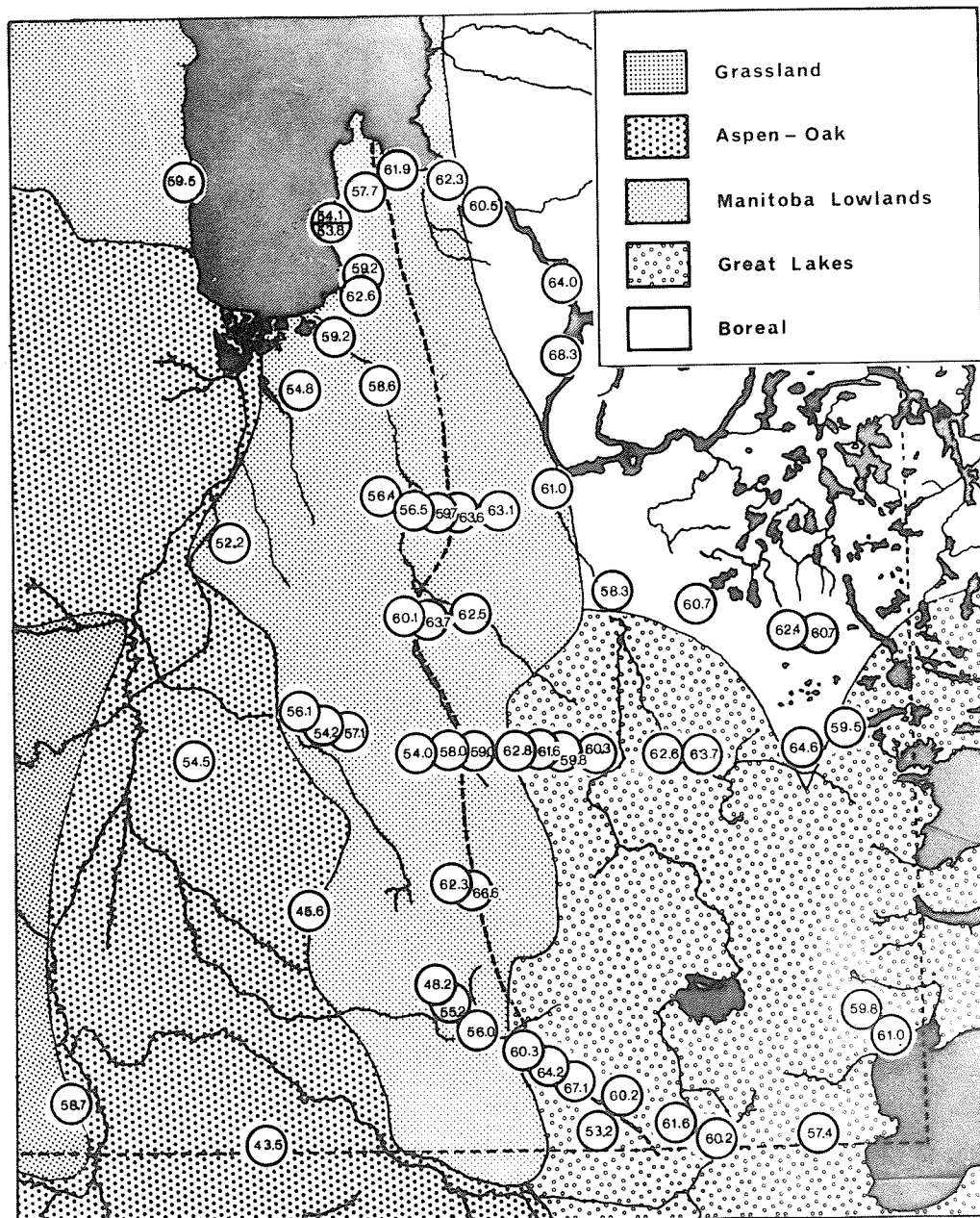


Figure 36. Map of southeastern Manitoba showing sample means for tibia/snout-vent lengths ratio for collections of male Bufo. Original values have been multiplied by 1000 to remove decimal.

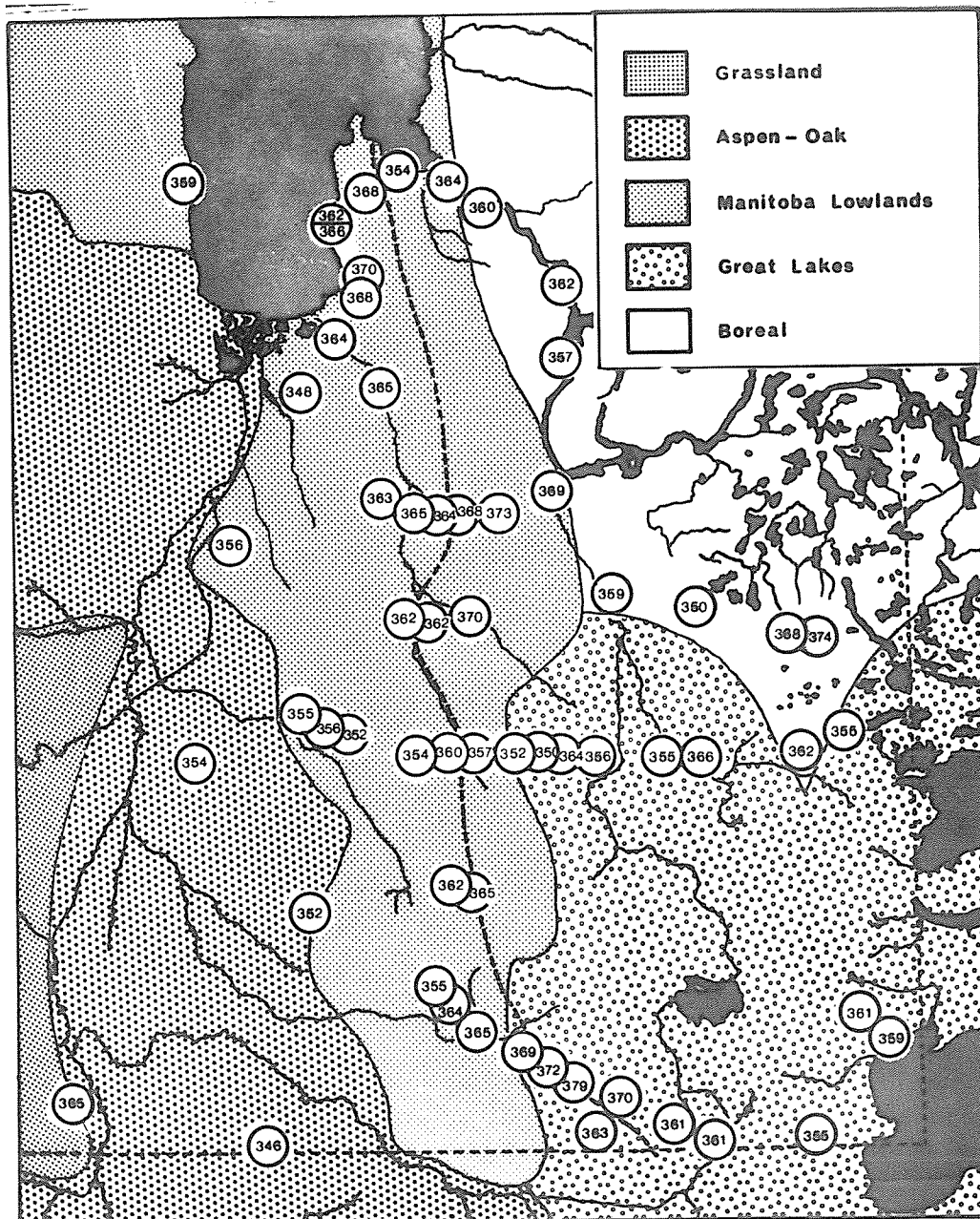


Figure 37. Map of southeastern Manitoba showing sample means for spot/snout-vent lengths ratio for collections of male Bufo. Original values have been multiplied by 1000 to remove decimal.

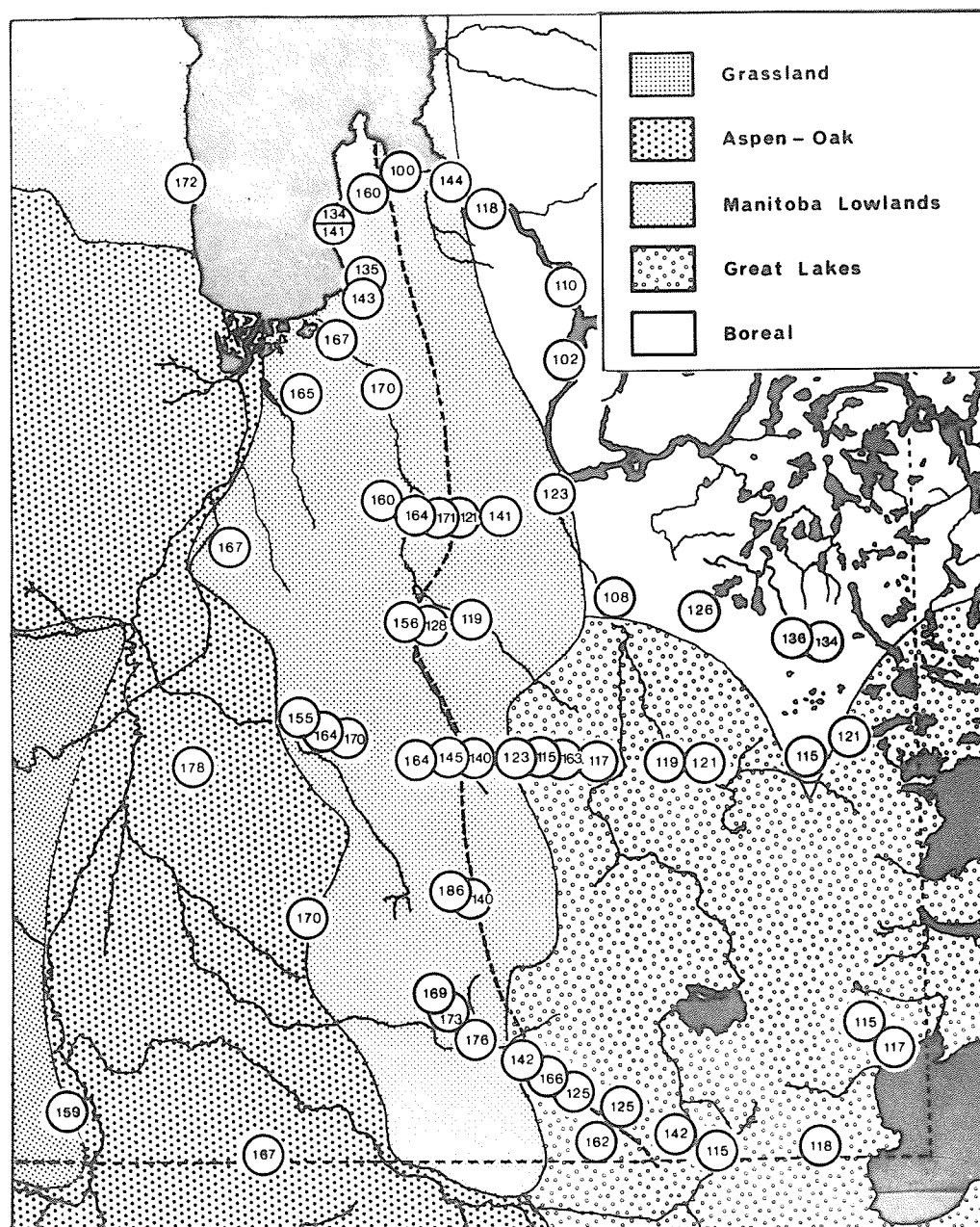


Figure 38. Map of southeastern Manitoba showing sample means for cranial crest posterior width/head width ratio for collections of male Bufo. Original values have been multiplied by 1000 to remove decimal.

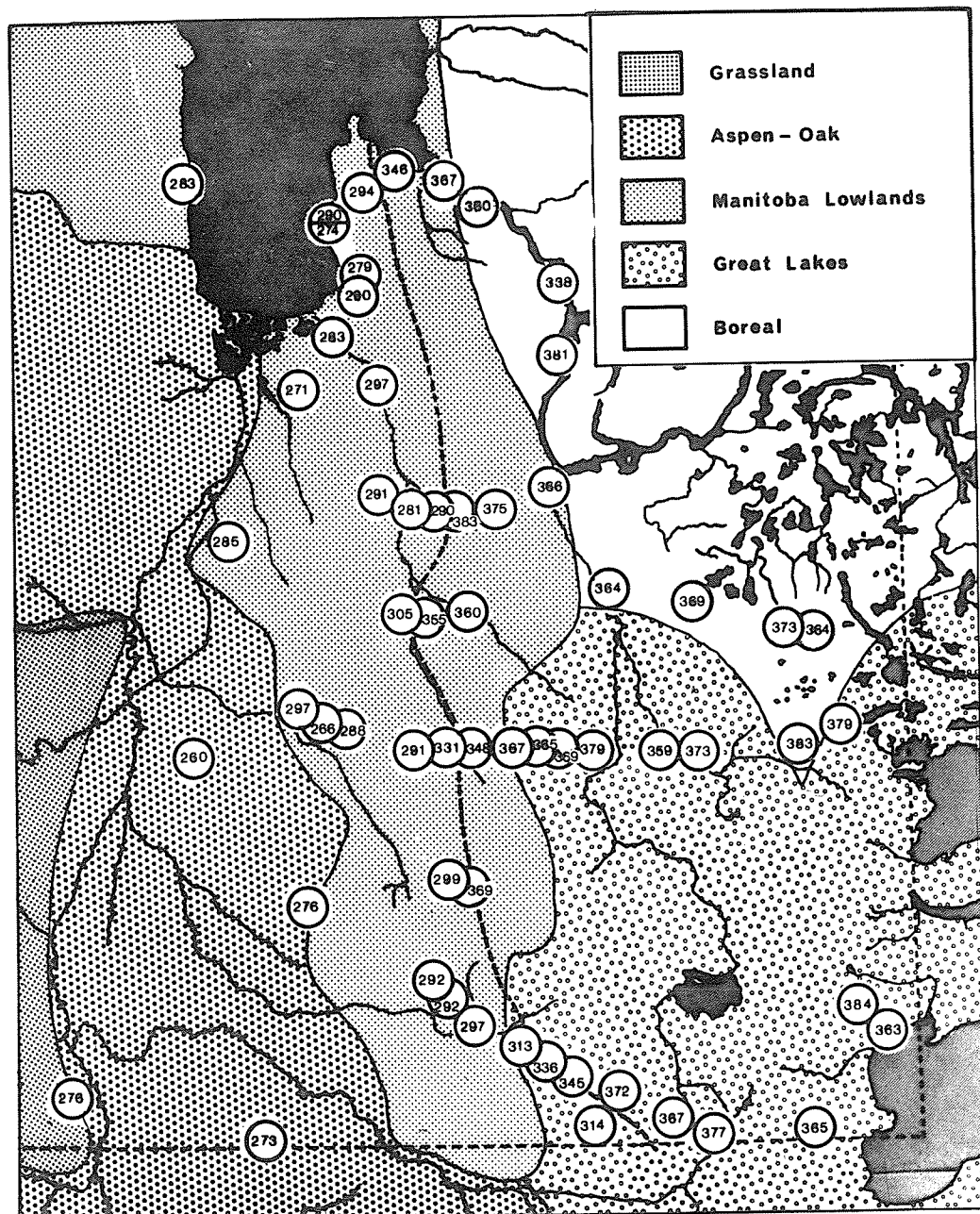
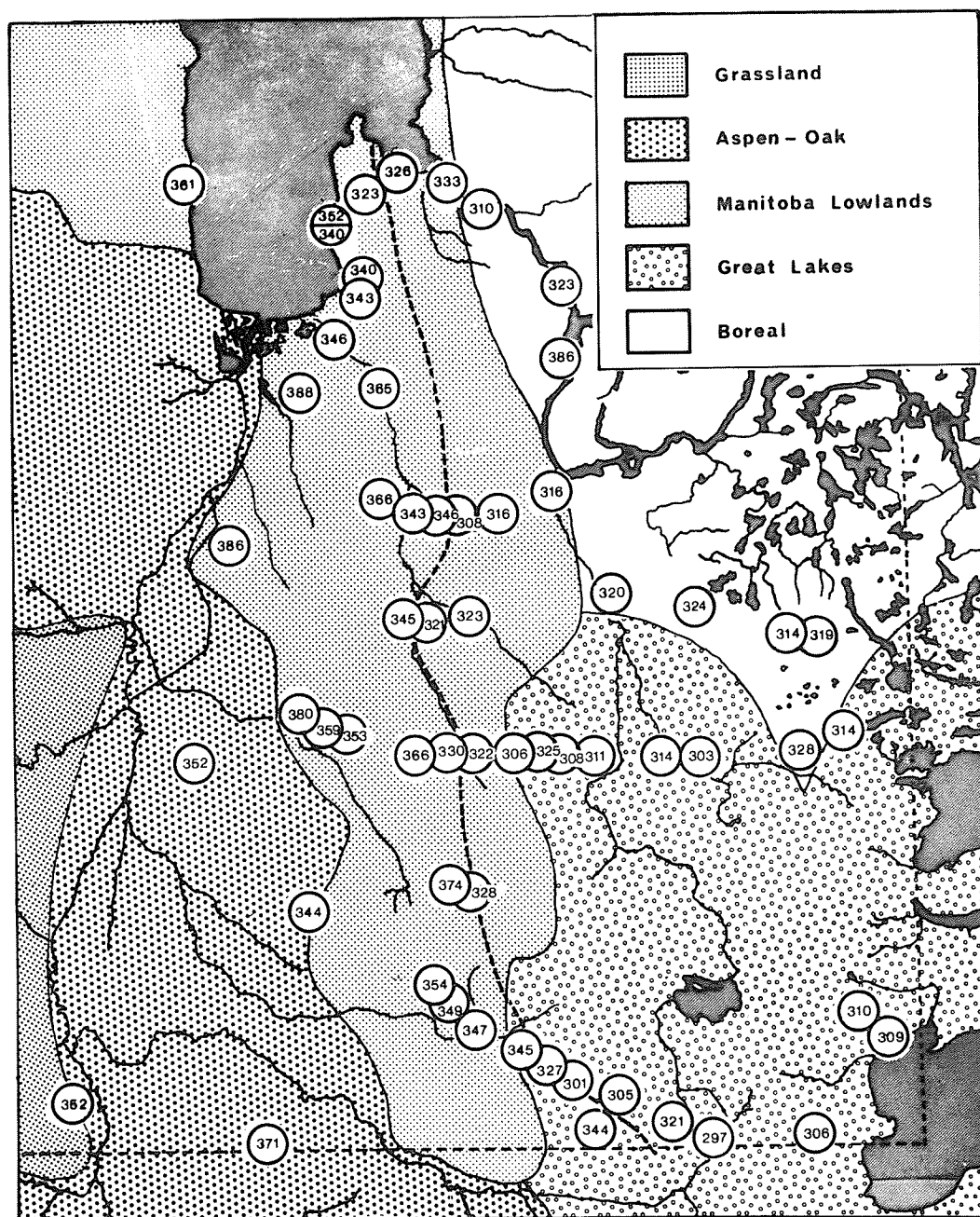


Figure 39. Map of southeastern Manitoba showing sample means for "spade" (outer metatarsal tubercle) width/tarsus length ratio for collections of male Bufo. Original values have been multiplied by 1000 to remove decimal.



Cranial crest score

Low mean values (1.0 and below) are evident to the west and high values (3.0) to the east. Values on or adjacent to the transition line plotted from the discriminant histograms are clearly intermediate and some intermediacy can be seen immediately east or west of this line.

Post-orbital score

Values near 1.0 are evident in the west and near 2.0 in the east, but there is much more variability in this score than in the cranial crest value within hemiophrys and americanus as earlier pointed out for the reference sample results, and it does not as clearly separate the taxa. However, the intermediate nature of populations within and to either side of the transition area is evident.

Venter score

In the reference sample comparisons, this character, as scored, did not separate hemiophrys and americanus. The variation between population samples across eastern Manitoba bears this out but there is a marked increase in values in eastern Manitoba where americanus is present and more often has a completely spotted or reticulated venter (see Figure 34).

Hemiophrys populations have generally lower values in this region than throughout their range and thus the transition zone does indicate a change in mean values from the low scores typical of western Manitoba hemiophrys to the higher scores of eastern

Manitoba americanus. This holds true despite the fact that western populations of americanus (including those in eastern Manitoba) have somewhat lower venter scores than those in the eastern portion of the range.

Snout-vent length

Mean population values are plotted in Figure 35. Values in the western portion of southeastern Manitoba are relatively low and this trend is most pronounced as the transition zone is approached. In the eastern portion the values are higher than in the west, but the body size increase is most pronounced along the transition line and falls off somewhat to the east. It is evident that there is a size decrease in hemiophrys populations, especially in the south where means for localities 44 and 50 are 45.6 and 43.5 respectively, the lowest mean size of all populations sampled throughout the study. The range of mature males varied between two collections pooled for the value of locality 44. A sample of 74 males collected 27 May 1968 ranged from 39.0 to 51.2, mean 44.7 mm, a sample of 12 taken at the same locality 12 June 1970 ranged from 48.8 to 56.0 mm. A sample of 37 males taken at locality 50 ranged from 38.1 to 47.9 mm. Generally in both species, population means to either side of the area of rapid transition were as large as any in eastern Manitoba, though greatly exceeded by some populations in the reference sample from the western portion of hemiophrys range and the eastern portion of americanus range (see Figure 29).

Tibia/snout-vent length

The values for this ratio in Eastern Manitoba populations are plotted in Figure 36. Over the entire eastern Manitoba area they range from 0.346 to 0.379, compared with the ranges of 0.351 to 0.385 in americanus and 0.341 to 0.381 in hemiohryys in the reference samples (Appendix II M). There are some notably low scores in the western portion of eastern Manitoba (note locality 50 at 0.346) and these are often associated with low snout-vent measurements.

Spot length/snout-vent

The values for eastern Manitoba populations are plotted in Figure 37. Means in the western portion of the area are clearly higher, and fall into the range (0.144 to 0.175) of the reference sample for hemiohryys. Means in the eastern portion and lower portion of the americanus reference sample range are typical of southern and intermediate populations (Appendix II N) though they are not as low as the Rondeau sample mean. The values on and near the transition zone are clearly intermediate.

Cranial crest - posterior width/head width

The population means are given in Figure 38. Values are clearly low in the western portion of eastern Manitoba and high in the eastern portion with an abrupt shift over the transition line. In general the western values are in the high portion of the hemiohryys reference sample range whereas the eastern values tend toward the lower portion of the americanus range (Appendix II D).

Outer metatarsal tubercle width/tarsus length

The mean values for eastern Manitoba populations are plotted in Figure 39. Generally values are high in the western portion of the range and lower in the eastern portion of the range. Comparison with reference samples values of Appendix II P shows the lack of a trend in the values within western Manitoba corresponds with the hemiophrys range in general but in the eastern portion the values approach the highest values of the character for americanus. The trend across the transition zone in eastern Manitoba is not as even as in some characters but the drop in ratio values is clearly evident, particularly to the west of the transition line.

Comparison of discriminant, univariate and ratio values

Table IV gives a comparison of the values for the localities on each transect where the most rapid change in values occurs, and shows their general agreement.

c. Discriminant function scores for females

The representation is meager compared with male samples, and only 2-4 individuals from the majority of localities, but a few large collections were available. Figures 40 and 41 give the histograms of individual scores from each locality represented. Most localities show individuals to the left or right of the bulk of the sample giving an impression of more intermediate localities, but where a significant number of specimens are available, such as localities 31-35-39 along the Trans-Canada, the intermediate zone

Table IV. Comparison of discriminant means and variances with selected univariate and ratio mean values for male Bufo samples on or immediately adjacent to contact zone.

Transect Number	Locality Number	Mean d.f.	d.f. Variance	Mean Boss	Mean post-orb.	Mean SV	Mean Spot/SV	Mean pcc/ Head	Mean Spade/ Tarsus
I	9	-10.1	31	1.1	1.3	57.7	160	294	323
	10	+7.2	44	2.9	1.8	61.9	100	346	326
II	18	-7.0	94	1.6	1.1	59.7	171	290	346
	19	+16.0	133	2.9	1.9	63.6	121	383	308
III	22	-7.0	87	1.7	1.6	60.1	156	305	345
	23	+11.3	110	2.9	1.9	63.7	128	355	321
IV	34	-1.8	97	2.5	1.8	58.0	145	331	330
	35	+5.4	90	2.5	1.8	59.0	140	348	322
V	46	+6.9	231	2.7	2.0	66.6	140	369	328
VI	54	-4.3	101	2.0	1.5	60.3	142	313	345
	55	+3.2	122	2.5	1.9	64.2	166	336	321
	56	+12.5	258	2.6	2.0	67.1	125	345	301

Figure 40. Histograms of individual discriminant function scores for collections of female Bufo for 11 collecting stations in southeastern Manitoba; 5-10 from northern transect, 15-20 from Beausejour transect, 23-28 from Vivian transect. An explanation of letters, scale, etc. is given in the legend for Figure 22.

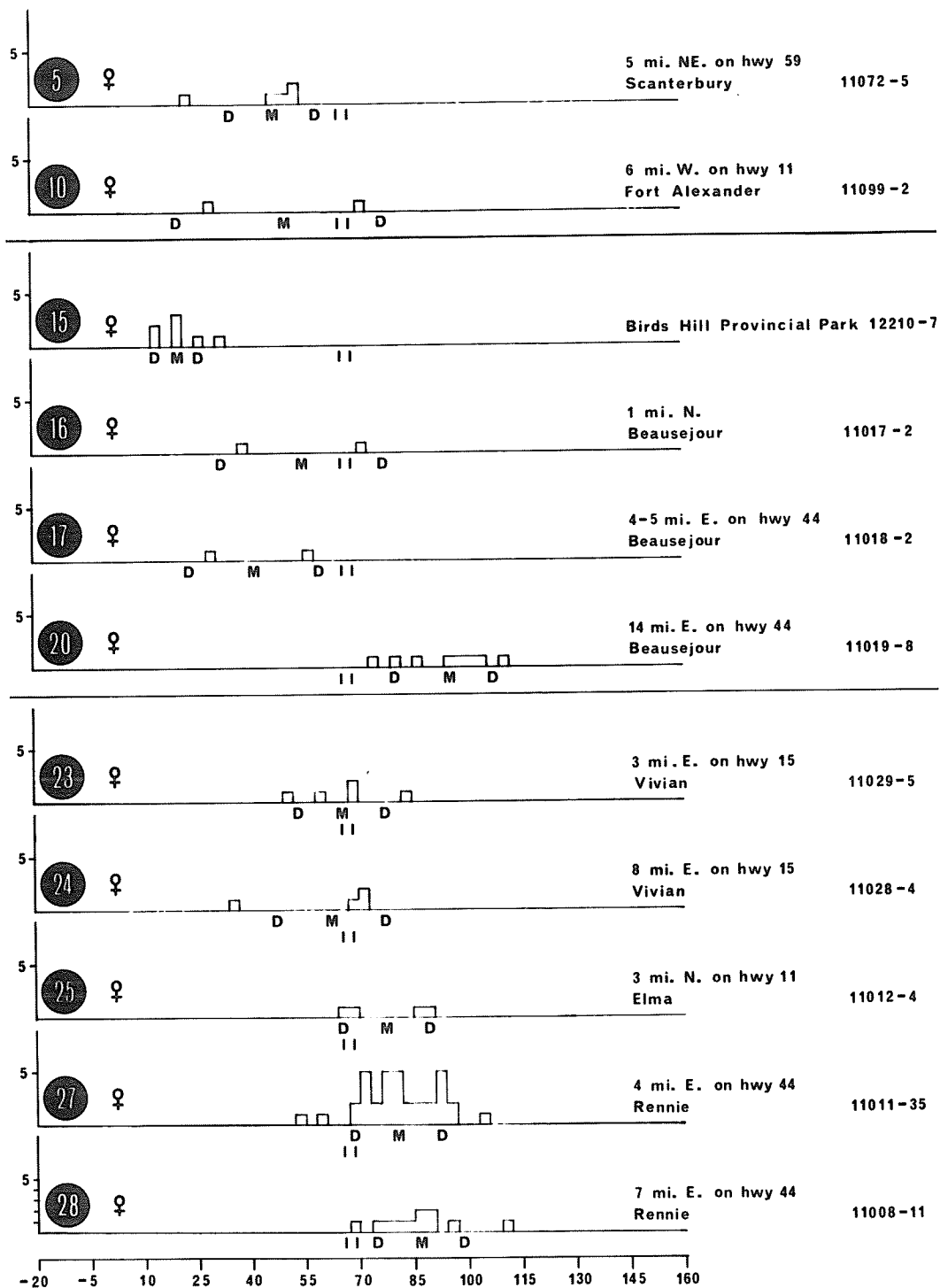
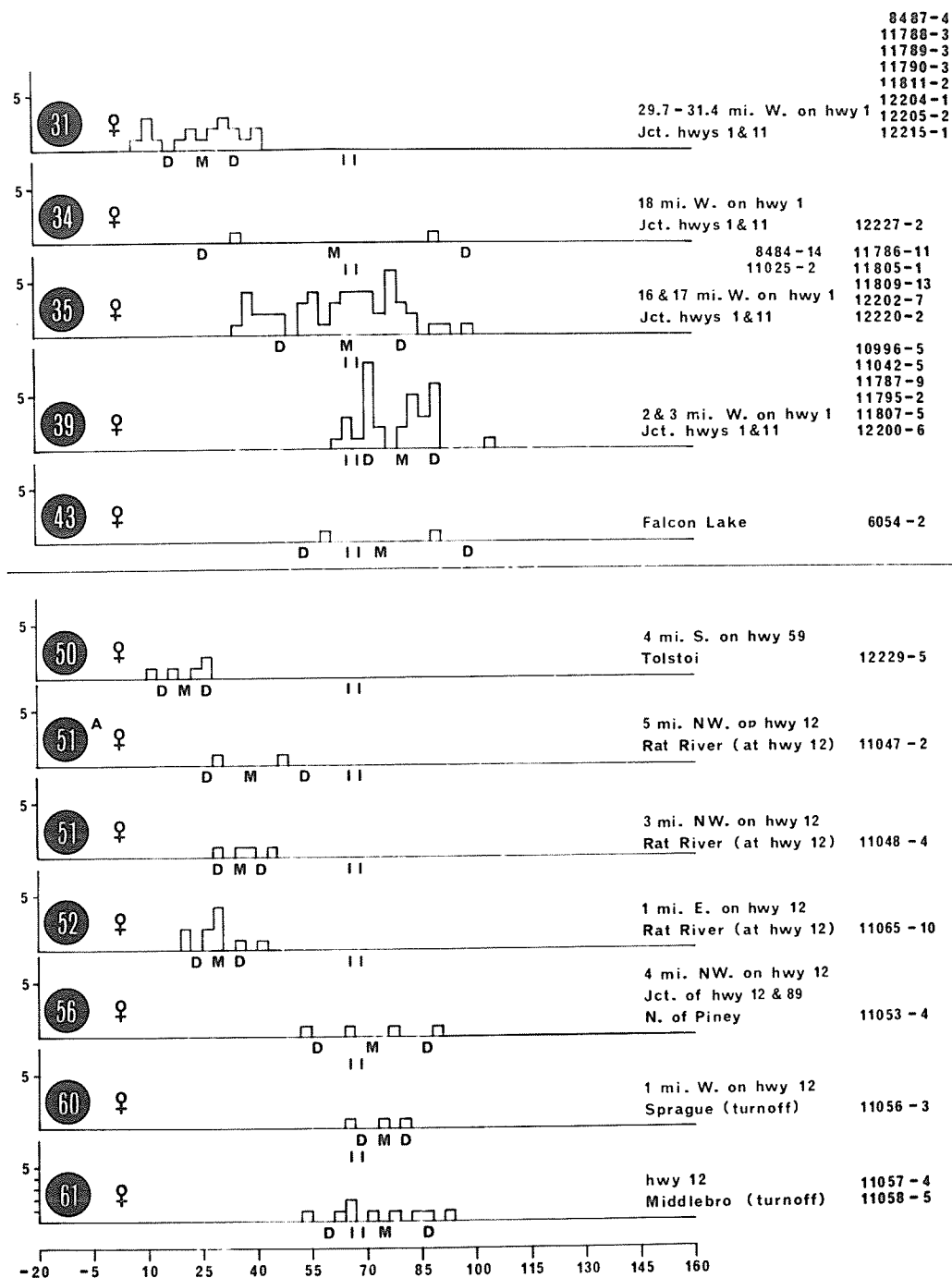


Figure 41. Histograms of individual discriminant function scores for collections of female Bufo for 12 collecting stations in southeastern Manitoba: 31-43 from the Trans-Canada Highway transect, 50-61 from the southern transect. An explanation of letters, scale, etc. is given in the legend for Figure 22.



is clearly in the same position as it was for males. The problem with females may be in the smaller reference sample used, although females may actually be more variable. The tendency for americanus localities in eastern Manitoba to show some individuals over the mid-point of the axis toward hemiohryys is most evident in locality 27 and locality 61, although neither of these has any individuals classifying as hemiohryys in the range of the reference sample (compare with Figure 18).

4. Breeding call analysis and comparisons

Tape recordings were obtained in the field of the breeding calls of 223 individual male toads (Figure 42). In the laboratory three call parameters were determined - pulse rate, dominant frequency and duration. The call sampling was done mainly along the Trans-Canada Highway transect, with additional populations sampled in the western half of eastern Manitoba as well as The Pas in western Manitoba and Oxdrift in western Ontario (Appendix III). The reference samples for both hemiohryys and americanus included populations from eastern Manitoba which are excluded from the morphological reference sample, and those in the "intermediate" sample included individuals recorded 21 to 17 miles west on Highway 1 of the junction of Highways 1 and 11.

Table V gives the results of correlation tests between each possible pair of variables and scatter plots of these are shown in Appendix IV, A-I.

The number of pulses per second increased directly with an

Figure 42. Audiospectrograms of portions of mating calls of male Bufo. For each example a portion of a single call in wide band (300 cps filter) display is given on the left and a "section" showing relative energy at different frequency levels at one selected point in the call is given on the right. The first display was used to determine pulse rate, the second to determine dominant frequency. Duration was timed from the tape.

- A. B. americanus NMNS 11793-1 (tape 24): 3 miles W on Hwy. 1 of Jct. Hwys. 1&11, Manitoba, 16 May 1969. Body temperature of toad 10.0°C , pulse rate 17/sec, dominant frequency 1483.33, duration 11.0 sec.
- B. B. americanus NMNS 11807-1 (tape 34): 3 miles W on Hwy. 1 of Jct. Hwys. 1&11, Manitoba, 26 May 1969. Body temperature of toad 23.5°C , pulse rate 45/sec., dominant frequency 2083.33, duration 7.1 sec.
- C. B. hemiophrys NMNS 11831-1 (tape 39): 31 miles W on Hwy. 1 of Jct. Hwys. 1&11, Manitoba, 3 June 1969. Body temperature of toad 10°C , pulse rate 27/second, dominant frequency 1879.17, duration 5.7 sec.
- D. B. hemiophrys NMNS 12249-2 (tape 64): 1-2 mi. S on Hwy. 75 of Letellier, Manitoba. Body temperature of toad 23.5°C , pulse rate 103/sec, dominant frequency 1849.17, duration 2.1 sec.

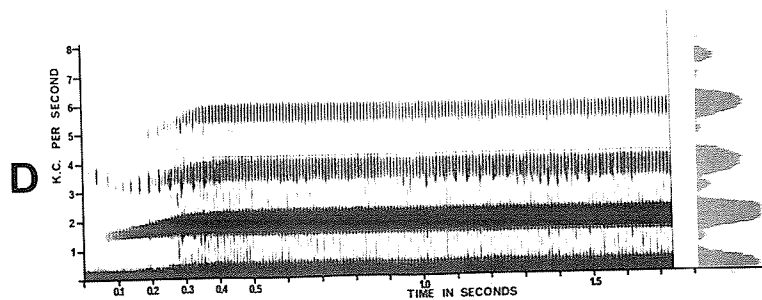
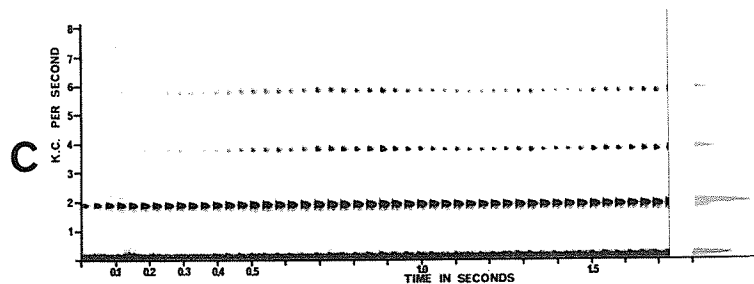
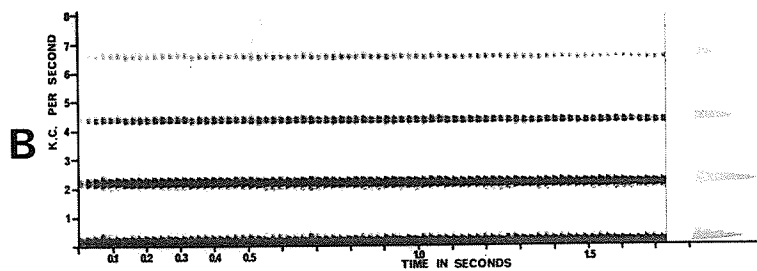
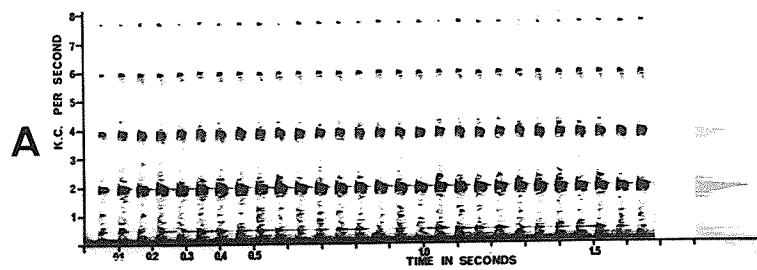


Table V. Correlation (r) and significance (P) values for comparison of variables in mating calls of Bufo americanus, B. hemiophrys, and "hybrid" populations.

A) Correlation with temperature.

	No. of Specimens	<u>Pulse Rate</u>		<u>Call Length</u>		<u>Dominant Frequency</u>	
		<u>r</u>	<u>P</u>	<u>r</u>	<u>P</u>	<u>r</u>	<u>P</u>
<u>B. americanus</u>	50	+0.959	< 0.001	-0.635	< .001	+0.416	< 0.01
Intermediate pop.	62	+0.583	< 0.001	-0.359	< 0.05	+0.272	< 0.05
<u>B. hemiophrys</u>	111	+0.929	< 0.001	-0.459	< .001	-0.036	>> 0.1

B) Correlation with snout-vent length.

<u>B. americanus</u>	50	+0.223	> 0.1	+0.170	>> 0.1	-0.196	>> 0.1
Intermediate pop.	62	-0.355	< 0.01	+0.267	< 0.05	-0.183	> 0.1
<u>B. hemiophrys</u>	111	-0.304	< 0.01	+0.018	>> 0.1	-0.246	< 0.05

Table V Continued

C) Correlation with pulse rate.

	<u>No. of Specimens</u>	<u>Call Length</u>		<u>Dominant Frequency</u>	
		<u>r</u>	<u>P</u>	<u>r</u>	<u>P</u>
<u>B. americanus</u>	50	-0.653	$\ll 0.001$	+0.409	$< 0.01 > .001$
Intermediate pop.	62	-0.588	$\ll 0.001$	-0.034	$\gg 0.1$
<u>B. hemiophrys</u>	111	-0.499	$\ll 0.001$	-0.027	$\gg 0.1$

D) Correlation between Call Length and Dominant Frequency.

	<u>r</u>	<u>P</u>
<u>B. americanus</u>	-0.235	< 0.01
Intermediate pop.	+0.130	$\gg 0.1$
<u>B. hemiophrys</u>	+0.031	$\gg 0.1$

increase in temperature in both americanus and hemiphrys samples as shown by the correlation coefficients (0.959 and 0.929 respectively). The relationship of temperature and call length is inverse and the correlation is weaker (-0.635 and -0.459 respectively) than with pulse rate. There is considerable variation from call to call within a single individual at any given temperature and the value used here is an average of several calls from each individual. The relationship of dominant frequency to temperature is positive and the correlation, although significant, is not high in americanus (+0.416, $P < 0.01$) but negative and not significant in hemiphrys (-0.036, $P \gg 0.1$). Intermediate populations show weaker correlation between pulse rate (0.583, $P < 0.001$), and call length (-0.359, $P < 0.05 > 0.01$) with temperature and are intermediate in the relationship of dominant frequency and temperature (0.272, $P < 0.05 > 0.01$).

Weak positive correlation was present in hemiphrys between snout-vent and pulse rate (+0.304, $P < 0.01$) but the correlation in americanus was not significant. In the intermediate populations the correlation was negative and weak (-0.335, $P < 0.01$). Call length showed a weak correlation with snout-vent length in intermediates (+0.267, $P < 0.05$) but was not significant in americanus or hemiphrys. Dominant frequency showed weak negative correlation and significance (-0.246, $P < 0.05$) in hemiphrys but was not significant in americanus or intermediates. Note that dominant frequency was not correlated with temperature,

but was weakly correlated with size, in hemiophrys.

Pulse rate correlation with call length was negative and weak but significant. Dominant frequency showed a weak positive correlation with pulse rate in americanus and an almost negligible negative correlation in intermediates, but was not significant in hemiophrys.

Call length in americanus showed a weak negative correlation with dominant frequency (-0.235 , $P < 0.01$) in americanus but no significant correlation in intermediates or hemiophrys. In hemiophrys in this study snout-vent length correlations with pulse rate (-0.304) and dominant frequency (-0.246) were significant ($P < 0.01$, $P < 0.05$ respectively).

Discriminant analysis of call variables and comparison with morphological discriminant scores

A discriminant function comparison was run using the three call variables (pulse rate, dominant frequency and duration) as well as temperature and snout-vent length to compare equal hemiophrys and americanus samples of 45 specimens each (Appendix III gives samples used). This comparison produced complete separation of the two taxa, and was based on the most geographically separated samples for which call data were available.

Application of the resultant weights (Table VI) to the individuals from the intermediate populations confirmed their intermediate position (Figure 43). Pulse rate made the largest

contribution to the separation between means (centroids).

Figure 44 plots the call discriminant vs. the morphological discriminant values for americanus, hemiphrys and intermediates. Table VII gives the results of correlation for each of the three units and the total sample. There is a positive correlation (+0.820, $P \ll 0.001$) within the whole sample, and a correlation (+0.552, $P \ll 0.001$) for morphology and call within the intermediate populations. However, within either hemiphrys or americanus there is no significant correlation between call and morphology, indicating they are varying independently within each taxon. These correlations (of total sample and intermediates) are evident despite the fact that a portion of the reference sample for call data includes populations where some introgression is evident in morphological characters, as demonstrated earlier.

5. Crosses reared in laboratory and field

During the 1969 field season, males and females were selected from three sites along the Trans-Canada Highway: west of its junction with Highway 11: 31-29 miles (hemiphrys), 17 miles west (intermediate) and 3 miles west (americanus). Crosses were attempted of every possible combination of males and females between these three populations (see Materials and Methods). In all, 61 pairings were tried but only 28 of these produced eggs, and in three of these the eggs did not develop, leaving only 25 successful crosses. However, these did include at least one cross of every possible combination.

Table VI. Discriminant weights and the weight contribution to the Mahalanobis generalized distance from the discriminant analysis of one morphological (snout-vent length), one physiological (body temperature) and three call (pulse rate, dominant frequency and duration) parameters for Bufo hemiophrys and B. americanus.

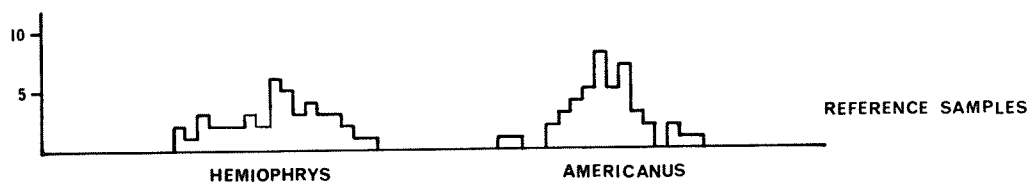
Character	Discriminant Weight	Mean for <u>B. americanus</u>	Mean for <u>B. hemiophrys</u>	Weight Contribution
Snout-vent length	+0.95150	60.216	55.111	+ 4.857
Body temperature	+3.42561	14.989	19.440	-15.248
Pulse rate	-1.23113	27.644	74.622	+ 57.836
Dominant frequency	+0.00477	1871.340	1847.407	+ 0.114
Duration	+0.92707	11.172	3.448	+ 7.160
Mean discriminant score:		93.885	54.719	

Mahalanobis generalized distance: 39.166

Table VII. Correlation (r) and significance (P) values for comparison of Morphological
Discriminant Function and Call Discriminant Function.

	No. sp.	r	P
<u>Bufo americanus</u>	50	-0.188	$\gg 0.1$
Intermediate populations	62	+0.552	$\ll 0.001$
<u>Bufo hemiophrys</u>	111	-0.178	$\langle 0.1 \rangle 0.05$
Pooled sample	223	+0.820	$\ll 0.001$

Figure 43. Histograms of call discriminant scores for individual Bufo in reference samples and in eight intermediate populations.



INTERMEDIATES
HWY I TRANSECT

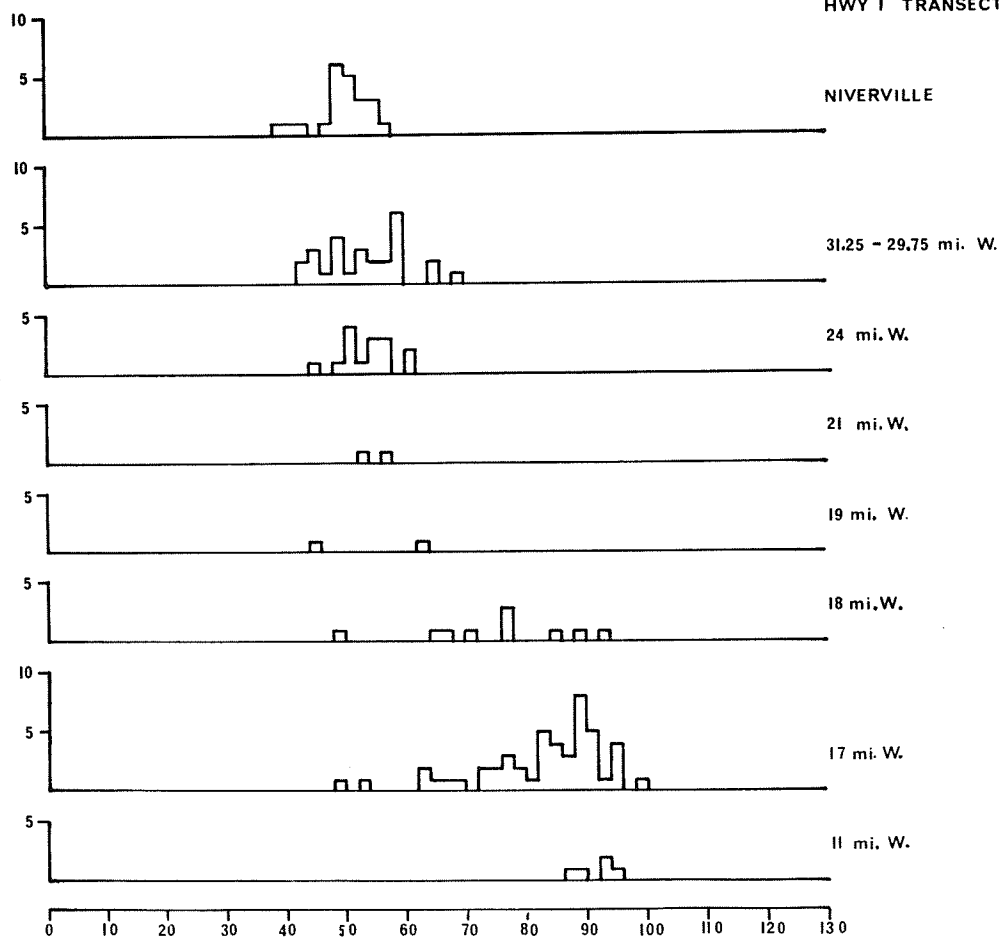
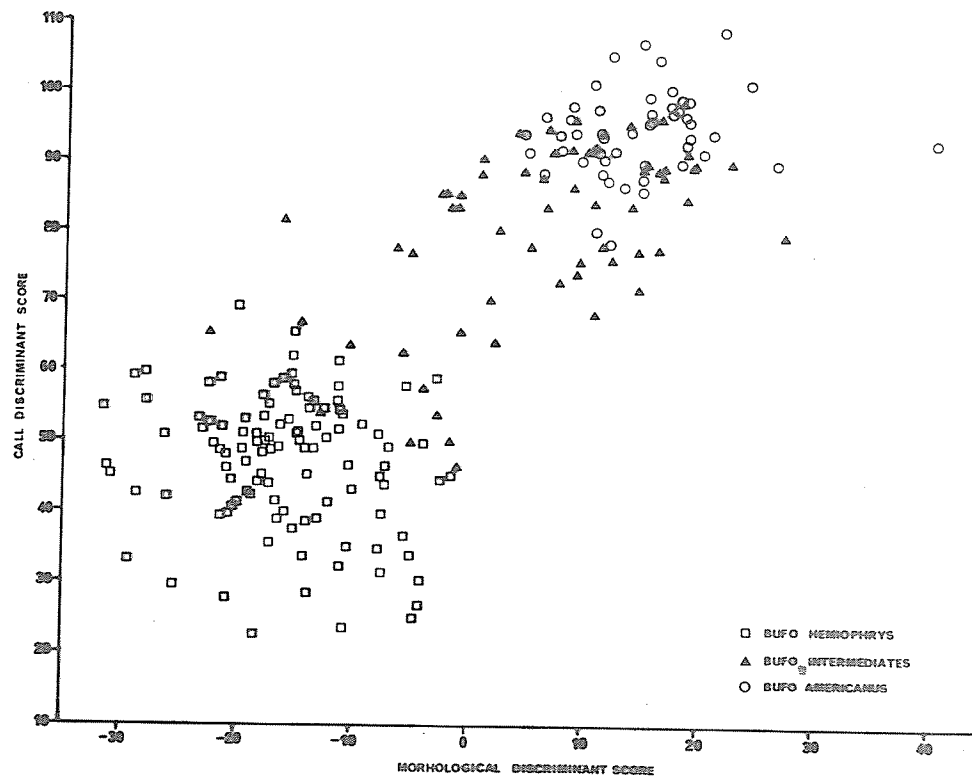


Figure 44. Relationship of call discriminant score to morphological discriminant score in three samples of Bufo (Appendix III gives localities and number of specimens).



The number of individuals metamorphosing in each cross, the date the cross was made and the dates of first and last transformation are given in Table VIII. The mean number of individuals transforming from all complete replicates (with some transforming individuals in laboratory and both ponds) are composed in Figure 45.

In the laboratory crosses using americanus females had high transforming success, and those using hemiohryps females had low success, with intermediate females yielding intermediate results.

The water used in the laboratory was pumped in from Shoal Lake (PC: K.W. Stewart) in eastern Manitoba, an area well within americanus territory. No similar marked contrasts existed within or between the field-raised replicates. A Chi-square test of pure americanus and pure hemiohryps between intermediate (17 mi.w.) and hemiohryps (31 mi.w.) ponds gives a value of $\chi^2=1.30$ which is not significant ($0.5 > p > 0.1$). The experiment lacks sufficient successful replicates, is unbalanced because of unequal replicates between crosses, and shows wide variation in transforming success between some replicates (a difference of as much as 28 individuals transformed). In addition some laboratory crosses took an abnormally long time to transform (eg. to Dec. 13 in AI-4!) possibly because of suboptimal temperatures or food.

Table VIII. Results of cross-mating and artificial rearing: number of individuals in each sample (50 eggs) which reached transformation.

A	<u>americanus</u> parent from 3 miles west on Highway 1 of junction Highways 1 & 11.
H	<u>hemiphrys</u> parent from 31-29 miles west on Highway 1 of junction Highways 1 & 11.
I	intermediate parent from 17 miles west on Highway 1 of junction Highways 1 & 11.

In each combination the first letter designates the female parent, the second the male; the number following the dash is the sequential number assigned a replicate within that cross combination. Missing numbers in sequence are crosses which failed to produce fertile eggs. Dates in parenthesis are: the date cross was made (after cross number) and dates of first and last individual to transform (after number reaching transformation).

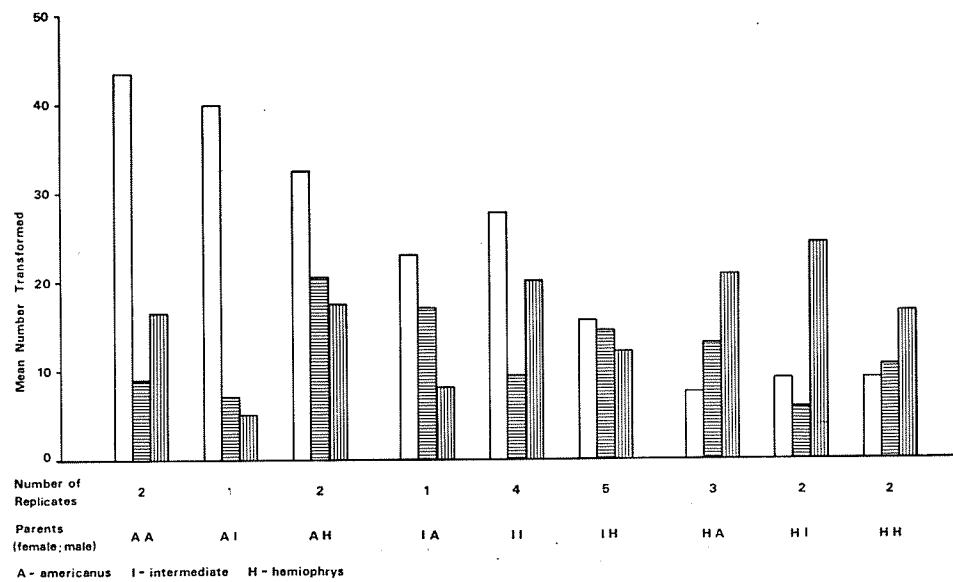
*Indicates a pre-hatching failure at a particular locality due to accident or obvious contamination (eg. fungal growth). In Figure 47 any replicate with a failure in laboratory or field is omitted completely in computing the mean.

Cross	<u>Number reaching transformation</u>		
	<u>Laboratory</u>	<u>17 miles west</u>	<u>31 miles west</u>
AA-1 (May 7)	45 (July 7-19)	11 (July 12-14)	11 (July 12)
AA-2 (May 16)	42 (July 8-Sept. 13)	4 (July 12)	22 (July 13-14)
AA-4 (May 27)	17 (July 14-24)	*	*
AA-5 (May 27)	44 (July 14-Aug. 25)	26 (July 14-16)	*
AI-4 (May 27)	40 (July 14-Dec. 13)	7 (July 15-16)	5 (July 14)
AH-1 (May 16)	47 (July 4-Aug. 11)	24 (July 13-16)	20 (July 13-18)
AH-4 (May 27)	18 (July 26-Oct. 2)	17 (July 15-19)	15 (July 13-17)

Table VIII Continued

Cross	Laboratory	Percentage reaching transformation	
		17 miles west	31 miles west
IA-5 (May 28)	23 (July 15-Aug.9)	17 (July 14)	8 (July 14-Aug. 8)
II-1 (May 25)	39 (July 3-Oct.5)	10 (July 14)	20 (July 13-17)
II-4 (May 27)	15 (July 11-Sept.1)	7 (July 13-16)	*
II-5 (May 27)	3 (July 16-28)	4 (July 15-16)	10 (July 14-19)
II-6 (May 28)	37 (July 13-Aug.31)	15 (July 14-16)	22 (July 14-29)
II-7 (May 29)	32 (July 18-Sept.29)	10 (July 14-16)	28 (July 15-Aug. 7)
IH-1 (May 16)	11 (July 7-14)	16 (July 12)	23 (July 14-25)
IH-4 (May 27)	2 (July 11)	18 (July 13-29)	4 (July 14-16)
IH-5 (May 27)	18 (July 14-Aug.25)	9 (July 14-16)	4 (July 13-14)
IH-6 (May 28)	21 (July 14-Sept.3)	15 (July 14-17)	25 (July 13-15)
IH-7 (May 28)	26 (July 18-Nov.12)	16 (July 14-16)	4 (July 14)
HA-1 (May 16)	3 (July 7-Aug.13)	14 (July 13-21)	13 (July 14-26)
HA-2 (May 16)	12 (July 9-19)	12 (July 12)	26 (July 13-15)
HA-3 (May 16)	8 (June 30-July 12)	13 (July 13-14)	23 (July 13-16)
HI-2 (May 16)	11 (July 10-30)	7 (July 14-16)	24 (July 13-15)
HI-3 (May 16)	7 (July 4-12)	5 (July 12)	25 (July 13-21)
HH-4 (May 27)	6 (July 17-Aug.25)	6 (July 14-15)	25 (July 14-31)
HH-5 (May 27)	12 (July 19-Sept.16)	15 (July 14-21)	8 (July 15-29)

Figure 45. Histogram of mean number of tadpoles reaching transformation (from samples of 50 eggs) for each combination of americanus, intermediate, and hemiophrys parents in laboratory (open bars), in pond 17 mi. W on Hwy 1 of jet Hwys 1 and 11 (horizontally hatched bars) and in pond 31 mi. W on Hwy of jet Hwys 1 and 11 (vertically hatched bars). The first letter given of each cross is the female parent, the second is the male.



6. Comparisons of hemiophrys and americanus with other Bufo in northern North America

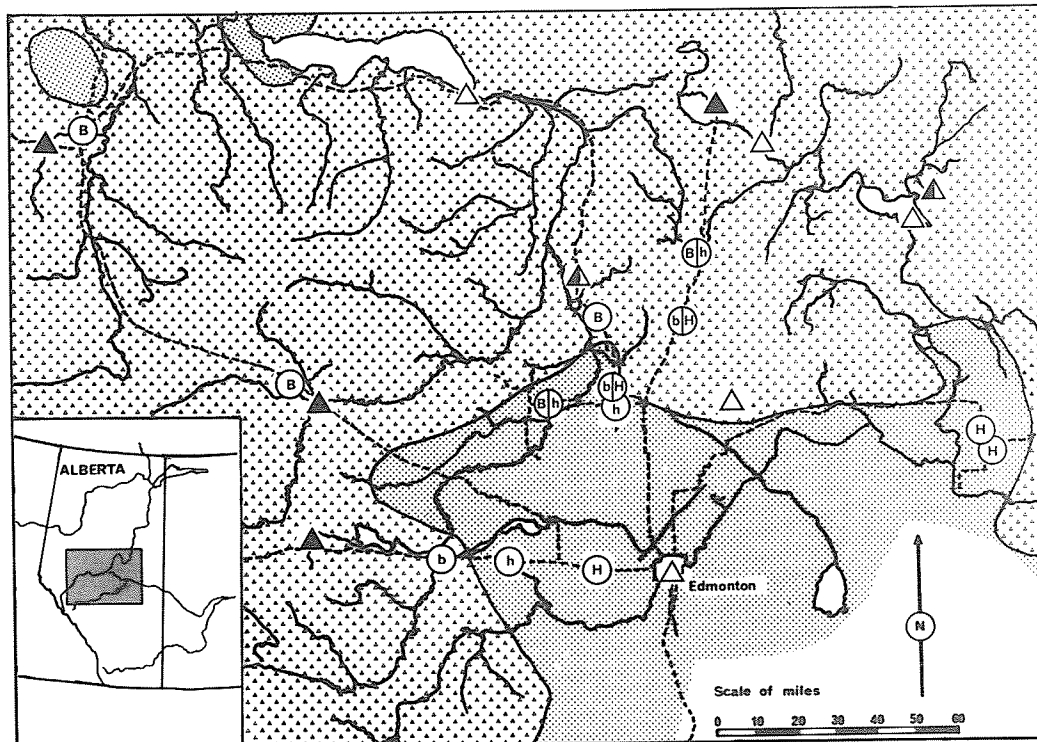
a) Hemiophrys - boreas

In 1965, the area north of Edmonton was examined during peak toad breeding and four breeding sites were found where boreas and hemiophrys were present in mixed choruses. Sites visited east of these contained only hemiophrys and west only boreas. (see Appendix I, Figure 46 and Table IX for localities). Examination of the collection of the University of Alberta Zoology Department documented a slightly more extensive overlap zone (Figure 46).

In two of the mixed chorus sites examined, boreas was the more common species (but one of these contained only 3 calling males), in the other two hemiophrys predominated. Mismatched pairs were found, usually a female of the less abundant species mated with a male of the predominant species. Only one individual suspected of being a hybrid was found, that in a pond (2.4 miles North of Westlock) where hemiophrys was the more common species.

To compare the two species and evaluate the suspected hybrid a discriminant comparison was made between 48 hemiophrys (NMNS (HS) 8521) and 60 boreas (NMNS (HS) 8530). The hemiophrys were from the same pond where the hybrid was taken, but because of the smaller sample of boreas available from this locality, a larger series from a nearby locality (2 mi W on Hwy 2 of Athabaska) was used. Measurements were those taken for the hemiophrys-americanus comparison, except that all characters involving

Figure 46. Map of west central Alberta showing the distributional relationship of Bufo boreas (B,b) and B. hemiophrys (H,h) in a narrow zone of sympatry. A capital letter indicates more than 20 males collected, a lower case letter, less than 20 males. All collections from breeding choruses. UA refers to collections in the University of Alberta, and NMNS to those in the National Museum of Natural Sciences.



Bufo boreas	NMNS	(B) < 20	(b) > 20;	UA	▲
Bufo hemiophrys	NMNS	(H) < 20	(h) > 20;	UA	△
Mixed population	NMNS	(Bh)		UA	▲△
Vegetation zones	Aspen Parkland		[Stippled Box]		
	Boreal Forest		[Cross-hatched Box]		

Table IX. Summary of localities where Bufo boreas and B. hemiophrys were found using the same breeding ponds in west-central Alberta during the 1965 field survey of the area.

	<u>B. boreas</u>		<u>B. hemiophrys</u>		<u>Hybrid</u>
	<u>males</u>	<u>females</u>	<u>males</u>	<u>females</u>	<u>male</u>
24 May 1965					
3 mi. W on Hwy 18 of Freedom (turnoff)	2	0	1	0	0
26 May 1965					
2.4 mi. N on Hwy 44 of Westlock	7	0	48	15	1
27 May 1965					
0.6 mi. SW on Hwy 2 of Perryvale (turnoff)	8	1	36	0	0
28 May 1965					
2 mi. W on Hwy 2 of Athabasca	60	5	4	1	0

the cranial crest had to be omitted since boreas usually lacks crests, or has crests so low that they are barely discernible and rarely can be measured with confidence. Several other characters which distinguish boreas and hemiohryys were not included in this comparison. Besides the lack of cranial crests, boreas also lacks a dark throat in the male, and possesses a prominent tarsal fold.

The discriminant weights, weight contributions to the Mahalanobis generalized distance, means for hemiohryys and boreas samples, and the measurements for the suspected hybrid are given in Table X.

The weight contributions indicate that "spade" width, nostril separation, size of tibia wart, body size, inner metatarsal tubercle width, tympanum diameter and tarsus length are making the largest contribution to separating the taxa in the analysis. B. boreas has a smaller spade, a larger inner metatarsal tubercle, a much larger tibial wart, somewhat larger snout-vent, smaller tympanum diameter and a longer tarsus. The mean of the discriminant scores for hemiohryys was +106.9462 (+78.16148 to +132.4602) and for boreas +19.47962 (+44.11512 to -14.2396). The suspected hybrid had a score of +63.0288. Histograms of the discriminant scores are given in Figure 47.

In addition to its intermediate score the hybrid had distinct, but open, cranial crests, with no indication of a hemiohryys-like central filling or posterior bridging. The anterior width of the cranial crest was 4.9 mm and the posterior 6.1 mm and on the americanus-hemiohryys boss scoring system it would be assigned a score of 3, clearly distinguishing it from the 0 or 1 score normal for a hemiohryys. The

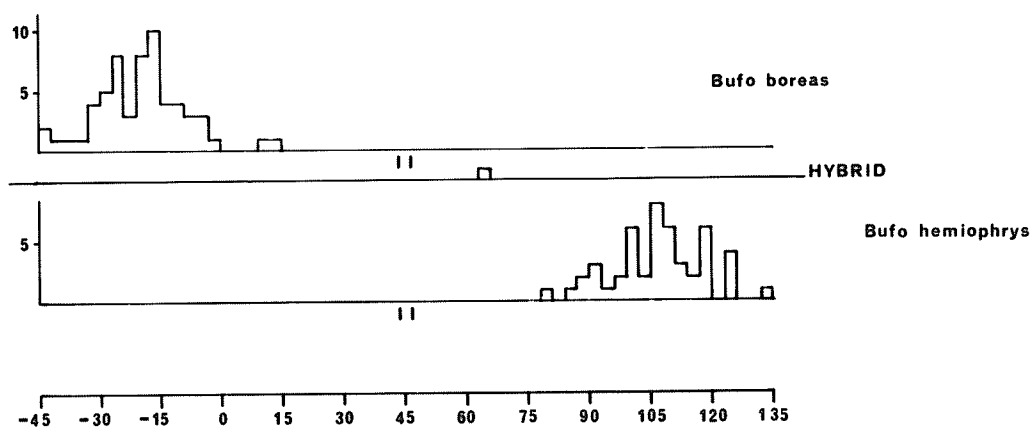
Table X. Discriminant weights and weight contributions to Mahalanobis generalized distance for each character for B. hemiophrys and B. boreas, mean discriminant scores for both species and values for a hybrid individual.

	Discriminant Weights	Mean for hemiophrys NMNS 8521	Mean for boreas NMS 8530	NMNS 8523	Weight Contribution
Snout-vent length	-4.842178	60.46243	62.88822	74.4	+11.74610697
Nostril separation	+29.637020	4.589577	3.9566610	4.5	+18.757774415
Nostril to parotoid (right)	+2.362891	11.87079	10.88994	13.8	+2.31641637
Nostril to parotoid (left)	-0.9124445	11.84996	10.93160	13.8	-0.837952531
Eyelid length	+1.16125	7.347894	7.429959	8.4	-0.095297981
Head width	+0.1174592	17.03535	17.29992	19.2	-0.031076181
Tympanum diameter	+11.6957	4.395826	3.734993	5.1	+7.728904518
Parotoid length (right)	+4.602726	10.9812	11.06492	13.1	-0.385340221
Parotoid length (left)	-4.331665	11.164530	11.10326	12.6	-0.265401115
Parotoid width (right)	+1.079502	6.929142	7.296618	8.2	-0.396691077
Parotoid width (left)	+2.935235	6.924962	7.326615	7.5	-1.178945943
Parotoid separation	+2.59703	9.358281	8.798274	11.1	+1.454354979
Spot length (right)	+1.009183	10.19787	9.464948	14.0	+0.739652423
Spot length (left)	-1.57778	10.19995	9.453274	12.5	-1.178090459
Spot width (right)	+0.3525454	4.904161	3.888327	4.2	+0.358127604
Spot width (left)	+1.497334	4.783328	4.029994	5.1	+1.127992612

Table X. continued.

	<u>Discriminant Weights</u>	<u>Mean for hemiphrys</u>	<u>Mean for boreas</u>	<u>NMNS 8523</u>	<u>Weight Contribution</u>
		NMNS 8521	NMNS 8530		
Wart width (right)	-2.970933	2.204161	3.179994	3.0	+ 2.899134462
Wart width (left)	-2.052994	2.22916	3.373328	2.9	+ 2.348970039
No. warts per spot (right)	+0.05815297	5.1875	3.216666	4	+ 0.114609851
No. warts per spot (left)	+1.092478	4.791666	3.233332	6	+ 1.702445612
Tibia length	+5.659762	22.22492	21.93157	25.3	+ 1.660291183
Largest wart on tibia (right)	-1.178755	2.272912	11.67494	3.1	+11.08268752
Largest wart on tibia (left)	-1.918204	2.164577	11.913280	2.8	+18.70000109
Tarsus length	-4.341073	14.23952	15.18993	16.9	+ 4.125799190
"Spade" width	+21.52207	5.177079	3.496661	5.3	+36.16607383
Inner metatarsal tubercle width	-16.23179	2.208327	2.686661	2.3	+ 7.764217038
Mean discriminant score (centroid):	+106.9462	+19.47962	+ 63.0288		
Mahalanobis generalized distance:	126.42582				

Figure 47. Histograms of discriminant function scores for reference samples of male Bufo boreas and B. hemiophrys and one natural hybrid between these taxa.



crest measurements, posterior wider than anterior, are the reverse of those typical for hemiphrys. Post-orbital crests were absent and therefore scored 0. The venter scored 5. The hybrid lacked the tarsal fold typical of boreas. Although the spot length does not differentiate the two species in this analysis, boreas often has spots than run together, in contrast to the distinct blotches typical of many hemiphrys.

b) Hemiphrys - cognatus

Only one mixed breeding chorus of B. hemiphrys and B. cognatus was found in the course of herpetofaunal surveys of the Canadian Prairie Provinces, but this was the only time when any breeding aggregation of B. cognatus was found during these surveys. B. cognatus breeds typically after heavy rains. On 28 May 1963 a large prairie slough 8.3 miles east on Highway 1 of the Piapot turnoff yielded 48 male and 4 female B. cognatus and 22 male and 2 female B. hemiphrys. No mismated pairs were noted.

B. cognatus is morphologically distinctive from hemiphrys. Males in the Piapot series ranged from 66.1 to 76.4 mm snout-vent length. Particularly obvious were the characteristically heavy cranial crests which unite on the nose and diverge strongly posteriorly. The postorbital crests are strongly developed, and the large, prominent dorsal blotches each contain 14 to 54 small warts. The outer metatarsal tubercle is large and broad. The venter is slightly spotted to unspotted (score values of 3(2), 2(12), and 0(34) among the males).

A discriminant analysis was made between with the 48 male B. cognatus from Piapot (NMNS (HS) 7110) and 22 male B. hemiphrys from

Piapot (NMNS (HS) 7111) pooled with an additional 36 males from nearby Tompkins (NMNS (HS) 6014). The discriminant weights, weight contributions, and mean values for each character are given in Table XI, and the discriminant scores presented as a histogram in Figure 48. This analysis confirms the distinctiveness of the two taxa and the lack of intermediates where they are sympatric.

The largest contributions to the separation between these taxa are made by the posterior cranial crest, cranial crest length, inner metatarsal tubercle, snout-vent length, spot width and number of warts per spot. B. cognatus is a larger toad than B. hemiophrys with much greater posterior divergence of the cranial crest, larger spots with more warts per spot, and a somewhat larger spade.

c) Hemiophrys - w. woodhousei and americanus - w. fowleri

Two small series, one each of w. fowleri (19 males taken 19 June 1973 at Long Point, Norfolk Co., Ontario) and w. woodhousei (10 males 22 June 1965, 7 males 23 June 1965 from $3\frac{1}{2}$ miles southeast of Stockton, Rooks County, Kansas) were available for comparison (see Appendix I).

The discriminant weights produced by the comparison of americanus and hemiophrys were applied to woodhousei and fowleri specimens to see where they would rank in comparison with these taxa. The results are given as histograms in Figure 49. Interestingly, they both score as intermediate between hemiophrys and americanus. Two subspecies produced nearly identical scores on the basis of the characters used. The variance in these samples, however, is low in contrast to the generally high variances of populations in the americanus and hemiophrys contact

zone. Other characters, which are not included in this comparison, effectively separate the subspecies of woodhousei and each from americanus and hemiophrys.

Table XI. Discriminant weights, weight contributions to Mahalanobis generalized distance, and means for each character for discriminant function analysis of Bufo hemiophrys and B. cognatus.

Character	Discriminant Weights	Mean B. cognatus	Mean B. hemiophrys	Weight Contribution
Snout-vent length	+ 1.9618	72.3333	63.0741	+ 18.165
Nostril separation	-10.0243	4.8292	4.6034	- 2.263
Cranial crest length (right)	+10.1456	13.9979	11.9741	+20.532
Cranial crest length (left)	-0.9120	14.0417	12.0690	- 1.799
Nostril to parotoid (right)	- 3.0633	14.8313	12.9345	- 5.810
Nostril to parotoid (left)	- 1.2635	14.8958	12.9586	- 2.448
Cranial crest width (anterior)	-21.5565	6.2792	5.5931	-14.789
Cranial crest width (posterior)	+17.8171	9.1292	4.6121	+80.481
Eyelid length	- 2.2461	8.5875	7.8138	- 1.738
Head width	+ 2.5182	21.1250	17.8052	+ 8.360
Tympanum diameter	+ 1.1682	5.0458	4.7552	+ 0.340
Parotoid length (right)	- 4.1194	10.7563	11.3224	+ 2.232
Parotoid length (left)	+ 3.2425	10.6896	11.4759	+ 2.550
Parotoid width (right)	- 5.9609	6.7646	7.1259	+ 2.154
Parotoid width (left)	+ 2.5110	6.6667	6.9828	- 0.794
Parotoid separation	+ 0.2284	13.9333	10.1948	+ 0.854

Table XI. continued.

Character	Discriminant Weights	Mean B. cognatus	Mean B. hemiophrys	Weight Contribution
Spot length (right)	- 0.2942	16.4396	9.8328	- 1.944
Spot length (left)	- 0.0370	14.9000	9.3328	- 0.206
Spot width (right)	+ 4.0041	7.6417	4.4724	+12.690
Spot width (left)	- 0.9240	7.3875	4.4052	- 2.756
Wart width (right)	+ 10.2861	2.0854	2.1328	- 0.487
Wart width (left)	- 2.9931	2.1104	2.1138	+ 0.010
No. of warts per spot (right)	+ 0.4006	28.2083	3.500	+ 9.898
No. of warts per spot (left)	+ 0.4401	25.7500	3.6207	+ 9.740
Tibia length	- 2.7946	25.6970	23.3534	- 6.552
Largest wart on tibia (right)	+ 0.5363	2.5042	2.2966	+ 0.111
Largest wart on tibia (left)	+ 1.5136	2.6312	2.4224	+ 0.316
Tarsus length	+ 2.4015	15.9792	14.4948	+ 3.565
"Spade" width	+ 3.7639	6.3917	5.4586	+ 3.512
Inner metatarsal tubercle width	+24.3866	2.9250	2.1724	+18.353
Mean discriminant scores (centroids):		249.605	97.228	
Mahalanobis generalized distance: 152.377				

Figure 48. Histograms of discriminant scores for reference samples of male Bufo hemiophrys and B. cognatus.

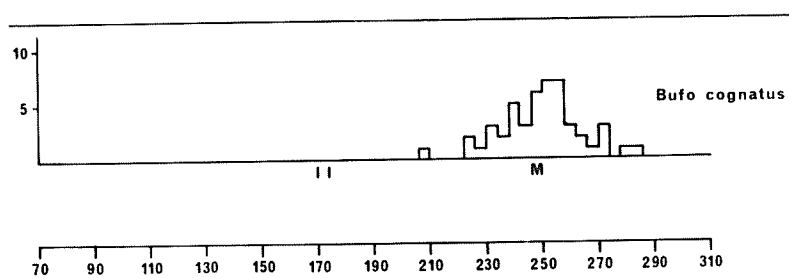
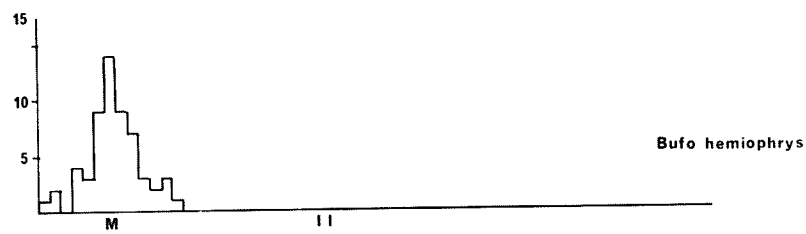
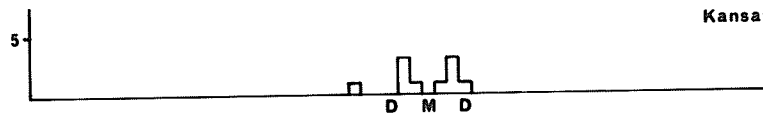
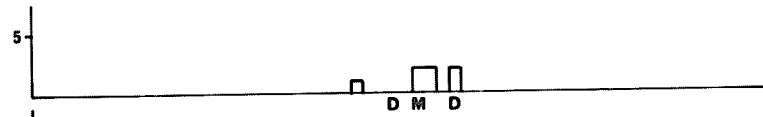


Figure 49. Histograms of discriminant scores for two samples of male Bufo woodhousei. Weights produced by the americanus-hemiophrys discrimination were used to complete these values.

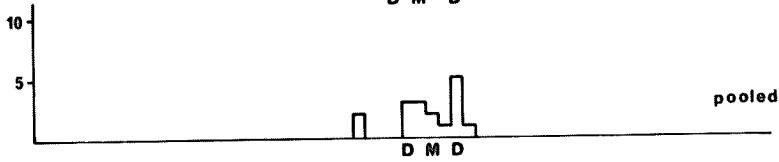
Bufo w. woodhousei
3.5 mi. SSE. Stockton, Rooks Co.
Kansas



8585-10

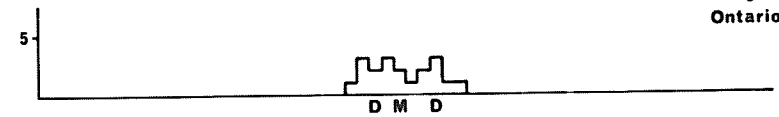


8591-7



pooled

Bufo w. fowleri
Long Point, Norfolk Co.
Ontario



15972-19

-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60

DISCUSSION

1. Habitat

a) Breeding habitat

In general, americanus populations seemed to be larger and males more clustered. In contrast, it was common to find hemiphrys males more dispersed, often in scattered small clumps of only two or three calling together. This contrast becomes obvious on examining the sample sizes in collections of the two taxa. Partly, this difference in the field is due to the fact that ponds are often larger and deeper in boreal forest and relatively smaller and shallower in aspen parkland and grassland. On the subjective basis of field experience throughout the range of both species, the impression exists that toads in the eastern part of northern North America occur in more extensive and densely populated breeding colonies than they do in the central region.

b) Summer habitat

Breckenridge and Tester (1961) reported from intensive studies of B. hemiphrys in Minnesota that it was found to live almost exclusively in or near the margins of prairie ponds. Field observations of hemiphrys over its Canadian range do not strongly support this as typical of hemiphrys populations in general. Samples of hemiphrys (such as the one through the Spruce Woods Forest Reserve in July 1960) have been taken of many individuals foraging well away from water. Surveys made in the summers of 1959-1967 along the margins of lakes, ponds, sloughs,

and potholes of all types throughout the range of hemiophrys seldom revealed large numbers of post-breeding or post-metamorphosing hemiophrys at such sites. Exceptions are the Wascana marsh at Regina, Saskatchewan, and the Delta marsh in Manitoba, where large summer series of all sizes of foraging hemiophrys were taken. Single adult hemiophrys were occasionally present at pond margins but usually occur there at the end of the breeding season. However, a large series of americanus of all sizes was taken along a lake margin in eastern Manitoba in August, and occasional adult americanus were found at pond margins near the end of breeding activity. If there is a tendency for hemiophrys to be restricted to pond margins more often than americanus it is not clearly indicated by present field data.

2. Morphology

a) Non-quantitative

Live Bufo americanus and B. hemiophrys are readily distinguished in the field but their differences are hard to quantify. Colour characteristics, not treated quantitatively, are helpful in this respect. B. americanus is usually more brightly coloured - with backgrounds of red, brown, yellow or green. B. hemiophrys is a more drab toad, usually greyish or dull brownish. A rusty-coloured phase occurs (Cook 1964c) but it is a more subdued colour than the red of americanus. Other good field characters, such as distribution of warts, spot size and cranial crest

features have been treated in detail in the morphological analysis. The difference between the calls of breeding males makes the two taxa easily distinguishable to the ear.

In field sampling across the east-west transects of the interbreeding zone the variety in morphology and vocalization gives the impression of sleight-of-hand by a master conjurer. In eastern Manitoba one can collect what appears to the eye and to the ear as "good" americanus, as recognizable as any from Prince Edward Island to western Ontario. If one misses the intermediate zone, then in the Aspen Parkland east of the Red River one can sample "good" hemiphrys, as recognizable as any from central Manitoba to Western Alberta. When the gap between the two is sampled, the change between breeding sites is subtle and almost imperceptible.

b) Quantitative

A major problem in any morphological comparison is finding an objective and practical method to evaluate and contrast variations. The discriminant function analysis used here has produced a high-resolution, repeatable analysis of morphological variation over the range of the parent taxa and through the transition zone between them.

The reference samples represent a good geographic dispersal for hemiphrys. Although they are relatively weak in material from North Dakota and the northern portions of Alberta and Saskatchewan, all major habitat types which its range covers are represented. The Laramie Valley (Wyoming) disjunct population is also included.

The sample largely avoids the southern periphery of the continuous range of hemiohryps and possible influence from the presumably allopatric B. w. woodhousei. The sample of americanus is drawn entirely from the northern portions of its range, largely to avoid its extensive area of geographic sympatry over the eastern United States with B. w. fowleri. Only two localities, Rondeau and Point Pelee parks in southern Ontario, are within that contact area. This restriction on the geographic area of samples used means that the discriminant analysis does not represent total americanus variation. However, the samples do cover three major habitat areas occupied by americanus in the northern part of its range. Western Ontario is the least well represented geographic area. Encompassed by the samples are areas supposedly occupied by the northern race B. a. copei (Logier and Toner 1961; Aston, Guttman and Buckley 1973; Conant 1975) and the transition between it and B. a. americanus.

In general, the variation in population mean discriminant values within taxa does not markedly follow geographic clines or forest zones though some trends are pointed out in the Results section. However, the wide area of low western scores for americanus and narrow area of high eastern scores for hemiohryps may be of some importance (see below). Most previous comparisons of B. americanus and B. hemiohryps (Breckenridge 1944, Blair 1957a, Underhill 1961) have agreed that these taxa are as morphologically distinct as good species, but sample size, geographic representation

and number of characters have not been very extensive.

A post-orbital score was used by Henrich (1968) as part of his basis for separating the two taxa. It is clearly shown here from the much more extensive geographic coverage of hemiophrys that post-orbital crests may be present on individuals well within hemiophrys range and neither their presence nor that of the intermediate conditions clearly establish an individual as intermediate.

The ventral scoring used here unfortunately encompasses only part of the ventral pattern variation exhibited in the two taxa, since it deals solely with the area covered by pigment. As Henrich (1968) pointed out, there is a tendency for hemiophrys to have lighter ventral markings, and he used a scoring system which attempted to evaluate this difference. However there is a large subjective element in any attempt to score relative intensity of markings, and this is especially true of trying to assign scores to intermediate specimens objectively. Furthermore, preservation may affect collections differently and there is a danger of scoring preservation differences rather than real phenotypic ones. Similarly, the sometimes obvious difference between individuals in the relative size of markings, spots or reticulations, has not been included in this assessment because of difficulties in obtaining objectivity. Differences are most obvious in the northern populations of americanus and this character does not have a prime importance in the general hemiophrys-americanus comparison.

It is notable in the ventral score results that northern americanus populations score high, southern score low. The tendency of Prince Edward Island toads to have a high ventral score has been noted previously by Cook (1967). The suggestion of Bleakney (1952) that there is a difference between Annapolis Valley populations and those outside this valley within Nova Scotia is not evident in comparing Wolfville (valley) and Musquodoboit (Halifax County) samples here.

There is great variation in mean snout-vent length in both taxa. The largest population mean lengths (71.8 for americanus and 70.8 for hemiphrys) are surprisingly close, although the smallest means (53.9 and 45.8 respectively) are more disparate. When individual maxima and minima are examined, 88.4 vs. 79.1 and 42.1 vs. 41.3 for americanus vs. hemiphrys respectively, the agreement is closest in the smallest size range. The discriminant analysis has responded to this variation by giving little weight to size in separating the taxa. The potential minimum size at maturity, and the maximum size attainable are, evidently, not that different between the two taxa. Much remains to be understood about geographic variation in size in toads, and the influence of local ecologic conditions and yearly variations in rainfall and the length of the effective growing period. Perhaps abundance of food and the size at transformation and the total hours per year of optimum foraging weather (in terms of temperature and moisture) have an important bearing on the question. Clear-cut north-south

or east-west gradations in either taxa are not apparent in the present samples, although there is a generalized trend for hemiophrys to be smaller in the east, and americanus to be large in the south and smaller in the north.

The relative tibia length has been thought to decrease toward the north in some anurans (Schmidt 1938, Bleakney 1974). This is not clear-cut in hemiophrys or americanus populations sampled, although there may be a trend in this direction in americanus. Jameson et al. (1973) has suggested for the Pacific Tree Frog (Hyla regilla) that various proportions are correlated with local climatic conditions.

The relative length of the spot (spot length divided by snout-vent length) is much more variable in americanus than in hemiophrys. Western and southern americanus populations have a smaller mean spot size than hemiophrys, though the entire americanus range encompasses the variation in hemiophrys. It is also noteworthy that there is a difference in dorsal spotting between northern americanus, where large (and often irregular) spots are present, and southern (and western) populations where smaller spots are more typical. The fact that western americanus are small spotted argues against the large spots in northern americanus being derived from contact with hemiophrys.

The separate discrimination for reference samples of females, though based on a much smaller numbers (59 americanus and 118 hemiophrys) than for males, also separated the two groups completely. The differences in weighting may be due to both the smaller

sample and the very uneven geographic distribution of female samples (see Appendix I). The Mahalanobis generalized distance between americanus and hemiophrys is 104.66 in the female analysis compared with a mean of 51.35 in the male discrimination, possibly indicating that larger body size accentuates the differences in measurements.

The discriminant scores for males from southeastern Manitoba clearly define a narrow zone of populations which are intermediate in mean value and have a wider dispersal of scores (as shown by the histograms and the high variance values) between reference sample populations of americanus and hemiophrys. This zone is only a few miles wide, and its narrowness most clearly indicated on the Trans-Canada transect where the most intensive sampling was conducted and there it is clearly 10 miles or less in width. The narrowness of the zone is also apparent in the other five transects. The histograms show no clear indication of bimodal or trimodal distribution which would have been indicative of hybrids mixed with "pure" examples of either or both parental types. The distribution presented seems best interpreted as complete interbreeding and back-crossing with no segregation into americanus or hemiophrys types within the interbreeding zones.

The lowered scores east and west of the narrow interbreeding zone could be interpreted as due to convergence in each taxon toward characters of the other as the intermediate zone is approached. This interpretation would require selection to be acting on the

natural variation within each of the hemiohryps and americanus stocks and would ignore the likelihood of gene flow from the intermediate zone. Discriminant scores of hemiohryps do not tend toward those for americanus over the northern part of the former's range where it is occurring in boreal coniferous forest nor do scores of northeastern americanus tend toward hemiohryps where americanus is also occurring in boreal coniferous forest. It may be more reasonable, because the lower scores grade relatively smoothly toward the area of intensive interbreeding, to postulate that they do reflect the direct effects of introgression from the contact area. The distribution of lowered mean scores only as far as Delta in central Manitoba, but all the way to Whitetop Creek, northeast of Moosonee in central Ontario, suggests that introgression is far more successful into americanus than into hemiohryps populations. That this is really occurring is further indicated by the observation of Logier (1928) of a specimen with "temporal crests" (cranial crests) resembling B. hemiohryps in a sample of toads from Lake Nipigon (geographically between Oxdrift and Whitetop Creek samples analysed here).

Characters analysed separately or in ratios also show the same pattern of abrupt change over a narrow zone that is evident in the multivariate analysis. Cranial crest score, cranial crest posterior width/head width, and "spade" width/tarsus length show the abrupt change in their means across this zone, as does the spot length/snout-vent length ratio. The latter value is different

between western americanus and all hemiophrys. It did not weight heavily in the discrimination but this may have been because of its broad variation over the whole of americanus distribution.

The comparison in Table IV shows intermediate populations on each of the six transects through eastern Manitoba and gives the mean discriminant score and variance together with the scored and measured univariate characters or ratios. These data clearly define the position of the interbreeding zone through eastern Manitoba.

The scores for females from southeastern Manitoba show a similar pattern to that obtained with the scores for males though the narrowness of the transition zone between the two taxa is not as well defined by the former. This may be partly due to the meager samples of females available, as the largest samples show the greatest agreement with the results obtained for males.

c) Selective advantage of taxonomic characters

B. hemiophrys has a major portion of its range in Aspen Parkland and Prairie regions and is therefore subject to more arid and exposed conditions and greater environmental fluctuation than B. americanus. The latter inhabits the mesic Great Lake-St. Lawrence, Acadian and Eastern Deciduous forests over much of its range. Both occur widely in the Boreal Forest in the northern portions of their ranges, and B. americanus continues into the Boreal-Tundra transition. Harper (1956, 1963) has pointed out that the northern limit of each is reached south of the limit of permafrost.

The appearance of the cranial crests is the most striking difference between B. hemiophrys and B. americanus. This difference may be related to selection pressures arising from differences in soil type and aridity in the respective environments of the two forms. Although experimental evidence is lacking, the cranial crests may function as the protection for the eyes, since the latter can be withdrawn into the head below the level of the crests. The solid or grooved boss formed by the crests of hemiophrys may be of advantage in emerging from the heavy prairie soils found over much of its range. Similar thickening, or thickening and filling in of the crests is found in the closely related B. w. woodhousei, the more distantly related B. cognatus, and in the Plains Spadefoot, Scaphiopus bombifrons, which belongs to a separate

family. All occur in prairie habitats over much or all of their ranges. In contrast the eastern forest-dwelling B. americanus has well-separated, relatively narrow crests (though they may be pronounced posteriorly) as does B. w. fowleri. The Eastern Spadefoot, Scaphiopus holbrooki, which typically occurs on light sandy soils, lacks a boss. The toad of the northern forests of western North America, B. boreas, either lacks cranial crests, or has only weak, low traces of them.

The relatively shorter tarsus of B. hemiophrys would result in shorter muscles in this portion of the leg, which may provide additional strength at the expense of some agility compared with B. americanus. This could be an advantage in digging in heavier prairie soils and for digging to greater depths to avoid aridity and frost. The relatively large spade in B. hemiophrys would also be an adaptation for more efficient digging in more difficult conditions. B. cognatus has a large spade as well, whereas this structure is relatively narrower in B. americanus and much narrower in B. boreas. The parallel reduction of the inner metatarsal tubercle that seems coincident with a large spade is noteworthy as the spadefoots (Scaphiopus), which are primarily arid-adapted, lack this tubercle entirely.

Tihen (1962a) postulated that the development of metatarsal spade and heavier limbs is associated with burrowing ability as an adaptation to arid conditions in the "americanus" group in which he includes B. cognatus.

Martin (1973) found the tibiofibula and femur of hemiohryns to be shorter and broader than all other members of the americanus group and the humerus shorter than it is in all but B. woodhousei. He suggested these proportions were due to selective pressures for efficient burrowing apparatus "in the cold environment" of this species.

Other characters, such as the nostril separation, head width and parotoid gland separation are related to a wider, stockier build in hemiohryns and cognatus compared with the forest-dwelling forms.

If B. americanus, exclusive of its northern-most populations, is compared with B. hemiohryns, the latter has larger dorsal blotches containing smaller and more numerous warts. B. cognatus shows a more extreme trend in this direction. However, northern B. americanus populations have large blotches and generally more warts per blotch. These northern toads may be more diurnal because of longer day length and cooler nights and occur in more open habitats than more southerly B. americanus. Larger blotches may provide a more contrasted pattern and better camouflage for a toad in open habitats. The more subdued colours in B. hemiohryns, where grey colouration is common basic background, is evident also in B. cognatus. Both lack the deep browns or reds that are evident in some B. americanus and B. boreas, and reach extreme development in northern populations of the former. Nevo (1973) in a study of color polymorphism in cricket frogs (Acris) found the frequency of

a grey morph increased, and red and green morphs decreased, along increasingly arid habitat gradients.

While there is general agreement that Bufo americanus americanus is a forest toad, some midwestern populations may show prairie adaptations. Smith (1961) designated it as a "prairie toad" in northern Illinois. However, he also expressed reservations about its equivalence to Bufo americanus Holbrook. Garman (1892) noted that an americanus he examined from Illinois had the cranial crests bridged posteriorly but did not indicate where in the State it had been collected. Smith (1961) regarded B. a. charlesmithi of southern Illinois as a forest toad. The variation and ecology of toads in this and adjacent states obviously deserves further attention, particularly as the post-glacial prairie peninsula once extended over the northern portion of Illinois.

3. Breeding call analysis and comparisons

Blair (1957a) has previously pointed out the distinctions and resemblances between the calls of B. americanus and B. hemiophrys and this comparison led him to postulate their close relationship. Henrich (1968) did not have call data available for his comparison of these taxa.

The samples for call analysis were restricted to a much smaller geographic area than those available for morphological analysis. Only those tapes recorded during 1969-1970 had body temperatures of calling toads recorded. Zweifel (1968) has stressed the importance of using actual body temperature in analysis of call parameters in preference to the air or water temperatures in the vicinity of a recorded animal. The body temperature of a toad may be close or identical to either that of the air or water in its vicinity. However, body temperature can also have an intermediate value because of recent movement from one medium to the other, and even adjacent toads may have different body temperatures.

The results obtained in this study of the relative effects of temperature and snout-vent length on the call parameters (pulse rate, dominant frequency and duration) are in general agreement with those of Zweifel (1968) who compared samples of 50 B. americanus from two populations in New Jersey and 63 B. woodhousei fowleri from New Jersey and New York populations. Zweifel found a strong positive correlation between temperature and pulse rate in both species and a negative correlation similar to the one reported

here for temperature and call length. He did find temperature and dominant frequency to be correlated for Northville, New Jersey, americanus ($r = +0.38$, $P < 0.05$) and this is quite similar to the americanus result in this study ($r = +0.416$, $P < 0.01$) but his Hayworth, N.J., sample showed a weak negative correlation which was not significant ($r = -0.08$, $P > 0.1$).

Zweifel's comparisons of body size with pulse rate, call length and dominant frequency for americanus are similar to the results of this study in that he did not find any significant correlation. (The later claim for this correlation by Licht 1976, notwithstanding). His results for fowleri in comparisons of body size, however, gave a significant correlation ($+0.47$, $P = 0.001$) with call length in the Hayworth sample and correlations with dominant frequency of -0.49 , ($P < 0.1 > 0.05$) in a Long Island sample and -0.59 ($P < 0.001$) in the one from Hayworth.

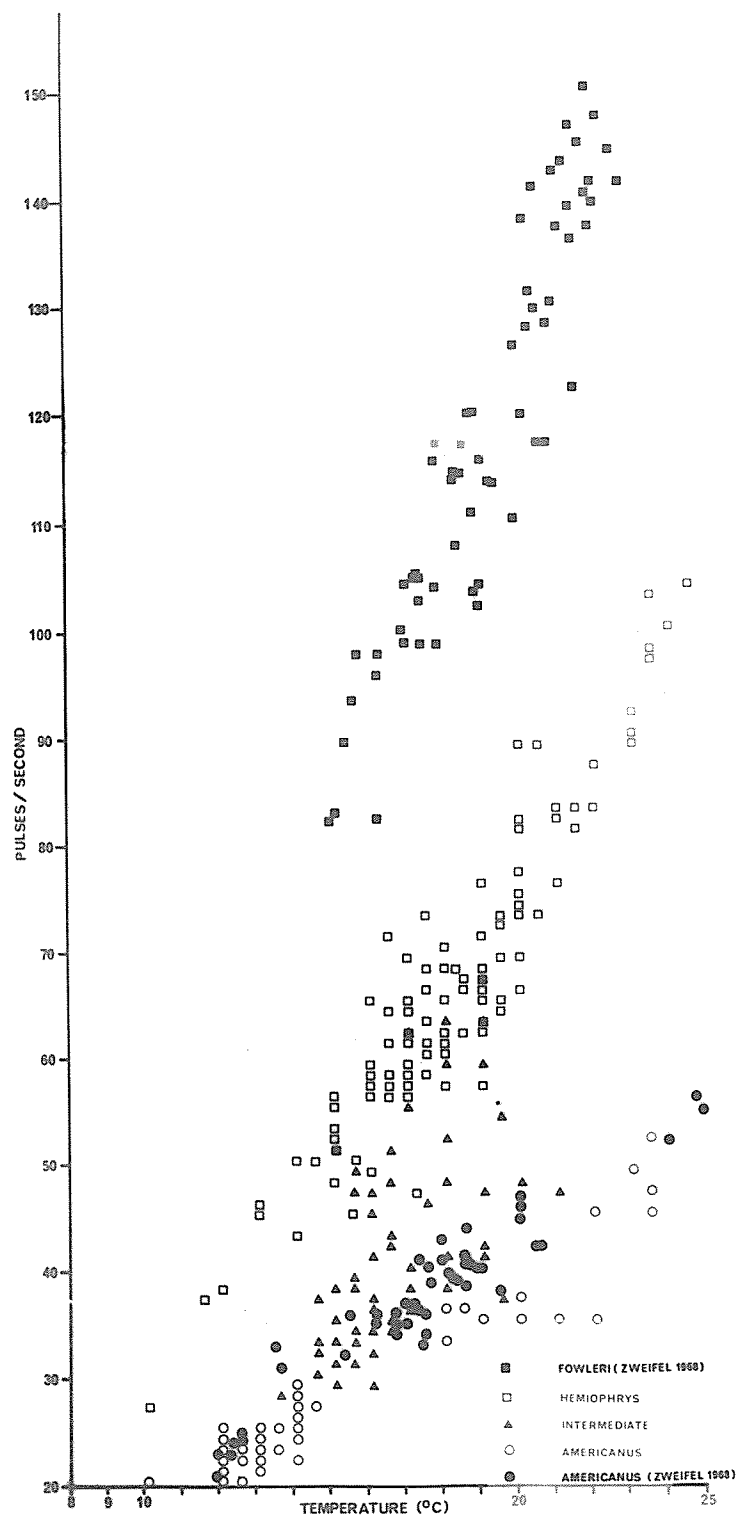
A comparison of Zweifel's values for the two most highly correlated variables, pulse rate and temperature, for his two americanus and two fowleri samples with samples analyzed in this study of americanus and hemiophrys shows the similarity of the New Jersey and Manitoba - western Ontario populations of americanus. As well, it indicates that hemiophrys occupies an intermediate position between americanus and fowleri (Figure 50).

The separation of taxa based on call and associated variables is equally as effective as the separation on strictly morphological variation. Intermediate populations show the wide variability

which is typical of the morphological analysis. However, in the call discrimination the body temperature of the toad has made a contribution, apparently because of the relatively fewer hemiophrys recorded at low readings.

The comparison of the discriminant scores for call and morphology indicates that call variation does not follow morphological variation within either taxon, but that in the intermediate populations there is a tendency for call type to correlate with morphological type. Within the contact zone hemiophrys-like toads morphologically tend to have hemiophrys-like calls and americanus-like morphologically tend to have americanus-like calls. The comparison of the total pooled sample shows the same correlation of call and morphology between taxa (Table VII).

Figure 50. Relationship between pulse rate and temperature in three samples of Bufo from this study (data from Figure 60) and two B. americanus and two B. w. fowleri populations from northern New Jersey and Long Island, New York (data from Zweifel 1968: Figure 8).



4. Crossbreeding experiments

The most important aspect in the results of these crosses is that all combinations can produce metamorphosing individuals. This supports the morphological evidence that natural intermediates are freely produced. Unfortunately time and facilities were not available to rear the metamorphosed toadlets to maturity and the experiments were terminated at transformation. However, the success of toads from intermediate localities, as well as those from the most separated localities, indicates that no absolute fertility barriers exist. The differences in results in some of the replicate crosses and the unequal number of replicates makes any conclusion based on relative success between combinations hazardous.

Previous hybridization experiments summarized in Blair (1972) involving hemiophrys and americanus have also demonstrated their ability to produce metamorphosing offspring. Porter (1968) demonstrated fertility between individuals from the Wyoming relict population of hemiophrys and those from North Dakota and Manitoba.

Blair (1972, Appendix H) reported two artificial crosses involving a female americanus and a male hemiophrys in which 85.2% and 34.9% of the resulting larvae metamorphosed. Three crosses involving a hemiophrys female and an americanus male gave 64.3, 93.9 and 43.5% fertilization of eggs. In two of the crosses 37.8% and 86.9% of the fertilized eggs hatched and 42.0 and 29.4% of the larvae reached metamorphosis.

5. Taxonomic conclusions

a) Comparisons of hemiophrys and americanus with other

Bufo in northern North America.

(i) Hemiophrys-boreas

In southern Alberta, B. boreas is restricted to the Rocky Mountains and their foothills, whereas hemiophrys occurs in Grassland and Aspen Parkland regions to the edge of the foothills. To date, no contact has been found in this region (unpublished results of herpetofaunal surveys of Canadian Prairie Provinces). However, north of Edmonton, B. boreas occurs west of the foothills in the Boreal Forest region and has a narrow area of sympatry with hemiophrys. To the northwest, only B. boreas occurs in Aspen Parkland islands of the Peace River region an area where, by habitat, hemiophrys would be expected.

Eastern Alberta boreas, although larger than hemiophrys at these localities, are markedly smaller than typical boreas from British Columbia (unpublished NMNS data). Also, although it has been often asserted that boreas lacks a true breeding call (Blair 1972 and elsewhere), populations in eastern Alberta have a distinct call, which has a low dominant frequency and is very slowly pulsed. It has been noted at Banff, High River, and in the overlap zone with hemiophrys (unpublished data). Although very distinct from the higher, rapidly-pulsed call of hemiophrys, the frequency of mismatings in mixed choruses throws doubt on its complete effectiveness as an isolating mechanism within a common

breeding pond. Different calls may, however, serve to differentially attract females and males when breeding ponds are distinct.

Blair (1972: Appendix H) reported two artificial crosses between female hemiphrys and male boreas in which 41.7% and 79.7% of the eggs were fertilized and 77.4% and 3.2% of the larvae metamorphosed. Only one cross involving a female boreas and a male hemiphrys was reported and this resulted in 98.2% fertilization and 81.8% hatching but none of the resulting larvae reached metamorphosis. No naturally occurring hybrids have been previously reported between these taxa.

The boreas-hemiphrys overlap without loss of distinctness and only rare survival of natural hybrids provides an interesting contrast to the americanus-hemiphrys interactions. However, the narrowness of the overlap, the apparent similarity in breeding time and site, and the closeness in body size may be indications that these two forms have not diverged enough to partition an area between them.

(ii) Hemiphrys-cognatus

B. hemiphrys and B. cognatus are sympatric in the short-grass prairie of southern Alberta and Saskatchewan and the adjacent Dakotas and western Minnesota. B. cognatus ranges south into Mexico (Stebbins 1966), is much more grassland adapted than B. hemiphrys and their sympatric area, which is much broader than that of B. hemiphrys and B. boreas, represents the area where B. cognatus reaches its northern limit and B. hemiphrys, with the

exception of the Wyoming relict, reaches its southern limit. In Minnesota, a natural hybrid between B. cognatus and B. hemiophrys has been reported (Brown and Ewert, 1971).

B. cognatus has a very long distinctive call resembling the sound of a pneumatic hammer (Conant, 1975). This call has tremendous carrying capacity and is strikingly distinctive from calls of the americanus group. The vocalization of males in this chorus could be heard at least a half a mile over the prairie from the road where we had stopped our vehicle. B. hemiophrys was not heard in this din until we were much closer to the pond.

Blair (1972b, Appendix H) has recorded one artificial cross of a female B. hemiophrys and a male B. cognatus where 68.0% of the eggs were fertilized, 73.5% of those fertilized hatched, but only 3.5% of the larvae metamorphosed. In addition, in one cross of a B. cognatus female and a B. hemiophrys male 33.3% of the eggs were fertilized but none hatched, all stopping in gastrula or neurula stage.

(iii) Hemiophrys-w. woodhousei and americanus-w. fowleri

The subspecies B. w. woodhousei is not known to extend into Canada and in the northern United States its range as currently understood is allopatric to that of hemiophrys.

Blair (1972: Appendix H) reported two crosses using a female hemiophrys and a male w. woodhousei. One of these resulted in 97.0% fertility, with 83.6% of the fertilized eggs hatching and 26.6% of the larvae reaching metamorphosis. For the other the

only data given are that seven larvae were obtained and 85.6% (or six of them) metamorphosed. In two crosses between a woodhousei female and a hemiphrys male given in the same report, 100% were fertilized and 24.4% of 600 larvae metamorphosed in the first and 31.2% of 279 larvae metamorphosed in the second. These values for hatching success are only marginally lower than those obtained with crosses between hemiphrys and americanus.

The subspecies B. w. woodhousei has prominent, usually somewhat parallel cranial crests, few warts per dorsal blotch and generally has an unspotted venter. Blair (1972) mentions that Great Basin populations are smaller and may have a cranial boss, but there is no published comparison of this variant with prominently-bossed hemiphrys. B. w. woodhousei occurs primarily in the great plains of the central United States, often in river valleys, and is sympatric over much of its range with cognatus, which tends to occupy the upland prairies (Timken and Dunlap 1965).

The other northern subspecies of woodhousei, B. w. fowleri, is eastern in distribution and largely sympatric with americanus. It is smaller than B. w. woodhousei and generally has more warts per dorsal blotch. Its intergradation with B. w. woodhousei has been documented by Meacham (1962). Underhill (1961b) has compared morphological variation between hemiphrys and w. woodhousei in South Dakota but his sample of the former was probably contaminated by introgression from americanus (cf. localities given by Underhill 1961 and Henrich 1968).

The call of w. fowleri has a faster pulse rate than hemiophrys (Figure 50). It is interesting to note that, while w. fowleri is intermediate between hemiophrys and americanus morphologically in the characters studied here, hemiophrys is the intermediate with respect to this call variable. The hemiophrys samples geographically closest to the northern limit of w. woodhousei (those from the prairie grassland of southern Alberta and southwestern Saskatchewan) have low mean discriminant scores (-30.8 to -26.9, see Figure 18). These give no indication of introgression from interbreeding with woodhousei further south as, if this was affecting southern hemiophrys scores it would presumably raise them. Another indication that interbreeding of woodhousei and hemiophrys is not widespread is the survival of the Wyoming disjunct of hemiophrys within woodhousei range. However, the narrowness of the contact zones between other Bufo taxa where complete interbreeding can occur leads one to be cautious in drawing any but tentative conclusions until adjacent populations can be analysed. The possibility does exist that introgression from woodhousei had some effect on the scores obtained by Henrich (1968) for his 160-mile intergradation zone between hemiophrys and americanus in South Dakota. Much of this zone is a southern extension of hemiophrys which is not only adjacent to americanus on its east but also to woodhousei on its west. Comparison with woodhousei was not made by Henrich. Bufo hemiophrys was considered a subspecies of B. woodhousei by Schmidt (1953) but Blair (1957a) presented convincing evidence for its

closer relationship to B. americanus.

Similarly, the mean discriminant value for B. americanus samples adjacent to the range of B. w. fowleri (+33.8 at Rondeau and +35.3 at Point Pelee) is high, perhaps even indicating divergence (character displacement of Brown and Wilson, 1956) between these forms in adjacent localities. Elsewhere in a wide area of geographic sympatry the two taxa are known to hybridize (e.g. Zweifel 1958) and selection against hybrids may be an important factor here.

b) Status of B. americanus copei and B. hemiophrys baxteri

(i) B. a. copei: northern B. americanus populations.

The validity of the northeastern Bufo americanus copei (Yarrow and Henshaw 1878) is open to question. Unmistakably there are populations of brightly coloured B. americanus with contrasted patterns accentuating reds and oranges in the northern portion of its range. These populations have also been characterized as having heavily pigmented venters and shorter hind legs. Such characters formed were the basis for the initial recognition of copei as a distinctive form, and for Gaige (1932) resurrecting it from synonymy. It was first described from James Bay material, but subsequently Trapido and Clausen (1938), Grant (1941), Vladykov (1941), Netting and Goin (1946), Backus (1954) and Harper (1956) extended its range across northern Quebec. Schueler (1973) reaffirmed the distinctive colouration of James Bay toads. Bleakney (1952) on the basis of relatively small samples applied the name to Nova Scotia populations outside the Annapolis Valley which had shorter tibias and more heavily pigmented venters. Subsequently,

however, Bleakney (1958) did not recognize subspecies within B. americanus in eastern Canada. Most recently, Ashton, Guttman and Buckley (1973) have extended the range of B. a. copei south over much of central Ontario and Quebec. However, B. a. copei characters are not pronounced in all northern individuals (Cook 1964, 1968). Earlier Logier (1952) and Logier and Toner (1955, 1961) presented a discussion of the validity of the race in which they pointed out the intermediate nature of supposedly diagnostic characters across a wide geographic area. Of all these papers, only Logier (1952) presented any statistical comparison of copei and americanus populations, and even in this account only relative leg length was so treated. In the present study neither tibia or tarsus length appear to be useful in defining a distinct northern race within B. americanus.

Two measurements of variation in the present analysis which do have bearing on the status of B. a. copei are the length of the dorsal spot and the ventral pigmentation score. Particularly high mean values for these characters are present in northern population samples: Whitetop, Moosonee, Lake Attila, Sept Iles, Mile 134, and Routhierville. To the south there is a wide zone of intermediates until the "typical" (southern) americanus with smaller dorsal spots and a less heavily pigmented venter is found. The variation in colouration seems to parallel these characters with the most brightly coloured toads in the north. The wide dorsal stripe and more contrasted pattern mentioned by most observers recognizing

copei are also evident in the material examined here and specimens with large dorsal blotches generally have these features as well. The dorsal "stripe" cited by previous authors seems to be an area largely defined by the dorsal blotches, and may therefore be highly irregular in width, varying with evenness of blotch edges and difficult to measure objectively. A "trace" mid-dorsal stripe can also be seen on many specimens which is very narrow with parallel edges, and is somewhat paler than the broad stripe. This narrow stripe is widespread in americanus group toads.

Northern B. americanus are apparently often strongly diurnal in habit (personal communications: Ross MacCulloch, northern Quebec; F.W. Schueler, James Bay) in contrast to the generally nocturnal or crepuscular behaviour, especially of adults, further south (FitzGerald and Bider, 1974, 65 m. NW of Montreal, Quebec).

Diurnal activity is to be expected in the north because of long day length and cool evening temperatures during the short summer. The bright colours and highly contrasted pattern of northern populations may be of selective advantage during diurnal activity because of the disruptive visual effect of such contrasts.

Because of the wide area which is transitional from north to south, there seems little justification in distinguishing northern populations as a distinct subspecies. As Logier and Toner (1955, 1956) have pointed out, the intergrade zone would be wider than the range of the northern race. Ashton, Guttman and Buckley attempted to resolve this difficulty by redefining copei to include

most of these intermediate populations but failed to provide evidence that this enlarged concept of copei could be adequately distinguished from southern americanus. Guttman (1975) failed to find distinctive isozyme distributions between "copei" and americanus. The reduction in blotch size and ventral pigmentation in americanus is toward its southern area of sympatry with B. w. fowleri. It is suggested here that the transition between "copei" and southern americanus is due in part to different selective pressures on extreme northern and southern populations resulting in a relatively smooth cline through the area where selection is presumably more neutral on pattern characteristics.

(ii) B. h. baxteri: the Wyoming disjunct of B. hemiophrys.

B. h. baxteri was named by Porter (1968) from a comparison of 34 Wyoming specimens and an equal number from a composite sample drawn from North and South Dakota and Manitoba. The differences used to define the subspecies were mainly statistical comparisons of certain morphological characters.

He concluded that Wyoming toads tended to be smaller, with shorter radio-ulna and tibio-fibula measurements, narrower heads, a more prominent boss and a narrower mid-dorsal stripe. Ulna length, boss height and mid-dorsal stripe were excluded from the present study. However, measurements of snout-vent length, tibia length and head width (remeasured on most of Porter's sample) do not distinguish Wyoming populations when considered against the

variation in populations from throughout the range hemiohryps. The relatively small differences found by Porter in the characters not measured here would probably not validate the distinctiveness of the Wyoming hemiohryps. The discriminant score mean for the Wyoming population is well within the range of score means over the rest of the geographic range of hemiohryps.

Criteria for subspecies vary widely between workers, but commonly a significant proportion of individuals within a geographic area have to be distinguishable from all other individuals in other areas to warrant taxonomic recognition (the so-called 75% rule). Smith (1974) has argued that an "abrupt geographical step" in the variation of at least one character should be present to warrant subspecies recognition. B. h. baxteri does not appear to meet either criterion for a subspecies. A disjunct distribution is not generally recognized as sufficient cause for erecting of a taxon unless distinguishing morphological or other characters can be clearly defined (Mayr 1963). The over-emphasis of the significance of its geographic isolation even led Packard (1972) to recommend its recognition as a full species. However Porter (1968) presented evidence that Wyoming toads were successful in crossing with Manitoba and Dakota toads under laboratory conditions. There is no reason to suppose they would not interbreed freely in nature if re-expansion of range brought the Wyoming population into contact with any other hemiohryps populations. Porter (1968) found only slight difference in the call of the Wyoming population. As he

did not record toad body temperatures exact comparison of his data with the variation presented here could not be attempted, but I do not believe that the differences appear significant.

c. The status of americanus and hemiophrys.

The contrast between results in the analysis of southeastern Manitoba samples compared with the reference samples from throughout the range of B. hemiophrys and northern B. americanus is evident. Clearly the distinction between the morphologically "good species", B. americanus and B. hemiophrys, disintegrates biologically across this zone, and extensive interbreeding and backcrossing are freely occurring in the apparent absence of strong isolating mechanisms. The narrowness of the contact zone between the taxa is emphasized by both morphological and call analysis. Its limited extent may be a function of the relative abruptness of the vegetation ecotone where it occurs. It may also be affected by the distance moved by individual toads in their lifetime.

This situation may be designated allopatric hybridization in the sense of Woodruff (1973). Both parental forms are distinct on either side of a zone populated by intermediate animals but pure individuals of either parent are not clearly distinguishable within the intermediate zone. This term contrasts with Woodruff's parapatric hybridization, where both parental forms are identifiable in an intermediate zone containing hybrids, and sympatric hybridization, where the ranges of the parental forms may extensively overlap and hybrids are produced in varying numbers. These interactions

represent various gradations between the relatively free and complete gene flow generally assumed to occur within a species and the absolute reproductive barriers (no gene exchange) between indisputably good species. The various natural interactions which demonstrate some, but incomplete, isolation between populations have been designated as semi-species, allospecies or incipient species as extensively reviewed by Amadon and Short (1976).

Mayr (1963, 1969, 1970) clearly regards cases where extensive interbreeding occurs between two essentially allopatric populations as evidence of their lack of reproductive isolation and concludes that such populations belong to the same species. Rising (1970) reviewed some of the inconsistent treatment of a selection of such taxa by many workers. Bigelow (1965) argued that reproductive isolation rather than interbreeding is the essential part of Mayr's definition and that the former could exist and be maintained even if interbreeding produces a range of recombinations of the parental genotypes in a hybrid zone. Although such forms may have no barriers to interbreeding where they meet, he reasoned, strong selection on either side of the hybrid zone which eliminates variants carrying genes of the other form would produce effective isolation. Short (1969) suggested that Bigelow ignored the differential action of natural selection on characteristics. One or a few genes from one differentiated population may be incorporated into another due to interbreeding and, as selection is unlikely to be so severe that some introgression is not occurring, complete

reproductive isolation is not likely to be maintained by selection where there are no barriers to free interbreeding. Only the sympatric occurrence of the two forms can be taken as an indication of reproductive barriers. Mayr (1963) largely discounted introgression as of any consequence between species in animals, as he regarded species' gene pools to be internally cohesive and coadapted that they are largely resistant to incorporating genes from other gene pools.

Sibley (1969) has provided a framework for taxonomic judgement of the varying grades of interbreeding situations. Evidence, or lack of it, for some degree of reduction in interbreeding as shown by at least some degree of sympatry is of paramount importance. The natural occurrence of some hybrid individuals or some geographic areas where complete interbreeding occurs (including isolated instances of local hybrid swarms) is weighed against the geographic extent or numerical preponderance of "pure" forms or populations in contact. If contact without extensive interbreeding predominates, the taxa have species status. But, if in the majority of contacts, interbreeding between distinct taxa produces a population in which all or nearly all individuals have some degree of intermediate characteristics the taxa are regarded as subspecies. Their gene pools are obviously not isolated.

Schueler and Rising (1976) provided a thoughtful analysis of cases where "hybridization" could be recognized, stressing the importance of mean, variance and modality of discriminant analysis

scores. Although they would regard results such as obtained here as indicating hybridization, it is unclear if they would consider species status a mandatory consequence.

In the present case, interbreeding is occurring between americanus and hemiophrys throughout their contact zone in eastern Manitoba. Henrich (1968) has clearly demonstrated the same situation at the opposite end of their contact, in South Dakota. Although Henrich's zone as presented is apparently much wider, his measure (a coefficient of similarity) may not be as precise for delimiting the contact zone as the discriminant function and therefore the results of the two studies are not exactly comparable. His sampling area ran in a NW-SE direction, and may be cutting the contact area on a diagonal, rather than at right angles, thus not evaluating the true width of contact. A similar effect is noticable in the "southern" transect presented here. It has been suggested earlier that analysis of the South Dakota contact should also consider possible introgression from B. w. woodhousei on the west, which could introduce additional variability, and perhaps broaden the apparent contact area.

Minnesota, in contrast to the northern and southern contact in Manitoba and South Dakota, respectively, has not received comparable analysis. That the contact zone there is narrow is apparent from statements by Breckenridge (1944), Blair (1957a), and Tester, Parker and Siniff (1965), who had no difficulty in assigning collections to one taxon or the other, and doubted any range overlap existed. Henrich included a few Minnesota specimens

in his analysis and found evidence of intermediacy of characters. Guttman (1969) found some transferrins with identical mobilities in Minnesota hemiphrys and americanus.

This evidence indicates a continuous interbreeding zone between the southern end of Lake Manitoba through Minnesota to northern South Dakota wherever B. hemiphrys has come in contact with B. americanus. The contact is centered on the ecotone between Great Lakes and Deciduous Forest with Parkland and Prairie (this study, Henrich 1968).

No evidence exists of primary isolating mechanisms (physical inability to physically mate or absolute sterility of hybrids) or strongly developed secondary isolating mechanisms (ecological or temporal) (Mayr, 1963).

The difference in calls may, however, be a weakly effective behavioral isolating mechanism. Licht (1976) in a study of B. americanus has suggested that female toads select mates, and has presented some observational evidence. He hypothesizes that call variables of vocal males allow females to select a male within a narrow size range. The evidence for call variables following precisely enough with male snout-vent length within a single taxon to allow such discrimination is weak (this study; Zweifel 1968). If the choruses in the intermediate zone consisted of clearly definable parental forms and hybrids, such discrimination by females might be reasonably postulated. However this is not the case. The majority of toads in the contact zone are intermediate

in both call and morphology, and no clear assortment into parental types and hybrids is evident. Mate selection by females, if it occurs here, may be effective at the edges of the contact zone in allowing a female to select a "pure" male, or chorus, instead of an hybrid individual or chorus of hybrids. This may increase in effectiveness with distance to the east and west of the zone. It is interesting to note in this regard that the hemiphrys males west of the contact zone are the smallest that occur (both in range and mean). Some character displacement (Brown and Wilson 1956) in size may presently be occurring in hemiphrys. This may account, in part, for the reduced extent of apparent introgression of americanus genes into hemiphrys populations.

It seems reasonable to assume that if hemiphrys characters are adaptations for relatively more arid environments, and if selection is most severe on toads in arid habitats because of recurrent drought then, "mesic-adapted" genes may be less able to penetrate westward than "arid adapted" genes can penetrate eastward. The geographic distribution of discriminate scores suggests that this could be true. Higher variances in discriminate scores on the eastern side of the contact zone, and the possible character displacement in size of hemiphrys to the west support the possibility of more restricted gene flow to the west than to the east of the contact zone. Tester, Parker and Siniff (1965) concluded, from behavioural studies, that americanus is less well adapted to avoid heat stress in open areas than hemiphrys. This could impose

further restriction on the westward flow of americanus genes.

In summation, although americanus and hemiophrys are clearly as distinct as good species in morphology and call, the lack of any recognizable sympatry between pure forms of each indicates a lack of isolation mechanisms where they contact each other. They cannot be considered as having isolated gene pools and should be treated as subspecies (Sibley 1961, Short 1969, Mayr 1963, Woodruff 1973, Amadon and Short 1976).

The morphological distinctness of americanus and hemiophrys and the high variability of hybrid populations indicates that their respective gene pools have incompatibilities and are perhaps more differentiated than most subspecies. Amadon and Short (1976) have used the term megasubspecies for well-marked subspecies known, or judged to be, approaching the status of a species. In trinomials designating a megasubspecies the species name is placed in parentheses, and the taxa here should be written

Bufo (americanus) americanus

Bufo (americanus) hemiophrys

This notation complements Amadon's (1966, 1968) suggestion that allospecies should be listed with the superspecies they are a member of placed in brackets. The distinction between allospecies and megasubspecies is developed from the discussion given earlier by Short (1969). When the designated forms contact a closely related form a predominance of allopatric hybridization in the sense of Woodruff (1973) indicates megasubspecies and a predominance

of parapatric or sympatric hybridization indicates allospecies.

6. Zoogeographic significance of the eastern Manitoba transition zone

The significance of location of the americanus - hemiophrys transition and its origin can be related to the general herpetofauna distribution patterns of northern North America.

Eastern Manitoba is the center of a transition between eastern and central herpetofaunas (Cook 1974). The americanus-hemiophrys contact line defined here also is the approximate western limit for at least two eastern species, the Blue-spotted Salamander, Ambystoma laterale, and the Spring Peeper, Hyla crucifer. It is the western limit for at least one central species, the Plains Garter Snake, Thamnophis radix. In addition, the wide intergrade zone between the central Red-sided Garter Snake, Thamnophis sirtalis parietalis, and the Eastern Garter Snake, T.s. sirtalis, which extends from extreme western Ontario to central Manitoba, is approximately centered on this line (Cook, unpublished data) and part of the overlap between the Gray Treefrogs Hyla chrysoscelis (western) and Hyla versicolor (eastern) in Manitoba occurs here (Stewart and Cook 1974, and unpublished data). This contact zone may be regarded as a zoogeographic suture zone in the sense of Remington (1968), a region where two faunas thought to be previously separated have re-established contact resulting in a variety of interactions. Because the northern faunas are poor in species diversity, the number of interactions are few. As pointed out above, they vary from the zone of sympatry of apparently good

species in Hyla versicolor and H. chrysoscelis to the wide area of subspecies intergradation between T. s. parietalis and T. s. sirtalis. The americanus-hemiophrys interaction is intermediate between these extremes, showing a sharply demarcated contact with limited introgression.

Western limits for widely distributed eastern amphibians and reptiles correspond to gradually increasing aridity from east to west (Cook 1974; see also Figure 77, p. 295 in Bryson and Wendland 1967), as do, in reverse, eastern limits for many central forms. A marked change in relative aridity in the Manitoba lowlands is indicated by the sharp break between largely coniferous forest and aspen parkland. The americanus-hemiophrys peak interbreeding seems centered in this area.

The central herpetofauna as defined by Cook (1974) is bounded by this eastern Manitoba suture-zone in the east and by the foothills of the Rocky Mountains in the west. The majority of amphibian and reptile species which range north into the Boreal Forest areas in each of the eastern, central, and western regions between these boundaries are specifically or subspecifically unique to each region. The transcontinental Boreal Herpetofaunal Zone postulated by Savage (1960) extending from Alaska to the Atlantic coast, seems based primarily on one species, the Wood Frog, Rana sylvatica, which is the sole taxon which ranges widely across all three regions. However, the Wood Frog is not restricted to Savage's supposed Boreal Zone but occurs far south of it in the

east continuously and in the west as disjunct populations. In the central region, all Boreal species (Rana sylvatica; the Leopard Frog, Rana pipiens; the Boreal Chorus Frog, Pseudacris triseriata maculata; B. (a.) hemiophrys and T. s. parietalis) also occur throughout the adjacent Aspen Parkland. In the east, four wide-ranging Boreal species, Ambystoma laterale, Hyla crucifer, and the Mink Frog, Rana septentrionalis, reach their western limit in eastern Manitoba at or near the Aspen Parkland boundary. None invades the Aspen Parkland. Two others, B. (a.) americanus and T. s. sirtalis, are replaced over this boundary by central subspecies. None of these five eastern forms occurs in the central Boreal Forest although suitable habitat appears to exist for them there. However, all central Boreal Forest species either show some range extension or genetic influence to the east. In R. pipiens no differences between central and eastern northern populations have been defined. In R. sylvatica a striped morph, common in central populations, shows a decreasing abundance from west to east in northern Canada. The number of individuals possessing a stripe shows its most rapid drop south and east of southern James Bay (Schueler and Cook, MS). Pseudacris t. maculata ranges east to Moosonee on southern James Bay. South of the Great Lakes it is replaced by an eastern subspecies, the Western Chorus Frog, P. t. triseriata. These forms do not contact in Ontario (Cook, 1964b; Weller and Palermo, 1976; and unpublished observations) but do southwest of Lake Superior (Conant 1975). This study presents

evidence of introgression in Bufo americanus from the central form (hemiophrys) east to James Bay in western Ontario. This area corresponds to the eastern range of Pseudacris t. maculata. The influence of T. s. parietalis into T. s. sirtalis populations is not known to be as extensive, apparently reaching its eastern limit in extreme western Ontario. These distributions of species and variations may indicate past isolation of central and eastern herpetofaunas, followed by the re-establishment of contact and subsequent eastward dispersal of some forms and gene flow from others primarily from the central region into the eastern region.

Most of the regions discussed here were covered by glacial ice during at least four successive continental glaciations (Flint 1971) each of which would have obliterated northern faunas and floras over their present ranges. It is generally assumed that much of the present northern fauna and flora was displaced southward in response to changing climatic conditions south of glaciation and survived south of the ice sheet in "gross refugia" (Lindroth 1969). The most recent continental glaciation, the Wisconsin, reached its maximum extent about 18,000 years ago (Prest, et al., 1969). Bryson and Wendland (1967), Flint (1971) and Terasmae (1973) present maps reconstructing climate and vegetation during the Wisconsin maximum which depict the entire glacial margin bordered to the south by tundra and boreal forest. However, recent evidence indicates the possibility of a region in Nebraska during peak glaciation of drifting sand dunes which may have been

treeless or even vegetationless (Wright 1971). If the area between these dunes and the glacier margin was occupied only by tundra, the Boreal Forest would be broken into eastern and central segments, assuming the southern edge of the dune areas was occupied by prairie vegetation. (Mengel, 1970, invokes this possible break in discussing zoogeography of bird taxa). Present central Boreal forms show a gradation of southern limits from Rana sylvatica which does not penetrate grassland habitats (Cook 1965b) to Pseudacris triseriata maculata which is well adapted to northern grassland but is replaced by related species in the southern prairies (Conant 1975). All would be excluded from a severely arid barrier. If the Boreal Forest was broken in central North America, central Boreal forms would be isolated along the eastern side of the Rocky Mountains and adjacent areas. Rana sylvatica and B. (a.) hemiophrys now have disjunct populations in southeastern Wyoming and the present ranges of Rana pipiens, Pseudacris and T. s. parietalis also include this area (Conant 1975, Stebbins 1966).

Although not a primary barrier, post-glacial Lake Agassiz which covered much of southern Manitoba (Mayer-Oaks, 1967) may have had an effect on the present distribution patterns. It has been postulated by Løve (1959) that the vegetation which first covered the lake bed after Lake Agassiz drained was grassland and another grassland interval occurred in the Hypsithermal. During either of these transitional periods, B. (a.) hemiophrys, assuming it had reached the area, may have been able to invade farther east

than at present. At least some of what has been interpreted here as eastward introgression of hemiophrys genes could be the result of initial hemiophrys invasion of the area, and subsequent swamping by americanus which spread into the area with the invading species from the eastern forest. However, it is unlikely that the postulated post-Agassiz invasion of hemiophrys was extensive enough to explain the far eastern extent (James Bay) of indications of introgression from hemiophrys.

The east-central faunal split may have occurred much earlier than the Wisconsin or more than once through successive Pleistocene glaciations. Tihen (1962b) tentively identified material from the Kansan glaciation in Kansas as similar to B. hemiophrys. Tihen (1972) has suggested that B. hibbardi from the Miocene-Pliocene boundry and the Middle Pliocene, and B. rexroadensis of the Upper Pliocene may represent temporal stages in a single population line leading to B. woodhousei. If this interpretation is correct it indicates the comparative antiquity of the americanus group and the long period available to differentiate distinctive forms. In a survey by Gelbach (1965) the oldest fossil assigned to other members of the americanus group is the tentative hemiophrys above.

Many cases of interbreeding between morphologically distinctive taxa have been explained in terms of comparatively recent human changes to the environment, particularly through land clearing for agriculture (Mayr 1963). In the present case, the interbreeding sampled is along east-west highways through a relatively well-

drained area (Warkentin 1967). Here road-side ditches provide new breeding sites across the transition zone. If call serves to attract females and/or other males to breeding sites (Bogart 1960) then lack of ponds before human alterations at the eastern forest-Aspen Parkland transition could have lessened the chance of a male coming to a pond in the "wrong" vegetation zone and of a female responding to the "wrong" chorus. East-west highways through the region are a comparatively recent development, and even east-west railways date back only about 100 years. However, the evidence for extensive eastern introgression argues against contact being recent enough to have been man-induced. A test of this hypothesis would be in the relatively unaltered area north of Lake Winnipeg where the nature of contact is yet unknown. Hemiphrys and americanus are present in the area, and their ranges are in close proximity. No adult preserved material is available. Harper (1963) noted what he recognized as calls of hemiphrys at Wabowden, and Vere Scott (personal communications based on his own observations and those of Leigh M. Nelson and Gordon Shaw) identified americanus from Warren's Landing and William River at the north end of Lake Winnipeg, based largely on the diagnostic cranial crests. In addition, I have examined three samples of toads collected from the area by F.W. Schueler (FWS 6870, 6873 and 6875 from Sipiwesk Lake, Jenpeg, and 7 km NW Jenpeg respectively). Unfortunately all are juveniles and because of their small size useful measurements can not be obtained on a significant number of them. The two

largest, from Sipiwesk Lake, appear to be B. hemiophrys.

7. Taxonomic significance of the americanus-hemiophrys relationship with respect to the other nominal species of the B. americanus group.

The B. americanus group of Blair (1972) consists of B. woodhousei, regarded as an early split from the remaining species, and the largely or completely allopatric B. terrestris, B. americanus, B. houstonensis, B. hemiophrys and B. microscaphus (Conant, 1975, and Stebbins 1966). B. woodhousei is known to hybridize naturally with all of these taxa except B. hemiophrys. A study of the region where contact between woodhousei and hemiophrys is probable through South Dakota, North Dakota and Montana is long overdue. B. terrestris and B. americanus may hybridize along a contact zone but the evidence is disputed (Netting and Goin, 1946; Neil, 1949). B. houstonensis in Texas and B. microscaphus in the southwest are disjunct from contact with other taxa in the americanus subgroup. B. houstonensis shows some features of both B. terrestris and B. americanus (Sanders 1953) but is commonly assumed to be a post-glacial isolate of B. americanus (Blair 1972). Blair (1972 and earlier papers) suggested an east-west split of a hemiophrys-microscaphus stock from the eastern americanus stock. Sanders (1961) postulated hybridization and introgression between americanus and an invasion of a broad-skulled toad, possibly of the B. valliceps group, as an explanation for the divergent osteological features of B. terrestris. A.P. Blair (1955) postulated introgression from a B. boreas stock to account for some morphological features

of B. microscaphus, although this view was not considered by W.F. Blair (1957b). This study shows that hybridization between hemiohryps and boreas results in a toad with well-separated, low, somewhat americanus-like crests. A previously defined subspecies of B. woodhousei, B. w. veletus in Texas and Louisiana, is now regarded as the product of hybridization between B. w. woodhousei, B. w. fowleri, and B. americanus (Conant 1975).

W.F. Blair (1972 and earlier papers) has shown that toads of the B. americanus group have a marked ability to hybridize in all combinations in the laboratory, and many F_1 individuals have proved fertile in backcrosses (Blair, 1963). Only B. woodhousei is sympatric to any extent with other species in the complex, and, although hybridization is common (as in B. americanus and B. w. fowleri) partial temporal isolation and ecological isolation (breeding site and non-breeding habitat) have developed and distinctness is often maintained over large areas of geographic sympatry. B. woodhousei seems to warrant its status as a species distinct from the others of the B. americanus group by the criteria of Short (1969). In addition, Jones (1973) has presented evidence that reproductive isolation may have intensified in 30 years in one area of sympatry of B. woodhousei and B. americanus. However, Loftus-Hills (1975) has questioned aspects of this analysis.

The status of the other species is more problematical. The case for conspecific status of americanus and hemiohryps, two of

the most morphologically distinctive taxa in the group, has been presented. The americanus - terrestris relationship has never been sufficiently analyzed. B. houstonensis has been regarded as the most weakly distinctive of the group (Blair 1972). The geographic isolation of houstonensis and microscaphus is not sufficient in itself to warrant separate species status for each. Laboratory hybridization studies (Blair 1972), ecology and the occurrence of natural hybridization of both B. houstonensis and B. microscaphus with woodhousei, suggest that if either were in contact with other americanus subgroup toads they would interbreed. It may be that the most pragmatic eventual classification to indicate relationships within the americanus subgroup will be to regard terrestris, americanus, houstonensis, hemiphrys and microscaphus as either allospecies or megasubspecies of americanus (or rather, of terrestris which is the oldest name, see Schmidt 1953). A choice between the two would be difficult but because of their similar interactions with woodhousei the latter may be best. Either would be consistent with the close relationships noted by many authors from many lines of evidence (Blair 1972) and place them into better evolutionary and zoogeographic perspective with other taxa of North American Bufo.

In part, a taxonomic decision on taxa pairs as closely related but distinctive as the pair analyzed here is often coloured by the speculative opinion of the taxonomist concerned as to the past events influencing the present situation and eventual outcome

of their interactions. Some assume that isolating mechanisms will inevitably develop and/or intensify (Remington 1968) and therefore the allocation of species-status is actually a prediction. Others regard any interbreeding as eventually breaking down the distinctiveness of present morphological differences with the eventual merging of the two forms. However, some zones of interbreeding along ecotones are known or assumed to remain for long periods without changing the taxa (Short 1970). The advantage of the Amadon and Short (1976) treatment is that it distinguishes this intermediate distinctiveness of the taxa and avoids the obscurity of conventional subspecies status (Rising 1970).

Because of the size and geographic extent of the samples used, the presumed objectivity of the discriminant analysis, and the repeatability of the measurements and scoring systems used, re-sampling in the future should be possible with results directly comparable to those presented. The weights generated should allow precise evaluation in 10, 25, 50 or more years of the then current status and perhaps provide an insight into exactly how, and if, the interbreeding and introgression affects future populations of these toads.

Obviously, the possible importance of such interactions in evolution could be in either adding additional variation for selection to act upon or as an intermediate step in eventual attainment of complete reproductive isolation. Only long-term studies, probably exceeding one or more human lifetimes will give

sound assessment of such situations.

Until now, many laboratory techniques have been applied to problems of interspecific classification in toads (Blair 1972). Too little attention has been paid to intraspecific variation (for a notable exception see Guttman 1975). Although natural introgression has often been invoked to explain variable results within taxa, insufficient attention has been paid to documenting its existence and extent. As Blair (1972) has suggested for interspecific studies, a multidisciplinary approach to intraspecific relationships involving extensive analysis of geographic variation in cytogenetic and biochemical characters as well as additional morphological comparisons of the type presented here are needed.

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APPENDIX I

List of collections measured

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
	Reference sample: <u>Bufo hemiophrys</u>			Males	Females
<u>Alberta</u>					
1A	Mikkwa River: 58°25', 114°46'	04 Jun 1968	<u>14045</u>	8	1
2A	2.4 mi N on Hwy 44 of Westlock	26 May 1965	<u>8521</u>	48	15
3A	6 mi SW on Hwy 2 of Perryvale (turnoff)	27 May 1965	<u>8527</u>	36	0
4A	½ mi E on Hwy 16 of Stony Plain	18 May 1965	<u>8502</u>	8	0
	0.5 mi E on Hwy 16 of Stony Plain	20 May 1965	<u>8505</u>	47	0
5A	3.3 mi SW of St. Paul	05 May 1965	<u>8547</u>	27	0
6A	3.2 mi N and 1.6 mi E of Jct 4th St. NE and NW Drive, Calgary	25 May 1966	<u>10332</u>	12	0
		20 May 1966	<u>10325</u>	3	0
		25 May 1966	<u>10333</u>	9	0
		Spring 1967	<u>10755</u>	12	0
7A	2.0 mi E on Hwy 1 of Strathmore	29 May 1963	<u>7115</u>	14	0
	2.9 mi. E on Hwy 1 of Strathmore	30 May 1963	<u>7116</u>	3	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS (HS) Catalogue #	Number of Specimens	
				Males	Females
Reference sample: <u>Bufo hemiophrys</u> continued					
<u>Alberta</u>					
	Strathmore	30 May 1963	<u>7117</u>	3	0
8A	2.2 mi NW on Hwy 1 of Brooks (turnoff)	20 May 1962	<u>6021</u>	11	1
	0.8 mi. NW on Hwy 1 of Brooks (turnoff)	20 May 1962	<u>6022</u>	31	6
	0.5 mi NW on Hwy 1 of Brooks (turnoff)	20 May 1962	<u>6023</u>	26	3
<u>Saskatchewan</u>					
1S	Shell River, 4.4 mi W on Hwy 3 of Prince Albert	21 Jun 1959 22 Jun 1959	<u>4049</u> <u>4068</u>	4 5	3 0
	Pond, 0.1 mi E of Shell River on Hwy 3	22 Jun 1959	<u>4060</u>	1	0
	Prince Albert	21 Jun 1959	<u>4052</u>	1	0
	Prince Albert	23 Jun 1959	<u>4072</u>	7	0
2S	6.5 mi E on Hwy 14 of Reward (turnoff)	13 May 1962	<u>6007</u>	40	3

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
Reference sample: <u>Bufo hemiophrys</u>					
<u>Saskatchewan</u>					
3S	14.5 mi E on Hwy 5 of Sutherland	28 May 1962	<u>6038</u>	44	7
	8.2 mi E on Hwy 5 of Sutherland	28 May 1962	<u>6040</u>	44	0
4S	Leach Lake at Hwy 9, 14.3 mi S of Yorkton	16 Jun 1959	<u>4033</u>	22	1
5S	8.3 mi E on Hwy 1 of Paipot (turnoff)	28 May 1963	<u>7111</u>	22	2
6S	3.3 mi W on Hwy 1 of Tompkins (turnoff)	16 May 1962	<u>6014</u>	36	7
7S	NW end of Buffalo Pound Lake	07 Jun 1959	<u>4007</u>	27	6
8S	Qu'Appelle River Valley, 1.8 mi W and 15.2 mi N of Belle Plaine	18 May 1961 19 May 1961 19 May 1961	<u>5263</u> <u>5267</u> <u>5265</u>	1 13 10	0 0 0
9S	2.3 mi E on Hwy 1 of Sintaluta (turnoff)	15 May 1965	<u>8496</u>	74	3

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
Reference sample: <u>Bufo hemiophrys</u> continued					
<u>Manitoba</u>					
1M	6-10 mi S on Hwy 10 of the Pas	07 Jun 1970	<u>12252</u>	14	0
2M	8 mi E on Hwy 10 of Minitonas (turnoff)	09 Jun 1970	<u>12256</u>	20	0
3M	0.5 mi N of Hwy 1 on Oberon (turnoff)	13 Jun 1960 14 Jun 1960	<u>4563</u> <u>4565</u>	3 5	2 14
4M	Delta Beach	04 Jun 1962	<u>6052</u>	49	15
5M	Northeast section, Spruce Woods Forest Reserve	20-21 Jul 1960	<u>4608</u>	34	26
<u>Wyoming</u>					
	Laramie River, 1.4 mi SE on Hwy 287 of Bosler	26 Jun 1965	8610	6	0
	Laramie River Valley	-	KRP*	30	3

*K.R. PORTER COLLECTION

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
Reference sample: <u>Bufo hemiophrys</u> Continued					
<u>North Dakota</u>					
1DU	41 mi E of Devil's Lake	27 May 1957	<u>3338</u>	9	0
Reference sample: <u>Bufo americanus</u>					
<u>Minnesota</u>					
1MU	4 mi S on Hwy 73 of Hibbing	26 May 1959	<u>3994</u>	30	0
<u>Ontario</u>					
10	Moosonee	05 Jun 1972	<u>14797</u>	18	1
20	Whitetop Creek, 12 mi NE of Moosonee	01 Jun 1972 02 Jun 1972	<u>14783</u> <u>14791</u>	21 39	0 0
30	Beaver Creek, 6 mi W on Hwy 17 of Oxdrift	31 May 1970	<u>12238</u>	54	0
40	Lake Kenogami	19 May 1963	<u>7076</u>	56	4
50	10½ mi E on Hwy 17 of North Bay	17 May 1963	<u>7064</u>	104	6
60	1 mi W of Carlsbad Springs, Carleton County	30 Apr 1963	<u>6958</u>	89	12

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
Reference sample: <u>Bufo americanus</u> Continued					
<u>Ontario</u>					
70	½ mi N of Rondeau Provincial Park, Kent County	17 Apr 1963	<u>6916</u>	Males 47	Females 3
80	Point Pelee National Park, Essex County	05 Apr 1967	<u>9684</u>	10	7
<u>Quebec</u>					
1Q	Lac Attila, James Bay	17 Jul 1973	<u>15851</u>	14	1
2Q	Mile 134, N of Seven Islands	10 Jun 1952	<u>2265</u>	14	0
		11 Jun 1952	<u>2269</u>	12	1
		17 Jun 1952	<u>2277</u>	1	0
		19 Jun 1952	<u>2278</u>	1	2
		21 Jun 1952	<u>2279</u>	1	0
		22 Jun 1952	<u>2280</u>	3	0
3Q	Seven Islands	19 May 1952	<u>2237</u>	24	2
		20 May 1952	<u>2238</u>	4	1
		21 May 1952	<u>2239</u>	1	2
		23 May 1952	<u>2240</u>	0	1
		24 May 1952	<u>2241</u>	1	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
Reference sample: <u>Bufo americanus</u> Continued					
<u>Quebec</u>					
3Q		25 May 1952	<u>2242</u>	Males 3	Females 1
		29 May 1952	<u>2251</u>	0	6
		01 Jun 1952	<u>2258</u>	5	3
4Q	Cap-des-Rosiers	03 Jun 1971	<u>13134</u>	54	1
5Q	Routhierville	10 May 1953	<u>2429</u>	22	2
<u>Prince Edward Island</u>					
1P	0.6 mi E of Roseneath	26 Apr 1959	<u>3964</u>	29	2
		10 May 1959	<u>3988</u>	20	0
<u>New Brunswick</u>					
1B	6 mi N of Moncton	8,9,12 May 1959	<u>8711</u>	53	1
<u>Nova Scotia</u>					
1N	1 mi E of Wolfville	17 May 1964	<u>8716</u>	55	0
2N	Musquodoboit Harbour	28 May 1964	<u>8729</u>	40	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
Eastern Manitoba <u>Bufo</u> Collecting Stations					
1	3 mi S on Hwy 9 of Gimli	15 Jun 1970	<u>12261</u>	1	0
	2 mi S on Hwy 9 of Gimli	15 Jun 1970	<u>12262</u>	1	0
	2 mi S on Hwy 9 of Gimli	15 Jun 1970	<u>12263</u>	21	0
2	1 mi SW on Hwy 59 of Libau (turnoff)	30 May 1968	<u>11071</u>	15	0
3	2 mi SW on Hwy 59 of Scanterbury	13 May 1968	<u>11077</u>	13	0
4	2 mi N on Hwy 12 of Brokenhead	24 May 1968	<u>11033</u>	1	0
		25 May 1968	<u>11038</u>	2	0
		25 May 1968	<u>11039</u>	11	0
		05 Jun 1968	<u>11100</u>	1	0
5	Ditches along Hwy 59 (at Patricia Beach turnoff) 5 mi NE on Hwy 59 of Scanterbury	31 May 1968	<u>11072</u>	23	5
	5 mi NE on Hwy 59 of Scanterbury	24 May 1968	<u>11032</u>	8	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
Eastern Manitoba <u>Bufo</u> collecting stations					
6	4 mi S on Hwy 12 of Grand Beach turnoff	24 May 1968	<u>11034</u>	30	0
7	Lagoon, west beach, Grand Beach Provincial Park	21 Jun 1968	<u>11196</u>	42	0
8	Lagoon extension, 1 mi from E Park Gates, Grand Beach Provincial Park	21 Jun 1968	<u>11197</u>	24	0
9	4 mi N on Hwy 12 of Grand Beach turnoff	25 May 1968	<u>11037</u>	18	0
10	6 mi W on Hwy 11 of Ft. Alexander	05 Jun 1968	<u>11099</u>	31	2
11	2 mi W on Hwy 11 of Ft. Alexander	05 Jun 1968	<u>11098</u>	12	0
12	1 mi W on Hwy 11 of Pine Falls	05 Jun 1968	<u>11095</u>	15	0
13	1 mi S on Hwy 11 of Great Falls	05 Jun 1968	<u>11094</u>	3	0
14	5 mi N on Hwy 11 of Lac du Bonnet	04 Jun 1968	<u>11093</u>	2	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
Eastern Manitoba <u>Bufo</u> collecting stations.					
<u>The Beausejour Transect</u>					
15	Bird's Hill Provincial Park	23 June 1968	<u>12209</u>	Males	1
		23 June 1968	<u>12210</u>	Females	0
16	1 mi N of Beausejour	15 May 1968	<u>11017</u>		7
17	4-5 mi E on Hwy 44 of Beausejour	15 May 1968	<u>11018</u>		2
18	7 mi E on Hwy 44 of Beausejour	15 May 1968	<u>11021</u>		2
19	9 mi E on Hwy 44 of Beausejour	15 May 1968	<u>11020</u>		0
20	14 mi E on Hwy 44 of Beausejour	15 May 1968	<u>11019</u>		0
21	11 mi S on Hwy 11 of Lac du Bonnet	04 Jun 1968	<u>11090</u>		8
<u>The Vivian Transect</u>					
22	1 mi E on Hwy 15 of Vivian	23 May 1968	<u>11030</u>		0
		26 May 1968	<u>11040</u>		0
23	Brokenhead River, 3 mi E on Hwy 15 of Vivian	22 May 1968	<u>11029</u>		5
24	7 mi E on Hwy 15 of Vivian	22 May 1968	<u>11027</u>		0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
Eastern Manitoba <u>Bufo</u> collecting stations					
<u>The Vivian Transect</u>					
24	8 mi E on Hwy 15 of Vivian	22 May 1968	<u>11028</u>	Males 49	Females 4
25	3 mi N on Hwy 11 of Elma	14 May 1968	<u>11012</u>	49	4
26	10 mi E on Hwy 4 of Jct. Hwys 4 & 11, E of Whitemouth	13 May 1965	<u>8483</u>	24	0
27	4 mi E on Hwy 44 of Rennie	14 May 1968	<u>11011</u>	34	35
28	7 mi E on Hwy 44 of Rennie	14 May 1968	<u>11008</u>	55	11
<u>The Trans-Canada Hwy transect</u>					
29	3 mi N on Hwy 59 of Niverville (turnoff)	30 May 1970 31 May 1970 31 May 1970 31 May 1970 31 May 1970	<u>12331</u> <u>12232</u> <u>12233</u> <u>12234</u> <u>12235</u>	18 3 9 2 5	0 0 0 0 0
30	Dufresne (turnoff at Hwy 1)	08 Jun 1962	<u>6086</u>	6	0
31	30.7 mi W on Hwy 1 of Jct. Hwys 1 & 11	08 Jun 1962	<u>6104</u>	10	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
<u>The Trans-Canada Transect</u>					
31	31.4 mi W on Hwy of Jct Hwys 1&11 W of Richer	14 May 1965	<u>8487</u>	19	4
	Dugout W of Lake Riviera turnoff, 3 mi E of Ste. Anne (turnoff)	27 May 1968	<u>11041</u>	43	0
	31½ mi W on Hwy 1 of Jct Hwys 1&11	15 May 1969	<u>11790</u>	5	3
		27 May 1969	<u>11811</u>	10	2
		04 Jun 1969	<u>11832</u>	5	0
		05 Jun 1969	<u>11847</u>	2	0
	31 mi W on Hwy 1 of Jct Hwys 1&11	19 Apr 1969	<u>11736</u>	1	0
		03 May 1969	<u>11765</u>	1	0
		07 May 1969	<u>11773</u>	3	0
		15 May 1969	<u>11788</u>	8	3
		03 Jun 1969	<u>11831</u>	3	0
		14 Jul 1969	<u>12001</u>	1	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
<u>The Trans-Canada Transect</u>					
33	22.6 mi W on Hwy 1 of Jct Hwys 1&11	11 Jun 1961	<u>5364</u>	32	0
	22 mi W on Hwy 1 of Jct Hwys 1&11	22 May 1968	<u>11026</u>	14	0
34	18 mi W on Hwy 1 of Jct Hwys 1&11	19 May 1970	<u>12208</u>	13	0
		24 May 1970	<u>12224</u>	13	0
		24 May 1970	<u>12227</u>	11	2
35	17.4 mi W on Hwy 1 of Jct Hwys 1&11	07 Jun 1962	<u>6064</u>	74	0
	16 mi W on Hwy 1 of Jct Hwys 1&11, E of Richer	13 May 1965	<u>8484</u>	36	14
			<u>10993</u>	6	0
	Brokenhead River, 17 mi W on Hwy 1 of Jct Hwys 1&11	11 May 1968	<u>10993</u>	6	0
		14 May 1968	<u>11013</u>	27	0
		21 May 1968	<u>11025</u>	17	2
	17 mi W on Hwy 1 of Jct Hwys 1&11	14 May 1969	<u>11786</u>	10	11
		22 May 1969	<u>11798</u>	15	0
		23 May 1969	<u>11800</u>	2	0
		23 May 1969	<u>11801</u>	4	0
		26 May 1969	<u>11805</u>	0	1

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS (HS) Catalogue #	Number of Specimens	
				Males	Females
		27 May 1969	<u>11809</u>	*23	13
		02 Jun 1969	<u>11826</u>	* 4	0
		04 Jun 1969	<u>11838</u>	* 4	0
		05 Jun 1969	<u>11839</u>	*16	3
		16 Jun 1969	<u>11884</u>	* 7	0
		18 May 1970	<u>12202</u>	23	7
		23 May 1970	<u>12220</u>	0	2
36	6 mi E on Hwy of Brokenhead River at Hwy 1 (- 11 mi W on Hwy 1 of Jct 1&11)	25 May 1969	<u>11802</u>	24	0
37	10.2 mi W on Hwy 1 of Jct of Hwys 1&11	11 Jun 1961	<u>5361</u>	9	0

* included in discriminant score mean and variance for this locality (35) but omitted from the univariate and ratio means which are based on the other 214 specimens from this locality.

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens
<u>The Trans-Canada Transect</u>				
38	10 mi W on Hwy 1 of Jct Hwys 1&11	12 May 1968	<u>10995</u>	4 0
39	3 mi W on Hwy 1 of Jct Hwys 1&11	13 May 1968	<u>10996</u>	68 5
		06 May 1969	<u>11775</u>	14 0
		14 May 1969	<u>11787</u>	11 9
		17 May 1969	<u>11794</u>	6 0
		18 May 1969	<u>11795</u>	5 2
		03 Jun 1969	<u>11830</u>	7 0
		17 May 1970	<u>12200</u>	13 6
	2 mi W on Hwy 1 of Jct Hwys 1&11	27 May 1968	<u>11042</u>	34 5
		26 May 1969	<u>11807</u>	16 5
40	4.6 mi E on Hwy 1 of Jct Hwys 1&11	10 Jun 1961	<u>5350</u>	35 0
41	8 mi E on Hwy 1 of Jct Hwys 1&11	13 May 1968	<u>10997</u>	7 0
42	6 mi W on Hwy 1 of Falcon Lake (turnoff)	13 May 1968	<u>10999</u>	7 0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
<u>The Trans-Canada Transect</u>					
43	Falcon Lake, Whiteshell Forest Reserve	05 Jun 1962	<u>6054</u>	114	2
	SW end Falcon Lake, Whiteshell Provincial Park	12 May 1965	<u>8477</u>	32	0
<u>The Marchand Transect</u>					
44	8 mi S on Hwy 12 of Steinbach	27 May 1968	<u>11044</u>	74	0
45	2 mi E on Hwy 210 of Marchand	12 Jun 1970	<u>12258</u>	12	0
		31 May 1968	<u>11079</u>	7	0
46	4 mi E on Hwy 210 of Marchand	31 May 1968	<u>11081</u>	9	0
		01 Jun 1968	<u>11088</u>	16	0
47	Refuse dump pond, Moose Lake, NW Angle Forest Reserve	16 Jun 1968	<u>11133</u>	5	0
		16 Jun 1968	<u>11136</u>	66	0
48	Birch Point, Buffalo Bay, Lake of the Woods, NW Angle Forest Reserve	17 Jun 1968	<u>11146</u>	17	0
		17 Jun 1968	<u>11151</u>	29	0

APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
<u>The Southern Transect</u>					
49	3 mi S on Hwy 75 of Letellier	04 Jun 1970	<u>12246</u>	12	0
	3½ mi S on Hwy 75 of Letellier	04 Jun 1970	<u>12247</u>	7	0
	4 mi S on Hwy 75 of Letellier	04 Jun 1970	<u>12248</u>	7	0
	1-2 mi S on Hwy 75 of Letellier	04 Jun 1970	<u>12249</u>	18	0
50	4 mi S on Hwy 59 of Tolstoi	30 May 1970	<u>12229</u>	37	5
51	5 mi NW on Hwy 12 of Rat River	28 May 1968	<u>11047</u>	0	2
	3 mi NW on Hwy 12 of Rat River at Hwy 12	28 May 1968	<u>11048</u>	55	4
52	Just E on Hwy 12 of Rat River at Hwy 12	29 May 1968	<u>11062</u>	6	1
	1 mi E on Hwy 12 of Rat River at Hwy 12	29 May 1968	<u>11065</u>	39	9
53	3 mi SE of Rat River at Hwy 12	30 May 1968	<u>11067</u>	9	0
54	1 mi NW on Hwy 12 of Menisino (turnoff)	30 May 1968	<u>11068</u>	28	0
55	7 mi NW on Hwy 12 of Jct Hwys 12 & 89, N of Piney	28 May 1968	<u>11051</u>	14	0

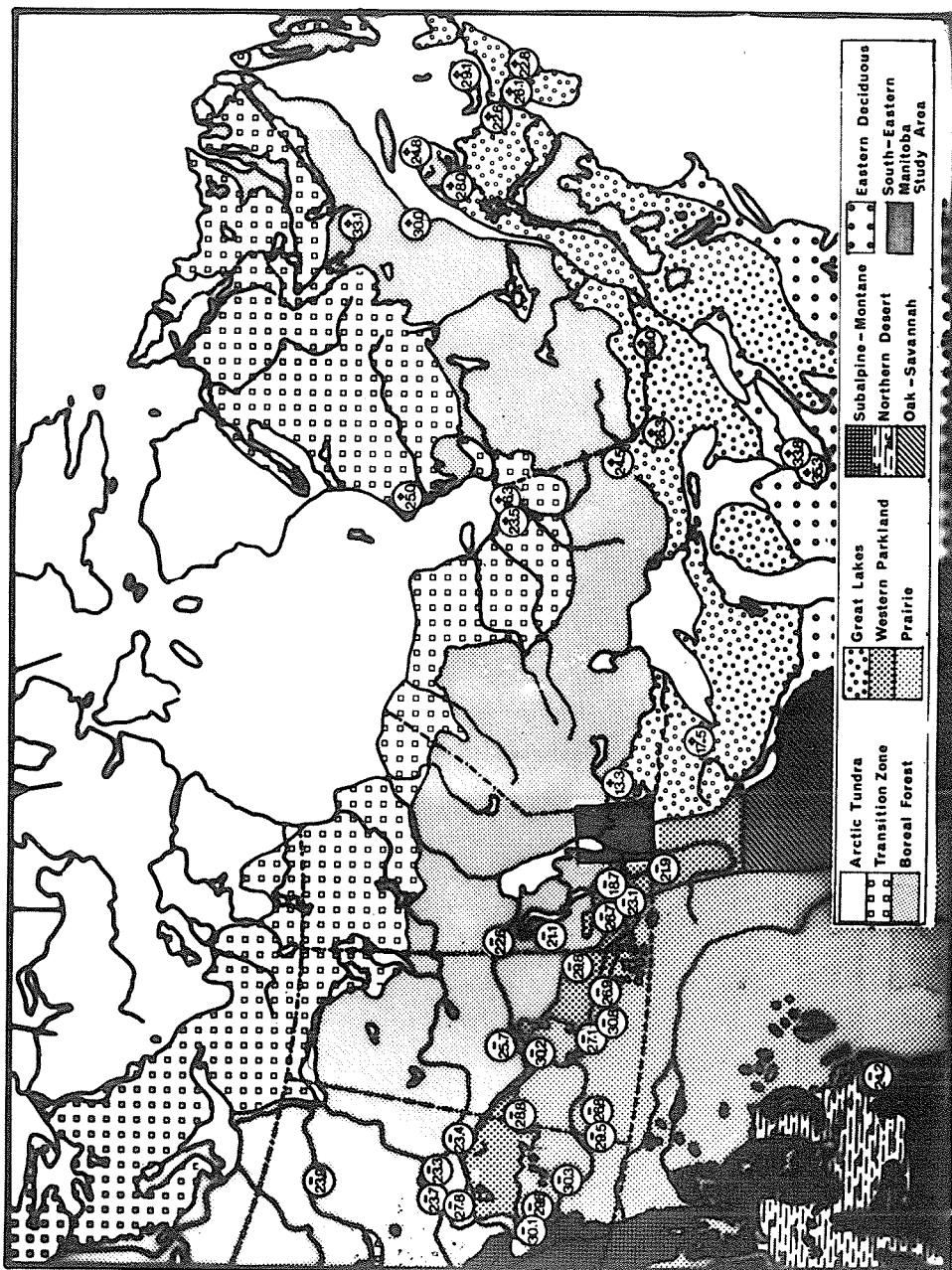
APPENDIX I (continued)

Locality Number	Locality	Collection Date	NMNS(HS) Catalogue #	Number of Specimens	
				Males	Females
<u>The Southern Transect</u>					
56	4 mi NW on Hwy 12 of Jct Hwys 12& 89, N. of Piney	28 May 1968	<u>11053</u>	20	4
57	3 mi S on Hwy 89 of Piney 3.5 mi S on Hwy 89 of Piney	30 May 1968 30 May 1968	<u>11069</u> <u>11070</u>	21 46	0 0
58	3 mi E on Hwy 12 of Jct Hwys 12&89, N of Piney	29 May 1968	<u>11060</u>	25	0
59	1 mi NW on Hwy 12 of South Junction turnoff	29 May 1968	<u>11055</u>	35	0
60	1 mi W on Hwy 12 of Sprague (turnoff)	29 May 1968	<u>11056</u>	69	3
61	$\frac{1}{2}$ mi E on Hwy 12 of Middlebro Hwy 12 at Middlebro turnoff	29 May 1968 29 May 1968	<u>11057</u> <u>11058</u>	26 42	4 4

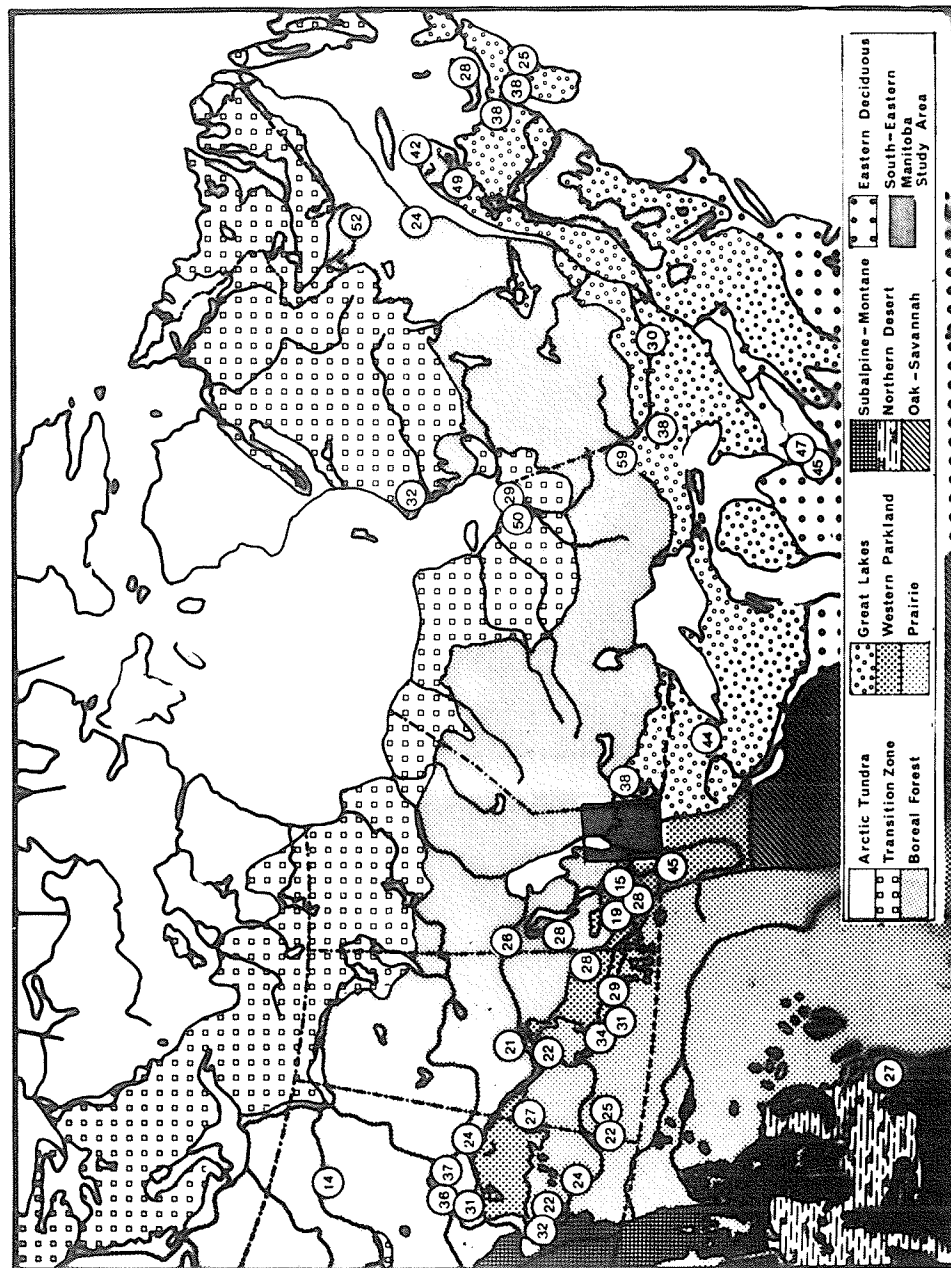
APPENDIX I (continued)

<u>Species/Locality</u>	<u>Collection Date</u>	<u>NMNS (HS) Cat. #</u>	<u>Males</u>
<u>Bufo woodhousei fowleri</u>			
<u>Ontario</u>			
Long Point, Norfolk Co.	19 June 1973	<u>15972</u>	19
<u>Bufo woodhousei woodhousei</u>			
<u>Kansas</u>			
3½ mi SSE of Stockton, Rooks Co.	23 June 1965	8585	10
	23 June 1965	<u>8591</u>	7
<u>Bufo boreas</u>			
2 mi W on Hwy 2 of Athabasca	28 May 1965	<u>8530</u>	60
<u>Bufo boreas x hemiophrys</u>			
<u>Alberta</u>			
2.4 mi N on Hwy 44 of Westlock	26 May 1965	<u>8523</u>	1
<u>Bufo cognatus</u>			
<u>Saskatchewan</u>			
8.3 mi E on Hwy 1 of Piapot (turnoff)	28 May 1963	<u>7110</u>	48

Appendix II. A: Map of central and eastern northern North America showing sample mean of discriminant function scores for reference collections of male B. hemiophrys and B. americanus.



Appendix II. B: Map of central and eastern northern North America
showing sample variance values for reference collections of male
B. hemiophrys and B. americanus.

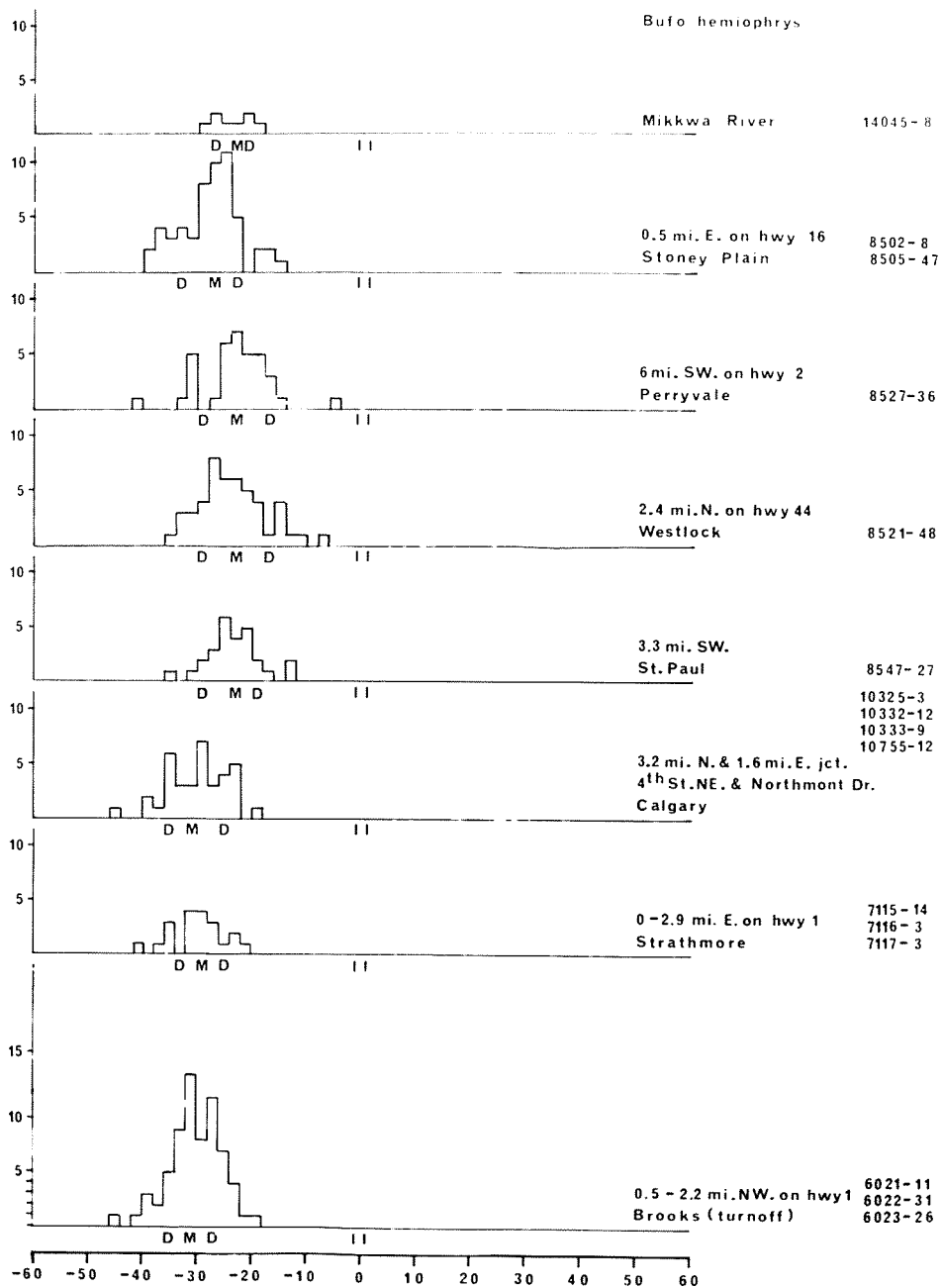


Appendix II. C: Histograms of individual discriminant function scores for eight Alberta reference collections of male B. hemiophrys.

The two vertical lines indicate the mid-point of the discriminant analysis between americanus and hemiophrys. M indicates the position of the mean for the sample and D is the position of one standard deviation on either side of the mean (the unequal distances apparent in some histograms are due to rounding values to fit the whole number scale units used). For each sample the locality designation is given, and to the right of it the catalogue number (National Museum of Natural Sciences unless otherwise indicated) followed by a dash and the number of specimens in the sample.

The scale at the bottom gives the discriminant score values, the one on the left the number of individuals.

ALBERTA
Bufo hemiophrys

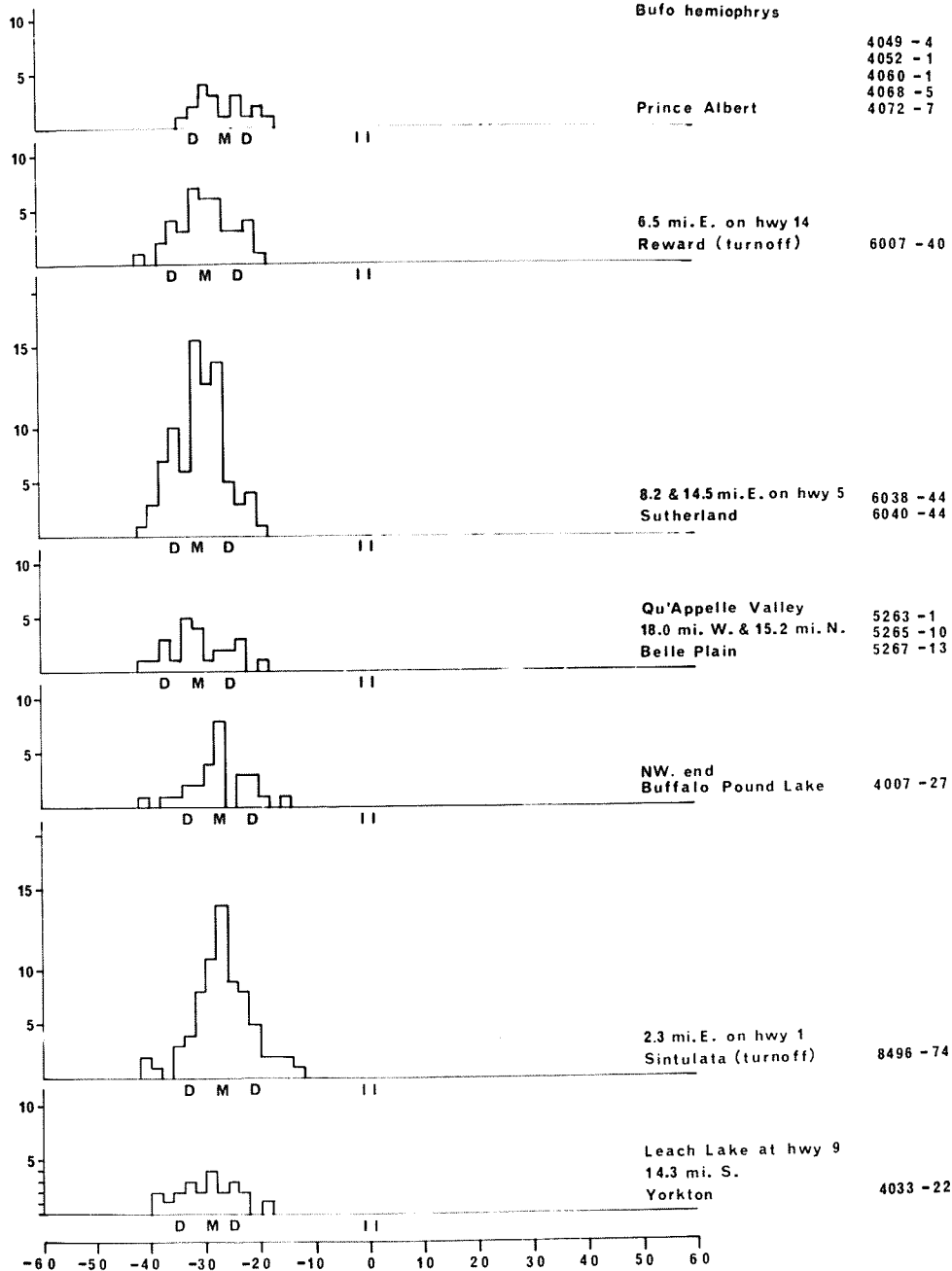


Appendix II. D: Histograms of individual discriminant function scores for seven Saskatchewan reference collections of male B. hemiophrys.

See Appendix II C legend for explanation of letters, scale, etc.

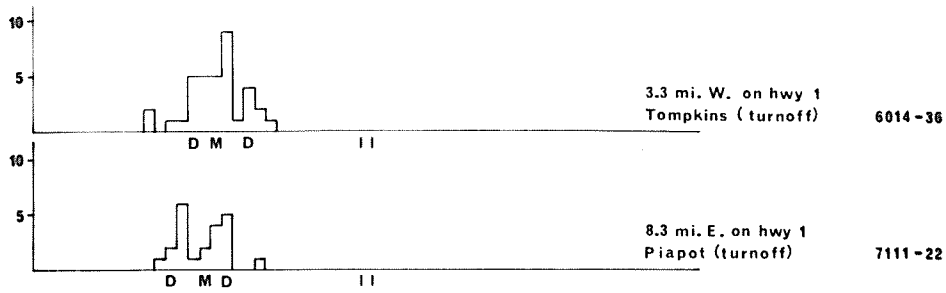
SASKATCHEWAN (1)

Bufo hemiophrys

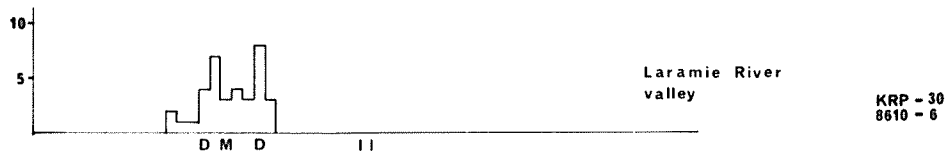


Appendix II. E: Histograms of individual discriminant function scores for two Saskatchewan and one Wyoming reference collections of male B. hemiophrys. See Appendix II C legend for explanation of letters, scale, etc.

SASKATCHEWAN (2)
Bufo hemiophrys



WYOMING, USA
Bufo

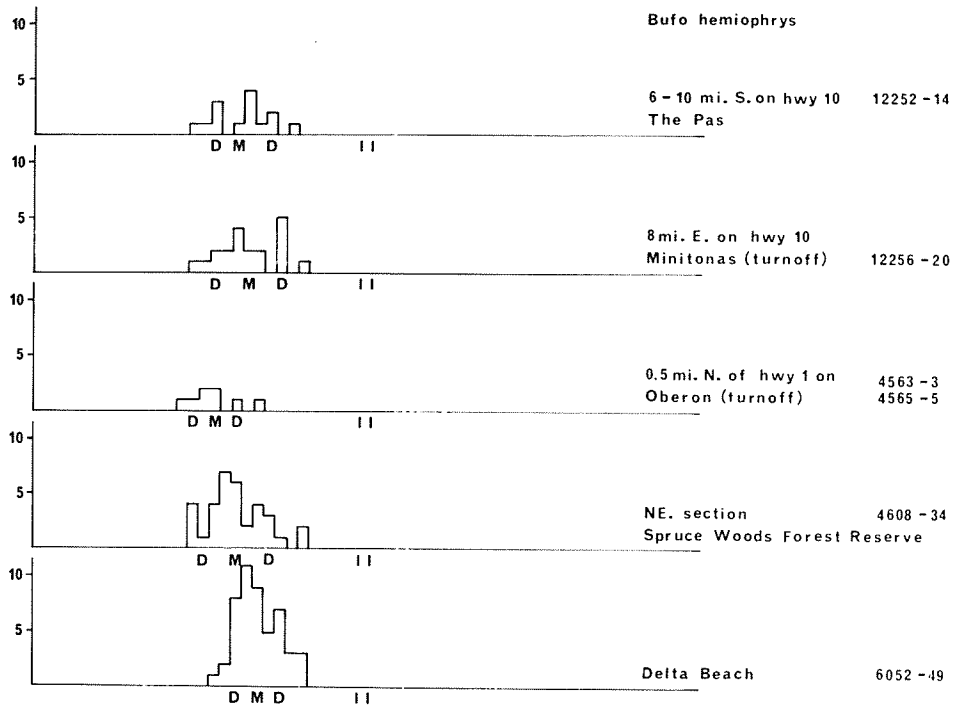


-60 -50 -40 -30 -20 -10 0 10 20 30 40 50 60

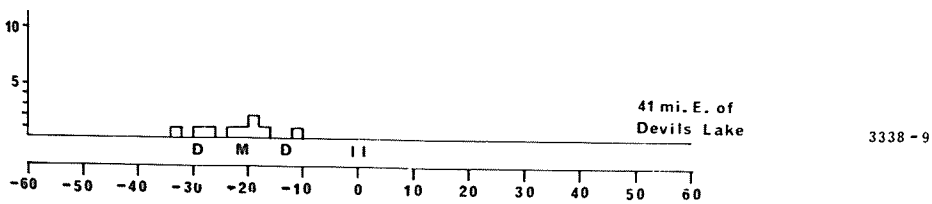
Appendix II. F: Histograms of individual discriminant function scores for five western and central Manitoba and one North Dakota reference collections of male B. hemiophrys. See Appendix II C legend for explanation of letters, scale, etc.

MANITOBA

Bufo hemiophrys



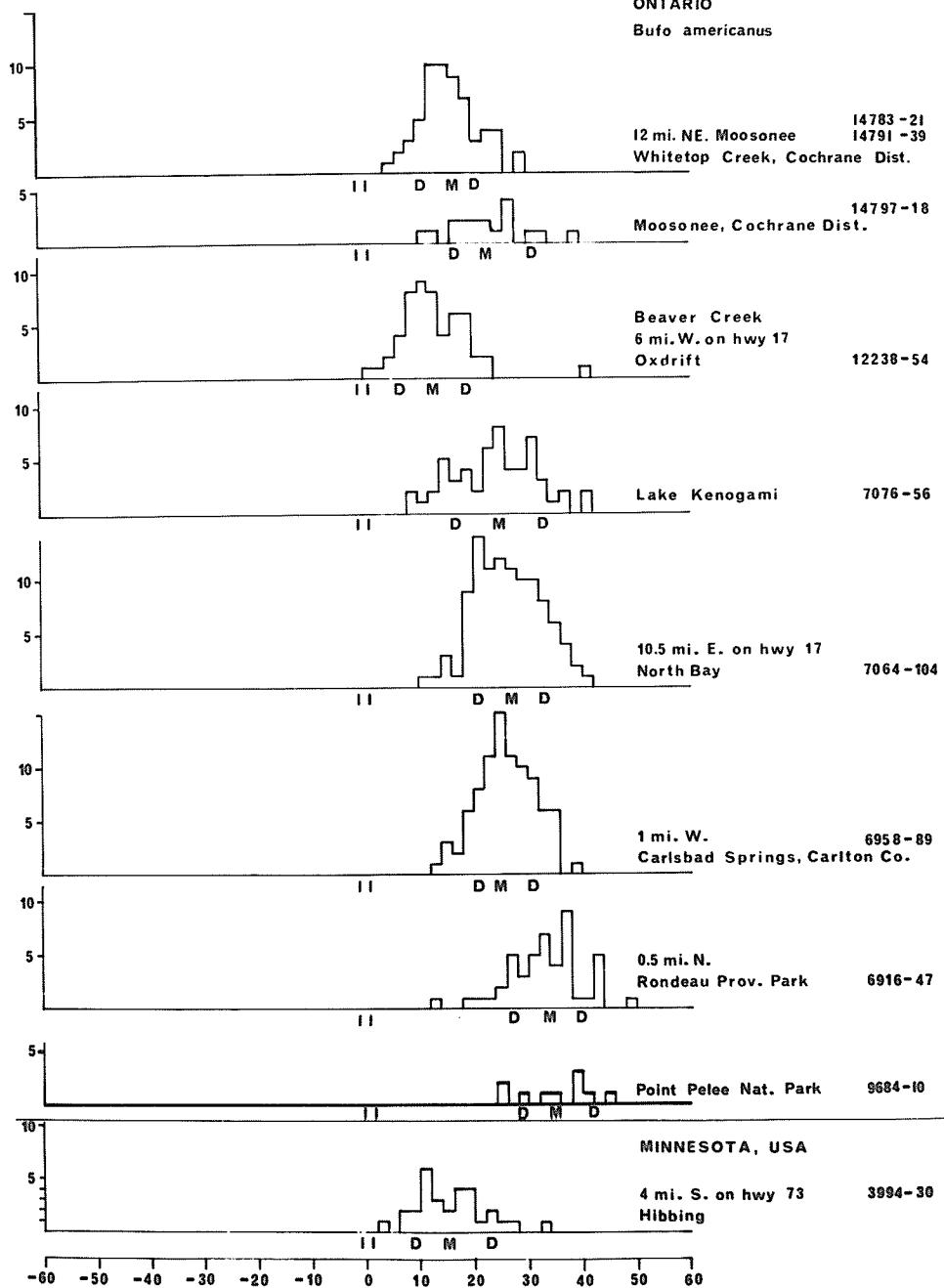
NORTH DAKOTA, USA



Appendix II. G: Histograms of individual discriminant function scores for seven Ontario and one Minnesota reference collections of male

B. americanus. See Appendix II C legend for explanation of letters, scale, etc.

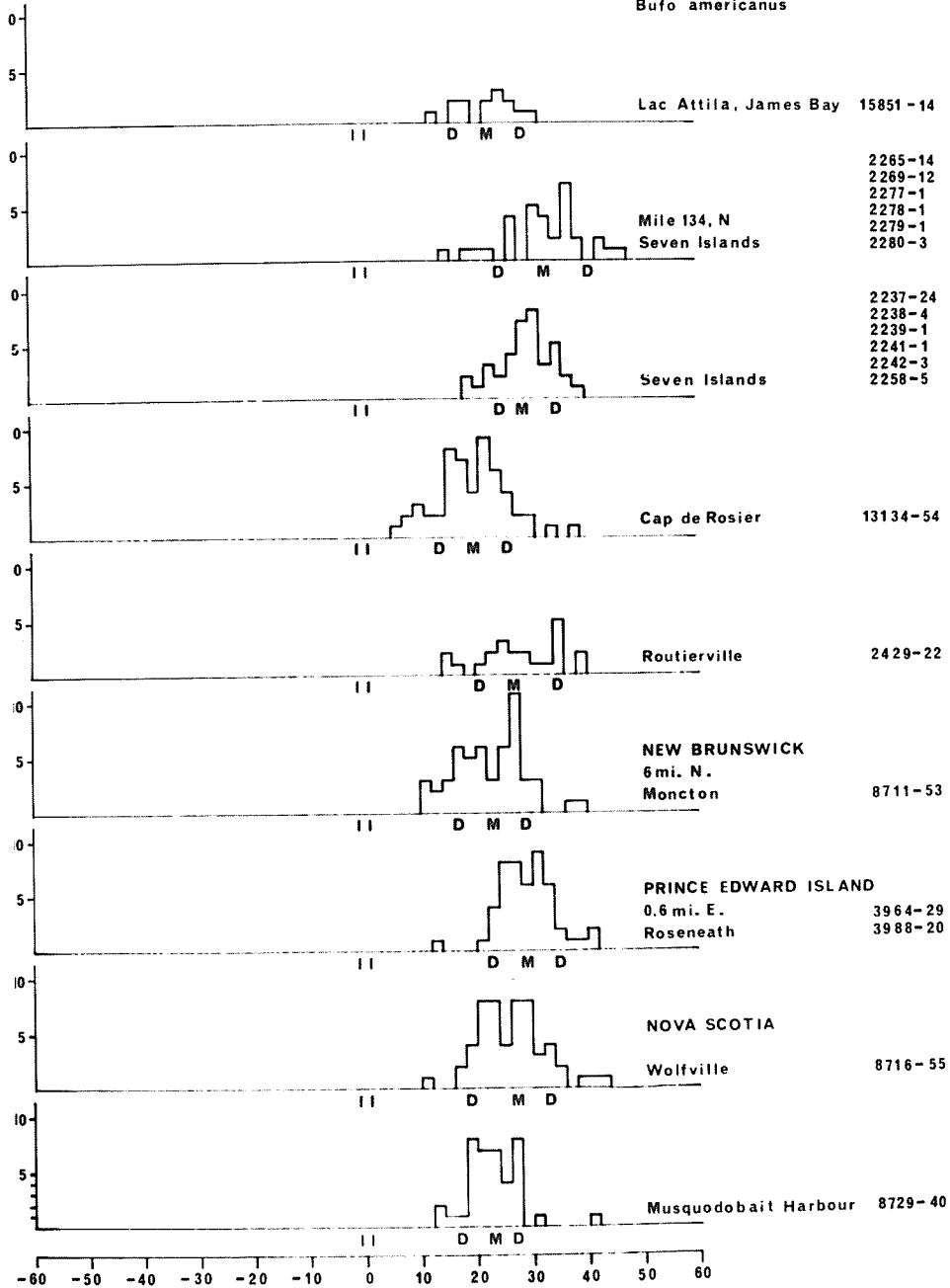
Bufo americanus



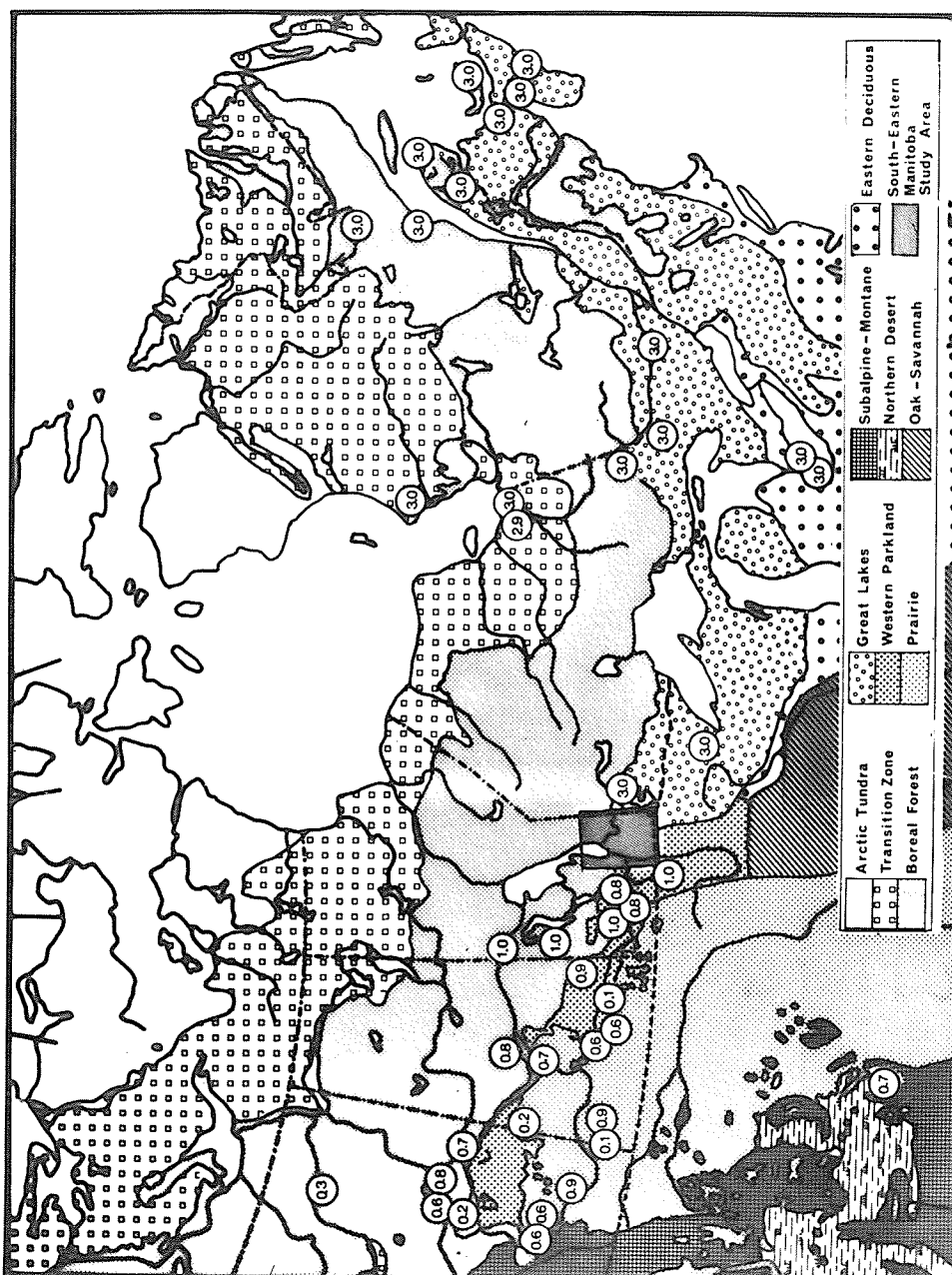
Appendix II. H: Histograms of individual discriminant function scores for five Quebec, one New Brunswick, one Prince Edward Island and two Nova Scotia reference collections of male B. americanus. See Appendix II C legend for explanation of letters, scale, etc.

QUEBEC

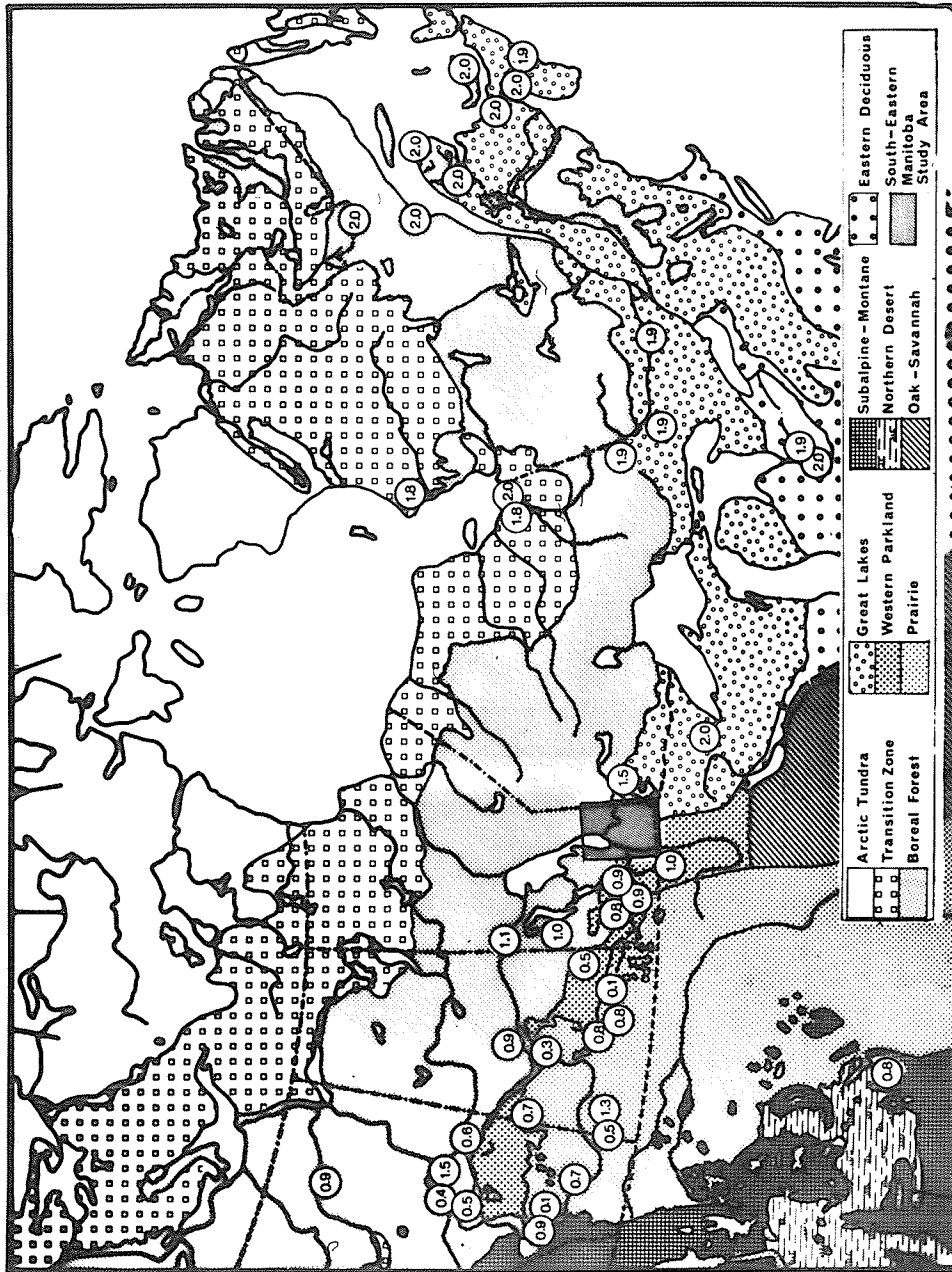
Bufo americanus



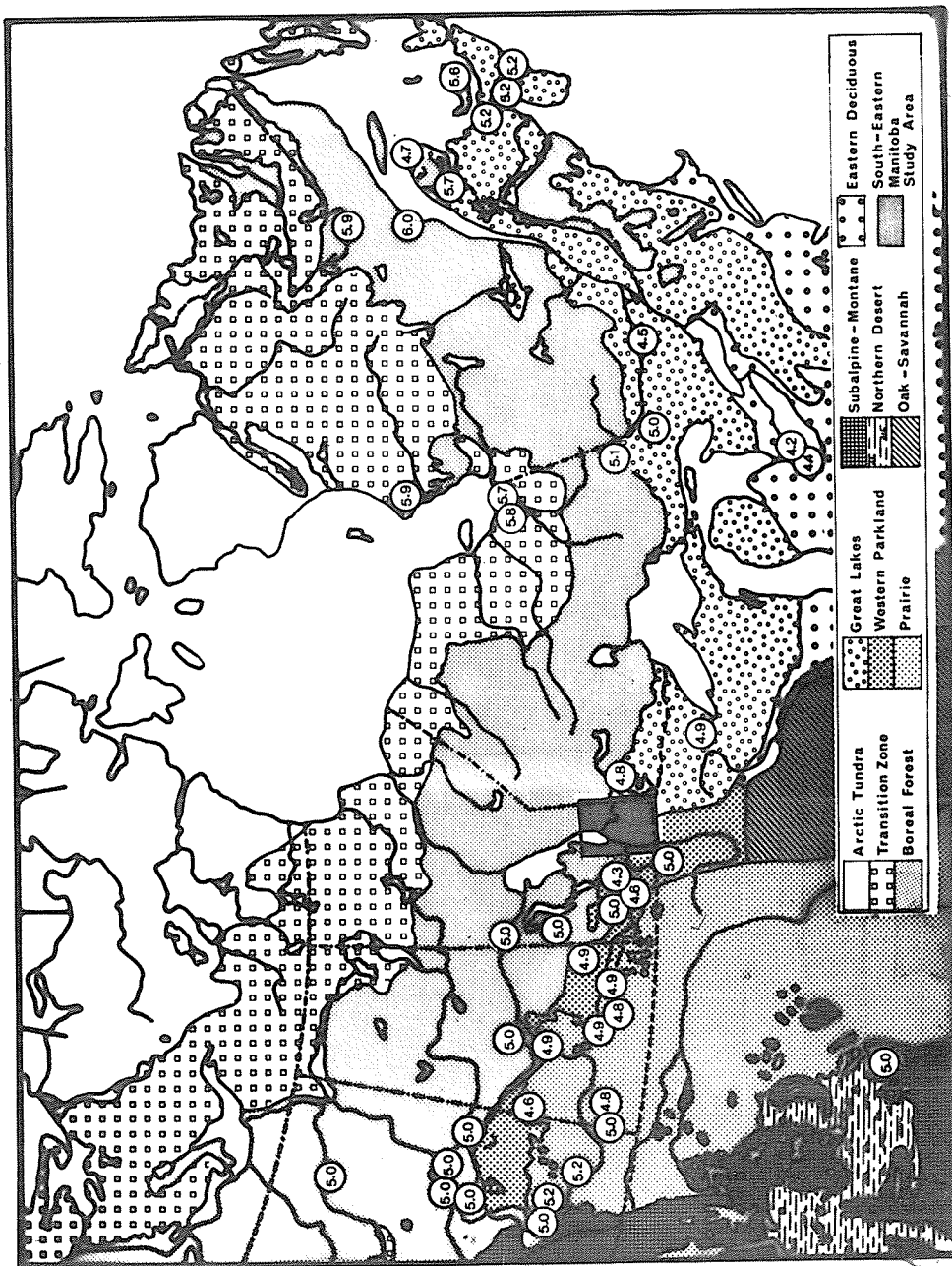
Appendix II. I: Map of central and eastern northern North America showing sample means of cranial crest scores for reference collections of male B. hemiophrys and B. americanus.



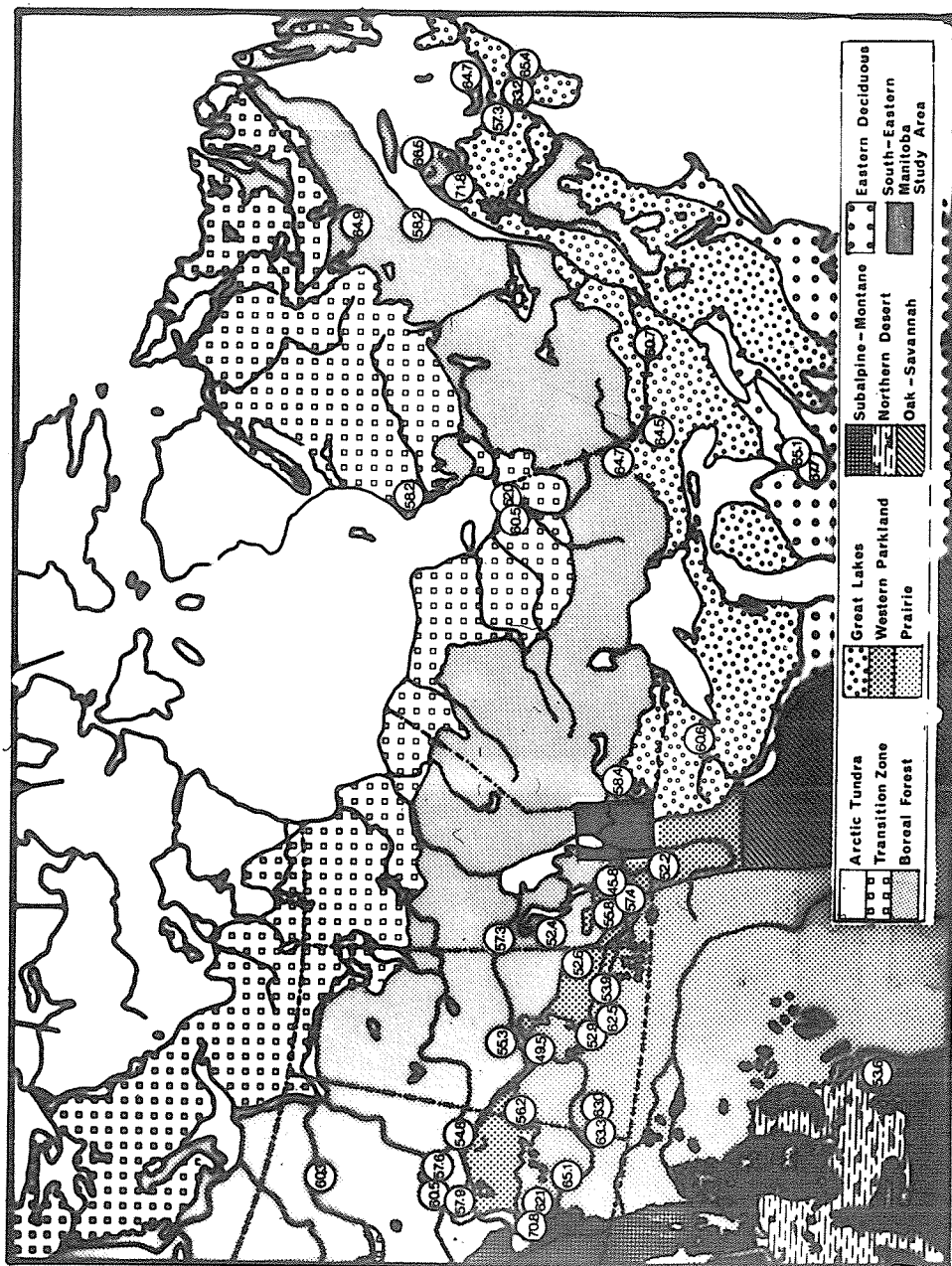
Appendix II. J: Map of central and eastern northern North America showing sample means for post-orbital crest scores for reference collections of male B. hemiophrys and B. americanus.



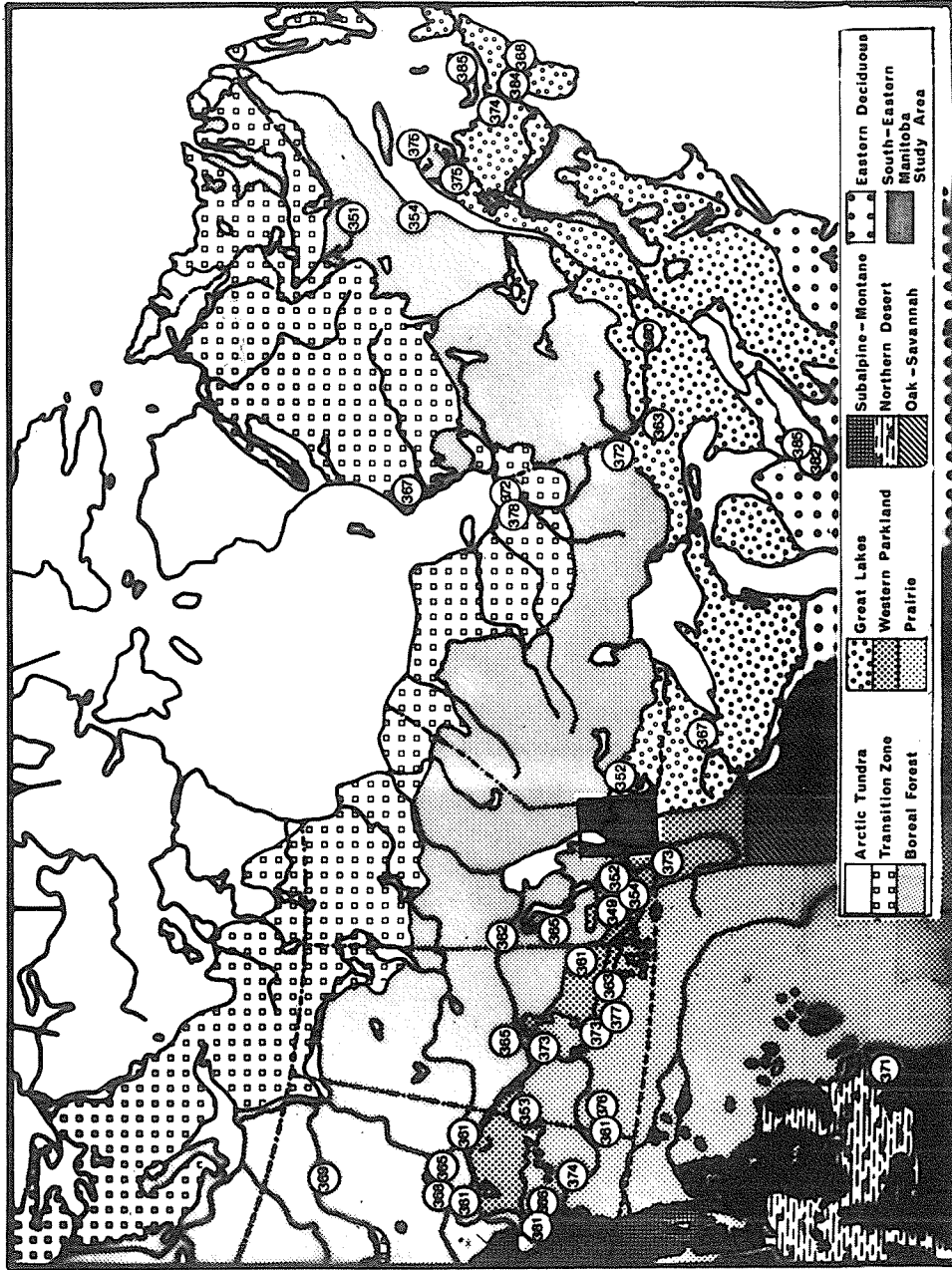
Appendix II. K: Map of central and eastern northern North America showing sample means for venter scores for reference collections of B. hemiophrys and B. americanus.



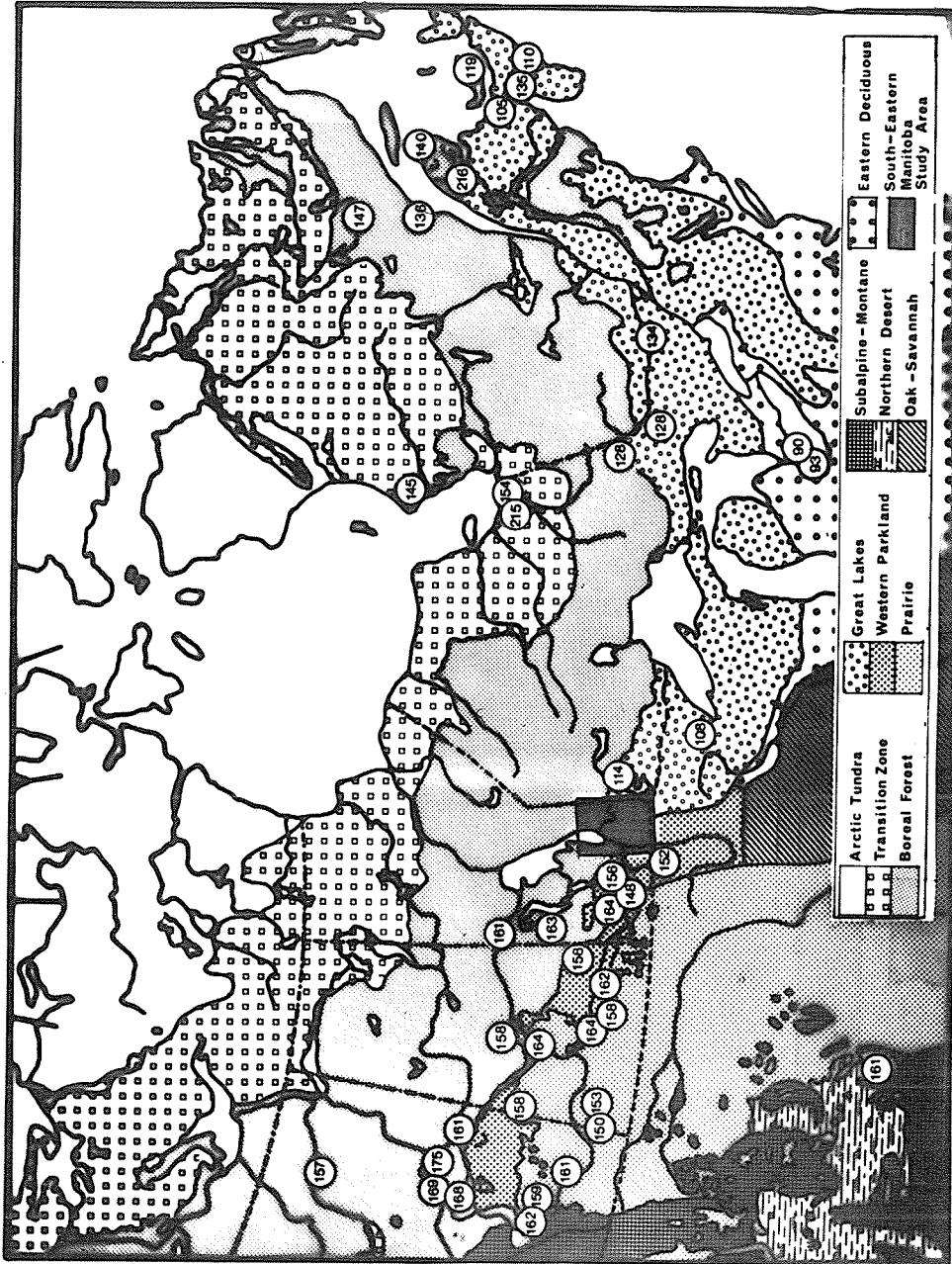
Appendix II. L: Map of central and eastern northern North America showing sample means for snout-vent length for reference collections of B. hemiophrys and B. americanus.



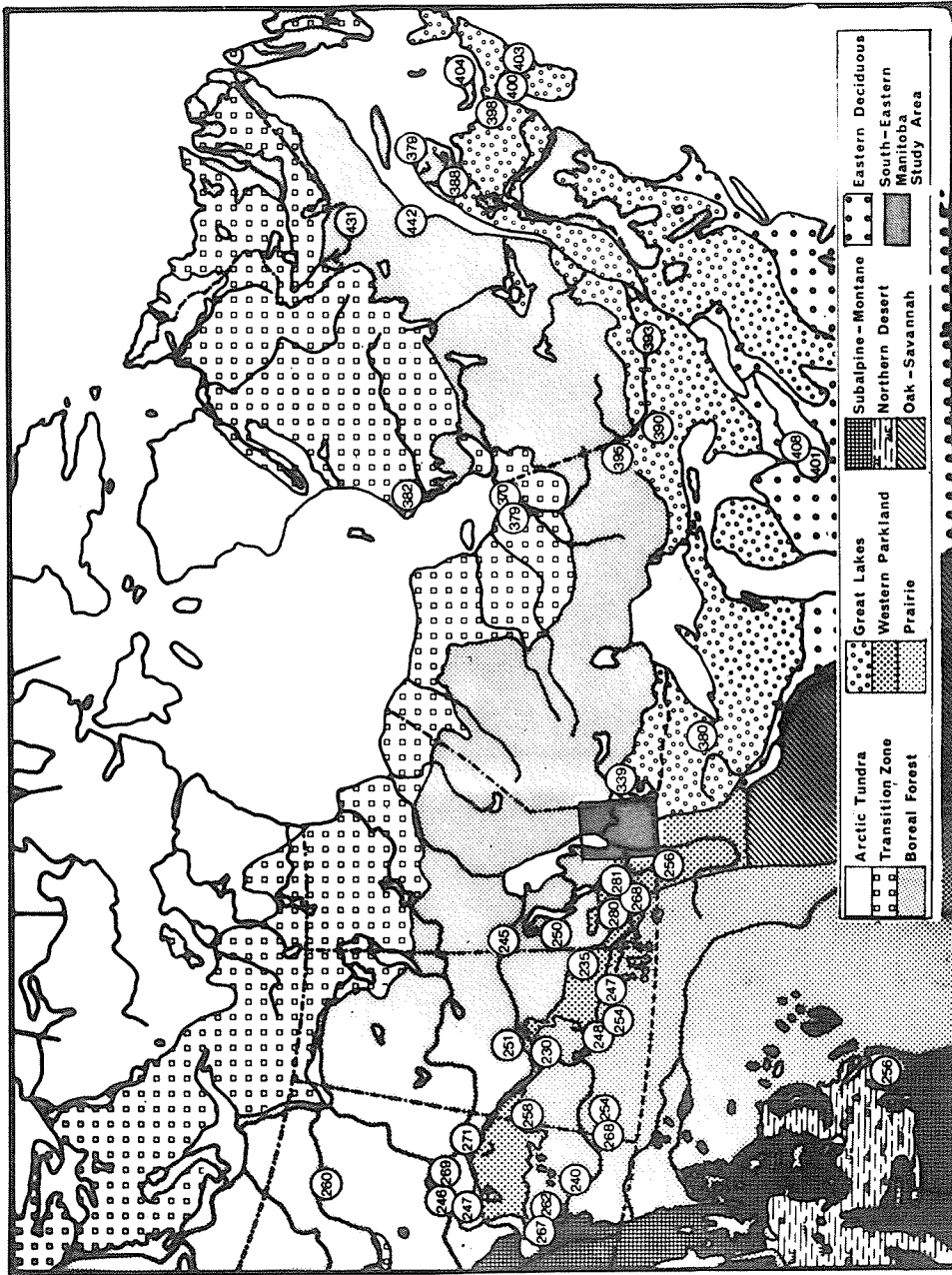
Appendix II. M: Map of central and eastern northern North America showing sample means for tibia/snout-vent lengths ratios for reference collections of B. hemiophrys and B. americanus. Each value has been multiplied by 1000 to remove the decimal point to simplify mapping.



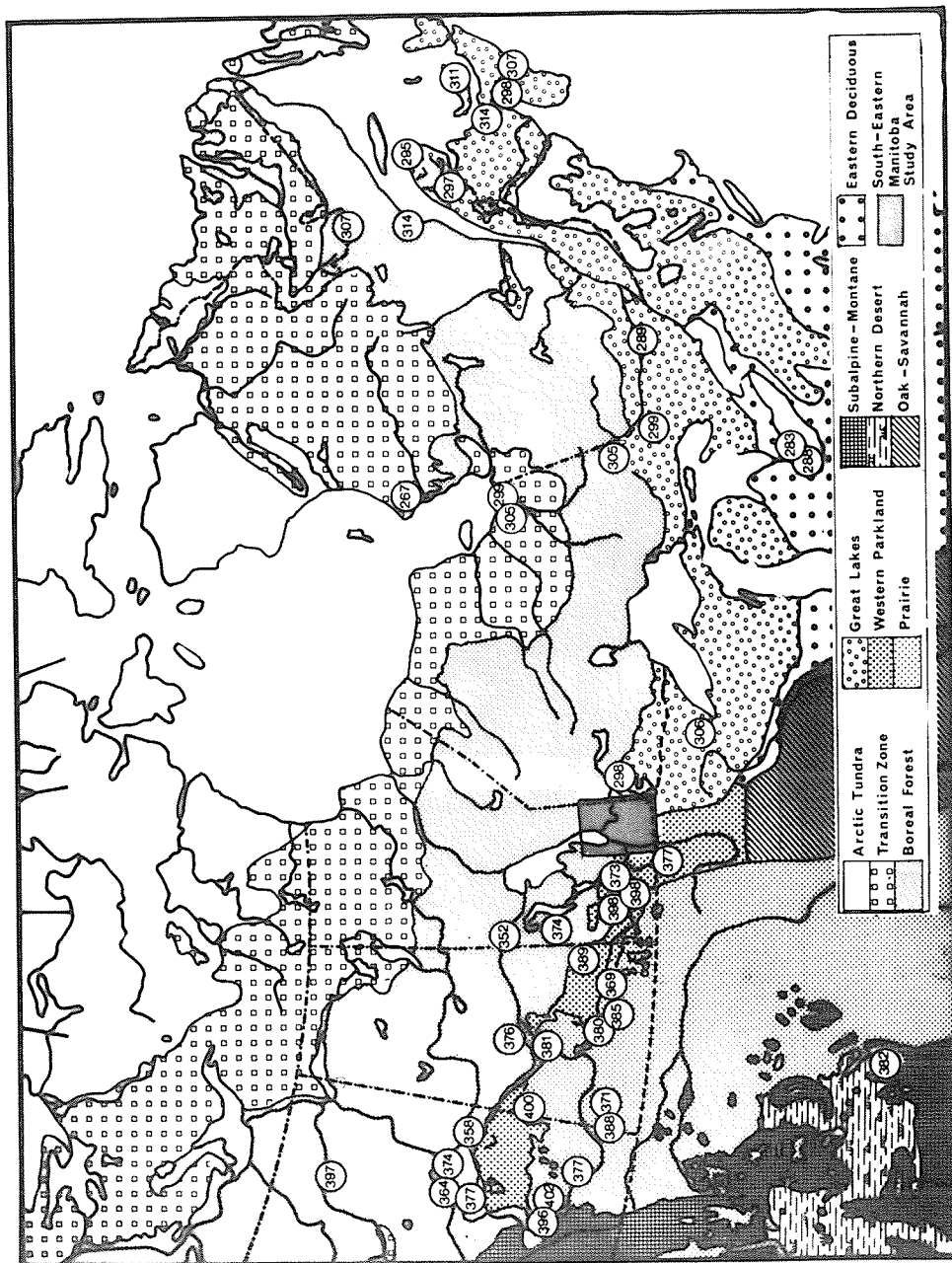
Appendix II. N: Map of central and eastern northern North America showing sample means for spot/snout vent lengths ratios for reference collections of B. hemiophrys and B. americanus. Each value has been multiplied by 1000 to remove the decimal point to simplify mapping.



Appendix II. O: Map of central and eastern northern North America showing sample means for cranial crest posterior width/head width ratios for reference collections of B. hemiophrys and B. americanus. Each value has been multiplied by 1000 to remove the decimal point to simplify mapping.



Appendix II. P: Map of central and eastern northern North America showing "spade" (outer metatarsal tubercle) width/tarsus length ratios for reference collections of B. hemiophrys and B. americanus. Each value has been multiplied by 1000 to remove the decimal point to simplify mapping.



APPENDIX III

List of localities and collections for which male breeding calls recorded.

Catalogue numbers given are the National Museum of Natural Sciences (Herpetology Section): lot number, followed by a dash and the individual specimen(s) number within that lot, followed (in parenthesis) by the tape catalogue number.

Most specimens recorded were also used in the morphological analysis and localities are numbered in Appendix I, and mapped in Figure 2 or Figure 3. At a few localities from which recordings were obtained, too few specimens were obtained to be worth including in the morphological analysis. Discriminant scores, however, were computed for these and have been used in the comparison of morphology and call in Figure 44. Included in the call reference samples are some localities in southeastern Manitoba excluded from the morphological reference samples.

<u>Locality</u>	<u>Number of Specimens</u>	<u>Date</u>	<u>Catalogue numbers</u>
<u>Bufo hemiophrys</u>			
*6-10 mi S on Hwy 10 of the Pas	7	7 June 1970	12252-1 to 6(68), -7(69)
*8 mi E on Hwy 10 of Minitonas turnoff	1	9 June 1970	12256-1(69)
*2 mi S on Hwy 9 of Gimili	8	15 June 1970	12263-1 to -8(70)
*3 mi S on Hwy 75 of Letellier	3	4 June 1970	12246-1 to -3(64)

APPENDIX III (continued)

*1-2 mi S on Hwy 75 of Letellier	17	4 June 1970	12249-1 to -4(64), -5 to -9(65), -10 to -14(66), -15, -16, -18(67)
*4 mi S on Hwy 59 of Tolstoi	7	30 May 1970	12229-1 to -7(54)
3 mi N on Hwy 59 of Niverville turnoff	12	30 May 1970	12231-1 to -5(55), -6 to -12(56);
	2	31 May 1970	12232-1, -2(57)
	4	31 May 1970	12233-1 to -3(57)-8(58)
	1	31 May 1970	12234-2(58)
	3	31 May 1970	12235-1, -3, -5(58)
*0.5 mi N on Hwy 75 of Glenlea	1	2 June 1970	12244-1(63)
1 mi N on Hwy 75 of Glenlea	3	1 June 1970	12243-1, -2, -4(62)
*4 mi N on Hwy 75 of Glenlea	1	1 June 1970	12241-1(61)
8 mi S on Hwy 12 of Steinbach	2	12 June 1970	12258-1, -2(70)
31 $\frac{1}{4}$ mi W on Hwy 1 of Jct Hwys 1&11	2	15 May 1969	11790-1, -2(24)
	2	4 June 1969	11832-1, -2(40)
31 mi W on Hwy 1 of Jct Hwys 1&11	3	3 June 1969	11831-1, to-3(39)
	5	21 May 1970	12214-2 to -6(49)
	2	22 May 1970	12218-1, -2(50)
30 mi W on Hwy 1 of Jct Hwys 1&11	1	22 May 1970	12215-1(50)
	3	22 May 1970	12217-1, -2, -4(50)
	1	12 June 1970	12257-1(69)
29 $\frac{3}{4}$ mi W on Hwy 1 of Jct Hwys 1&11	2	27 May 1969	11810-1, -2(36)
	1	27 May 1969	11812-1(36)
	2	28 May 1969	11815-1, -2(37)
	2	5 June 1969	11840-1, -2(42)

APPENDIX III (continued)

24 mi W on Hwy 1 of Jct Hwys 1&11	2	23 May 1970	12219-1, -2(51)
	4	23 May 1970	12221-1 to -4(51)
	9	23 May 1970	12222-1 to -9(52)

Total B. hemiophrys

111

* specimens used in call discrimination(45 specimens).

Intermediate populations

21 mi W on Hwy 1 of Jct Hwys 1&11	2	29 June 1969	11922-1, -2(43)
19 mi W on Hwy 1 of Jct Hwys 1&11	1	24 May 1970	12223-1(52)
	1	24 May 1970	12226-1(53)
18 mi W on Hwy 1 of Jct Hwys 1&11	6	24 May 1970	12224-1, -2(5), -3 to -6(53)
	3	24 May 1970	12227-1 to -3(54)
	1	24 May 1970	12228-1(54)
17 mi W on Hwy 1 of Jct Hwys 1&11	2	23 May 1969	11800-1, -2(30)
	1	23 May 1969	11801-1(31)
	9	27 May 1969	11809-1, -3(34), -4 to -9(35), -10(36)
	3	2 June 1969	11826-1 to -3(38)
	4	4 June 1969	11838-1 to -4(40)
	4	5 June 1969	11839-1 to -4(41)
	2	16 June 1969	11884-1, -2(42)
	23	18 May 1970	12202-1 to -5(45), -6 to -11(46), -12 to -17(47), -18 to -23(48)

Total intermediate Bufo

62

APPENDIX III (continued)

Bufo americanus

6 mi E of Brokenhead River at Hwy 1
(-11 mi W on Hwy 1 of Jct Hwys 1&11)

5 25 May 1969 11802-1 to -5(33)

*2-3 mi W on Hwy 1 of Jct Hwys 1&11

3 19 May 1969 11796-1 to -3(27)

5 26 May 1969 11807-1 to -5(34)

*3 mi W on Hwy 1 of Jct Hwys 1&11

7 6 May 1969 11775-1, -2, -3, -6, -7, -9, -12
(23)

1 12 May 1969 11783-1(24)

1 16 May 1969 11793-1(24)

5 17 May 1969 11794-1 to -5(25)

5 18 May 1969 11795-1 to -5(26)

8 17 May 1970 12200-1 to -8(44)

Ontario

*6 mi W on Hwy 17 of Oxdrift

10 31 May 1970 12238-3 to -6(59), -7 to -12(60)

Total Bufo americanus

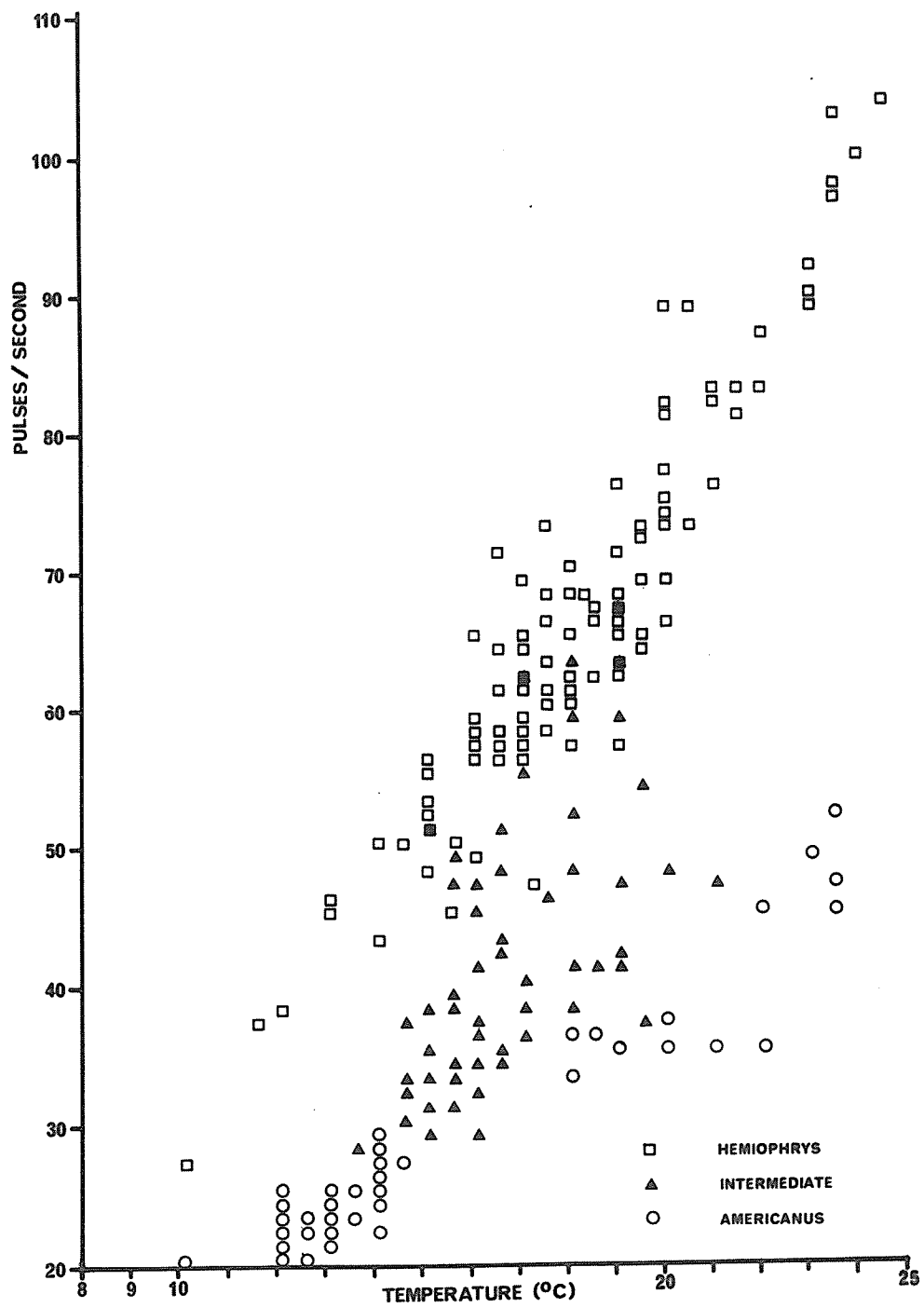
50

Total Bufo

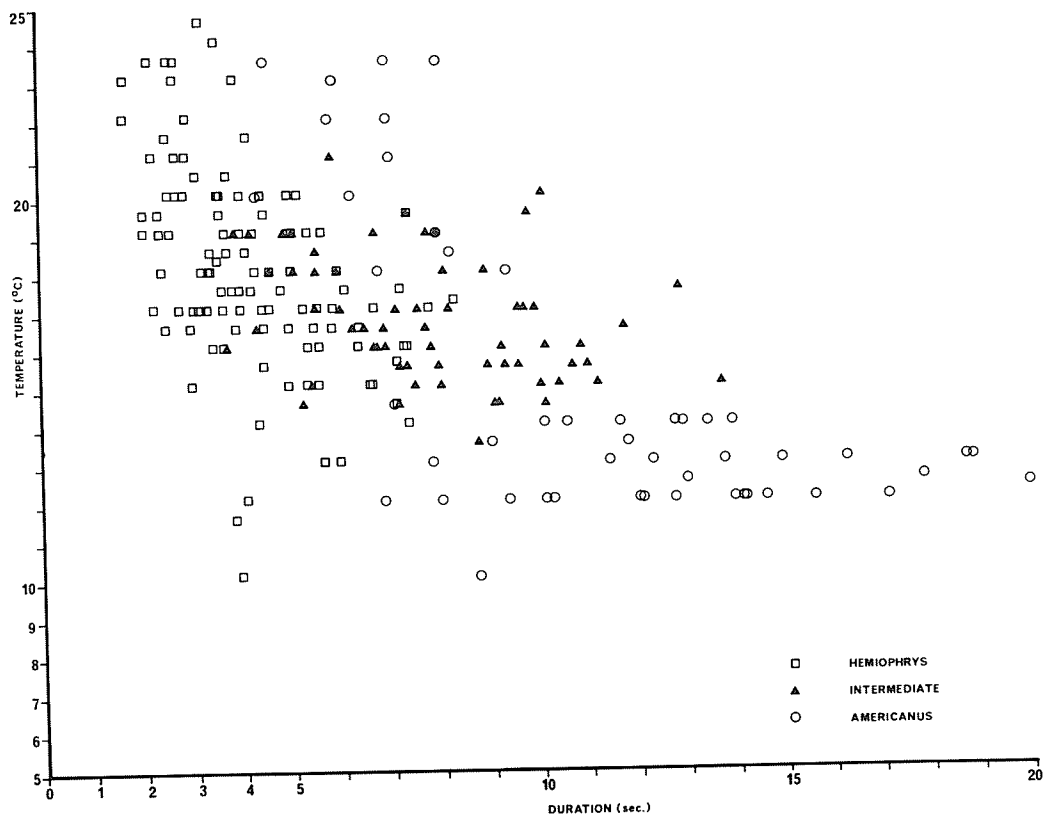
223

* included in call discrimination (45 specimen)

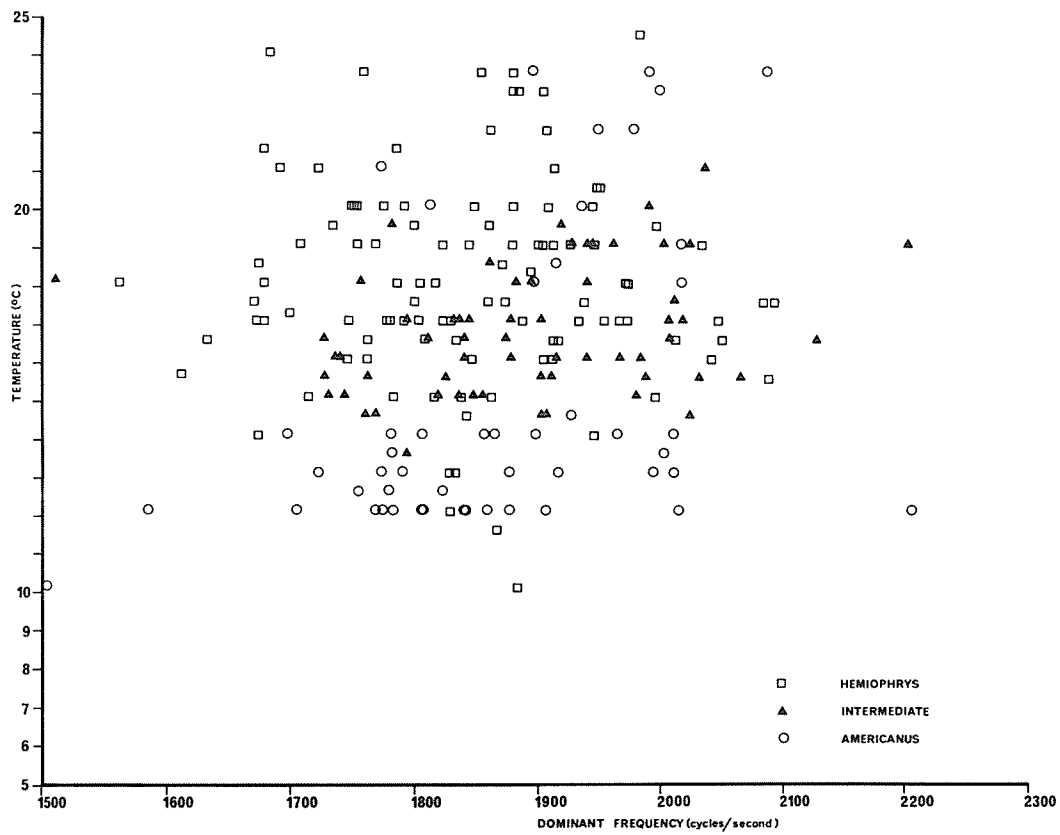
Appendix IV.A. Relationship of pulse rate to temperature in three samples of Bufo (collecting stations and number of toads recorded are listed in Appendix III). Solid squares indicate intermediate and hemiphrys individuals with identical values.



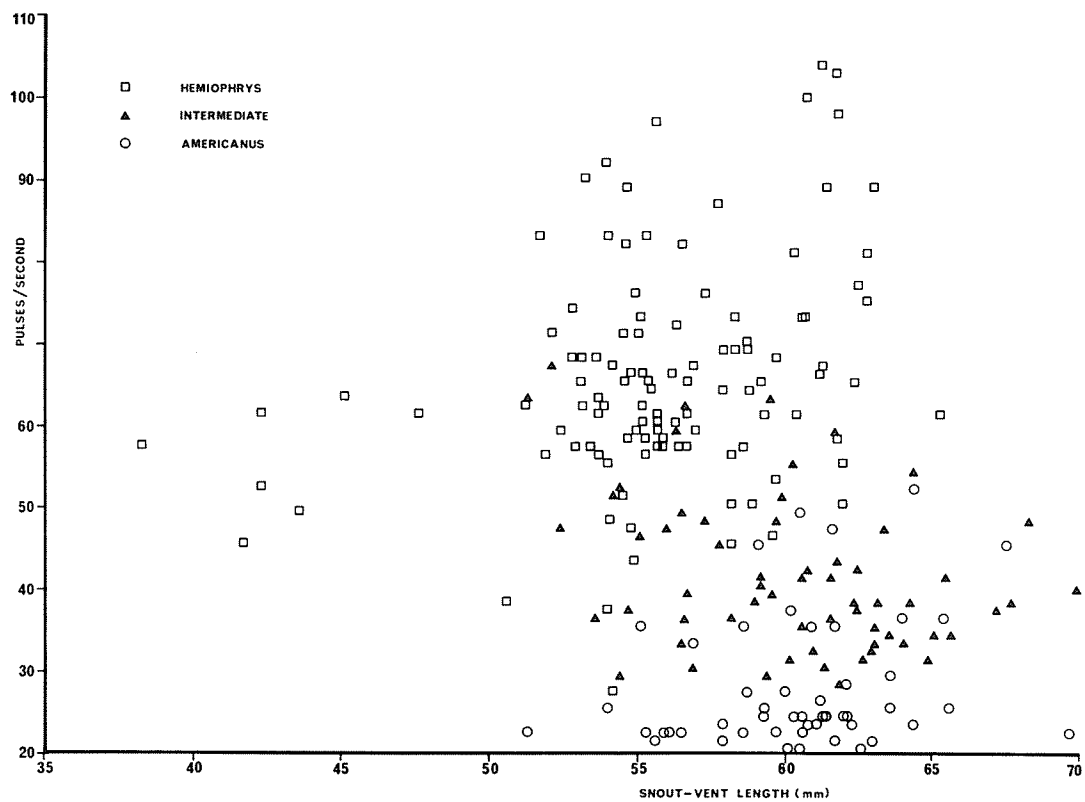
Appendix IV.B. Relationship of duration to temperature in three samples of Bufo (Appendix III gives localities and number of specimens).



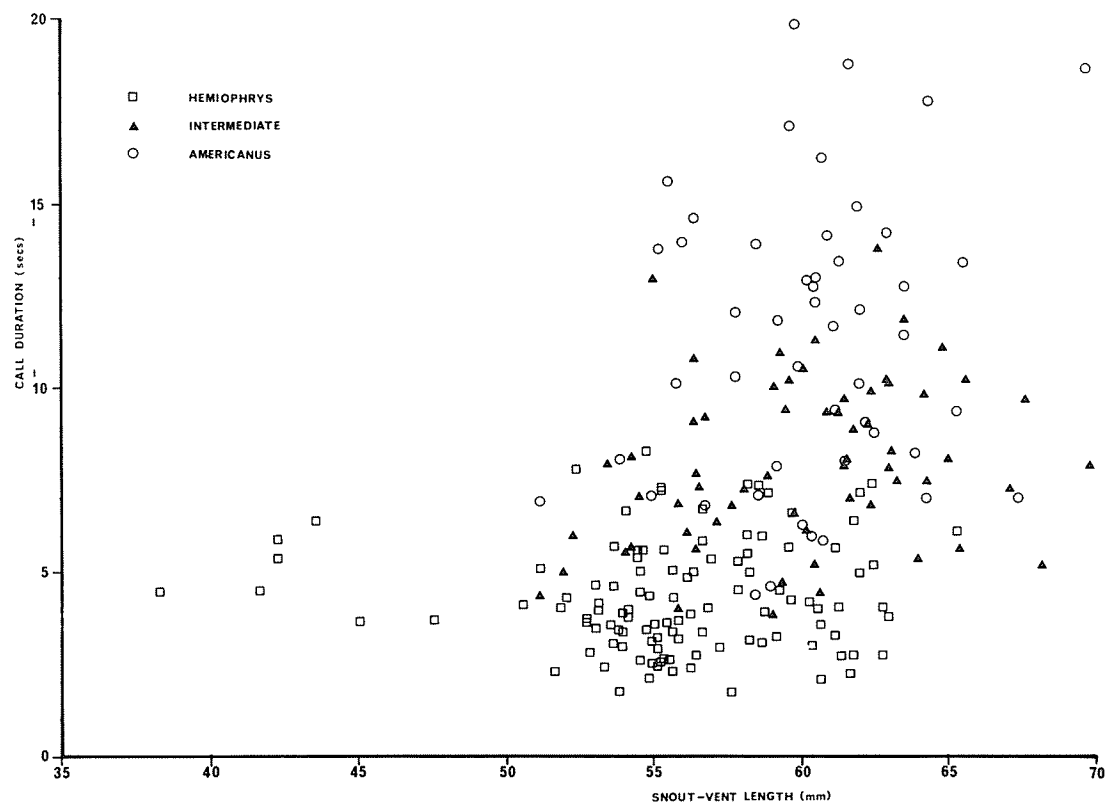
Appendix IV.C. Relationship of dominant frequency to temperature in three samples of Bufo (Appendix III gives localities and number of specimens).



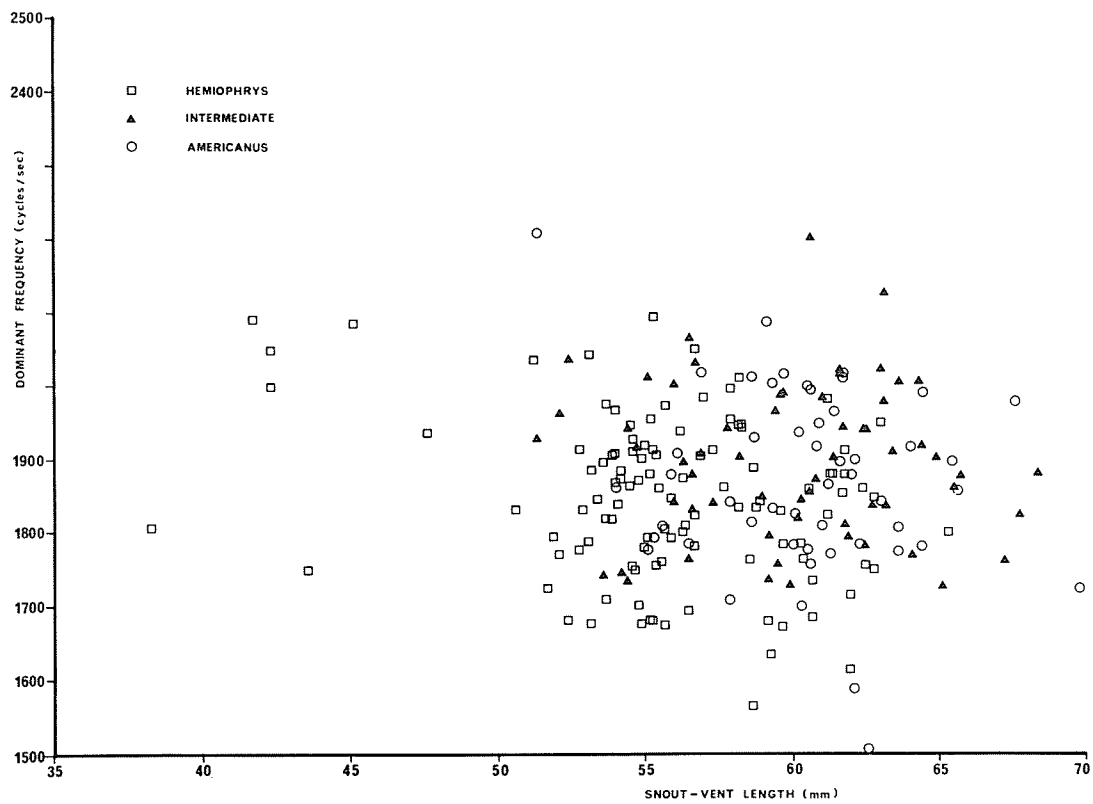
Appendix IV.D. Relationship of pulse rate to snout-vent length in three samples of Bufo (Appendix III gives localities and number of specimens).



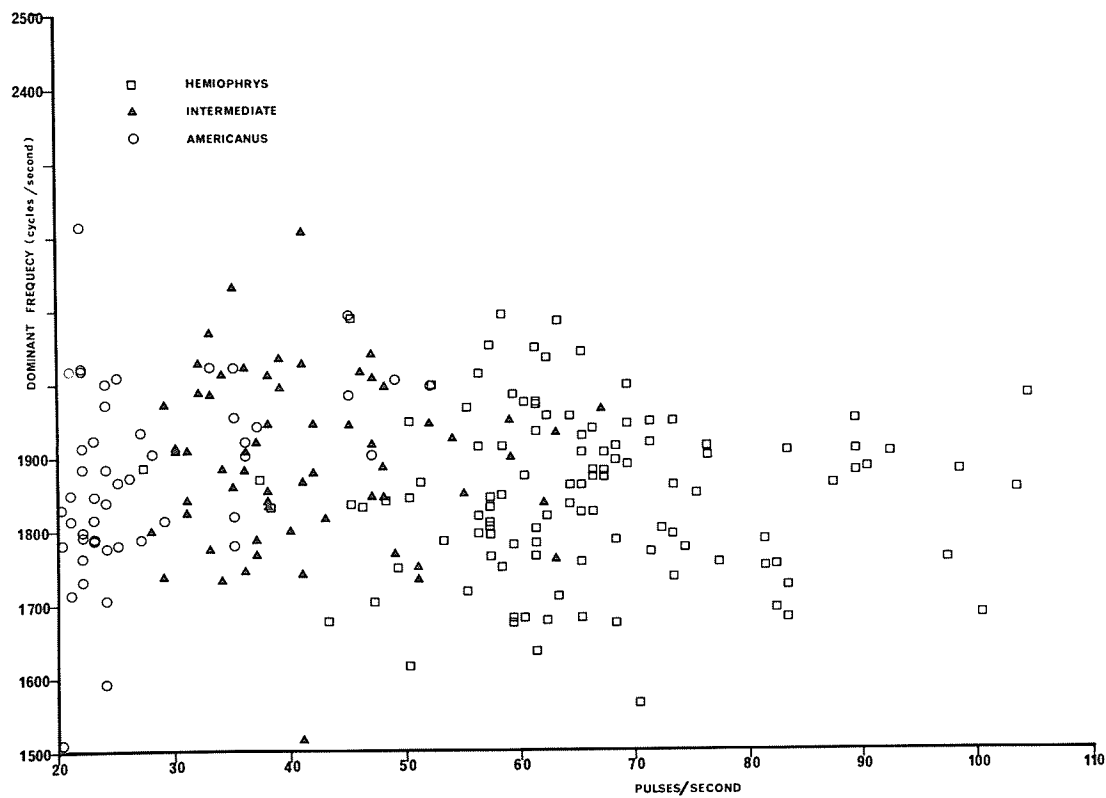
Appendix IV.E. Relationship of call duration to snout-vent length in three samples of Bufo (Appendix III gives localities and number of specimens).



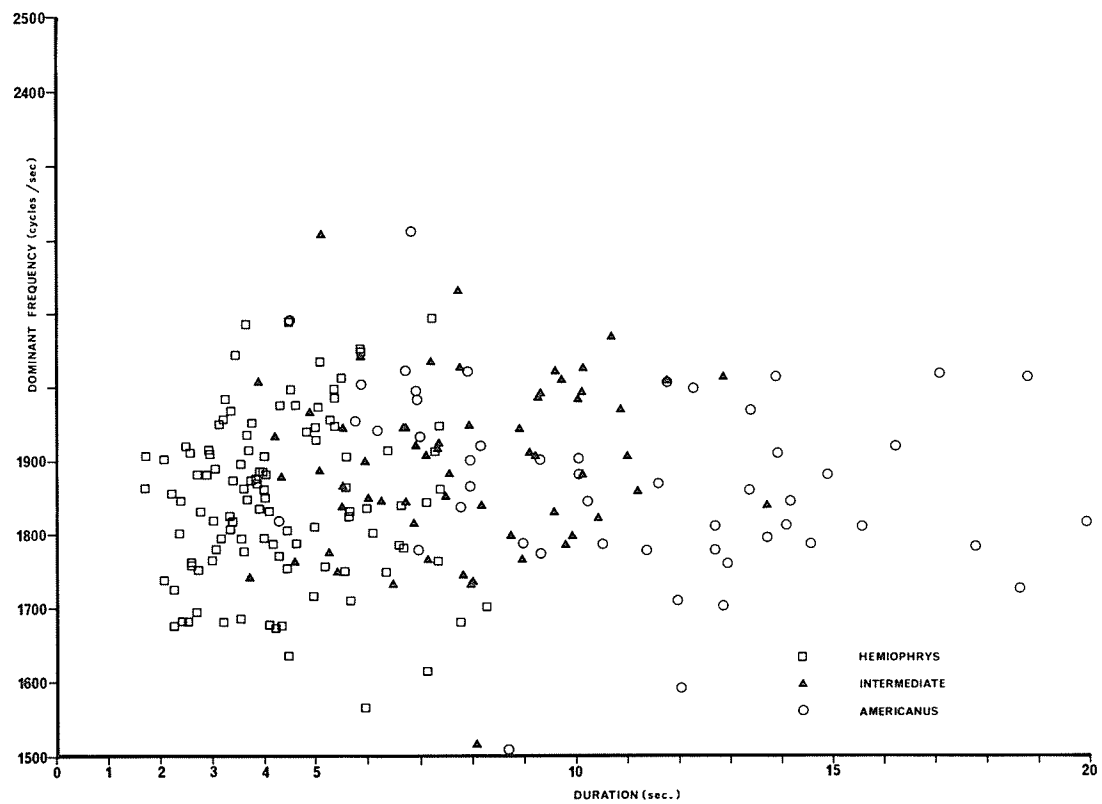
Appendix IV.F. Relationship of dominant frequency to snout-vent length in three samples of Bufo (Appendix III gives localities and number of specimens).



Appendix IV.G. Relationship of dominant frequency to pulse rate in three samples of Bufo (Appendix III gives localities and number of specimens).



Appendix IV.H. Relationship of dominant frequency to duration in three samples of Bufo (Appendix III gives localities and number of specimens).



Appendix IV.I. Relationship of pulses per second to duration in three samples of Bufo (Appendix III gives localities and number of specimens).

