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METHODS

STUDY SITES

All study sites were located between the towns of Okanagan Falls ($49^{\circ} 21' N$, $119^{\circ} 36' W$) and Oliver ($49^{\circ} 10' N$, $119^{\circ} 37' W$), about 20 km apart in the Okanagan Valley of British Columbia (Figure 3). The physical features and vegetation of this region were described by Woodsworth (1981), and the habitat is typical of semiarid habitats further south (Woodsworth et al., 1981).

The locations of the sites where I monitored the foraging activity of bats are given in Figure 3; and are described in Table 1 and Figure 4. The Okanagan River is part of an irrigation system that is the sole source of water for the extensive agriculture of the valley. At site 20 the river was about 20 m wide and turbulent. At sites downstream (south) from site 20 the river was wider (40 to 60 m), and the waterflow slower and less turbulent. Waterflow in the river is regulated by numerous small weirs. I stood midway across four of these weirs and observed bats foraging upstream and downstream. Near weirs the river was deeper upstream (about 1.5 m) and less turbulent, than downstream (usually < 1 m deep).

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A GENETIC, MORPHOLOGICAL, AND ECOLOGICAL INVESTIGATION
OF A PUTATIVE HYBRID ZONE BETWEEN MYOTIS LUCIFUGUS
AND MYOTIS YUMANENSIS

by

© Robert Maxwell Herd, B.Sc., M.Sc.

A thesis submitted to the Faculty of Graduate
Studies and Research in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Department of Biology
Carleton University
Ottawa, .Canada
January, 1983

The undersigned hereby recommend to the Faculty
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ABSTRACT

In the Okanagan Valley of British Columbia Myotis lucifugus and Myotis yumanensis are sympatric and some individuals are intermediate between the typical morphology of either species. Previous studies in the western United States had suggested that some bats of intermediate appearance could be hybrids. In a survey of the electrophoretic variation at 31 protein loci I did not detect hybrids, supporting their status as distinct biological species. A discriminant analysis using external and skull characters was performed on the same specimens used in the electrophoretic survey. Skull characters gave the better separation of the two species, although using this technique, 13 bats (14% of 93 bats examined) were designated as intermediate and seven bats as putative hybrids. These specimens were not hybrids, and similar morphology reflects overlap of extremes of intraspecific morphological variation.

I observed marked differences in the ecology of the two species. Myotis lucifugus were trapped in the western foothills adjacent to the Okanagan River, and observed foraging in all habitats I visited. Myotis yumanensis were only trapped in the valley,

and preferred to forage over flowing water.

The composition of the diets of M. lucifugus and M. yumanensis changed over the course of this study. There were significant differences in the proportions of insect taxa consumed by the two species, probably associated with differences in the habitats in which each species foraged. There were no agonistic interactions between the two species, each preferentially formed maternity colonies and clustered with conspecifics.

Parturition in M. lucifugus commenced after mid-June and most female bats did not produce offspring until age two years, a reproductive pattern typical of M. lucifugus in northern parts of their range. Parturition in M. yumanensis commenced in early June and their female offspring gave birth during the following summer. This reproductive pattern is typical of bats from a southern latitude, and together with habitat associations, suggest that M. yumanensis did not enter the Okanagan Valley until post-glacial boreal forests (which were probably already occupied by M. lucifugus) had been replaced by the semi-arid habitats presently found in the valley.

ACKNOWLEDGEMENTS

This thesis was energetically supervised by Prof. M. B. Fenton. I also thank the other members of my advisory committee, Dr G. R. Carmody, Dr S. B. Peck, and Dr P. Weatherhead, for their advice and guidance.

Larry Speers shared with me his expertise in electrophoresis and introduced me to the computer, Dr. B. V. Peterson arranged for the identification of bat ectoparasites, and Rowland Thomas provided invaluable statistical advice. Many residents of the Okanagan Valley provided me with information on the habits of local bats and allowed access to their property. I thank all these people.

I especially thank Denise Herd for her support and encouragement, and for her invaluable assistance, in the laboratory, in the field, and in the preparation of this thesis.

Christina Fernandez assisted with the electrophoresis and preparation of museum specimens. Denise Herd, Karen Furlonger, and Shirley Rake were field assistants in the Okanagan Valley. This study was supported by an operating and equipment grant from NSERC to M. B. Fenton.

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INTRODUCTION

Hybrids have long been of interest to biologists, particularly as they are seen as antithetical to the biological species concept. Hybrids may represent incomplete evolution of reproductive isolating mechanisms or a breakdown of reproductive isolation and may occur even between "good species". However, the viability and fertility of hybrids may be reduced or absent (Ferguson, 1980). Hybridization between species with a parapatric distribution (occupying contiguous geographical areas with no or only a narrow zone of overlap) can provide clues to the mechanisms by which new species are formed. The varying levels of genetic and morphological differentiation across hybrid zones challenges our concept of a species, and can be used to infer the time and course of speciation (Futuyma, 1979).

The little brown bat, Myotis lucifugus Le Conte, and the yuma bat, Myotis yumanensis Allen, are sympatric throughout much of the western United States and in southern British Columbia (Figure 1). Over most of their range the two species are morphologically distinct, but in four areas of sympatry (two in the southwestern United States; Harris, 1974; Parkinson, 1979; one in Washington; Barbour and Davis, 1969;

and one in southcentral Brazil Columbia; Woodsworth, 1981) there are bats whose appearance is intermediate between the usual morphology of either species.

Previous studies in the southwestern United States have suggested that some bats of intermediate appearance may result from an overlap of intraspecific variation, or might be evidence of occasional hybridizations (Harris, 1974; Parkinson, 1979).

In these putative hybrid zones the two species may have only recently become sympatric, following range expansions after the retreat of late Pleistocene glaciers, and reproductive isolation may not be complete (Harris, 1974; Parkinson, 1979). These studies used a discriminant analysis of external and skull morphology, and could not achieve a complete separation of the two species. Confirmation of hybridization was not possible using statistical methods, and lack of reproductive isolation was not proven (Parkinson, 1979).

The ecological relationships between M. lucifugus and M. yumanensis remain unclear. Parkinson (1979) noted that the two species are morphologically and ecologically distinct in most areas of sympatry. However, M. l. carissima Thomas and M. y. sociabilis Grinnell were morphologically convergent and ecologically similar in the areas she examined

(northeastern California and southcentral Oregon).

The relationships of these two bats will remain obscure until the extent of the hybridization is determined and they can be separated accurately in the field (Woodsworth, 1981).

This study is a genetic, morphological, and ecological investigation of a putative hybrid zone between M. l. carissima and M. y. sociabilis in the Okanagan Valley of southcentral British Columbia. To determine the extent by which the two forms differ genetically, and if there is any genetic evidence of hybridization, I used the techniques of protein electrophoresis. The extent of morphological variation in the two forms was assessed by morphometric analysis using specimens assigned to either species on the basis of the results from the electrophoresis. I subsequently sought differences in their ecology through intensive trapping and field observations of foraging behaviour.

THE PROBLEM OF FIELD IDENTIFICATION

Throughout their range most bats can be identified using external characters alone. Myotis lucifugus is of medium size, with a forearm length (FA) of 34 to 41 mm and sleek glossy pelage varying in colour from pale tan through reddish brown to dark brown. Myotis yumanensis is slightly smaller (FA 32 to 38 mm) and has shorter and duller fur ranging in colour from light tan to dark brown (Barbour and Davis, 1969). In the Okanagan Valley the pelage of M. lucifugus was often not glossy. However the hairs were usually darker at the base, particularly on the shoulders, than in M. yumanensis; and the skin on the ears and nose-pad of M. lucifugus was darker than that of M. yumanensis (Figure 2). To conduct ecological investigations on the two species, determination of the specific status of the bats was essential. I used a simple antibody test to rapidly identify bats which I could not confidently identify by their external appearance.

The Antibody Test

The basis for the test was the time taken for lysis of erythrocytes in a small sample of blood from the bat in question, using complement fixation with antibodies produced against erythrocytes of M. lucifugus. Erythrocytes of one species display marked antigenic properties when introduced into animals of another species. The antibodies elicited are directed against determinants in the cell membrane. Variations in molecular structure of the proteins, lipids, and carbohydrates in the red cell membranes of different species are responsible for their large spectrum of antigenic determinants (Garvey et al. 1977). The pathway of immune hemolysis and the release of hemoglobin from an erythrocyte following the reaction of specific antibodies with antigenic determinants of the cell membrane is outlined in Garvey et al., 1977.

Antibodies were raised in guinea pigs by giving them intraperitoneal injections of erythrocytes from M. lucifugus. Heparinized blood samples were obtained

from M. lucifugus collected in Ontario. Erythrocytes were separated from plasma by centrifugation and if not used immediately were stored in a solution of trisodium citrate-ethylene glycol (VandeBerg and Johnston, 1977) and washed once with saline prior to use. Erythrocytes from about 50 μ L of blood were resuspended in 2 mL sterile saline for injection via the intraperitoneal route, which in rabbits receiving sheep-erythrocytes results in the highest mean peak titer (Garvey et al., 1977), and were given every second or third day for three weeks. Detectable levels of "anti-lucifugus" antibody were present in the plasma of the guinea pigs after eight weeks. Heparinized blood samples from the guinea pigs were centrifuged and the plasma carefully removed to avoid inclusion of cellular material. This was the only purification step required. Guinea pig plasma containing the "anti-lucifugus" antibodies was stored at -20°C and retained its activity for at least eight months.

In the field two 5 μ L blood samples were taken up in micropipettes from a small incision in the interfemoral vein of the bat being examined. One blood sample was mixed with about 100 μ L saline and served as a control, the other with an equal volume of test solution. The latter was made by diluting guinea pig plasma with saline (usually x20) to produce immune hemolysis of erythrocytes of M. lucifugus in about 10 min. The erythrocytes of M. yumanensis presumably

offer fewer binding sites for the "anti-lucifugus" antibodies and more time is required for their lysis. The actual time taken for lysis of erythrocytes varied with each batch of test solution, but for each batch, the time required for lysis of erythrocytes from M. yumanensis was typically twice that for erythrocytes from M. lucifugus, and there was no overlap in the times for each species. Variation between batches probably reflects variations in antibody titer of plasma from different guinea pigs and from the same guinea pig on different days. I included at least one bat of whose specific identity I was confident, to "calibrate" each test. For twelve tests, the mean of the longest times to hemolysis for erythrocytes from M. lucifugus was 12.2 min (SD = 4.5 min) and differed significantly ($t = 7.5$, $p < 0.001$) from the mean of the shortest times to hemolysis for erythrocytes from M. yumanensis ($X = 19.4$ min, $SD = 6.0$ min). This test did not harm the bat, and was suitable for field use.

During the summer of 1982 I tested 129 of 965 M. lucifugus and M. yumanensis captured. Based on their external appearance I tentatively identified 85 of the tested bats: 75 (88%) of these identifications were confirmed by the test. Many of these tentative identifications were made hurriedly and in the knowledge that they were to be checked by this antibody test. In retrospect I suggest that about 95% of the bats I captured could have been correctly identified on

the basis of their external appearance. Importantly, all tentative identifications made on bats with external appearances typical of either of the two species were confirmed by this test. The assignment of two bats by the antibody test was checked by protein electrophoresis, both had been correctly identified as M. lucifugus.

METHODS

STUDY SITES

All study sites were located between the towns of Okanagan Falls ($49^{\circ} 21' \text{ N}$, $119^{\circ} 36' \text{ W}$) and Oliver ($49^{\circ} 10' \text{ N}$, $119^{\circ} 37' \text{ W}$), about 20 km apart in the Okanagan Valley of British Columbia (Figure 3). The physical features and vegetation of this region were described by Woodsworth (1981), and the habitat is typical of semiarid habitats further south (Woodsworth et al., 1981).

The locations of the sites where I monitored the foraging activity of bats are given in Figure 3; and are described in Table 1 and Figure 4. The Okanagan River is part of an irrigation system that is the sole source of water for the extensive agriculture of the valley. At site 20 the river was about 20 m wide and turbulent. At sites downstream (south) from site 20 the river was wider (40 to 60 m), and the waterflow slower and less turbulent. Waterflow in the river is regulated by numerous small weirs. I stood midway across four of these weirs and observed bats foraging upstream and downstream. Near weirs the river was deeper upstream (about 1.5 m) and less turbulent, than downstream (usually $< 1 \text{ m}$ deep).

STUDY ANIMALS

For my genetic and morphological investigations 57 M. lucifugus carissima and 45 M. yumanensis sociabilis were collected in the Okanagan Valley during the summers of 1980 to 1982. The bats collected included individuals whose external morphology was typical of either M. lucifugus or M. yumanensis, but most bats had external morphologies that I judged at the time of capture to be in some way intermediate between those typical of either species. Thus I expected this series of specimens to include putative hybrids. To establish patterns of genetic variation in M. lucifugus the following bats were used: six M. lucifugus lucifugus Le Conte from New York State and 25 from Ontario. With the exception of one M. l. carissima used in the genetic investigation, all bats were judged to be adults, having fully ossified finger joints which were knobby (Barbour and Davis, 1969; Kunz and Anthony, 1982).

All specimens are deposited in the National Museum of Natural Sciences, Ottawa (catalogue numbers 45901 to 46033). In addition to specimens collected by myself, for the discriminant analysis of morphology I examined specimens collected in the Okanagan Valley and held by Canadian museums: Royal Ontario Museum (Toronto): specimen numbers 20042, 20046, 29465, 29466, 29468, 29498 - 29500.

National Museum of Natural Sciences (Ottawa):

specimen numbers 12776, 12777, 28686, 28688, 28689,
28691, 28693, 28694, 28696 - 28698, 28700, 28701,
28703, 28723, 28725 - 28727, 28731, 28732, 28736,
28739, 28740, 28742, 28744, 28746 - 28748, ML1,
ML3, ML5, ML16, ML70, DBC351 - 357, DBC361, DBC364,
DBC366 - 367, DBC370, DBC373.

Cowan Museum (University of British Columbia, Vancouver):

specimen numbers 473, 474, 548 - 554, 456, 458, 459,
3748, 3749 - 3756, 3758, 3759, 3761 - 3764,
3768, 5422, 5451 - 5454.

GENETIC INVESTIGATIONS

Most of the proteins used in the electrophoretic survey of genetic variation were extracted from the breast muscles of bats I had captured and later sacrificed in the laboratory, or from bats which died during capture and had been promptly frozen (-20°C). The other proteins came from plasma and erythrocytes of bats sacrificed in the laboratory. The latter bats were anaesthetized with ether before exposing the heart and cutting the aorta. Blood was taken up in a heparinized Pasteur pipette, plasma separated from erythrocytes by centrifugation and stored at -60°C . Erythrocytes were washed with saline and stored (-20°C) suspended in two volumes of trisodium citrate-ethylene glycol (VandeBerg and Johnston, 1977).

The enzymes and non-enzymatic proteins of 31 loci were examined using either continuous polyacrylamide gel electrophoresis or horizontal starch electrophoresis (Table 2). Polyacrylamide gel electrophoresis was performed in a vertical gel electrophoresis cell (Model EC570, E-C Apparatus Corporation, Philadelphia, Pennsylvania), run at 400 V for 1.5 to 2.5 h, and cooled by continually circulating refrigerant (-6°C). Starch gel electrophoresis was performed as described by May et al. (1979) employing 15% starch gels (Electrostarch Co., Marri^{son}, Wisconsin), run at

voltages (200 to 250 V) and times (3 to 5 h) predetermined to yield optimal resolution of the proteins under study, and cooled by circulating refrigerant through plates placed on top of the gels.

The crude protein extracts used for polyacrylamide electrophoresis were the supernatants after centrifugation at 15,600 g for five minutes of: breast muscle ground in three volumes of 0.1 M phosphate buffer pH 7.5 containing 0.1 M 2-phenoxyethanol and 0.25 M sucrose; or plasma mixed with an equal volume of above buffer; or washed erythrocytes lysed in three volumes of water with 0.1 mL 2-mercaptoethanol added per 10 mL water. Transferrins were examined in plasma following precipitation of other proteins (Chen and Sutton, 1967). For starch gel electrophoresis muscle was ground in water, centrifuged and the supernatant applied to filterpaper wicks which were then inserted into the gel, whereas erythrocytes were ground onto the paper wicks prior to their application (VandeBerg and Johnston, 1977).

The stains used to visualise the proteins examined are given in Table 3. Loci were considered to be monomorphic if the frequency of their most

common allele was greater than 0.99. Two indices were calculated to quantify genetic divergence between the three populations of M. lucifugus (New York, Ontario, British Columbia) and M. yumanensis from British Columbia: genetic distance, D (Nei, 1972), and genetic similarity, S (Rogers, 1972).

MORPHOLOGICAL INVESTIGATION

The specimens of M. lucifugus and M. yumanensis from the Okanagan Valley from the above genetic investigation were used to evaluate morphological differences between the two species. Only adult bats were used. Based on its genotype, as determined by electrophoresis, each specimen could be unequivocally assigned to M. lucifugus or M. yumanensis, prior to discriminant analysis. For this analysis I took a series of nine external and 11 skull measurements. Six external measurements were taken with a millimeter ruler to the nearest mm: total length (TL); length of tail (TV); hindfoot (HF); ear from notch (EAR); tragus from notch (TRAG); wingspan (WING). Three external measurements (FA; third metacarpal, MC3; fifth metacarpal, MC5) were dry measurements. The average decrease in FA of 64 specimens during drying was 0.17 mm (SD = 0.14) or 0.49% (SD₄ = 0.40). These three lengths and the following skull measurements were taken with dial calipers accurate to 0.05 mm: greatest length of skull (GSL); mastoid breadth (MASTB); breadth of braincase (BB); least interorbital breadth (IB); occipital depth (OD); condylocanine length (CCL); greatest maxillary breadth (MAXB); rostral width (RW); length of the maxillary tooth row (MTL); length of P4M3 series (PML); braincase depth (BD). Above skull

measurements, except BD, are defined by Harris (1974) and van Zyll de Jong (1979); for BD see Figure 5.

A stepwise discriminant analysis (BMD Programme .7M) was performed on the two species, first using all 20 measurements, then only the external measurements and finally only the skull measurements. A discriminant analysis uses the morphometric data to achieve a maximal separation of the two groups. Based on the observed character values multiplied by a series of corresponding weights, scores for each specimen are calculated and projected onto an axis (the canonical variable axis). The BMD Programme used a leave-one-out or "jackknife" procedure, whereby the discriminant analysis was performed leaving one individual out and then the a posteriori probability (P) of membership in either species for that individual was computed. Because there is an equal probability of assigning an individual to either species by chance alone, I considered individuals to be correctly assigned if $P > 0.90$, and designated individuals assigned to either species with $0.90 > P > 0.50$ as morphological intermediates. The three analyses were performed with the sexes combined, and repeated for females only. I had too few males to permit separate discriminant analysis.

ECOLOGICAL INVESTIGATIONS

Differences in the ecology of M. lucifugus and M. yumanensis were investigated by:

(i) intensive trapping of bats to assess distribution, timing of reproduction, diet, and to mark individual bats.

This procedure provided opportunities for miscellaneous observations on behaviour;

(ii) Observations on the number of foraging bats in different habitats throughout the night.

(i) Trapping

I trapped bats using mist-nets (9m x 2m; 38mm mesh), Tuttle traps (Tuttle, 1974), and hand-nets.

Trapping was conducted from 6 May to

25 August 1982, in areas along the Okanagan River

(340 m above mean sea level) and in the western

foothills to 600 m. Captured bats were identified, using the antibody test where necessary, and the following data recorded: bodyweight (to nearest

0.5 g); age (juvenile or adult); FA; and reproductive

condition. Females were judged to be nonparous

(nipples rudimentary and giving rise to body hair)

or parous (having given birth; Racey, 1974).

Parous bats were pregnant (determined by palpation),

lactating (milk could be squeezed from a teat), or

post-lactating (enlarged teat surrounded by bare skin

indicative of recent suckling, no milk expressable). Distended cauda epididymides and enlarged testes were indicative of spermatogenesis in male bats (Racey, 1974). Bats were marked with an individually numbered aluminium bat band (Gey Band and Tag Company, Inc.). Myotis lucifugus were additionally marked with a split celluloid band (A. C. Hughes, England) covered with red Scotchlite (R) reflective tape; males on the right FA, females on the left. Myotis yumanensis were similarly marked using yellow tape, allowing me to subsequently recognise either species and sex of these bats in flight.

Some bats captured at the same site and on the same night, within two hours after sunset, were held in individual plastic cups for two to four hours and their faeces collected and stored in 70% ethanol. For 68% of these bats I analysed 10 faecal pellets, and from those bats which produced fewer than 10 pellets, I analysed from three to nine pellets. Insect remains in the faeces were identified to order, and in the case of Diptera, into Chironomidae and other Diptera, using the method of Belwood and Fenton (1976).

I estimated the number of insects per pellet and the total number of insects of each taxa per sample, and calculated the percentages of each insect taxa in the sample. The diversity of prey items consumed was estimated using the diversity index ($D_v = - \sum_{i=1}^S p_i \cdot \log p_i$) of

Emlen (1973). Conspicuous ectoparasites were collected from some of the above bats, stored in 70% ethanol, and forwarded to the Biosystematics Research Institute, Agriculture Canada, for identification.

The search-phase echolocation calls (Simmons et al., 1979) of known bats (light-tagged; Buchler, 1976) were recorded in the field using a broadband microphone (QMC SM1) and Racal (Lockheed) Store 4D tape recorder operated at 0.76 m. s^{-1} (frequency response to 150 kHz). Calls of similar amplitude were analysed in the laboratory with a Kay Sona Graph model 7029A and Princeton Applied Research model 4513 fast Fourier transform (FFT) spectrum analyser..

(ii) Observations of banded bats

Between 13 June and 19 August 1982 I monitored the foraging activity of M. lucifugus and M. yumanensis at 20 sites located in five areas (Figure 2, Table 1) along the Okanagan Valley. These sites were selected after trapping revealed both species to be in the valley whereas only M. lucifugus was present in the adjacent foothills. Each area enclosed a site on a rocky bluff adjacent to water, at least one site by water and a site in or by forest adjacent to the rocky bluff and water (not at McIntyre Bluff).

I used a hand-held, sealed-beam spotlight (approximately 200,000 candle-power) to observe the bats and was able to identify the species and sex of banded bats. This technique was evaluated by Woodsworth (1981) and I followed his guidelines to minimize disturbance to the bats by the spotlight.

Three assistants and I worked in pairs and simultaneously monitored foraging activity at two sites for 15 min, then allowed ourselves 10 min to move to adjacent sites and commence another 15 min of observations. We repeated this procedure five times throughout the night, beginning 70 min after sunset and finishing 70 min before sunrise, at which times bats were foraging and it was sufficiently dark for us to see reflective bands in the spotlight beam.

The initial and final five minutes of each 15 min period were spent watching bats. In the intervening five minutes we monitored feeding activity with a QMC mini Bat Detector tuned to 40 kHz. We quickly learned to recognize the erratic flight patterns of foraging bats, as distinct from the rapid, straight flight of transient bats. We alternated duties and partners to reduce bias in our observations. Little bias was apparent when on four occasions we independently estimated the number of foraging bats at site 16 (mean = 60, 53, 25, 21 bats) as indicated by the mean CV of our estimates, 14.5% (range: 12 to 16%). For each site and time period we estimated the total

number of foraging bats present and the numbers of banded bats of each species and sex.

Foraging bats were conspicuous at sites by water and we were able to monitor adjacent upstream and downstream sites within the same 15 min period. We limited our range of observation to 50 m, this being about the maximum distance at which we spotted banded bats at sites away from water. At the latter sites bats were less conspicuous and we used the directional sensitivity of the bat detectors to localize bats in these places. Within each area we monitored all the sites in one night, and over four consecutive nights visited all areas (McIntyre Bluff and Gallagher Lake were monitored on the same night). Areas were visited in a randomized order, and the order of visitation to pairs of adjacent sites alternated between successive nights (but constant during any one night). Observations were made during all phases of the moon and under comparable weather conditions; observations were not made during heavy rain or strong winds and we did not commence observations when the air temperature was below 15°C at sunset. When observations could not be completed for an entire night, they were repeated the next night if possible, or alternatively not used in the analysis of observations.

STATISTICAL ANALYSIS

With the exception of tests for "goodness of fit" and independence, based on the G-statistic (Sokal and Rohlf, 1969), all statistical analyses were computed using the June, 1981 revision of BMDP Statistical Software (Department of Biomathematics, University of California, Los Angeles).

A probability (p) of 0.05 or less was required to reject null hypotheses (H_0). Other abbreviations

used are: X (mean); SD (standard deviation);

CV (coefficient of variation, $CV = X/SD$ times 100);

n.s. (not significant).

RESULTS

GENETIC INVESTIGATION

Myotis lucifugus and M. yumanensis were monomorphic at 17 loci, with different alleles fixed at three of the loci (Table 4). There was no evidence of hybridization between M. lucifugus and M. yumanensis and each species could be uniquely characterized on the basis of the alleles present at the monomorphic loci. The three populations of M. lucifugus were genetically similar to each other (mean $S = 0.96$, Table 5), and shared many common alleles which were rare in the genetically distinct population of M. yumanensis (mean S between the species = 0.70).

The sample sizes of populations 2, 3, and 4 (Table 4) were sufficiently large to test if the populations were in Hardy-Weinberg equilibrium at their polymorphic loci. Myotis yumanensis was in Hardy-Weinberg at all 14 polymorphic loci ($G = 0.37$ to 2.2, n.s.). Both populations of M. lucifugus were in Hardy-Weinberg equilibrium at most polymorphic loci ($G = 0.50$ to 3.8 and 0.01 to 3.1 respectively, n.s.), except for ME-1 in population 2 ($G = 6.1$, $p < 0.05$), and ADA ($G = 12$, $p < 0.005$)

6PGD-1 ($G = 5.3$, $p < 0.05$), and PMI ($G = 5.6$, $p < 0.05$) in population 3, with only ADA not approaching equilibrium. Male M. lucifugus ($n = 6$ bats) fixed for the A allele whereas male M. yumanensis ($n = 2$) fixed for the B allele; no male heterozygotes were observed. The females in both populations were in Hardy-Weinberg equilibrium ($G = 3.3$ and 0.72 respectively, n.s.), and I suggest the ADA locus is sex-linked in these species. Hardy-Weinberg equilibrium indicates random mating of bats within each of the populations examined, and that the allelic frequencies will remain unchanged over subsequent generations (Futuyma, 1979).

MORPHOLOGICAL INVESTIGATIONS

Males tended to be smaller than conspecific females (Table 6) but the differences for 19 of the variables measured were not statistically significant in M. lucifugus ($t = 0.03$ to 2.4 , n.s.; BMD Program 3D using separate variances), and only MASTB differed significantly between the sexes ($t = 2.15$, $p < 0.05$). Male M. yumanensis were significantly smaller than females in MC5 ($t = 3.9$, $p < 0.05$), BB ($t = 3.2$, $p < 0.05$), and CCL ($t = 3.3$, $p < 0.05$), but not in other measurements ($t = 0.03$ to 2.4 , n.s.). The ranges of all 20 measurements of conspecific bats of each sex overlapped.

There were significant differences between the two species in the means for five (of nine) external variables measured and nine (of 11) skull measurements (Table 7). Myotis lucifugus was generally larger than M. yumanensis, but the height of the skull (OD, BD) was less than that of M. yumanensis. However, for each of the 20 variables, the range of measurements for individuals of each species overlapped, and no measured variable gave an absolute separation of the two species. The mean CV for the nine external measurements of M. lucifugus was 5.5% and

for M. yumanensis was 5.3%, and for the 11 skull measurements, 2.5% and 2.2% respectively. Most external variables had CVs typical of those for linear dimensions of mammals (Simpson et al., 1960), whereas CVs for most skull measurements were smaller than usual, and agree with Parkinsons (1979) suggestion that members of the genus Myotis are apparently less variable than other mammals, at least in some dimensions.

The discriminant performed were not able to completely separate the specimens into the two species groups (Table 8). Performing the analysis with the sexes combined and using all 20 variables measured, or using only the 11 skull measurements, yielded similar results: 86% of M. lucifugus and 94 or 86% of M. yumanensis were correctly assigned ($P > 0.90$). When repeated for female bats alone, the percentage of correct assignments were similar, suggesting that sexual dimorphism was not responsible for the incomplete separation of the two species. External measurements alone gave a poor resolution of the species: only 27% of M. lucifugus and 26% of M. yumanensis were assigned with $P > 0.90$. Length of the dried forearm and length of tragus were the only external measurements with significant discriminant

ability, but the large overlap of the ranges of each (Table 7) restricts their use in species assignment. Bats with a FA $\leq$ 34.1 mm (34.2 mm measured on fresh specimens) were most likely M. yumanensis ($P > 0.90$). If FA $>$ 35.8 mm (36.0 mm on fresh specimens) the bat was probably M. lucifugus ($P > 0.90$). The larger CVs of external measurements, compared to skull measurements, suggest that they would not be expected to yield a separation of the two species similar to that based on skull measurements.

Bats designated as intermediate based on skull measurements ($P > 0.90$) have a morphological score on the canonical variate axis (Figure 6) between the scores of bats correctly assigned. Thirteen bats (14% of 93 bats examined) were designated as intermediate. Examination of the study-skins of these bats showed that 11 were not intermediate in appearance, but two M. yumanensis had dark pelages similar to those of M. lucifugus. The canonical variable scores for both species approximated a normal distribution (Figure 7), indicating that the bats examined represent random samples from populations of each species.

To identify possible hybrids Parkinson (1979) arbitrarily set $0.60 > P > 0.50$ as a hybrid criterion, by which three percent of the bats she examined were

designated as hybrids, compared to 0.8% (Harris, 1974) and eight percent (seven bats) of my specimens. Most of the 87 specimens of M. lucifugus and M. yumanensis held by Canadian museums, and assigned using the results of my discriminant analysis of skulls, were assigned with $P > 0.90$, but 17% (15 bats) were designated intermediate ($0.90 > P > 0.60$) including one bat (1%) designated as a hybrid ($0.60 > P > 0.50$).

Ratios of variables have been used in an attempt to describe "shape" independent of size (Neff and Marcus, 1980). I performed another discriminant analysis using skull measurements (other than GSL) expressed as a ratio to GSL as additional variables. Separation between the two species was not improved, and in view of the statistical and conceptual difficulties implicit in the use of ratios (Albrecht, 1978), I did not proceed with their use.

ECOLOGICAL INVESTIGATIONS

(i) Trapping

I trapped both species along the Okanagan River and in areas adjacent to it within about 20 m elevation of the river. I trapped M. lucifugus but no M. yumanensis (Table 9) in the adjacent foothills, to elevations of about 20m above the river. The number of bats of each species present in either area was independent of the presence of the other species ($G = 3.4$, n.s.) indicating that the observed distribution of bats was not due to interactions between the two species. Fewer bats were trapped in the foothills reflecting both a disproportionately small amount of trapping-time spent in the foothills after this pattern emerged, and lower levels of bat activity there.

There were significant temporal differences in the reproductive status of females of each species (Table 10, Figure 8). During May, June and August, reproductive status of females in trapping samples differed between species. Major reproductive events (pregnancy and lactation) in most M. yumanensis occurred two weeks in advance of these reproductive events in M. lucifugus. By August all M. yumanensis trapped were parous, whereas throughout August about

one-fifth of adult M. lucifugus trapped were nonparous. Most nonparous M. lucifugus trapped during July had dull pelage, whereas the appearance of parous females were typical of their species.

In June, there was no statistical difference between the species in the proportion of breeding to non-breeding males ($G = 0.07$, n.s.). The proportion of adult males showing evidence of spermatogenesis increased during July and August (Table 10). In July, the proportion of breeding to non-breeding males differed between the species ($G = 95$, $p < 0.005$), which suggests that some male M. yumanensis attain breeding condition before most male M. lucifugus.

Although I could not enter maternity colonies, I placed Tuttle traps outside some of the bats exit holes. During May, bats of both species were unequally distributed between two maternity colonies located in cabins 10 m apart (Table 11). I suspect that some bats not from the cabins were also trapped which reduced the apparent extent of segregation. Ninety one percent of the bats trapped outside a third maternity colony (19 June) were M. lucifugus (Table 11). When a Tuttle trap was set overnight, bats captured had usually arranged themselves in small clusters within the trap-bag by morning. I examined the species composition of seven clusters

trap-bags containing similar numbers of bats of each species. The clusters did not consist of equal numbers of each species ($t = 6$, $p < 0.01$), and on average 80% of the bats in a cluster were conspecifics (range 60 to 100%).

Prey consumed by M. lucifugus and M. yumanensis changed over the course of this study (Table 12 and 13). Faeces collected from adults and juveniles of both species during August revealed significant differences in the proportions of insect taxa consumed by the four classes of bat (Table 14). Trichoptera were the major prey of both species. Adult M. lucifugus consumed more Diptera than adult M. yumanensis, which relied more on Ephemeroptera. Prey selected by juveniles of both species were quite similar to each other and to those of adult M. lucifugus. Juveniles of both species and adult M. lucifugus showed similar diversities of prey consumed, whereas adult M. yumanensis had a less diverse diet. Ectoparasites collected from some bats of each species were similar (Table 15).

There were significant differences in the frequency of emitted sounds with maximum energy, the lowest frequency, and the highest frequency, in the search-phase echolocation calls of M. lucifugus compared to those of M. yumanensis (Table 16). The patterns of frequency change over time of their calls are similar (Figure 9).

(iii) Observations of banded bats

Observations were made at each site for two to five nights prior to 26 July, and throughout three nights after 26 July. Levels of bat foraging activity (banded and unbanded bats) were consistently high (> 60 bats observed per night) at sites 15, 16, 19, and 20, in the Okanagan Falls area (Table 17). At sites by water (sites 2, 5, 6, 8, 9, 14, 15, 16, 17, 18, 20 but not at site 1) I observed moderate (> 20 bats per night) and high levels of activity. Only two land sites (7 and 19) had levels of activity similar to those observed at sites by water. Site 7 was classed a "bluff" site (Table 1) but all bats observed there flew along the irrigation flume present at this site (Figure 3H). Site 19, by a bluff, had high levels of activity as mentioned above.

Foraging bats appeared oblivious of nearby bats. On fewer than five occasions I observed up to four bats pursue the same moth and I did not observe agonistic interactions between the bats.

A distinct change in the foraging behaviour of the bats was noticed on 26 July and persisted through the summer. Bats foraged in different places, such as over trees and high (often more than 10 m) above

water and adjacent fields, and some were observed foraging in pairs. These changes in behaviour occurred just before juvenile bats first appeared in trapping samples of foraging bats (29 July was the first capture of juveniles of both species). Based on this change in behaviour I have divided my observations into two periods for analysis: observations made (a) prior to 26 July and (b) after 26 July.

Foraging activity by banded bats was not constant over the night during early summer. Before 26 July, more bats of both species and both sexes were seen foraging before midnight rather than after (Table 18, Figure 10). Foraging activity was more evenly distributed throughout the night from 26 July onwards. The numbers of bats of each species seen foraging throughout the night were independent of the numbers of bats of the other species, or sex present, both before and from 26 July onwards ($G = 0.62$ to 5.6 , n.s.).

The numbers of bats of each species seen foraging were associated with observation site, both prior to 26 July ($G = 176$, $p < 0.005$) and from 26 July onwards ($G = 81$, $p < 0.005$), indicating that bats of each species were more abundant at some sites than at others.

I considered significant differences between the observed numbers of bats of each species seen foraging at each site, from those expected under the H_0 that

the numbers of bats seen of each species should be the same at all sites, as indicative of site (and habitat) preference. Throughout the summer M. lucifugus preferred to forage over the lake at site 6 whereas M. yumanensis mainly foraged over the irrigation flume at site 7 (Table 19), less than 100 m away. This preference for habitat was more pronounced during early summer, as M. lucifugus were not observed at site 7 until after 26 July. In the Okanagan Falls area, M. yumanensis were more active over the slow-flowing water at sites 15 and 16, and over fast-flowing water at site 20, although preference for the latter site was only significant after 26 July. In the same area M. lucifugus preferred to forage over the bluff at site 19, and after 26 July, more M. lucifugus began to forage over the slow-flowing water at site 16.

The numbers of female bats of each species were associated with site ($G = 140$, $p < 0.005$ before 26 July, $G = 96$, $p < 0.005$ from 26 July onwards), and the patterns of foraging activity for female bats (Table 20), were quite similar to those for the sexes combined (Table 19), and is consistent with both sexes foraging in similar habitats. Too few males were seen to permit analysis of their pattern of foraging activity.

My observations, considered over all sites, support the following generalizations on habitat preference of the two species.

Habitat: over fast-flowing water (site 20)

Both species foraged in this habitat which was preferred only by M. yumanensis. Most M. lucifugus seen at this site in late summer foraged around the ponderosa pines present at this site, while M. yumanensis foraged low over the river. M. lucifugus were more active over an adjacent bluff (site 19).

Habitat: over slow-flowing water (site 1, 2, 5, 8, 9, 15, 16, 17, 18)

Both species foraged in this habitat which was preferred by M. yumanensis. At sites 8 and 9 only M. yumanensis was observed in the early summer, when they were not active on the adjacent bluff (site 10) occupied by M. lucifugus. This segregation weakened after 26 July but these habitat preferences of the two species were still evident. Myotis yumanensis was observed in greater than expected numbers at sites 15 and 16 during the early summer, and continued to preferentially forage at site 15 after 26 July. However at site 16 M. lucifugus became the more common bat after 26 July. As noted above M. yumanensis preferentially foraged throughout the summer over the irrigation flume at site 7. Neither species was more active in the habitat at sites 1, 2, 5, 17, and 18.

Habitat: over lake (sites 6 and 14)

As noted above M. lucifugus were most active over a lake, while M. yumanensis foraged over an adjacent (100 m) irrigation flume at site 7, this site included bluff and ponderosa pine forest. Neither species was more active at site 14.

Habitat: over bluff (sites 4, 7, 10, 12, 19)

Myotis lucifugus forage over bluffs and M. yumanensis do not. At Okanagan Falls, M. lucifugus preferentially foraged over the bluff (site 19) and M. yumanensis foraged mainly over the adjacent Okanagan River (site 20). The few banded bats observed foraging at site 4, 10, and 12, were predominantly M. lucifugus and among the few bats trapped at these sites, two were M. lucifugus; I did not trap M. yumanensis. Bats were not observed on the bluff at site 7, rather they foraged exclusively over the irrigation flume at this site.

Habitat: Forest (sites 3, 7, 11, 13, 20).

Myotis lucifugus were more commonly observed in this habitat than were M. yumanensis, but few bats of either species appeared to use this habitat. During late summer, most M. lucifugus at site 20 foraged among ponderosa pines while all M. yumanensis foraged low over the surface of the water.

SYNOPSIS OF RESULTS

1. There was no genetic evidence of hybridization between M. l. carissima and M. y. sociabilis.
2. Morphological intermediates of each species represent an overlap in intraspecific variation.
3. Myotis yumanensis was only present in the Okanagan Valley, whereas M. lucifugus was present both in the valley and in the adjacent foothills.
4. There were significant temporal differences in the reproductive status of female bats, and at the end of the breeding season all M. yumanensis were parous whereas four-fifths of M. lucifugus were parous.
5. Bats were unequally distributed between maternity colonies, and preferentially clustered with conspecifics.
6. Prey consumed by M. lucifugus and M. yumanensis changed over the course of this study, and in late summer there were significant differences in the proportions of insect taxa in the diets of adults and juveniles, and between the two species.
7. Ectoparasites collected from some bats of each species were similar.
8. The search-phase echolocation calls of the two species differed, but their patterns of frequency change over time were similar.
9. Myotis lucifugus foraged in a greater variety of habitats than did M. yumanensis, the latter bat foraging mainly over flowing water.

DISCUSSION

GENETIC AND MORPHOLOGICAL INVESTIGATIONS

The ranges in FA and GSL of bats I examined from British Columbia were similar to those reported for the two species in a putative hybrid zone in northeastern California and southcentral Oregon (Parkinson, 1979). The measurements overlapped but were generally smaller than those reported for bats from another putative hybrid zone in the southwestern United States (Harris, 1974). They are within the range reported for the two species over their entire geographical distribution (Parkinson, 1979). Males tended to be smaller than conspecific females but the differences were not significant. Parkinson (1979) did not observe a consistent pattern of significant sexual differences among the bats she examined. Significant sexual dimorphism in external measurements (FA, length of head and body) of M. lucifugus and M. yumanensis, contrasted with a lack of sexual dimorphism in skull measurements on the same specimens examined by Williams and Findley (1979).

As in previous studies of these two species (Harris, 1974; Parkinson, 1979), morphometric data did

not permit confident identification of all specimens. Thirteen bats (14% of bats examined) were designated as intermediate in morphology by the discriminant analysis of skull measurements with an a posteriori probability (P) of correct assignment equal to 0.90. Just over one percent of the 354 bats examined by Harris (1974) were designated as intermediates, whereas Parkinson (1979) judged 14% of the 409 specimens she examined as intermediate ($0.90 > P > 0.50$). I found the best discriminator between the two species was MASTB, followed by BD, GSL, MAXB, OD, and RW, whereas Parkinson (1979) identified condylo-premaxillary length (CONP) of her specimens as the most reliable feature. In her study CONP was highly correlated with GSL, suggesting that GSL should also be a good discriminator, as it was in my study. Harris (1974) also observed MASTB to be the best discriminator between the two species.

Bats designated as intermediates may represent individuals from overlapping portions of the intraspecific ranges of variation of the two forms or may be hybrids (Harris, 1974). To identify hybrids Parkinson (1979) arbitrarily set $0.60 > P > 0.50$ as a hybrid criterion, but statistical criteria related to morphology do not confirm hybridization as they do not prove lack of reproductive isolation (Parkinson, 1979). In the present study bats of intermediate

morphology are shown to be the extremes of intraspecific variation (Figure 6). I did not detect any electrophoretic evidence of hybridization between populations of M. lucifugus and M. yumanensis from the Okanagan Valley, British Columbia. Bats from each species fixed for different alleles at three monomorphic loci. The genetic similarity between the two species ($S = 0.70$) is within the range reported for congeneric species of rodents (S : 0.31 to 0.99; Avise, 1974) and bats. The average S between four species of Eptesicus was 0.67 (0.62 to 0.79; Arnold et al., 1980), and between two species of Macrotis 0.60 (0.60 to 0.67; Greenbaum and Baker, 1976). It is well known that the electrophoretic methods employed underestimate true genetic distances (Avise et al., 1980). As I did not find any evidence for gene flow in the samples of M. lucifugus and M. yumanensis I studied, it is highly probable that these forms are reproductively isolated and therefore distinct biological species.

The use of the discriminant analysis of morphology to identify hybrids has been questioned on theoretical and practical grounds (Neff and Smith, 1979). Particularly relevant in the situation involving overlap of intraspecific variation is their observation that discriminant analysis lacks robustness relative to one

of its basic assumptions: the correct a priori assignment of specimens to groups. This lack of robustness negates the method's usefulness for the assignment of unknown specimens where the bounds of the groups are not clear (Neff and Smith, 1979).

There are few studies of morphologically similar animals showing that the results of discriminant analysis are concordant with the genetic structure as revealed by electrophoretic survey of protein variation (Ferguson, 1980). In a recent study of hybridization between races of pocket gophers (Thomomys bottae), Patton et al., (1979) noted that the discriminant analysis of morphometric characters could not identify the actual proportion of hybrids revealed by protein electrophoresis. In an earlier study of hybridization between two species of pocket gopher, Patton (1973) observed generally good concordance between the two methodologies used to detect hybrids, namely karyotypic (genetic) and morphologic. However, without the karyotypic data several individuals would have been incorrectly designated as hybrids (Patton, 1973). The preceding is presented to highlight the need for extreme caution in interpreting intermediates (by discriminant analysis) as evidence of hybridization between morphologically similar species.

Other genetic and morphological characters of M. lucifugus and M. yumanensis which have been studied include the karyotype, morphology of the baculum, and hair structure. The karyotype of 14 species of Myotis from North America, including M. lucifugus and M. yumanensis, are identical (Baker and Patton, 1967).. Bickham (1979) reported the G-band karyotypes of 11 species of North American Myotis: eight species, including M. yumanensis had identical G-band karyotypes. Myotis lucifugus was not examined and I have forwarded specimens of M. lucifugus and M. yumanensis to Bickham for G-band karyotyping. The shape of the baculum of M. l. lucifugus is distinctly different to that of M. y. sociabilis (Krutzsch and Vaughan, 1955). The cuticular scale pattern of hair from all North American species of Myotis are similar, except for M. velifer (Miles, 1965).

ECOLOGICAL INVESTIGATIONS

I observed marked differences in the ecology of the two species. Myotis yumanensis had a more restricted distribution within the study area, and foraged in fewer types of habitat, than M. lucifugus. The two species exhibited differences in diet and reproduction, and displayed behavioural preferences favouring association with conspecifics.

Individual M. lucifugus were trapped in the western foothills adjacent to the Okanagan River, and were observed foraging in all habitats I visited along the valley. By contrast, individual M. yumanensis were only trapped in the valley, and foraged over water, particularly moving water. The species displayed consistent patterns of habitat association, particularly in the areas of Okanagan Falls and Gallagher Lake (see summary in RESULTS).

Observations made during previous studies of these two species in the Okanagan Valley differed from those reported here. Fenton et al., (1980) suggested that both species feed over fast-flowing water but M. yumanensis feed mainly along the banks and near the canopy, and M. lucifugus over midstream. However, these observations were made in two weeks and involved few marked individuals. Woodsworth (1981) did not find

evidence of habitat or temporal preferences for either species, but noted that the extreme morphological similarity of some individuals may have resulted in many incorrect identifications (Woodsworth, 1981, and pers. comm.).

My observations are consistent with what is known of these species in other areas. Myotis lucifugus forages over water, and if water is not close by, it forages in open areas among trees (Barbour and Davis, 1969). In southern Ontario, New Hampshire, and Nova Scotia M. lucifugus feeds initially along margins of lakes and streams, and later in the evening over water. Individuals often vary their hunting patterns (Fenton and Barclay, 1980). Myotis lucifugus was observed to forage in woods, and over and around the periphery of a small lake and ponds in New York (Buchler, 1980). In eastern Missouri, M. lucifugus foraged in a variety of edge and forest situations (La Val et al., 1977). Myotis lucifugus and Myotis yumanensis are associated with relatively large bodies of permanent water in the southwestern United States, but M. lucifugus tends to be common in woodlands and pine forests at middle elevations, whereas M. yumanensis is much more common in lowland deserts and grasslands (Findley, 1969). Barbour and Davis (1969) note that "M. yumanensis seems to be more closely associated with water than any other North American species of bat". Maintenance of

different patterns of habitat usage was passive, I did not observe any agonistic interactions between the species.

The changes in foraging behaviour observed from 26 July onwards coincided with the entry of juvenile bats into the foraging populations. I propose that the different patterns of foraging I observed were attributable to these inexperienced juvenile bats, and present the following observations in support of this proposition:

1. I observed differences in the diet of juvenile and adult bats. Differences in the diet of juvenile and adult M. lucifugus have been interpreted as indicating that juveniles are not as efficient feeders as adults (Belwood and Fenton, 1976; Anthony and Kunz, 1977). However, as these bats are opportunistic feeders (see discussion below), the differences in diet also suggest that juvenile bats, at least during some stage of their development, forage in different places to adult bats and encounter a different array of prey.)

2. In New York, Buchler (1980) observed that juvenile M. lucifugus may fly outside their maternity roosts from age 18 days, but emerge after the adults have left to forage and do not attain adult-like flight and foraging ability until four to six weeks of age.

Patterns of habitat usage by different species of bats may be monitored remotely if the species can be identified by their echolocation calls (Bell, 1980). In the laboratory I was able to detect differences between recorded search phase echolocation calls of M. lucifugus and M. yumanensis, but these differences were not sufficiently large to permit identification of either species in the field. The pattern of frequency change over time provided by a period meter display of search phase echolocation calls is a versatile field method for identifying many species (Fenton and Bell, 1981), but after examining several sequences of calls recorded from known individual M. lucifugus and M. yumanensis, M. B. Fenton and G. P. Bell agreed that the two species could not be routinely identified by this method.

The composition of the diets of M. lucifugus and M. yumanensis changed over the course of this study. These changes appeared to reflect qualitative changes in the composition of the nocturnal insect fauna, as indicated by shifts in the prevalence of insect taxa attracted to my flashlight. In early May I observed very few Trichoptera and at this time Trichoptera formed a negligible portion of the

diet of either species. Ephemeroptera were not observed until mid-July when they became very abundant, and by the end of July they formed a substantial portion of the diets of both species; previously neither species had eaten them.

The statistically significant differences in the proportions of insect taxa in the diets of M. lucifugus and M. yumanensis probably reflect differences in foraging habitat. Both species have similar jaw morphologies suggesting potential similarity in food habits (Freeman, 1981).

Myotis lucifugus foraged in more habitat types than M. yumanensis, and individuals of each species concentrated their feeding in different habitats within the same area. These differences correspond with the greater diversity of prey items in the faeces of M. lucifugus, and the differences in the proportions of insect taxa consumed. Species of Myotis, including M. yumanensis, were opportunistic feeders in Arizona and exploited local insect faunas by preying upon the most abundant insects within the

appropriate size range (Fenton and Morris, 1976; Vaughan 1980). Correspondance between prey selected by M. lucifugus and the insects available in the largest numbers has been demonstrated in southern Ontario and New Hampshire (Belwood and Fenton, 1976; Anthony and Kunz, 1977).

The first lactating M. lucifugus was trapped 21 June suggesting that the first parturition in this species occurred a few days earlier. The last capture of a pregnant M. lucifugus was 4 July. Parturition date for M. lucifugus vary with latitude and also show considerable variation within a colony (Barbour and Davis, 1969). Parturition commences later in M. lucifugus at more northerly latitudes (Schowalter et al., 1969).

Pregnant bats were trapped on 10 July at Pennask Lake, about 90 km north of, and at an elevation 1000 m higher than my study area in the Okanagan Valley. In Alberta parturition commences about 15 June and continues to 15 July (Schowalter et al., 1979; Fujita, in litt.).

Parturition dates reported for M. lucifugus from areas at more southerly latitudes include: southern Ontario 7 June to 5 July (Fenton, 1970); Vermont, 7 June to 10 July (Barbour and Davis, 1969); New Hampshire, 6 June to 6 July (Kunz, in litt.); New York, end of June to mid July (Wimsatt, 1945); northeastern Ohio, 7 June to 17 July (Smith, 1954, fide Fenton, 1970); southern Illinois, 17 May to 12 July; Kentucky, 21 May

to 21 June (Barbour and Davis, 1969).

There is a trend towards nonbreeding by juvenile female M. lucifugus at higher latitudes (Schowalter et al., 1979). In central Alberta the majority of female M. lucifugus do not bear young as yearlings (bats born during the previous summer), and these nonparous yearling bats probably comprise the majority of unsuckled bats (Schowalter et al., 1979). In New England about half the yearling M. lucifugus are nonparous (Davis and Hitchcock, 1965). Further south in Indiana and New Mexico most juvenile M. lucifugus are thought to be reproductively active, as nonparous females were almost entirely absent from maternity colonies (Humphrey and Cope, 1976; O'Farrell and Studier, 1975).

In the Okanagan Valley about one fifth of female M. lucifugus remained nonparous throughout the summer, and during July the appearance of most of these bats differed slightly from that typical of the parous M. lucifugus. I propose that most of these nonparous bats were yearlings, and predict that most of the nonparous bats I trapped and banded this summer will next summer be parous and have a more typical appearance. This proposition is supported by the observation that M. lucifugus banded two years prior to my study, and recaptured from June onwards

(N = 10), were parous and had a typical M. lucifugus appearance.

The first lactating M. yumanensis was trapped on 19 June. However, the majority of births in M. yumanensis must have occurred earlier than in M. lucifugus, indicated by the high proportion of M. yumanensis pregnant (69%) or lactating (21%) during June when most M. lucifugus (58%) still appeared nonparous. The last pregnant M. yumanensis trapped was on 28 June, considerably earlier than the last pregnant M. lucifugus. The preceding observations suggest that the parturition period of M. yumanensis commences before that for M. lucifugus, probably beginning in early June and finishing by the end of the month. To the south, parturition dates reported for M. yumanensis are: California, late May to mid June (Dalquest, 1947, fide Barbour and Davis, 1969); Arizona, mid June (Barbour and Davis, 1969); Texas, late May and early June (Easterla, 1973). The latitudinal variation in commencement of parturition discussed above for M. lucifugus, is not apparent in M. yumanensis.

Throughout August all female M. yumanensis trapped were parous, and this is consistent with observations in the southern part of their range.

Commissaris (1959) reported that all the bats were parous in a sample of 30 M. yumanensis examined in New Mexico on 15 June. In a sample of M. yumanensis examined from a large maternity colony in a Nevada cave on 11 July, all the bats were pregnant or with newborn young (Hall, 1946, fide Barbour and Davis, 1969). The dearth of nonparous female bats late in the summer suggests that juvenile female M. yumanensis are parous during the parturition period following their birth.

There are two reproductive strategies displayed by these bats, which I shall call the southern strategy and the northern strategy. The southern strategy involves earlier parturition, and females bear young in their first year. Myotis lucifugus and M. yumanensis follow the southern strategy in southern parts of their ranges. The northern strategy describes the pattern of reproduction I observed in M. lucifugus. Parturition begins a little later than in the south, and females do not bear young in the parturition period following their birth.

Clearly these two reproductive strategies are not exclusive, but rather represent the two ends of a latitudinal gradient, probably reflecting differences in the number of frost-free days available for

parturition and growth prior to hibernation. Both a later commencement of parturition and an abbreviation of the period of parturition are observed in more northern populations (Schowalter et al., 1979).

Juvenile M. lucifugus entering hibernation weigh less than adults (Fenton, 1970; Schowalter, 1980), and have smaller accumulations of fat than adults (Davis and Hitchcock, 1965; Ewing et al., 1970).

Juveniles with energy reserves inadequate to sustain the rigors of migration and hibernation may succumb. Deferment of reproduction until their second year may reflect allocation of most of their energy reserves to maintenance and survival during their first winter and spring (Burnett and Kunz, 1982). In northern parts of their range most M. lucifugus have entered hibernation by mid September (Fenton, 1970; Schowalter, 1980), whereas at this time in New Mexico M. lucifugus and M. yumanensis were still present at a nursery roost and were gaining body weight and fat reserves (Ewing et al., 1970). In southern parts of their range the longer warm season may permit juveniles to attain sexual maturity in their first autumn.

Assuming that: (i) most adult M. lucifugus females give birth to a single young each summer, and that there is an equal sex ratio among the newborn bats (Humphrey and Cope, 1976);

(ii) yearling females do not give

birth during their first summer, as discussed above;

and (iii) similar winter survival rates for juvenile and adult females;

then by the end of summer the bat population should consist of equal numbers of yearling and adult females. During August I trapped about four times as many adult females as I did yearling females. This discrepancy may indicate that some yearling females produce young, in which case I counted them as being adult bats. That the ability of juveniles to give birth during their first summer may be facultative requires further research. Alternatively, if the survival rate of juvenile bats during their first year is lower than the annual adult survival rate, then relatively fewer yearlings would be observed during the summer. A lower survival rate among juvenile bats supports the suggestion that the accumulation of adequate energy reserves prior to hibernation is a problem for juvenile M. lucifugus, and that deferment of reproduction until their second year may be a response to conserve energy reserves during their first winter. In Indiana and Kentucky, studies based on the recovery of marked individuals have estimated the survival rate of juvenile M. lucifugus females during their first year to be lower than the annual survival rate of adult females after one year of age (Humphrey and Cope, 1976).

At present M. lucifugus is more widespread than M. yumanensis, and in British Columbia M. lucifugus occurs in the boreal forests at higher elevations, and in the drier interior valleys such as the Okanagan Valley. In the Okanagan Valley the reproductive pattern of female M. lucifugus follows the northern strategy described above, a strategy which appears to have evolved in response to the shorter frost-free period of northern latitudes. Myotis yumanensis presently occurs through the western United States into southern British Columbia. In the southwestern United States it is not a bat of boreal forests (Findley, 1969), and presumably did not enter the Okanagan Valley until post-glacial boreal forests (probably already occupied by M. lucifugus) were replaced, about 8,000 years ago, by the semi-arid habitats presently found in the valley (Heusser, 1965; Wright, 1971). The reproductive pattern of female M. yumanensis reflects an origin in a region with a longer warm period, presumably at more southern latitudes. If the two species have only recently become sympatric in the Okanagan Valley, this may in part, account for their morphological similarity. In areas of long-term sympatry in the southwest and Pacific northwest of the United States, these species are morphologically distinct (Parkinson, 1979). Character displacement, at least in skull dimensions, occurs in M. lucifugus and is influenced by the

number of other kinds of Myotis present.
(Findley and Jones, 1967).

The two species were trapped in unequal numbers outside buildings containing maternity colonies. That M. lucifugus and M. yumanensis may roost in different parts of these buildings could not be determined. Most other species of bats occupy separate maternity colonies, although exceptions include species that roost in different parts of the same shelter (Kunz, 1982). The moderate density of human settlement has provided many potential roosting sites for bats in the Okanagan Valley. Recaptures of marked bats, and reports by local residents, suggest that bats often use roosts for only short periods, often only a few days, and that the bats have a repertoire of several roosts. To the south in California, Nevada, and New Mexico, both species occasionally share roosts (Ewing et al., 1970; Parkinson, 1979). Perhaps potential roosts sites for maternity colonies are limited in these areas, for most Nearctic species usually do not share roosts (Humphrey, 1975; Kunz, 1982). Bats preferentially clustered with conspecifics in the bag of Tuttle traps suggesting that although we may sometimes have difficulty distinguishing the two species, the bats recognize their conspecifics, and prefer to be near them.

In his recent review of the feeding ecology of insectivorous bats, Fenton (1982) reported no "convincing evidence that bats specialize by the timing of activity, diet, use of habitat, foraging strategy or morphology". In the present study of two morphologically similar species of bats, most M. lucifugus and M. yumanensis routinely foraged in different habitats. The separation of the two species by habitat preference was not absolute, but did appear sufficient for the two species to encounter and consume different insect prey. Thus their habitat preference could reduce competition between the two species. Studies of jaw morphology (Freeman, 1981) suggested that the two species might be expected to consume similar diets, and a simple trapping survey of the bats present in the Okanagan River valley would not have revealed the observed pattern of habitat use. More detailed ecological studies of other bat species may reveal hitherto unsuspected specializations in feeding ecology. Indeed, the technique I used to monitor foraging bats, by the use of reflective coloured bands and a spotlight, was developed by Woodsworth (1981) who used it to demonstrate that Myotis leibii and Myotis californicus often foraged in different habitats in the Okanagan Valley.

CONCLUSIONS

1. I did not find any genetic evidence of hybridization between M. l. carissima and M. y. sociabilis supporting their status as distinct biological species. Morphological intermediates of each species represent an overlap in intraspecific variation.
2. Myotis lucifugus has broader habitat associations than M. yumanensis, the latter being closely associated with flowing water.
3. Myotis yumanensis forages mainly over flowing water, whereas M. lucifugus forages over quieter water, and among trees and over rocky bluffs. Association with different habitats did not appear to be a result of agnostic interactions between species as none was observed.
4. The two species preferred to associate with their own kind in maternity colonies and clusters.
5. Prey consumed by both species changed over the summer, and the diets of the two species differed in the relative proportions of insect taxa consumed, presumably due to foraging in different habitats.

6. The search phase echolocation calls of the two species differ, but not sufficiently to facilitate routine identification of the species in the field by a period meter display of their search phase echolocation calls.

7. Puberty in M. yumanensis commences in early June and females reach sexual maturity in their first year. Myotis lucifugus give birth later than M. yumanensis, and the females do not give birth until their second summer.

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Table 1. Descriptions of observation sites at which the foraging activity of bats was monitored. Sites are grouped according to habitat, for locations see Figure 3, photographs of sites are given in Figure 4.

Site Description

Habitat: over fast-flowing water

- 20 Observations were made from west bank. River was bordered by rocky bluff (west bank, see site 19) and agricultural land (east bank). Ponderosa pine (Pinus ponderosa) and willows (genus Salix) were common downstream, as were the shrubs: sumac (Rhus glabra); greasewood (Sarcobatus vermiculatus); and Saskatoon berry (Amelanchier cusickii).

Habitat: over slow-flowing water

- 15,16 Upstream (15) and downstream (16) from a weir. Upstream bordered by willows, water birch (Betula occidentalis) and cattails (Typha latifolia). Downstream bordered by agricultural land; few trees or shrubs along banks.
- 17,18 Upstream (17) and downstream (18) from a weir. River bordered by agricultural land; few trees or shrubs along riverbanks.
- 1,2 Upstream (1) and downstream (2) from a weir. River bordered by agricultural land; few trees or shrubs along riverbanks.
- 5 Observations were made from the west bank. River bordered by agricultural land; few trees or shrubs along riverbanks.
- 8,9 Upstream (8) and downstream (9) from a weir. Upstream the west bank bordered mainly by water birch, downstream by by northern black cottonwood (Populus trichocarpa). East bank bordered by agricultural land; few trees or shrubs along bank.

continued . . .

Table 1 Cont.

Habitat: over lakes

- 14 Vaseux Lake. Observations were made from shore, on bats foraging over this shallow (about 1m deep) lake. Water birch, willows, and cattails along shoreline.
- 6 Gallagher Lake is a deep lake with a steep rocky bluff bordering its eastern shore. Observations were made from western shore. Shore was lined by scattered ponderosa pine, willows, and cottonwood.

Habitat: bluffs

- 19 Bluff was a 60 to 80° slope 120m high and 200m wide. Observations on bats foraging on the bluff were made from a road cut into the steep hillside 10m above the Okanagan River. The slope is a mosaic of rocky outcrops, small ponderosa pines, greasewood, Saskatoon berry, and redstem ceanothus (Ceanothus sanguineus).
- 4 Observations were made on bats foraging on the steep hillside above a road 10m above the Okanagan River. The slope was gentler and had fewer rocky outcrops than at site 19. Dominant trees were Douglas fir (Pseudotsuga menziesii) and Douglas maple (Acer glabrum var. douglasii), and the shrubs redstem ceanothus and Saskatoon berry were common.
- 12 Talus slope (30° incline and 150m long) at base of a 50m cliff. Observations were made from halfway up slope. A few Saskatoon berry shrubs were present.
- 10 Talus slope (40° incline and 100m long) at base of a 100m high rock face. Observations were made from halfway up slope. Sparse vegetation was dominated by Douglas maple and redstem ceanothus.
- 7 Observations were made at the base of a talus slope (40° incline and 50m long) below a 100m high rock face. An irrigation flume (2m wide) cut across the base of the slope. At the base of the slope ponderosa pine was the dominant tree; Douglas maple, sumac, and Saskatoon berry were common shrubs.

continued . . .

Table 1 Cont.

Habitat: forest

- 20 This site (see above) included a strand of ponderosa pine.
- 3 Observations were made at northern edge of a strand of trees at the base of a steep slope (site 4 above), 20m west of Okanagan River. Most common trees were northern black cottonwood and water birch.
- 13 A gully in an area of grassland and scattered ponderosa pines, at the base of a 20m cliff.
- 11 Open ponderosa pine forest at base of talus slope (site 12 above).
- 7 This site (see above) bordered a ponderosa pine forest.

Table 2. Proteins and electrophoretic conditions employed in surveying 31 loci in Myotis lucifugus and M. yumanensis.

Tissues are breast muscle (M), plasma (P), and erythrocytes (E). Rf = relative anodal mobility of alleles. Gels were either polyacrylamide (A) or starch (S). Buffers were: CM - Clayton and Tretiak (1972); R - Ridgeway et al. (1970); TC = 1 L contained 2.77 g Tris, 1.10 g citric acid, pH 8.0; TBE = 1 L contained 9.83 g Tris, 0.76 g boric acid, 0.56 g disodium ethylenediamine tetraacetate, pH 9.0. Where indicated 10 mg of cofactor was added per gel and per 1 L electrode buffer (NAD = nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate).

<u>Protein</u>	<u>Tissue</u>	<u>Locus</u>	<u>Allele</u>	<u>Rf</u>	<u>Gel</u>	<u>Buffer</u>
Adenosine deaminase	M	ADA	A	100	S	R
			B	120		
Alcohol dehydrogenase	M	ADH	A	-100	6%A	TC+NAD
			B	-70		
Adenylate kinase	M	AK	A	100	7%A	TBE
Aspartate aminotransferase	M	AAT	A	10	S	R
			B	100		
Creatine kinase	M	CK	A	100	S	R
Diaphorase	M	DIA	A	100	S	R
Esterase	P	EST-1	A	100	7%A	TC
			B	90		
	M	EST-2	A	100	7%A	TC
			B	80		

continued . . .

Table 2 Cont

Protein	Tissue	Locus	Allele	Rf	Gel	Buffer
General protein	P	GP-1	A	100	7% A	TBE
	M	GP-2	B	100	7% A	TBE
				120		
Glucose-6-phosphate dehydrogenase	E	G6PD-1	A	100	S	R+NADP
			B	80		
	M	G6PD-2	A	100	S	CM+NADP
			B	80		
	M	G6PD-3	A	-100	S	CM+NADP
Haemoglobin	E	Hb	A	100	S	R+NADP
Isocitrate dehydrogenase	M	IDH-1	A	100	S	R+NADP
	M	IDH-2	A	-100	S	R+NADP
Lactate dehydrogenase	M	LDH-1	A	100	S	CM+NADP
			B	140		
	M	LDH-2	A	-100	S	CM+NADP
Malate dehydrogenase	M	MDH-1	A	100	S	CM+NADP
	M	MDH-2	A	-100	S	CM+NADP
Malic enzyme	M	ME-1	A	100	S	R
			B	160		
	M	ME-2	A	-100	S	R
6-Phosphogluconate dehydrogenase	M	6PGD-1	A	100	S	CM+NADP
			B	70		
	M	6PGD-2	A	-100	S	CM+NADP
Phosphoglucose isomerase	M	PGI-1	A	-100	S	CM+NADP
			B	-150		
	M	PGI-2	A	100	S	CM+NADP
			B	80		
			C	120		

Continued . . .

Table 2 Cont.

Protein	Tissue	Locus	Allele	Rf	Gel	Buffer
Phosphoglucomutase	M	PGM	A	100	S	R
			B	140		
Phosphomanno- isomerase	M	PMI-1	A	90	S	CM+NADP
			B	100		
			C	110		
	M	PMI-2	A	-100	S	CM+NADP
Superoxide dismutase	M	SOD	A	100	S	CM+NADP
			B	300		
Transferrin	P	Trf	A	100	7%A	TBE

Table 3. Stains used to visualize proteins examined in Myotis lucifugus and M. yumanensis.

Quantities are for 50 mL of stain solution. Where indicated, MTT (10 mg) was used in place of NBT. $MgCl_2$ (100 mg) was included in stains using NADP. Agar overlay indicates that the stain was prepared as a 1% agar overlay.

ADP = adenosine diphosphate; MTT = 3-(4,5-Dimethylthiazol-2)-2,5-diphenyltetrazolium bromide; NADH = B-dihydropyridine

nucleotide, reduced form; NBT = p-nitro blue tetrazolium;

PMS = phenazine methosulfate; other abbreviations are given in Table 2.

<u>Protein</u>	<u>NBT/PMS</u> (10mg/2mg)	<u>Cofactor</u> (10mg)	<u>Stain buffer</u> (Tris-HCl)	<u>Other components</u>
ADA	MTT/PMS		0.05M, pH8.5	20 mg Arsenic acid 30 mg Adenosine 3 μ Nucleoside phosphorylase 1.5 μ Xanthine oxidase Agar overlay
ADH	X	NAD	0.05M, pH8.5	1 mL Ethanol 1 mL 2-Propanol
AK	X	NADP	0.05M, pH8.0	50 mg ADP 100 mg Glucose 250 μ Hexokinase 100 μ G6PD
AAT				May et al. (1979)
CK	X	NADP	0.05M, pH8.5	25 mg Phosphocreatine 10 mg Glucose 10 mg ADP 30 μ Hexokinase 30 μ G6PD Agar overlay
DIA			0.05M, pH8.5	5 mg NADH 10 mg MTT 1 mg 2,6-Dichloro-phenolindol phenol Agar overlay

continued . . .

Table 3 Cont.

Protein	NBT/PMS	Cofactor	Stain buffer	Other components
EST				50 mg Fast garnet GBC salt dissolved in 50 mL 0.1M phosphate buffer pH6.1
				20 mg α -naphthyl-acetate in 2 mL 1:1 of acetone and water
GP				0.25% Coomassie brilliant blue R in 5:5:1 of methanol:water: acetic acid
G6PD	X	NADP	0.04M, pH8.0	200 mg Glucose-6-phosphate
Hb				No stain required
IDH	X	NADP	0.04M, pH8.0	100 mg DL-isocitric acid
LDH	X	NAD	0.04M, pH8.5	5 mL 1M DL-Na-lactate
MDH	X	NAD	0.04M, pH8.5	5 mL 1M DL-Na-malate
ME	X	NADP	0.05M, pH8.5	10 mL 1M DL-Na-malate
6PGD	X	NADP	0.04M, pH8.0	100 mg 6-Phosphogluconic acid
PGI	MTT/PMS	NADP	0.05M, pH8.0	25 mg D-fructose-6- phosphate
				10 μ G6PD
				Agar overlay
PGM	X	NADP	0.04M, pH8.0	200 mg α -D-glucose-1- phosphate
				1 mg α -D-glucose-1,6- diphosphate
				50 μ G6PD
PMI	MTT/PMS	NADP	0.05M, pH8.0	20 mg D-mannose-6- phosphate
				50 μ PGI
				30 μ G6PD
				Agar overlay
SOD	X	NAD	0.05M, pH8.0	Additional 25 mg NBT and 2 mg PMS
Trf				Stained as per GP

Table 4. Allelic frequencies for 31 loci of Myotis lucifugus and M. yumanensis. Numbers in parentheses indicate the number of bats examined differed from N.

	<u>M. lucifugus</u>			<u>M. yumanensis</u>
	New York	Ontario	British Columbia	British Columbia
Population	1	2	3	4
Sample size, N	6	25	57	45

1. Loci monomorphic in M. lucifugus and M. yumanensis

Locus Allele

AK	A	1.00	1.00	(54) 1.00	(44) 1.00
AAT	A	1.00	1.00	1.00	-
	B	-	-	-	1.00
CK	A	1.00	1.00	(55) 1.00	(39) 1.00
DIA	A	1.00	1.00	1.00	1.00
GP-1	A	(0) -	1.00	(32) 1.00	(29) 1.00
G6PD-3	A	1.00	(17) 1.00	1.00	1.00
Hb	A	(0) -	(19) 1.00	(33) 1.00	(29) 1.00
IDH-1	A	1.00	1.00	1.00	1.00
IDH-2	A	1.00	1.00	1.00	1.00
LDH-2	A	1.00	1.00	(38) 1.00	(30) 1.00
MDH-1	A	1.00	1.00	(55) 1.00	(39) 1.00
MDH-2	A	1.00	1.00	(55) 1.00	(39) 1.00

continued . . .

Table 4 Cont.

Population	1	2	3	4
Locus Allele				
ME-2	A	1.00	1.00	(38) 1.00
6PGD-2	A	1.00	1.00	(56) 1.00
PGI-1	A	1.00	1.00	-
	B	-	-	1.00
PMI-2	A	1.00	1.00	1.00
SOD	A	1.00	1.00	-
	B	-	-	1.00

2. Loci coding for polymorphic proteins

ADA	A	1.00	(18) 1.00	(45) 0.79	(31) 0.06
	B	-	-	0.21	0.94
ADH	A	0.75	0.64	(39) 0.63	(34) -
	B	0.25	0.36	0.37	1.00
EST-1	A	(0) -	0.62	(17) 0.68	(12) 1.00
	B	-	0.38	0.32	-
EST-2	A	1.00	(22) 0.93	(46) 0.84	(29) 0.14
	B	-	0.07	0.16	0.86
GP-2	A	0.92	0.84	(55) 0.92	(44) 0.91
	B	0.08	0.16	0.08	0.09
G6PD-1	A	(0) -	(4) 0.88	(21) 0.79	(10) 0.60
	B	-	0.12	0.21	0.40
G6PD-2	A	0.33	(17) 0.65	(47) 0.77	(40) 0.74
	B	0.67	0.35	0.23	0.26

continued . . .

Table 4 Cont.

Population		1	2	3	4
Locus	Allele			(38)	(30)
LDH-1	A	0.75	0.84	0.37	-
	B	0.25	0.16	0.63	1.00
ME-1	A	0.08	0.22	0.33	-
	B	0.92	0.78	0.67	1.00
6PGD-1	A	0.83	0.94	0.78	1.00
	B	0.17	0.06	0.22	-
PGI-2	A	0.83	0.82	0.74	0.99
	B	0.17	0.18	0.26	-
	C	-	-	-	0.01
PGM	A	0.92	0.94	0.91	0.35
	B	0.08	0.06	0.09	0.65
PMI-1	A	-	-	0.02	1.00
	B	1.00	1.00	0.96	-
	E	-	-	0.02	-
Trf	A	(0)	0.78	0.91	0.91
	B	-	0.22	0.08	0.09
	C	-	-	0.01	-

Table 5. Genetic distance (D) and genetic similarity (S) between populations of Myotis lucifugus and M. yumanensis.

Values above diagonal are D, below are S. Number of loci used in calculating D and S is indicated by superscripts.

Population	<u>M. lucifugus</u>			<u>M. yumanensis</u>
	New York	Ontario	British Columbia	British Columbia
	1	2	3	4
1	-	0.01 ²⁶	0.02 ²⁶	0.36 ²⁶
2	0.96	-	0.01 ³¹	0.30 ³¹
3	0.93	0.95	-	0.26 ³¹
4	0.67	0.70	0.72	-

Table 6. External and skull measurements of female and male Myotis lucifugus and M. yumanensis from British Columbia. All measurements are in millimeters. Abbreviations are explained in text.

		<u>M. lucifugus</u>			<u>M. yumanensis</u>		
		female		male	female		male
				7			5
no. of bats	45	X	Range	X	X	Range	X
TL	83.0		77-90	82.6	81.3	72-85	78.8
TV	36.8		33-41	37.1	36.1	31-40	35.4
HF	9.0		8-11	9.1	8.8	7-10.5	8.9
EAR	14.4		12-17	14.6	14.2	12-16	13.8
TRAG	7.8		4.5-9.5	7.9	7.8	4.5-9	7.9
FA	35.7		34.1-37.9	35.2	34.6	32.2-35.8	34.0
WING	244		228-265	240	238	222-249	234
MC3	33.1		31.2-33.5	32.9	32.4	31.0-33.7	31.7
MC5	30.9		29.0-33.0	30.4	30.4	29.0-31.9	29.8
GSL	13.9		13.2-14.5	13.9	13.6	12.9-14.4	13.4
MASTB	7.45		7.15-7.75	7.39	7.24	6.95-7.45	7.16
BB	7.05		6.70-7.45	7.05	7.02	6.75-7.25	6.91
IB	3.75		3.40-4.10	3.78	3.70	3.50-3.90	3.74
OD	4.89		4.55-5.15	4.86	5.05	4.65-5.25	4.99

continued . . .

Table 6 Cont.

	female		male		female		male	
	X	Range	X	Range	X	Range	X	Range
CCL	12.40	11.90-13.00	12.27	11.90-12.45	12.12	11.60-12.60	11.86	11.65-12.05
MAXB	5.53	5.25-5.90	5.50	5.25-5.7	5.37	5.05-5.55	5.25	5.15-5.45
RW	3.51	3.30-3.65	3.60	3.45-3.75	3.40	3.20-3.55	3.37	3.30-3.45
MTL	5.15	4.95-5.50	5.10	5.05-5.35	5.03	4.75-5.20	4.98	4.90-5.05
PML	3.67	3.50-4.00	3.70	3.60-3.70	3.59	3.40-3.75	3.55	3.45-3.60
BD	6.05	5.75-6.35	6.06	5.95-6.25	6.20	5.90-6.65	6.27	6.05-6.45

Table 7. External and skull measurements of 52 Myotis lucifugus and 42 M. yumanensis from British Columbia. All measurements are in millimeters. Abbreviations are explained in text.

	<u>M. lucifugus</u>		<u>M. yumanensis</u>		t-value
	X	Range	X	Range	
TL	82.8	77-90	81.0	72-85	3.1, $p < 0.05$
TV	36.8	33-41	36.0	31-40	1.9, n.s.
HF	9.0	8-11	8.8	7-10.5	1.5, n.s.
EAR	14.4	12-17	14.2	12-16	1.3, n.s.
TRAG	7.8	4.5-9.5	7.9	4.5-10	0.3, n.s.
FA	35.67	33.90-37.90	34.50	32.20-35.80	6.6, $p < 0.001$
WING	244	224-265	238	222-249	4.0, $p < 0.001$
MC3	33.14	31.3-35.55	32.33	31.00-33.75	4.6, $p < 0.001$
MC5	30.87	29.05-33.89	30.35	29.00-31.90	3.1, $p < 0.005$
GSL	13.90	13.25-14.55	13.55	12.95-14.45	5.5, $p < 0.001$
MASTB	7.44	7.15-7.75	7.23	6.90-7.45	7.7, $p < 0.001$
BB	7.05	6.70-7.45	7.01	6.75-7.25	1.6, n.s.
IB	3.75	3.40-4.10	3.71	3.50-3.90	1.7, n.s.
OD	4.88	4.55-5.15	5.04	4.65-5.25	5.7, $p < 0.001$
CCL	12.38	11.90-13.00	12.09	11.60-12.60	5.7, $p < 0.001$
MAXB	5.53	5.25-5.90	5.35	5.05-5.55	6.1, $p < 0.001$
RW	3.52	3.30-3.75	3.40	3.20-3.55	7.4, $p < 0.001$
MTL	5.16	4.95-5.50	5.03	4.75-5.20	5.3, $p < 0.001$
PML	3.66	3.50-4.00	3.58	3.40-3.75	3.9, $p < 0.001$
BD	6.05	5.75-6.35	6.21	5.90-6.65	4.9, $p < 0.001$

Table 8. Number of bats correctly assigned (a posteriori probability, $P > 0.90$) in discriminant analyses of Myotis lucifugus and M. yumanensis from British Columbia.

Variables in parentheses are those chosen by the analysis for entry into discriminant functions, in order of decreasing discriminant ability (U-statistic).

	<u>M. lucifugus</u>			<u>M. yumanensis</u>		
	N in	P > 0.90		N in	P > 0.90	
	sample	N	%	sample	N	%
Analysis based on:						
20 measurements of morphology						
Sexes combined	50	43	86	35	33	94
(MASTB, BD, MTL, OD, MAXB, FA)						
Females	43	36	84	31	29	94
(MASTB, BD, MTL, OD, FA, MAXB)						
Nine external measurements						
Sexes combined (FA, TRAG)	51	14	27	35	9	26
Females (FA, TRAG)	44	13	30	31	6	19
11 skull measurements						
Sexes combined	51	44	86	42	36	86
(MASTB, BD, GSL, MAXB, OD, RW)						
Females	44	37	84	37	31	84
(MASTB, BD, GSL, MAXB, OD)						

Table 9. Composition of bats trapped in the Okanagan Valley and in the adjacent foothills, during the spring and summer of 1982.

	<u>Myotis lucifugus</u>			<u>M. yumanensis</u>		
	Females	Males	Both sexes	Females	Males	Both sexes
A. In Okanagan Valley						
	453	99	552	221	26	247
(57	12	69		28	3	31) percent
B. In foothills						
	24	5	29	0	0	0
(83	17	100		0	0	0) percent

Table 10. Reproductive status of Myotis lucifugus and M. yumanensis trapped in the Okanagan Valley of British Columbia during the spring and summer of 1982.

	Females				Males			
	Number trapped	Non-parous	Pregnant	Lactating	Post-lactating	Number trapped	Non-breeding	Breeding
May								
<u>M. lucifugus</u>	155	99	1	0	0	14	100	0
<u>M. yumanensis</u>	160	94	6	0	0	3	100	0
June								
<u>M. lucifugus</u>	245	58	40	2	0	55	96	4
<u>M. yumanensis</u>	159	10	69	21	0	16	94	6
July								
<u>M. lucifugus</u>	116	18	1	75	6	51	67	33
<u>M. yumanensis</u>	130	3	0	77	20	20	45	55
August								
<u>M. lucifugus</u>	22	23	0	27	50	13	38	62
<u>M. yumanensis</u>	4	0	0	50	50	2	50	50

Table 11. Composition of bats trapped outside maternity colonies.

A. Two adjacent cabins near Gallagher Lake, British Columbia.

	North cabin	South cabin
<u>Myotis lucifugus</u>	36	56
<u>M. yumanensis</u>	18	117

Bat species are associated with cabins ($G=44$, $p < 0.005$)

B. House by Vaseaux Lake, British Columbia.

	Females	Males
<u>M. lucifugus</u>	91	4
<u>M. yumanensis</u>	9	1

2 OF / DE 2

Table 12. Diet of adult Myotis lucifugus in the Okanagan Valley of British Columbia during the spring and summer of 1982.

Values represent percentages of each insect taxa recovered in monthly faecal samples. Frequency of insect taxa in faeces was associated with date ($G = 460$, $p < 0.005$).

Date	May 7	July 4	July 30	August 5
Insects				
Coleoptera	0	0	0.4	0
Lepidoptera	2.3	1.3	1.9	1.2
Trichoptera	1.1	54	62	35
Ephemeroptera	0	0.3	22	3.6
Diptera				
Chironomidae	82	20	7.2	20
Other	15	25	6.4	40
No. bats in sample	8	24	15	15

Table 13. Diet of adult Myotis yumanensis in the Okanagan Valley during the spring and summer of 1982.

Values represent percentages of each insect taxa recovered in the monthly faecal samples. Frequency of insect taxa in faeces was associated with date ($G = 460$, $p < 0.005$).

Date	May 7	July 4	July 30
Insects			
Coleoptera	0	3.5	0.3
Lepidoptera	0	2.4	0
Trichoptera	0	72	62
Ephemeroptera	0	0	32
Diptera			
Chironomidae	76	4.5	0.7
Other	24	18	5.1
No. bats in sample	4	7	14

Table 14. Diet of Myotis lucifugus and M. yumanensis in the Okanagan Valley during the summer of 1982.

Values represent percentages of each insect taxa recovered in faecal samples from bats trapped 30 July to 22 August. Frequency of insect taxa in faeces was associated with class of bat ($G = 95, p < 0.005$).

Insects	<u>M. lucifugus</u>		<u>M. yumanensis</u>	
	Adult	Juvenile	Adult	Juvenile
Coleoptera	0.2	0	0.3	0
Lepidoptera	1.5	0	0.3	0.2
Trichoptera	50	42	60	34
Ephemeroptera	12	8	30	10
Diptera				
Chironomidae	14	20	3	23
Other	25	30	6	32
Diversity, D_v	0.57	0.54	0.43	0.57
No. bats	30	6	15	21

Table 15. Ectoparasites of 14 Myotis lucifugus and 10 M. yumanensis from the Okanagan Valley, British Columbia.

Ectoparasite	No. of bats with parasite	
	<u>M. lucifugus</u>	<u>M. yumanensis</u>
<u>Basilia forcipata</u> Ferris (Diptera: Nyteribiidae)	12	4
<u>Cimex pilosellus</u> Horvath (Hemiptera: Cimicidae)	1	0
<u>Spinturnix bakeri</u> Rudnick (Acarina: Spinturnicidae)	1	4
<u>Neotrombicula</u> sp. (Acarina: Trombiculidae)	1	0

Table 16. Characteristics of the search phase echolocation calls of Myotis lucifugus and M. yumanensis.

Data were obtained by FFT analysis of recorded echolocation calls; values are means with ranges (in parentheses) of machine-averaged spectra for 16 calls from each of N bats.

Species	<u>M. lucifugus</u>	<u>M. yumanensis</u>	
N	8	6	
	(kHz)	(kHz)	
Frequency with maximum energy	45.3 (42.6-48.3)	55.7 (51.6-58.8)	t=5.6, p < 0.001
Lowest frequency	33.5 (29.3-37.0)	38.5 (34.7-42.1)	t=3.4, p < 0.01
Highest frequency	79.2 (60.6-91.7)	87.6 (72.2-97.5)	t=4.5, p < 0.001

Table 17. Numbers of foraging bats observed at twenty sites in the Okanagan Valley during the Summer of 1982.

Site	No. nights of observation	Total no. bats seen	Bats seen per night	
			mean	SD
South Weir area				
1	7	61	9	11
2	7	282	40	28
3	6	59	10	8
4	7	63	9	7
5	6	155	26	14
Gallagher Lake area				
6	7	271	39	20
7	7	157	22	15
McIntyre Bluff area				
8	5	103	21	17
9	5	160	32	17
10	5	43	9	6
Vaseaux Lake area				
11	7	83	12	11
12	7	34	5	6
13	8	37	5	7
14	8	272	34	23
Okanagan Falls area				
15	8	704	88	49
16	8	1365	171	82
17	7	235	34	18
18	7	202	29	16
19	8	555	69	59
20	7	606	87	56

Table 18. Mean number of banded bats observed foraging throughout the night. Time III corresponds to midnight. Data are mean (SD) number of bats seen per night during 78 nights of observation prior to 26 July and during 60 nights after 26 July. H_0 tested is that the number of bats observed was constant over the five sampling times.

	Time of night					
	I	II	III	IV	V	H _O
A. Sexes combined						
<u>Myotis lucifugus</u>						
prior to 26 July	0.81 (1.25)	0.58 (0.96)	0.43 (0.88)	0.29 (0.69)	0.31 (1.12)	G = 28, p < 0.005
26 July onwards	0.73 (1.18)	0.78 (1.43)	0.90 (1.26)	0.62 (1.03)	0.65 (1.13)	G = 3.7, n.s.
<u>M. yumanensis</u>						
prior to 26 July	1.23 (1.88)	0.91 (1.37)	0.91 (1.43)	0.74 (1.11)	0.69 (1.17)	G = 15, p < 0.005
26 July onwards	0.72 (0.98)	0.75 (1.16)	0.90 (1.43)	0.76 (1.31)	0.71 (1.06)	G = 1.7, n.s.
B. Females						
<u>M. lucifugus</u>						
prior to 26 July	0.71 (1.15)	0.56 (0.94)	0.38 (0.87)	0.25 (0.54)	0.29 (1.01)	G = 27, p < 0.005
26 July onwards	0.70 (1.17)	0.57 (0.93)	0.68 (1.00)	0.52 (0.91)	0.55 (1.11)	G = 2.7, n.s.

continued . . .

Table 18 Cont.

	I	II	III	IV	V	H _O
<u>M. yumanensis</u>						
prior to 26 July	1.03 (1.53)	0.81 (1.19)	0.75 (1.16)	0.62 (0.90)	0.60 (1.05)	G = 12, p < 0.01
26 July onwards	0.57 (0.85)	0.55 (0.87)	0.77 (1.14)	0.63 (1.06)	0.57 (0.89)	G = 3.0, n.s.
C, Males						
<u>M. lucifugus</u>						
prior to 26 July	0.09 (0.29)	0.03 (0.16)	0.05 (0.22)	0.04 (0.34)	0.03 (0.16)	no test - too few observations
26 July onwards	0.03 (0.18)	0.22 (0.67)	0.20 (0.55)	0.10 (0.35)	0.10 (0.35)	G = 12, p < 0.05
<u>M. yumanensis</u>						
prior to 26 July	0.21 (0.57)	0.10 (0.31)	0.16 (0.49)	0.12 (0.36)	0.09 (0.35)	G = 4.9 n.s.
26 July onwards	0.15 (0.40)	0.20 (0.23)	0.13 (0.47)	0.13 (0.43)	0.15 (0.40)	G = 1.1, n.s.

Table 19. Banded bats observed per night foraging at twenty sites in the Okanagan Valley during the summer of 1982. No test indicates too few bats were seen for statistical test.

26 July onwards

Before 26 July.

Site	<u>M. lucifugus</u>			<u>M. yumanensis</u>			<u>M. lucifugus</u>			<u>M. yumanensis</u>			Differed from expected
	mean	SD		mean	SD		mean	SD		mean	SD		
1	1.2	2.7		1.2	2.2	no test	0	0		0.7	1.8		no test
2	6.2	5.6		7.7	5.7	G=1.9, n.s.	3.7	3.0		3.7	4.0		G=0.0, n.s.
3	0	0		0	0	no test	0	0		0.3	1.3		no test
4	0	0		0	0	no test	0.3	1.3		0	0		no test
5	2.7	2.6		2.0	2.5	G=0.9, n.s.	3.3	4.5		2.0	3.2		G=0.6, n.s.
6	14	9.4		3.2	3.4	G=61, p<0.005	12	5.6		3.0	3.7		G=17, p<0.005
7	0	0		4.2	4.7	G=11, p<0.005	1.0	2.8		6.0	4.7		G=12, p<0.005
8	0	0		1.0	2.1	no test	1.8	2.5		2.3	4.7		no test
9	0	0		4.0	3.2	no test	2.7	2.6		3.3	3.1		G=0.0, n.s.
10	0.5	1.6		0	0	no test	0.3	1.3		0.3	1.3		no test

continued . . .

Table 20. Banded female bats observed per night foraging at twenty sites in the Okanagan Valley during the summer of 1982. No test indicates too few bats seen for statistical test.

26 July onwards									
Before 26 July									
Site	<u>M. lucifugus</u>		<u>M. yumanensis</u>		<u>M. lucifugus</u>		<u>M. yumanensis</u>		Differed from expected
	mean	SD	mean	SD	mean	SD	mean	SD	
1	1.2	2.7	1.2	2.2	0	0	0.3	1.3	no test
2	6.2	5.6	7.25	5.2	3.7	3.0	2.3	2.6	G=0.5, n.s.
3	0	0	0	0	0	0	0	0	no test
4	0	0	0	0	0.3	1.3	0	0	no test
5	2.3	2.6	2	2.5	3.0	3.7	2.0	3.2	G=0.3, n.s.
6	13.5	8.6	3.2	3.4	12	5.6	2.3	2.6	G=20, p<0.005
7	0	0	3.7	3.9	1.0	2.8	6.0	4.7	G=10, p<0.005
8	0	0	1.0	2.1	1.8	2.5	0.9	2.0	no test
9	0	0	4	3.2	2.7	2.6	3.0	3.2	G=0.0, n.s.
10	0.5	1.6	0	0	0.3	1.3	0.3	1.3	no test

continued . . .

Before 26 July	26 July onwards
1. The first 100 cases were reported in the first 10 days of the outbreak. 2. The first 100 cases were reported in the first 10 days of the outbreak. 3. The first 100 cases were reported in the first 10 days of the outbreak. 4. The first 100 cases were reported in the first 10 days of the outbreak. 5. The first 100 cases were reported in the first 10 days of the outbreak. 6. The first 100 cases were reported in the first 10 days of the outbreak. 7. The first 100 cases were reported in the first 10 days of the outbreak. 8. The first 100 cases were reported in the first 10 days of the outbreak. 9. The first 100 cases were reported in the first 10 days of the outbreak. 10. The first 100 cases were reported in the first 10 days of the outbreak.	1. The first 100 cases were reported in the first 10 days of the outbreak. 2. The first 100 cases were reported in the first 10 days of the outbreak. 3. The first 100 cases were reported in the first 10 days of the outbreak. 4. The first 100 cases were reported in the first 10 days of the outbreak. 5. The first 100 cases were reported in the first 10 days of the outbreak. 6. The first 100 cases were reported in the first 10 days of the outbreak. 7. The first 100 cases were reported in the first 10 days of the outbreak. 8. The first 100 cases were reported in the first 10 days of the outbreak. 9. The first 100 cases were reported in the first 10 days of the outbreak. 10. The first 100 cases were reported in the first 10 days of the outbreak.

Site	<u>M. lucifugus</u>			<u>M. yumanensis</u>			<u>M. lucifugus</u>			<u>M. yumanensis</u>			Differed from expected
	mean	SD		mean	SD		mean	SD		mean	SD		
11	0.2	1.1		0	0		1.3	2.3	0	0			no test
12	0.2	1.1		0	0		0	0	0	0			no test
13	0	0		0	0		0	0	0	0			no test
14	2.0	2.9		2.2	2.9		1.3	1.0	4.0	2.1			G=3.1, n.s.
15	1.2	3.3		13	9.2		1.7	2.4	14	7.2			G=30, p<0.005
16	3.0	1.6		9.4	7.8		17	4.9	10	5.5			G=3.9, p<0.05
17	0.5	1.5		3.2	3.4		3.0	3.2	1.0	2.1			G=2.1, n.s.
18	1.0	2.6		3.0	4.1		2.7	2.6	4.7	3.5			G=1.1, n.s.
19	3.6	5.1		3.0	4.1		6.0	6.6	3.7	4.8			G=1.2, n.s.
20	4.7	5.5		13	6.6		3.3	4.1	6.0	5.4			G=1.8, n.s.

Figure 1. Distributions of Myotis lucifugus and Myotis yumanensis in North America.

Myotis lucifugus is distributed within the bold boundaries, M. yumanensis within the shaded regions. Distributions and subspecies are based on Hall (1981).

1. M. l. alascensis Miller   2. M. l. pernox Hollister
3. M. l. lucifugus Le Conte   4. M. l. carissima Thomas
5. M. l. relictus Harris   6. M. l. occultus Hollister
7. M. y. saturatus Miller   8. M. y. sociabilis Grinnell
9. M. y. oxalis Dalquest   10. M. y. yumanensis Allen
11. M. y. lambi Benson

Figure 2. Myotis yumanensis (left) and M. lucifugus (right) from the Okanagan Valley, British Columbia. Note the darker fur of M. lucifugus, particularly on the shoulders, and that the ears and nose-pad of M. lucifugus are darker than those of M. yumanensis.

Figure 3. Map of study area showing observation sites (● + number), see Table 1 for site descriptions; bodies of water (wavy lines) and bluffs (hatched lines). The insert shows the location of the study area.

Figure 4. Photographs of the observations sites.

Sites are indicated by encircled number.

A. Okanagan Falls area: site 19 and site 20.

B. Okanagan Falls area: site 15 and site 16.

C. Okanagan Falls area: site 17 and site 18.

D. South weir area: sites 1, 2, 3, 4, and 5.

E. Vaseux Lake area: sites 11, 12, and 14.

F. McIntyre bluff area: sites 8, 9, and 10.

G. Gallagher Lake area: site 6 and site 7.

H. Gallagher Lake area: site 6.

Figure 5. Measurement of braincase depth, BD.

Figure 6. Canonical variate scores of known individual Myotis lucifugus and M. yumanensis from British Columbia based on discriminant analysis of 11 skull measurements. Each L or Y represents a specimen of M. lucifugus or M. yumanensis; P is a posteriori probability of species membership.

Figure 7. Expected normal values for the canonical variable scores (from Figure 6) of Myotis lucifugus (L) and M. yumanensis (Y) from British Columbia. A linear relationship indicates that the canonical variable scores are normally distributed (lines are fitted by eye; Sokal and Rohlf, 1969).

Figure 8. Reproductive status of female Myotis lucifugus and M. yumanensis trapped during spring and summer, 1982. See Table 10 for further details.

Figure 9. Patterns of frequency change over time in the search phase echolocation calls of Myotis lucifugus and M. yumanensis. Additional data are given in Table 16.

Figure 10. Temporal pattern of foraging activity of Myotis lucifugus and M. yumanensis in the Okanagan Valley during the summer of 1982.

See Table 18 for details.

END

3 1 0 8 18 4

FIN