Morphology of gartersnake populations in the Wisconsin zone of overlap between *Thamnophis butleri* and *Thamnophis radix*

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Executive Summary

Butler's Gartersnake, *Thamnophis butleri*, was described in 1889 (Cope 1889). This species is closely related to the Plains gartersnake, *Thamnophis radix*, but is ecologically more specialized and narrowly distributed (Rossman et al. 1996). These two species are distinguished morphologically through color, pattern, scale count, and size differences, except in a narrow hybrid zone in southeastern Wisconsin (Casper 2003), a finding mirrored by genetic studies (Burghardt et. al 2006; Fitzpatrick et al. 2008b). The present study applied prior morphological analysis techniques (Casper 2003) to evaluate more precisely how animals in a subset of populations of special management concern in this putative hybrid zone scored on eight morphologic characters. A total of 452 snakes were examined and scored for phenotypic variation from fourteen populations in the putative hybrid zone, and five reference groups removed from the hybrid zone. Strong *T. butleri* phenotypes were present in all hybrid zone populations, albeit at differing proportions. Phenotypic *T. butleri* individuals thus are likely present throughout the hybrid zone in some proportion, but in genetically segregated hybrid populations. Combined with genetic information, these findings may better inform conservation policy.

Introduction

Butler's gartersnake, *Thamnophis butleri* (Cope 1889), is listed as Threatened in Wisconsin and Ontario, Canada, and as Endangered in Indiana. The decline of *T. butleri* is attributed mainly to habitat loss and degradation, with its preferred habitat (i.e., wet meadows and prairies) being developed for commercial and residential purposes in many areas (Vogt 1981; Joppa and Temple 2005). In Waukesha and Milwaukee counties, conflicts between urban development and habitat conservation have been particularly common. Effective conservation planning has been hampered by lack of data on several important aspects of snake biology. One of the most critical issues arises from difficulties in distinguishing *T. butleri* (the protected taxon) from the plains gartersnake, *T. radix* (a close relative with no legal status). This difficulty is a result of their close similarity (Rossman et al. 1996; Alfaro and Arnold 2001; de Queiroz et al. 2002) and the presence of hybrids with intermediate traits (Albright 2001; Casper 2003; Kirby 2005; Fitzpatrick et al. 2008b). Here I used morphological traits to evaluate character scores of individuals from populations with known or suspected hybridization.

Thamnophis butleri and *T. radix* are closely related (Ruthven 1908; Conant 1950), with molecular phylogenetic studies showing several mitochondrial DNA loci to have nearly identical sequences (Alfaro and Arnold 2001; de Queiroz et al. 2002; Burghardt et al. 2006). Rossman et al. (1996) suggested that *T. butleri* is a neotenic (dwarfed) derivative of *T. radix*, which may explain the similarity in genetics between the two, given that any divergence probably occurred relatively recently. Ecologically and behaviorally there are also differences between the two taxa. For example, *T. radix* is a prey generalist and eats earthworms, amphibians, fish, rodents, and even birds. *T. butleri*, in contrast, eats virtually nothing but earthworms and leeches in the wild (although it will often eat fish and amphibians in captivity), further supporting their close relationship (Burghardt 1969; Halloy & Burghardt 1990; Rossman et al. 1996).

Previous studies of Wisconsin *T. butleri* and *T. radix*, have confirmed that the two taxa are very closely related, that they do hybridize where their geographic ranges overlap, and that they maintain distinctiveness in size, shape, appearance, and gene frequencies (Casper 2003; Burghardt et al. 2006; Fitzpatrick et al. 2008b). Burghardt et al. (2006) surveyed mitochondrial DNA variation across both taxa in Wisconsin and neighboring states. They identified a region, dubbed the IZ for "indeterminate zone", where additional sampling was needed to understand the genetic/taxonomic status of the gartersnakes. The IZ largely corresponds to a hybrid zone identified by the occurrence of morphologically intermediate snakes (Casper 2003) and confirmed by DNA analysis showing individuals with mixtures of alleles derived from *T. butleri* and *T. radix* (Fitzpatrick et al. 2008b). The IZ in Milwaukee and eastern Waukesha counties is a fairly narrow (2-8 km) east-west band where suitable *T. butleri* habitat occurs. The goal of this study was to assess the phenotypic variation of gartersnakes at specific sites in the IZ chosen by the Wisconsin Department of Natural Resources (WDNR), to assist in conservation planning.

Methods

Sampling and Animal Care

Samples from the IZ were collected by the WDNR, with 252 snakes supplied from 15 sites, although 17 of these snakes were from Site TC which is actually outside of the IZ (Table 1; Figure 1). Note that PCN and PCS sites were populations residing along the same stream and separated from each other by one roadway, across which gene flow undoubtedly occurs along the stream flowing under the roadway. Data from another 200 snakes were utilized as reference samples and were drawn from field work conducted over the past ten years, and examination of museum specimens. These included 80 *T. radix* from three Illinois counties; 55 *T. butleri* from the three northernmost Wisconsin counties determined as *T. butleri* from Casper (2003); 45 *T. butleri* from twelve Michigan counties; and 20 *T. butleri* from Lucas County, Ohio (Table 1; Figure 1). The Ozaukee County, Wisconsin, data set included some unpublished data from Paul Nys taken in the mid-1990s. The target sample size per IZ site was 20 snakes, representing a compromise between statistical rigor for concurrent molecular research (Fitzpatrick et al. 2008a) and collecting constraints.

Snakes collected by WDNR personnel were delivered to a lab facility where they were housed in aquaria for approximately 1-3 weeks while processing was completed. While housed, snakes were kept on clean paper substrates, had constant access to fresh water, and were fed earthworms twice a week. The facilities were kept at 65-85 degrees F, and a bank of south windows in the room supplied a natural photoperiod and opportunities for solar basking. Snakes were released at their capture sites by WDNR personnel within a few days of being processed.

Morphological Methods

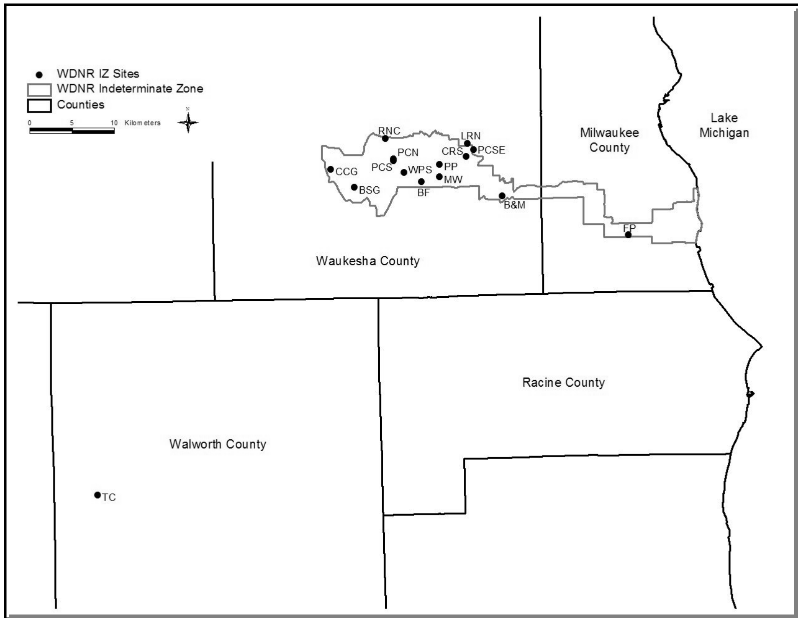
Morphological analyses repeated methods from Casper (2003). Eight characters were examined and scored as shown in Table 2, and additional data were recorded to assist in segregating specimens into sex and age classes (sex, snout-vent length, tail length, number of subcaudals). Supralabials and infralabials were counted and summed. On preserved specimens ventrals and subcaudals were directly counted. On live specimens the ventor was xeroxed with the snake in a transparent plastic bag, or digitally photographed by placing the snake on a transparent plexiglass plate with a foam cushion over the snake and inverting the sandwiched snake for photography. Ventral and subcaudal counts were then obtained from the xerox or photo. Snout-vent and tail length were measured by gently aligning snakes along a meter stick. To determine sex external tail morphology was used, and where this was inconclusive, living specimens were probed and preserved specimens dissected. Degree of supralabial barring and development of the post-cranial crescent were scored on a qualitative five class scale from no black pigment present to very strongly pigmented. Degree of dorsal spotting was similarly scored on a five class scale from no spotting discernable (thoroughly black ground color) to very strongly spotted (light brown ground color). Width of the lateral stripe was scored by determining the scale rows included in the stripe at a point approximately one fourth of the body length posterior to the head, and resulted in eight character classes. Vertebral stripe color was described in live specimens only, as a comparative value within each specimen (unicolor or bicolor). Measurements were made with a ruler or digital calipers.

Table 1: Study Sampling Sites

Sample	Site	N	State	County
IZ	B&M	16	WI	Waukesha
IZ	BF	18	WI	Waukesha
IZ	BSG	21	WI	Waukesha
IZ	CCG	4	WI	Waukesha
IZ	CRS	14	WI	Waukesha
IZ	FP	21	WI	Milwaukee
IZ	LRN	1	WI	Waukesha
IZ	MW	18	WI	Waukesha
IZ	PCN	23	WI	Waukesha
IZ	PCS	23	WI	Waukesha
IZ	PCSE	5	WI	Waukesha
IZ	PP	25	WI	Waukesha
IZ	RNC	20	WI	Waukesha
IZ	TC	17	WI	Walworth
IZ	WPS	26	WI	Waukesha
T. radix	IL	37	IL	Champaign
T. radix	IL	40	IL	Cook
T. radix	IL	3	IL	Will
T. butleri	WI	7	WI	Fond du Lac
T. butleri	WI	47	WI	Ozaukee
T. butleri	WI	1	WI	Sheboygan
T. butleri	MI	2	MI	Alpena
T. butleri	MI	2	MI	Arenac
T. butleri	MI	2	MI	Bay
T. butleri	MI	1	MI	Clinton
T. butleri	MI	1	MI	Eaton
T. butleri	MI	2	MI	Genesee
T. butleri	MI	4	MI	Huron
T. butleri	MI	1	MI	Jackson
T. butleri	MI	8	MI	Livingston
T. butleri	MI	3	MI	Monroe
T. butleri	MI	2	MI	Presque Isle
T. butleri	MI	17	MI	Wayne
T. butleri	OH	20	OH	Lucas

Table 2. Morphological characteristics examined and taxonomic assignments per Casper (2003)

Character	Description	*T. butleri* state	*T. radix* state
Post-cranial crescent	A vertically positioned, crescent shaped, black mark interrupts the lateral stripe just posterior to the mandible; score degree of development 1 (least) to 5 (most).	1, 2	4, 5
Supralabial barring	The posterior edge of the supralabials is edged with black pigment, giving the appearance of vertical bars along the supralabial sutures; score degree of development 1 (least) to 5 (most).	1, 2	5
Dorsal spotting	Strength of dorsal spotting was scored, 1 (least) to 5 (most), based on how distinct spots were from the ground color. The darker the ground color, the more obscured the spots.	1, 2	4, 5
Stripe color	Stripes were scored either uni-colored, or bi-colored. When uni-colored, all 3 stripes are nearly the same color and shade. When bi-colored, the vertebral (dorsal) stripe is a different color than the lateral stripes. Typically, in bi-colored individuals, the vertebral stripe is more orange than are the lateral stripes, therefore darker in shade. Questionable or very slight color differences were left unscored.	uni-colored	bi-colored
Lateral stripe position	The position (dorsal scale row number) of the scale rows involved in the lateral stripe, with data taken between the level of the 10th and 20th ventral scales. If the stripe color just barely touched a scale row it was scored in parentheses, indicating very weak character presence.	1-2-3-4, 2-3-4, (2)3, 2-3(4)	3(4), 3-4
Ventral scale count	Count ventral scales from first one in contact with the lowermost dorsal scale row on both sides to, but not including, the anal plate.	<145 males <140 females <140 sex undetermined	>149 males >149 sex undetermined >144 females
Supralabial scale count	Count supralabials, sum left and right.	<13.5	>14.5
Infralabial scale count	Count infralabials, sum left and right.	<17	>19.5



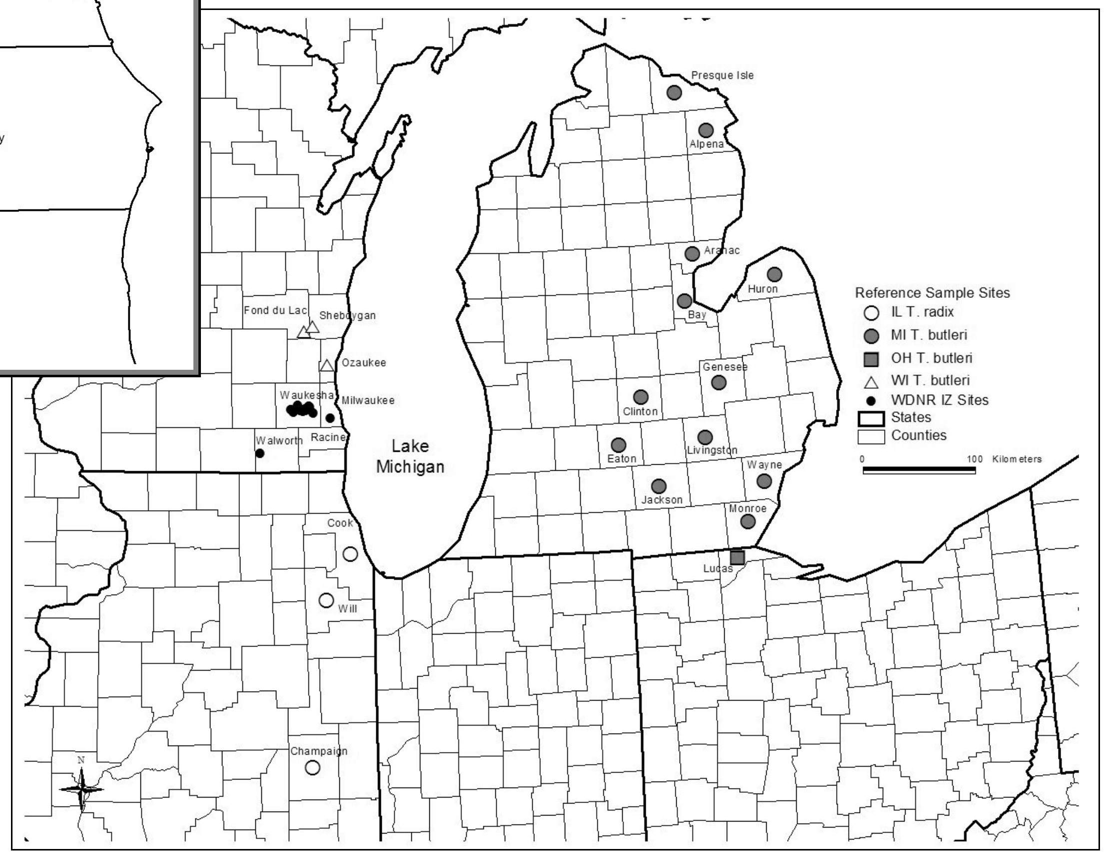


Fig. 1. Approximate localities of study sites.

Data Analysis

For each individual snake, the number of characters scoring within the prior determined *T. butleri* range (Casper 2003) was divided by the total number of characters scored, for a measure of *T. butleri* likeness (TBL) for each individual. For example, if five of eight characters scored within the *T. butleri* range, and the remaining three characters were outside the *T. butleri* range, then the likeness index (TBL) of the individual is 5/8 or 0.625. The TBL values were then averaged for each population. Snakes with less than five characters scored were omitted from analyses.

Results

Mean TBL values for each population are presented in Table 3 and Figure 2. For this analysis the *T. radix* reference group was pooled (all Illinois samples), and *T. butleri* reference samples were pooled into three groups – all Wisconsin, all Michigan, and all Ohio. TBL values for individual snakes are given in Appendix A. The proportion of individuals in each population exhibiting a strong *T. butleri* phenotype (TBL>0.7) is given in Table 3. TC and the *T. radix* reference samples contained no such individuals, while the Michigan *T. butleri* reference sample is 100% TBL>0.7. Samples containing more than 70% strong *T. butleri* phenotypes were: B&M, BF, PCN, PCS, PP, and the Wisconsin and Ohio *T. butleri* reference samples. Samples containing less than 50% strong *T. butleri* phenotypes were: BSG, FP, TC and the *T. radix* reference samples.

Discussion

Mean TBL values suggest that all samples from the IZ except Site TC are similar to *T. butleri* reference sites. Site TC groups with the *T. radix* reference sample, and is in Walworth County, Wisconsin, which is not within the previously described putative hybrid zone (Figure 1; Casper 2003; Burghardt et. al 2006; Fitzpatrick et al. 2008b). Examination of individual and average snake TBL scores reveals that phenotypic variation within suspected hybrid populations can be substantial, and that the *T. radix* and the Michigan *T. butleri* reference samples had very little variation on the eight characters examined. Ohio and Wisconsin *T. butleri* reference samples had phenotypic variation similar to most IZ sites in magnitude, but the proportion of snakes exhibiting high TBL scores substantially exceeded that of all but one of the IZ samples (PCN).

Ignoring the three sites with low sample sizes, every site examined contained snakes with TBL scores of at least 0.875 – well within the conventional parameters used to make a *T. butleri* identification (Vogt 1981; Rossman et al. 1996). This suggests that other populations proximal to those examined here will also harbor *T. butleri* individuals in some proportion. Previous studies have documented bimodal distributions of *T. butleri* and *T. radix* genomes within these hybrid populations (Fitzpatrick et al. 2008a, 2008b), and this finding is now supported by morphological evidence. The distribution of unique mtDNA haplotypes revealed by earlier work (Burghardt et al. 2006) also appears to correspond with variation revealed by morphology, which may explain the higher variation revealed here in the Ohio and Wisconsin *T. butleri* reference samples, but further analysis is warranted.

These findings suggest that the geographic range of Wisconsin *T. butleri* extends somewhat further south than previously thought, into southern Milwaukee and Waukesha counties, but that populations are mixed and hybridizing in this region while still maintaining genetic distinctiveness (Fitzpatrick et al. 2008b). Sampling more widely in transects perpendicular to the IZ would reveal more precisely where the change-over to predominantly one or the other taxon begins. Both the stability and geographic limits of this hybridizing but genetically segregated system are unknown. Morphological evidence suggests that it has been stable for some decades, as evidenced by confusing old museum specimens (Cope 1950; Casper 2003). A post-glacial secondary contact zone has been postulated for this region, where evolution is actively underway, which may explain this variation (Burghardt et al. 2006; Fitzpatrick et al. 2008b). Additional information of conservation and taxonomic relevance may be obtained by studying in a comparable manner the phenotypic and molecular variation in *T. radix* and *T. butleri* in Ohio, where the two species have been in contact in recent historic times and share some mtDNA haplotypes not found in other populations of either species (Burghardt et al. 2001; Burghardt et al. 2006).

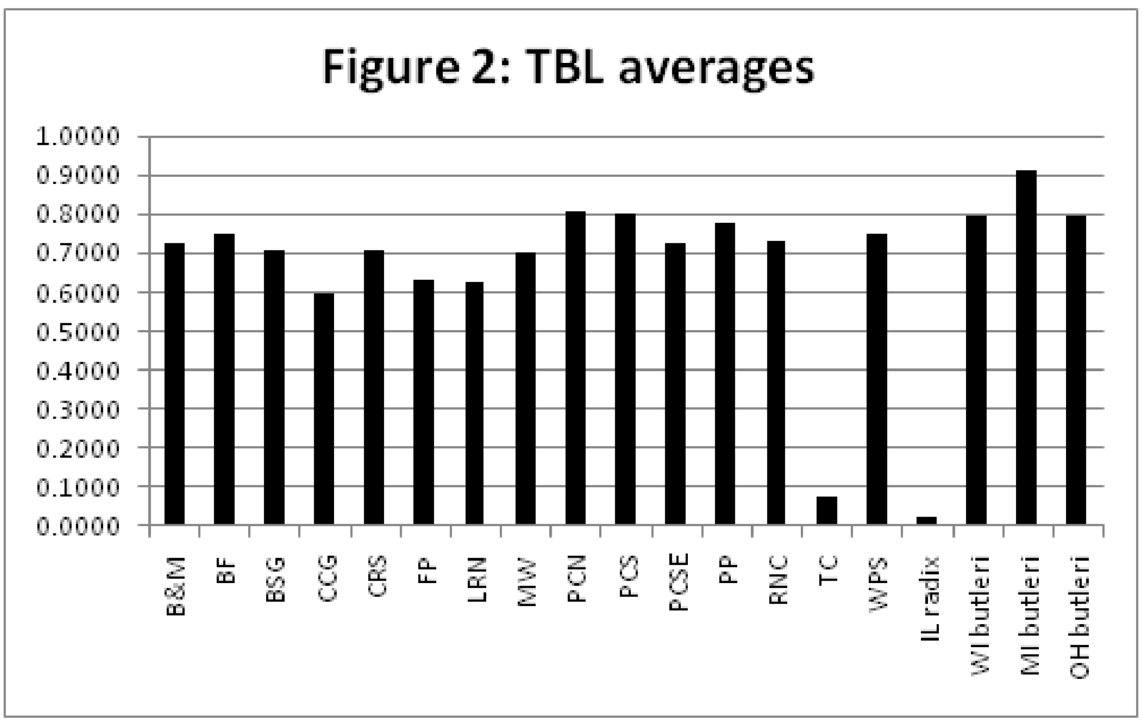
Whether or not segregated *T. butleri* and *T. radix* gene pools will persist over time in these hybridizing populations is a more difficult, but important question. The mechanisms maintaining the genetic segregation in these hybridizing populations remain unknown, and in need of investigation. Mating systems are a likely causal mechanism, perhaps enabled through pheromone differences. It is not clear if land use (i.e. habitat quality) and conservation practices might affect the genetic dynamics of mixed populations. The conservation value of mixed populations depends on both genetic and demographic stability. For example, if *T. radix* genotypes tend to displace *T. butleri*, then sites with predominantly *T. butleri* ancestry might have more potential to contribute to the species recovery. In Wisconsin, existing populations are more isolated in the north (where habitat patches are generally smaller and fewer), and less isolated in the IZ (where habitat patches are generally larger and more numerous). Moreover, unique genetics are concentrated in the IZ (Burghardt et al. 2006). It follows that a conservation focus on the northern populations may better preserve the *T. butleri* genome without hybrid influence, but may have more demographic risk (owing to these more isolated and smaller sites), while a focus on the IZ may have less confidence in preserving the *T. butleri* genome owing to the hybridization issue, but may be more likely to succeed demographically (as a consequence of more, and more connected, habitat availability). Finally, if preserving genetic diversity is a conservation objective, the IZ populations are where a high proportion of unique genetic combinations are concentrated (Burghardt et al. 2001; Burghardt et al. 2006).

Spatial conservation planning decisions may be better informed if a firm relationship between genetic makeup and morphology can be established. This may allow the use of many of the available museum specimens collected decades ago to investigate the stability of the system over time, and would be a more efficient means of evaluating existing populations than molecular methods.

Table 3: Morphological results by site

Site	Ave. TBL*	Sample Size	Max	Min	Spread	% of sample TBL>0.7
B&M	0.7266	16	1.0000	0.5000	0.500	75.0%
BF	0.7500	18	1.0000	0.5000	0.500	72.2%
BSG	0.7083	21	1.0000	0.5000	0.500	42.9%
CCG	0.5938	4				
CRS	0.7054	14	0.8750	0.5000	0.375	57.1%
FP	0.6344	21	0.8750	0.3750	0.500	33.3%
LRN	0.6250	1				
MW	0.7014	18	1.0000	0.5000	0.500	61.1%
PCN	0.8098	23	1.0000	0.5000	0.500	91.3%
PCS	0.8020	23	1.0000	0.5714	0.429	82.6%
PCSE	0.7250	5				
PP	0.7750	25	1.0000	0.5000	0.500	72.0%
RNC	0.7313	20	1.0000	0.6250	0.375	60.0%
TC	0.0735	17	0.3750	0.0000	0.375	0.0%
WPS	0.7500	26	1.0000	0.5000	0.500	61.5%
IL radix	0.0253	80	0.2000	0.0000	0.200	0.0%
WI butleri	0.7961	55	1.0000	0.5000	0.500	85.5%
MI butleri	0.9103	45	1.0000	0.7143	0.286	100.0%
OH butleri	0.7973	20	1.0000	0.5000	0.500	85.0%

* - TBL, *T. butleri* likeness score, see text



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Appendix A: Individual snake TBL scores

Site	Snake	TBL Score	% of Sample >0.7
B&M	B&M 10	0.5000	
B&M	B&M 1	0.6250	
B&M	B&M 12	0.6250	
B&M	B&M 14	0.6250	
B&M	B&M 11	0.7500	
B&M	B&M 13	0.7500	
B&M	B&M 15	0.7500	
B&M	B&M 16	0.7500	
B&M	B&M 2	0.7500	
B&M	B&M 3	0.7500	
B&M	B&M 4	0.7500	
B&M	B&M 6	0.7500	
B&M	B&M 7	0.7500	
B&M	B&M 8	0.7500	
B&M	B&M 9	0.7500	
B&M	B&M 5	1.0000	75.0%
BF	BF5	0.5000	
BF	BF9	0.5000	
BF	BF15	0.6250	
BF	BF17	0.6250	
BF	BF8	0.6250	
BF	BF10	0.7500	
BF	BF11	0.7500	
BF	BF12	0.7500	
BF	BF14	0.7500	
BF	BF18	0.7500	
BF	BF3	0.7500	
BF	BF4	0.7500	
BF	BF1	0.8750	
BF	BF16	0.8750	
BF	BF2	0.8750	
BF	BF6	0.8750	
BF	BF7	0.8750	
BF	BF13	1.0000	72.2%
BSG	BSG14	0.5000	
BSG	BSG20	0.5000	
BSG	BSG1	0.6250	
BSG	BSG11	0.6250	
BSG	BSG13	0.6250	
BSG	BSG16	0.6250	
BSG	BSG17	0.6250	
BSG	BSG19	0.6250	
BSG	BSG4	0.6250	
BSG	BSG6	0.6250	
BSG	BSG7	0.6250	
BSG	BSG8	0.6250	

Site	Snake	TBL Score	% of Sample >0.7
BSG	BSG10	0.7500	
BSG	BSG12	0.7500	
BSG	BSG21	0.7500	
BSG	BSG5	0.7500	
BSG	BSG15	0.8750	
BSG	BSG2	0.8750	
BSG	BSG3	0.8750	
BSG	BSG18	1.0000	
BSG	BSG9	1.0000	42.9%
CCG	CCG1	0.5000	
CCG	CCG3	0.5000	
CCG	CCG2	0.6250	
CCG	CCG4	0.7500	
CRS	CRS6	0.5000	
CRS	CRS14	0.6250	
CRS	CRS2	0.6250	
CRS	CRS5	0.6250	
CRS	CRS7	0.6250	
CRS	CRS9	0.6250	
CRS	CRS1	0.7500	
CRS	CRS10	0.7500	
CRS	CRS11	0.7500	
CRS	CRS13	0.7500	
CRS	CRS3	0.7500	
CRS	CRS8	0.7500	
CRS	CRS12	0.8750	
CRS	CRS4	0.8750	57.1%
FP	FP16	0.3750	
FP	FP19	0.3750	
FP	FP13	0.5000	
FP	FP18	0.5000	
FP	FP21	0.5000	
FP	FP4	0.5000	
FP	FP7	0.5714	
FP	FP1	0.6250	
FP	FP12	0.6250	
FP	FP2	0.6250	
FP	FP20	0.6250	
FP	FP3	0.6250	
FP	FP8	0.6250	
FP	FP9	0.6250	
FP	FP10	0.7500	
FP	FP14	0.7500	
FP	FP15	0.7500	
FP	FP17	0.7500	
FP	FP11	0.8750	
FP	FP5	0.8750	
FP	FP6	0.8750	33.3%

Site	Snake	TBL Score	% of Sample >0.7
IL radix	BDV-A	0.0000	
IL radix	BDV-B	0.0000	
IL radix	BDV-C	0.0000	
IL radix	BDV-D	0.0000	
IL radix	BDV-F	0.0000	
IL radix	90	0.0000	
IL radix	91	0.0000	
IL radix	92	0.0000	
IL radix	93	0.0000	
IL radix	94	0.0000	
IL radix	96	0.0000	
IL radix	97	0.0000	
IL radix	98	0.0000	
IL radix	99	0.0000	
IL radix	100	0.0000	
IL radix	101	0.0000	
IL radix	102	0.0000	
IL radix	103	0.0000	
IL radix	105	0.0000	
IL radix	106	0.0000	
IL radix	107	0.0000	
IL radix	108	0.0000	
IL radix	109	0.0000	
IL radix	110	0.0000	
IL radix	111	0.0000	
IL radix	112	0.0000	
IL radix	113	0.0000	
IL radix	05-IL-0010A	0.0000	
IL radix	05-IL-0010D	0.0000	
IL radix	05-IL-0010F	0.0000	
IL radix	1694	0.0000	
IL radix	2076	0.0000	
IL radix	2077	0.0000	
IL radix	33462	0.0000	
IL radix	17601	0.0000	
IL radix	17602	0.0000	
IL radix	1173	0.0000	
IL radix	1174	0.0000	
IL radix	1176	0.0000	
IL radix	1177	0.0000	
IL radix	1178	0.0000	
IL radix	1179	0.0000	
IL radix	12394	0.0000	
IL radix	1391	0.0000	
IL radix	15779	0.0000	
IL radix	15780	0.0000	
IL radix	16226	0.0000	
IL radix	16227	0.0000	

Site	Snake	TBL Score	% of Sample >0.7
IL radix	16228	0.0000	
IL radix	16262	0.0000	
IL radix	16360	0.0000	
IL radix	16361	0.0000	
IL radix	16362	0.0000	
IL radix	16394	0.0000	
IL radix	164	0.0000	
IL radix	165	0.0000	
IL radix	16534	0.0000	
IL radix	167	0.0000	
IL radix	41317	0.0000	
IL radix	5091	0.0000	
IL radix	5107	0.0000	
IL radix	5108	0.0000	
IL radix	5109	0.0000	
IL radix	5110	0.0000	
IL radix	5112	0.0000	
IL radix	84417	0.0000	
IL radix	84418	0.0000	
IL radix	00004	0.1250	
IL radix	BDV-E	0.1429	
IL radix	95	0.1429	
IL radix	104	0.1429	
IL radix	05-IL-0010B	0.1429	
IL radix	05-IL-0010E	0.1429	
IL radix	05-IL-0010L	0.1429	
IL radix	62362	0.1429	
IL radix	1392	0.1667	
IL radix	166	0.1667	
IL radix	41081	0.1667	
IL radix	2197	0.2000	
IL radix	5111	0.2000	0.0%
LRN	LRN1	0.6250	
MI butleri	46527	0.7143	
MI butleri	BEL-15	0.7500	
MI butleri	37723	0.7500	
MI butleri	156133	0.7500	
MI butleri	156134	0.7500	
MI butleri	BEL-2	0.8571	
MI butleri	BEL-3	0.8571	
MI butleri	BEL-4	0.8571	
MI butleri	BEL-5	0.8571	
MI butleri	BEL-7	0.8571	
MI butleri	BEL-8	0.8571	
MI butleri	FN1453	0.8571	
MI butleri	BEL-10	0.8750	
MI butleri	BEL-14	0.8750	
MI butleri	BEL-16	0.8750	

Site	Snake	TBL Score	% of Sample >0.7
MI butleri	BEL-9	0.8750	
MI butleri	33460	0.8750	
MI butleri	37724	0.8750	
MI butleri	46529	0.8750	
MI butleri	118085	0.8750	
MI butleri	155966	0.8750	
MI butleri	156071	0.8750	
MI butleri	156072	0.8750	
MI butleri	205026	0.8750	
MI butleri	31628	0.8750	
MI butleri	46532	0.8750	
MI butleri	BEL-1	1.0000	
MI butleri	BEL-11	1.0000	
MI butleri	BEL-12	1.0000	
MI butleri	BEL-13	1.0000	
MI butleri	STER-2	1.0000	
MI butleri	33461	1.0000	
MI butleri	33473	1.0000	
MI butleri	33474	1.0000	
MI butleri	33475	1.0000	
MI butleri	37725	1.0000	
MI butleri	72346	1.0000	
MI butleri	118086	1.0000	
MI butleri	142861	1.0000	
MI butleri	156132	1.0000	
MI butleri	31627	1.0000	
MI butleri	46526	1.0000	
MI butleri	46530	1.0000	
MI butleri	46531	1.0000	
MI butleri	46533	1.0000	100.0%
MW	MW18	0.5000	
MW	MW3	0.5000	
MW	MW4	0.5000	
MW	MW8	0.5000	
MW	MW10	0.6250	
MW	MW11	0.6250	
MW	MW14	0.6250	
MW	MW1	0.7500	
MW	MW13	0.7500	
MW	MW15	0.7500	
MW	MW16	0.7500	
MW	MW17	0.7500	
MW	MW2	0.7500	
MW	MW5	0.7500	
MW	MW9	0.7500	
MW	MW6	0.8750	
MW	MW7	0.8750	
MW	MW12	1.0000	61.1%

Site	Snake	TBL Score	% of Sample >0.7
OH butleri	TOL-5	0.5000	
OH butleri	33434	0.5714	
OH butleri	TOL-17	0.6250	
OH butleri	1717	0.7500	
OH butleri	TOL-12	0.7500	
OH butleri	TOL-13	0.7500	
OH butleri	TOL-14	0.7500	
OH butleri	TOL-18	0.7500	
OH butleri	TOL-4	0.7500	
OH butleri	TOL-7	0.7500	
OH butleri	TOL-8	0.7500	
OH butleri	TOL-1	0.8750	
OH butleri	TOL-11	0.8750	
OH butleri	TOL-15	0.8750	
OH butleri	TOL-16	0.8750	
OH butleri	TOL-3	0.8750	
OH butleri	TOL-6	0.8750	
OH butleri	TOL-10	1.0000	
OH butleri	TOL-2	1.0000	
OH butleri	TOL-9	1.0000	85.0%
PCN	PCN 17	0.5000	
PCN	PCN 1	0.6250	
PCN	PCN 10	0.7500	
PCN	PCN 11	0.7500	
PCN	PCN 12	0.7500	
PCN	PCN 16	0.7500	
PCN	PCN 21	0.7500	
PCN	PCN 3	0.7500	
PCN	PCN 4	0.7500	
PCN	PCN 5	0.7500	
PCN	PCN 7	0.7500	
PCN	PCN 13	0.8750	
PCN	PCN 14	0.8750	
PCN	PCN 15	0.8750	
PCN	PCN 19	0.8750	
PCN	PCN 2	0.8750	
PCN	PCN 22	0.8750	
PCN	PCN 6	0.8750	
PCN	PCN 8	0.8750	
PCN	PCN 9	0.8750	
PCN	PCN A	0.8750	
PCN	PCN 18	1.0000	
PCN	PCN 20	1.0000	91.3%
PCS	PCS 18	0.5714	
PCS	PCS 17	0.6250	
PCS	PCS 20	0.6250	
PCS	PCS 8	0.6250	
PCS	PCS 11	0.7500	

Site	Snake	TBL Score	% of Sample >0.7
PCS	PCS 12	0.7500	
PCS	PCS 14	0.7500	
PCS	PCS 19	0.7500	
PCS	PCS 21	0.7500	
PCS	PCS 22	0.7500	
PCS	PCS 3	0.7500	
PCS	PCS 9	0.7500	
PCS	PCS 1	0.8750	
PCS	PCS 13	0.8750	
PCS	PCS 15	0.8750	
PCS	PCS 2	0.8750	
PCS	PCS 23	0.8750	
PCS	PCS 4	0.8750	
PCS	PCS 5	0.8750	
PCS	PCS 7	0.8750	
PCS	PCS 10	1.0000	
PCS	PCS 16	1.0000	
PCS	PCS 6	1.0000	82.6%
PCSE	PCSE 4	0.5000	
PCSE	PCSE 3	0.6250	
PCSE	PCSE 2	0.7500	
PCSE	PCSE 5	0.7500	
PCSE	PCSE 1	1.0000	
PP	PP4	0.5000	
PP	PP5	0.5000	
PP	PP11	0.6250	
PP	PP14	0.6250	
PP	PP19	0.6250	
PP	PP2	0.6250	
PP	PP3	0.6250	
PP	PP17	0.7500	
PP	PP20	0.7500	
PP	PP22	0.7500	
PP	PP7	0.7500	
PP	PP8	0.7500	
PP	PP1	0.8750	
PP	PP10	0.8750	
PP	PP12	0.8750	
PP	PP13	0.8750	
PP	PP16	0.8750	
PP	PP18	0.8750	
PP	PP21	0.8750	
PP	PP23	0.8750	
PP	PP24	0.8750	
PP	PP25	0.8750	
PP	PP6	0.8750	
PP	PP9	0.8750	
PP	PP15	1.0000	72.0%

Site	Snake	TBL Score	% of Sample >0.7
RNC	RNC1	0.6250	
RNC	RNC16	0.6250	
RNC	RNC17	0.6250	
RNC	RNC2	0.6250	
RNC	RNC3	0.6250	
RNC	RNC4	0.6250	
RNC	RNC8	0.6250	
RNC	RNC9	0.6250	
RNC	RNC10	0.7500	
RNC	RNC13	0.7500	
RNC	RNC14	0.7500	
RNC	RNC18	0.7500	
RNC	RNC19	0.7500	
RNC	RNC20	0.7500	
RNC	RNC5	0.7500	
RNC	RNC7	0.7500	
RNC	RNC12	0.8750	
RNC	RNC15	0.8750	
RNC	RNC6	0.8750	
RNC	RNC11	1.0000	60.0%
TC	TC1	0.0000	
TC	TC11	0.0000	
TC	TC12	0.0000	
TC	TC13	0.0000	
TC	TC15	0.0000	
TC	TC16	0.0000	
TC	TC2	0.0000	
TC	TC4	0.0000	
TC	TC5	0.0000	
TC	TC9	0.0000	
TC	TC10	0.1250	
TC	TC14	0.1250	
TC	TC17	0.1250	
TC	TC3	0.1250	
TC	TC6	0.1250	
TC	TC8	0.2500	
TC	TC7	0.3750	0.0%
WI butleri	1700	0.5000	
WI butleri	WEECS-1	0.5000	
WI butleri	WEECS-4	0.5000	
WI butleri	RH-02-1	0.5000	
WI butleri	RH82004	0.6250	
WI butleri	1701	0.6250	
WI butleri	FS-8JN1	0.6250	
WI butleri	GP00-1	0.6250	
WI butleri	GSC 00036	0.7143	
WI butleri	23353	0.7143	
WI butleri	26288	0.7143	

Site	Snake	TBL Score	% of Sample >0.7
WI butleri	26289	0.7143	
WI butleri	30263	0.7143	
WI butleri	1699	0.7500	
WI butleri	2247	0.7500	
WI butleri	27A/D	0.7500	
WI butleri	1680	0.7500	
WI butleri	1705	0.7500	
WI butleri	1708	0.7500	
WI butleri	Flood 1	0.7500	
WI butleri	WEE-8JN1	0.7500	
WI butleri	WEE-8JN2	0.7500	
WI butleri	WEE-8JN3	0.7500	
WI butleri	WEECS-2	0.7500	
WI butleri	WEECS-3	0.7500	
WI butleri	WEECS-7	0.7500	
WI butleri	WEECS-9	0.7500	
WI butleri	11848	0.8571	
WI butleri	32540	0.8571	
WI butleri	33962	0.8750	
WI butleri	22F	0.8750	
WI butleri	1702	0.8750	
WI butleri	1703	0.8750	
WI butleri	1707	0.8750	
WI butleri	1709	0.8750	
WI butleri	1711	0.8750	
WI butleri	1712	0.8750	
WI butleri	Flood 2	0.8750	
WI butleri	FS-8JN2	0.8750	
WI butleri	MP-1	0.8750	
WI butleri	OZ-04-8	0.8750	
WI butleri	WEE-8JN4	0.8750	
WI butleri	WEECS-10	0.8750	
WI butleri	WEECS-11	0.8750	
WI butleri	WEECS-12	0.8750	
WI butleri	WEECS-13	0.8750	
WI butleri	WEECS-5	0.8750	
WI butleri	WEECS-6	0.8750	
WI butleri	WEECS-8	0.8750	
WI butleri	1698	1.0000	
WI butleri	1692	1.0000	
WI butleri	1704	1.0000	
WI butleri	1706	1.0000	
WI butleri	1710	1.0000	
WI butleri	1	1.0000	85.5%
WPS	WPS15	0.5000	
WPS	WPS18	0.5000	
WPS	WPS19	0.5000	
WPS	WPS20	0.5000	

Site	Snake	TBL Score	% of Sample >0.7
WPS	WPS1	0.6250	
WPS	WPS16	0.6250	
WPS	WPS21	0.6250	
WPS	WPS25	0.6250	
WPS	WPS6	0.6250	
WPS	WPS7	0.6250	
WPS	WPS10	0.7500	
WPS	WPS12	0.7500	
WPS	WPS13	0.7500	
WPS	WPS17	0.7500	
WPS	WPS2	0.7500	
WPS	WPS24	0.7500	
WPS	WPS22	0.8750	
WPS	WPS23	0.8750	
WPS	WPS26	0.8750	
WPS	WPS4	0.8750	
WPS	WPS5	0.8750	
WPS	WPS9	0.8750	
WPS	WPS11	1.0000	
WPS	WPS14	1.0000	
WPS	WPS3	1.0000	
WPS	WPS8	1.0000	61.5%