Supplemental Material

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Introduction

Inferences from phylogenetic comparative methods depend upon accurate resolution of evolutionary history. One potential problem is incomplete lineage sorting, which can lead to misleading phylogenetic relationships due to retention and stochastic sorting of polymorphisms (Maddison and Knowles 2006). Intraspecific studies are more likely to be affected by incomplete lineage sorting than analyses among species because incomplete lineage sorting is more likely at shallow time depths. To account for lineage sorting at shallow time depths, simulation studies suggest that sampling more individuals is better than sampling more loci to resolve the species tree (Maddison and Knowles 2006). In our study, we made a limited assessment of whether retention of sequence polymorphism within recently separated populations is likely to obscure phylogenetic relationships. We did this by comparing the phylogenies obtained when a single individual versus multiple individuals were sequenced per population.

Here we provide our methods and analysis for our "complete" data set, which included sequences for multiple individuals from each population. The main paper only includes our "reduced" data set, which contained sequence from only a single individual from each population. The large reduction in the number of taxa in the reduced data set allowed for more efficient and thorough methods of tree reconstruction. Thus, we give preference to the relationships and bootstrap values obtained from the reduced data set phylogeny. The complete dataset was analyzed to assess whether sequence polymorphism within a population suggested any alternative topologies to those found in the reduced data set.

Methods

PCR and sequencing conditions

The mitochondrial gene cytochrome b (cyt b) and some flanking regions were amplified with the primers IguaCytob_F2 (5'-CCACCGTTGTTATTCAACTAC-3') and IguaCytob_R2 (5'-GGTTTACAAGACCAATGCTTT-3') to give a 1174 base pair (bp) fragment. These primers were designed from a complete *Iguana iguana* mtDNA sequence (Genbank accession #AJ278511). Individual polymerase chain reactions (PCR) for cyt b were 30 μl in total volume and contained 20 ng

of genomic DNA, 0.133 μM of each primer, 0.8 mM of each dNTP, 4.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 1.5 U Taq DNA polymerase (New England Biolabs). PCR conditions were denature at 94°C for 5 minutes, then cycle 35 times at 94°C for 30 seconds, 50°C for 30 seconds, 72°C for 1.5 minutes, and then a final hold at 72°C for 5 minutes.

A 666 bp fragment of the mitochondrial gene ATPase 6 was amplified with the primers ScelopATP_F1 (5'-GAACCTGACCATGACACTAAG-3') and ScelopATP_R1 (5'-GCTTGGTGGGTCATTATACG-3'). These primers were designed from a complete *Sceloporus occidentalis* mtDNA sequence (Genbank accession #AB079242). Individual PCR reactions for ATPase 6 were 24 μl in total volume and contained 20 ng of genomic DNA, 0.133 μM of each primer, 0.4 mM of each dNTP, 3.5 mM $MgCl_2$, 25 mM KCl, 10 mM Tris-HCl (pH 8.3), and 1.6 U Taq DNA polymerase. PCR conditions were denature at 94°C for 5 minutes, then cycle 35 times at 94°C for 30 seconds, 48°C for 30 seconds, 72°C for 1 minute, and then a final hold at 72°C for 5 minutes.

For cyt b, internal sequencing primers (UtaCytob_F3: 5'- CCCGATTCTTTACCTTCCACT-3', UtaCytob_R3: 5'-GAGGCTAGTCCTGTTGGATTG-3') were used to obtain full sequence from both strands. Other internal primers (UtaCytob_F4: 5'-TCAGTTGACAACGCAAC-3', UtaCytob_R4: 5'-GGATTTTGTCTGTGTTTGA-3', UtaCytob_R5: 5'-GGAATGGGAYTTTGTCTG-3') were used when sequence variation prevented the use of the original internal primers.

Three individuals were sequenced from each population except Anacapa Island (4 individuals), Santa Cruz Island (4 individuals), and Los Banos (5 individuals). Most individuals in the complete data set were sequenced according to the same conditions reported in the main paper. However, some individuals (37 of 127 ingroup individuals) had ATPase 6 sequences that were only 599 bp because they were amplified with the primers in Trépanier and Murphy (2001). In addition, for cyt b, some individuals (45 of 127) were not sequenced for the full 1174 bp with internal primers because 1130 bp of sequence was reliably obtained using only IguaCytob_F2 and IguaCytob_R2. Similarly, for ATPase 6, some individuals (34 of 127) were only sequenced with the ScelopATP_R1 primer because 633 bp of quality sequence was obtained from a single strand. Slightly shortened sequences should have minimal impacts upon the resolution of relationships among populations. All populations had at least one sample that was 1174 bp long for cyt b and 666 bp long for ATPase 6 (i.e. complete sequence for both genes). For cyt b, the 20 bp missing for some individuals fell in a conserved region of the gene, which minimizes any effects. Individuals missing the maximum possible sequence across both genes would only have 16 fewer parsimony informative characters out of the 486 parsimony informative characters observed for ingroup taxa.

Unique haplotypes of the combined sequence of ATPase 6 and Cyt b were identified using the program DNAsp v. 4.10 (Rozas et al. 2003). Phylogenies for the complete data set only included unique haplotypes.

Maximum likelihood analyses were conducted using PAUP* version 4.0b10 (Swofford 2002). The analysis of our complete data set used stepwise addition of taxa, NNI branch swapping, and the TIM+I+G model, as selected by Modeltest 3.7 (Posada and Crandall 1998). The parameters for the model were: Base=(0.3572 0.2951 0.0770), Nst=6, Rmat=(1.0000 19.7758 0.6769 0.6769 10.4236), Rates=gamma, Shape=0.9072, Pinvar=0.5250. Branch support was assessed by 100 bootstrap replicates with a neighbor joining starting tree and with NNI branch swapping. For comparison, the analysis of our reduced data set used stepwise addition of taxa, TBR branch swapping, and the TIM+I+G model selected by Modeltest. The parameters for the model were: Base=(0.3553 0.2966 0.0780), Nst=6, Rmat=(1.0000 18.7530 0.6206 0.6206 9.7984), Rates=gamma, Shape=0.9637, and Pinvar=0.5376. Branch support for the reduced data set was assessed by 1000 bootstrap replicates, with a neighbor joining starting tree and TBR branch swapping.

Bayesian trees were constructed using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The data set for the Bayesian analysis was partitioned by

gene, and by codon position within the two genes. Models of evolution for the six partitions were selected using MrModeltest 2.2 (Nylander 2004). For the complete data set, the models used were: Cyt b position 1: nst = 6, rates = invgamma; Cyt b position 2: nst = 6, rates = propinv; Cyt b position 3: nst = 6, rates = invgamma; Atp position 1: nst = 6, rates = gamma; Atp position 2: nst = 2, rates = propinv; Atp position 3: nst = 6, rates = gamma. This analysis was run for 5 million generations, with a temperature of 0.1 and 4 heated chains. A burnin of 500000 generations was used, by which time stationarity was achieved. We ran the analysis five times and compared the parameter values of each run to verify that the analyses did not become stuck on local optima. The reduced data set used all the same parameters as the complete data set, except that the temperature was set to 0.2 and Cyt b position 1 had rates = gamma.

Results

There were 99 unique haplotypes out of the 127 sequences obtained for *U. stansburiana* (Supplemental Fig. 1, see below). In most instances, haplotypes were shared among individuals within a population. Only two haplotypes were present in multiple populations. One of these was shared between Pisgah and Power, two populations that are four miles apart. The other haplotype (labeled as "Horseridge 1 + 2, Burns 1, Vantage 1, Lovelock 3" in Fig. 3) was widely distributed in populations from Nevada to Washington. This area is at the northern end of the distribution of *U. stansburiana*, and there is little phylogenetic resolution throughout the region.

There was a strong influence of geography on the clustering of haplotypes (Supplemental Fig. 1, see below). Of the 41 populations sampled, 18 had all their haplotypes form a monophyletic group, often with good support values. Even when population haplotypes were not monophyletic, they often were exclusive to a single population, grouped only with geographically close populations, and formed a monophyletic group when neighboring populations are included (e.g. Supplemental Fig. 1: Corral Hollow and Los Banos in Northern, CA).

Phylogenetic analysis using the reduced data set (Fig. 3, main paper) closely matched the relationships found using the complete data set, with just two differences (Supplemental Fig. 1). The first instance involves relationships within the Central, South CA clade. The reduced data set analysis has Corn Springs as sister to a clade containing the Granite Mtns., Pisgah, and Power. In addition to this topology, the complete analysis suggested that Granite Mtns. and Corn Springs could be sister to one another, or that the whole Central, South CA clade might best be represented as a polytomy. Both of these alternative possibilities were tested in our comparative analyses. The second difference between data sets involves the placement of the Mercury population, which the reduced data set places sister to Lytle Ranch, which belongs to the Utah, Nevada clade (Fig. 3, main paper). In addition to this topology, the complete data set includes two haplotypes from Mercury that grouped with the closest neighboring population, Daylight Pass, which belongs to the East CA clade (Supplemental Fig. 1). The affinity of the Mercury population is thus ambiguous and the analysis suggests that the Mercury population may be part of an intergrade zone where divergent clades meet, as was suggested by Ballinger and Tinkle (1972). Both alternative placements of Mercury were included in the comparative analyses.

Discussion

The complete data set analysis (Supplemental Fig. 1) only suggested two alternative topologies to the reduced analysis (Fig. 3, main paper). The general agreement between data sets suggests that retention of polymorphism may not be a serious problem in resolving mtDNA relationships among *Uta* populations. If retention of polymorphism within population was a problem, then population haplotypes would have been related to different, possibly geographically distant populations. Contrary to this, almost half of the populations had all their individual sequences form a monophyletic grouping, and most populations were most closely related to the geographically closest population.

There are two caveats to our analysis of incomplete lineage sorting. First, only a limited number of individuals (3-5) were sequenced per population, and sequencing more individuals could reveal new haplotypes with different evolutionary histories. However, given the strong geographic clustering of haplotypes in our data, it seems unlikely that new haplotypes would result in large changes in topology. Second, sampling multiple individuals can only help resolve problems due to retention of ancestral polymorphism, and cannot address problems of lineage sorting at deep nodes in the phylogeny where mtDNA haplotypes have been stochastically fixed. This is potentially a more serious problem, and future studies could benefit from sequencing multiple nuclear loci to obtain gene trees independent from the mtDNA tree presented here.

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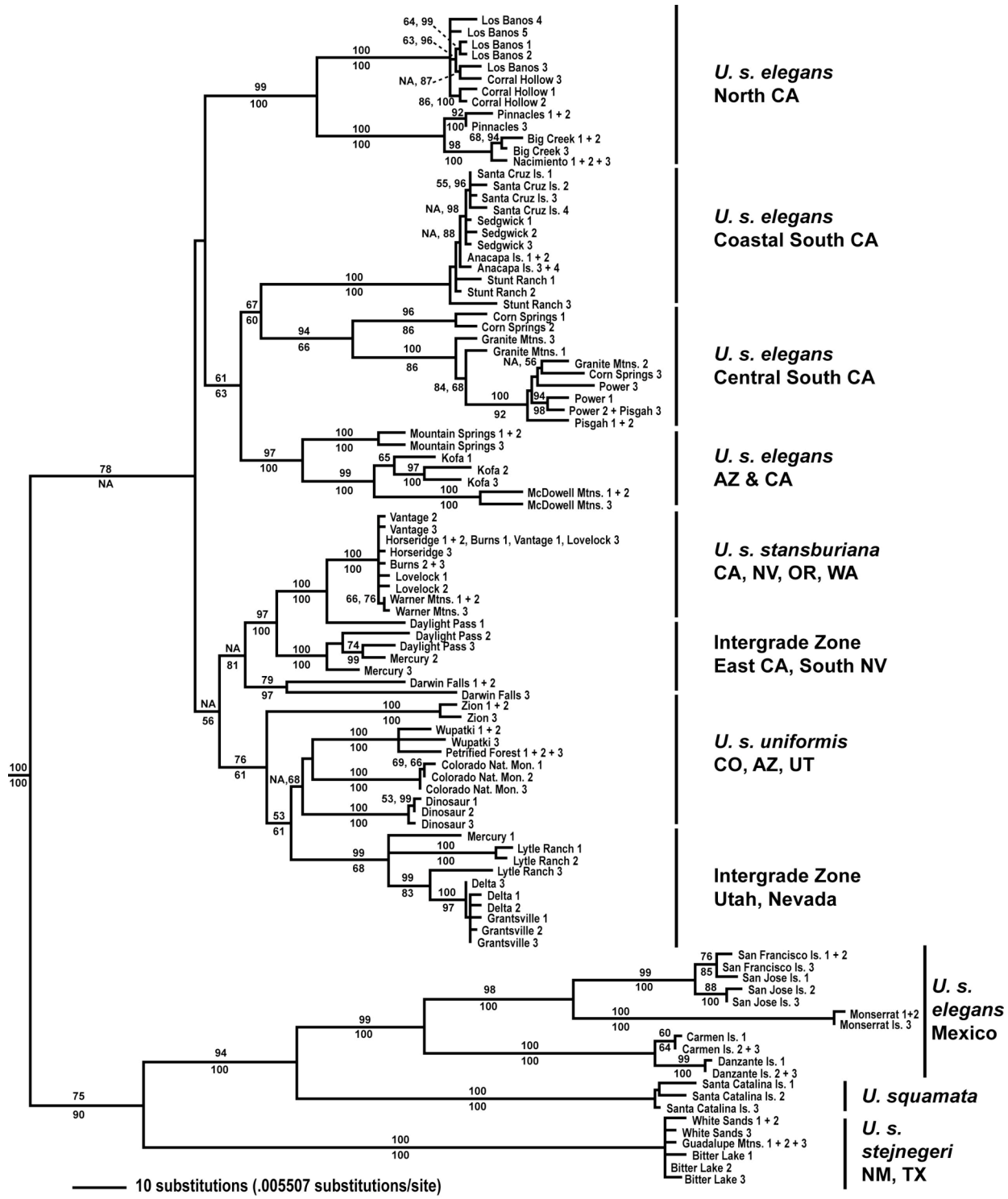

Supplemental Figure 1. Phylogram of *U. stansburiana* populations from the complete data set that included multiple individuals per population. Only unique haplotypes are shown, but branches with multiple numbers (e.g. 1 + 2) indicate the number of individuals that had an identical haplotype. The haplotype labeled #1 for each population corresponds to the single sequence used to reconstruct relationships among populations in Fig. 3 in the main paper. Numbers above a branch show likelihood bootstrap values out of 100 replicates and numbers below a branch show Bayesian posterior probabilities. Where space did not allow, these values are shown adjacent to a node, with the likelihood bootstrap value given first. A value of "NA" is given when one of the analyses did not give a value above 50. Geographic groupings of the populations are shown to the right and labeled with their respective subspecies. The population on Santa Catalina Island is considered a different species (*Uta squamata*). The tree has been pruned of outgroup taxa to allow better resolution of genetic distances within *U. stansburiana*.

Supplemental Table 1. Population locations and years sampled. The North and West columns give GPS coordinates for each population. Note that Los Banos has been continuously sampled since 1990 and Anacapa Island has been continuously sampled since 2000. A single representative year's data for each of these two populations is given in order to provide equal sample sizes. Three individuals were sequenced from each population except Anacapa Island (4 individuals), Santa Cruz Island (4 individuals), and Los Banos (5 individuals).

Population Number	Population Name	State	North	West	Years Data Collected
1	Corral Hollow	CA	37.63967	-121.48714	2004
2	Los Banos	CA	36.9962	-121.05068	2003
3	Pinnacles National Monument	CA	36.49351	-121.17299	2003
4	Big Creek University of California Reserve	CA	36.09435	-121.60294	2002
5	Nacimiento Road	CA	35.98917	-121.41258	2004, 2005
6	Sedgwick University of California Reserve	CA	34.73853	-120.02652	April 2002, June 2002
7	Santa Cruz Island, Channel Islands National Park	CA	34.04341	-119.57002	2003
8	Anacapa Island, Channel Islands National Park	CA	34.01325	-119.37053	2003
9	Stunt Ranch U.C. Reserve & Cold Creek Preserve	CA	34.10737	-118.65247	2002, 2007
10	Power (Off-Lava Site)	CA	34.81902	-116.33904	2003, 2004, 2005
11	Pisgah Lava Flow	CA	34.76157	-116.36576	2003, 2004, 2005
12	Granite Mountains University of California Reserve	CA	34.78786	-115.63743	2002, 2003
13	Corn Springs	CA	33.62182	-115.31622	2003
14	Mountain Springs	CA	32.67333	-116.09756	2006
15	Kofa National Wildlife Refuge	AZ	33.25277	-114.21918	2002, 2004
16	McDowell Mountains	AZ	33.66594	-111.86942	2006
17	White Sands National Monument	NM	32.79397	-106.21497	2002, 2004
18	Guadalupe Mountains National Park	TX	31.924	-104.99785	2002, 2004
19	Bitter Lake National Wildlife Refuge	NM	33.57756	-104.38543	2002, 2004
20	Carmen Island	BCS, Mexico	26.02136	-111.1628	2005
21	Danzante Island	BCS, Mexico	25.77868	-111.25669	2005
22	Monserrat Island	BCS, Mexico	25.67637	-111.02169	2005
23	Santa Catalina Island	BCS, Mexico	25.6101	-110.78635	2005
24	San Jose Island	BCS, Mexico	24.89715	-110.5846	2003
25	San Francisco Island	BCS, Mexico	24.8259	-110.58136	2003
26	Petrified Forest National Park	AZ	34.96414	-109.7891	2002, 2004
27	Wupatki National Monument	AZ	35.5308	-111.32377	2002, 2003
28	Zion National Park	UT	37.17331	-113.03378	2003, 2004
29	Colorado National Monument	CO	39.10119	-108.70588	2002, 2004
30	Dinosaur National Monument	UT	40.4198	-109.18961	2002, 2004
31	Mercury	NV	36.58605	-115.99556	2003
32	Lytle Ranch	UT	37.08341	-113.92692	2003
33	Delta	UT	39.6953	-113.09665	2004
34	Grantsville	UT	40.59868	-112.53988	2004
35	Darwin Falls, Death Valley National Park	CA	36.32103	-117.52014	2003, 2004
36	Daylight Pass, Death Valley National Park	NV	36.80285	-116.91518	2003, 2004
37	Lovelock	NV	40.26784	-118.53888	2003, 2004
38	Warner Mountains	CA	41.35359	-120.13028	2006
39	Burns	OR	43.43668	-118.92795	2006
40	Horseridge	OR	43.9608	-121.04621	2003, 2006
41	Vantage	WA	46.89992	-119.94836	2003, 2006

Supplemental Table 2. Tail break frequency data. N = number of individuals, M = Males, F = Females. The difference between the frequency of male tail breaks and female tail breaks (last column) gives our measure of sex-biased tail breaks.

Population Number	Population Name	Years Data Collected	N Females	N Males	M Tail Break Freq.	F Tail Break Freq.	M Tail - F Tail
2	Los Banos	2003	35	37	0.297	0.171	0.126
5	Nacimiento Road	2004, 2005	24	34	0.235	0.250	-0.015
7	Santa Cruz Island	2003	28	34	0.500	0.214	0.286
8	Anacapa Island	2003	59	55	0.782	0.559	0.222
9	Stunt Ranch	2002, 2007	24	20	0.6	0.353	0.247
10	Power (Off-Lava Site)	2003, 2004, 2005	52	55	0.291	0.365	-0.074
11	Pisgah	2003, 2004, 2005	44	47	0.255	0.432	-0.176
12	Granite Mountains	2002, 2003	16	21	0.211	0.438	-0.227
14	Mountain Springs	2006	8	21	0.238	0.000	0.238
15	Kofa	2002, 2004	12	30	0.333	0.333	0.000
16	McDowell Mountains	2006	11	21	0.381	0.273	0.108
17	White Sands Natl. Mon.	2002, 2004	21	27	0.407	0.286	0.122
18	Guadalupe Mountains	2002, 2004	12	19	0.474	0.333	0.140
19	Bitter Lake	2002, 2004	15	20	0.400	0.067	0.333
21	Danzante Island	2005	31	19	0.684	0.419	0.265
22	Monserrat Island	2005	22	7	0.286	0.136	0.149
23	Santa Catalina Island	2005	29	23	0.565	0.448	0.117
26	Petrified Forest	2002, 2004	10	15	0.267	0.400	-0.133
27	Wupatki Natl. Mon.	2002, 2003	15	11	0.455	0.333	0.121
28	Zion National Park	2003, 2004	14	22	0.500	0.429	0.071
29	Colorado Natl. Mon.	2002, 2004	17	34	0.412	0.235	0.176
30	Dinosaur Natl. Mon.	2002, 2004	19	24	0.167	0.211	-0.044
31	Mercury	2003	15	20	0.550	0.467	0.083
32	Lytle Ranch	2003	20	15	0.600	0.450	0.150
34	Grantsville	2004	16	25	0.160	0.313	-0.153
35	Darwin Falls	2003, 2004	21	15	0.267	0.238	0.029
36	Daylight Pass	2003, 2004	13	21	0.324	0.308	0.016
38	Warner Mountains	2006	13	17	0.294	0.231	0.063
40	Horseridge	2003, 2006	18	26	0.462	0.444	0.017
41	Vantage	2003, 2006	20	31	0.323	0.200	0.123

Supplemental Table 3. Sexual size dimorphism (SSD) observed within populations. Size data were restricted to a single sampling trip to avoid problems of averaging body size across multiple sampling trips with different time points of growth. Thus, sample sizes for some populations are smaller than those reported in Supplemental Table 2. Two-step SSD is equal to one when the sexes are equal in size, is greater than one when there is male-biased SSD, and is less than one when there is female-biased SSD. N = number of individuals. M = Males and F = Females. SVL = Snout-vent length, which is a measure of body size.

Population Number	Population Name	Year	N Females	N Males	F Mean SVL	M Mean SVL	2-Step SSD
1	Corral Hollow	2004	35	15	53.043	55.967	1.055
2	Los Banos	2003	35	37	52.886	57.568	1.089
3	Pinnacles Natl. Mon.	2003	9	15	50.778	53.400	1.052
4	Big Creek	2002	5	7	48.400	49.286	1.018
5	Nacimiento Road	2005	21	21	46.905	52.571	1.121
6	Sedgwick	June, 2002	12	21	47.750	49.952	1.046
7	Santa Cruz Island	2003	28	34	47.214	51.265	1.086
8	Anacapa Island	2003	59	55	51.356	58.473	1.139
9	Stunt Ranch	2007	17	20	49.147	52.050	1.059
10	Power (Off-Lava Site)	2005	43	46	45.163	46.326	1.026
11	Pisgah	2005	30	28	45.667	48.679	1.066
12	Granite Mountains	2002	16	18	43.500	44.833	1.031
13	Corn Springs	2003	6	6	43.167	46.333	1.073
14	Mountain Springs	2006	8	21	46.250	51.690	1.118
15	Kofa	2004	7	18	45.857	48.611	1.060
16	McDowell Mountains	2006	11	21	48.591	52.643	1.083
17	White Sands Natl. Mon.	2004	11	13	45.818	49.231	1.074
18	Guadalupe Mountains	2002	6	14	42.667	47.286	1.108
19	Bitter Lake	2004	9	13	46.889	50.923	1.086
20	Carmen Island	2005	12	9	48.083	54.389	1.131
21	Danzante Island	2005	31	19	49.194	54.658	1.111
22	Monserrat Island	2005	22	7	46.818	53.000	1.132
23	Santa Catalina Island	2005	29	23	48.862	53.391	1.093
24	San Jose Island	2003	7	7	45.571	50.429	1.107
25	San Francisco Island	2003	9	8	44.333	48.500	1.094
26	Petrified Forest	2004	8	11	48.375	47.727	0.986
27	Wupatki Natl. Mon.	2003	14	10	46.500	47.800	1.028
28	Zion National Park	2003	8	17	49.000	51.235	1.046
29	Colorado Natl. Mon.	2004	12	20	45.250	45.500	1.006
30	Dinosaur Natl. Mon.	2004	9	19	45.333	45.474	1.003
31	Mercury	2003	15	20	47.267	49.900	1.056
32	Lytle Ranch	2003	20	15	47.000	49.267	1.048
33	Delta	2004	7	8	45.429	46.063	1.014
34	Grantsville	2004	16	25	50.813	49.480	0.973
35	Darwin Falls	2004	14	11	43.929	47.000	1.070
36	Daylight Pass	2004	7	18	48.714	50.111	1.029
37	Lovelock	2003	8	8	47.375	47.000	0.992
38	Warner Mountains	2006	13	17	45.692	47.794	1.046
39	Burns	2006	31	19	49.484	50.553	1.022
40	Horseridge	2006	11	20	42.000	44.600	1.062
41	Vantage	2006	13	21	45.962	47.833	1.041

Supplemental Table 4. Clutch size data for *Uta* populations. Year = the year the clutch size data were gathered. N = number of individuals. SD = one standard deviation around the mean. A "–" in the published location column indicates that the population sampled in our study (first two columns) exactly corresponds to the population originally sampled for clutch size data. Locations that are not exact matches are geographically close to the populations that we sampled and the exact location is listed. See the literature cited section of the main paper for the clutch size data references.

Unpublished clutch size data from Corl and Sinervo & Wilson was collected as follows. Female follicular development was assessed by abdominal palpation. Lizards with enlarged follicles were housed in individual terraria filled halfway with a mixture of three parts sphagnum peat moss to one part sterile sand that was kept moist with the regular addition of water. Each terrarium contained a small dish of sand on which the lizards could bask under a heat lamp suspended above the terrarium. Terraria were inspected daily for the presence of eggs.

Population Number	Population Name	Year	N	Mean Clutch	SD	Published Location	Source of Data
1	Corral Hollow	1988-1989	16	4.6		-	Sinervo and Licht, 1991
2	Los Banos	1988-1989	35	6.3		-	Sinervo and Licht, 1991
5	Nacimiento	2005	7	3.29	0.49	-	Corl, unpublished data
8	Anacapa Island	2000-2003	129	4.287	1.06	-	Comendant, unpublished data
9	Stunt Ranch	2007	9	3.22	0.67	-	Corl, unpublished data
10	Power	2005	17	4.412	0.71	-	Corl, unpublished data
11	Pisgah	2005	9	4.556	0.88	-	Corl, unpublished data
13	Corn Springs	1988	7	4.857	1.21	-	Sinervo & Wilson, unpublished data
16	McDowell Mtns.	1965, 1966	50	4.00		Phoenix South Mtn., AZ	Parker and Pianka, 1975
17	White Sands	1970	16	3.31		Dona Ana County, NM	Parker and Pianka, 1975
19	Guadalupe Mtns.	1963	20	4.4		Kermit, TX	Tinkle et. al., 1970
20	Carmen Is.	?	22	2.55	0.07	-	Case, 2002
21	Danzante Is.	?	24	2.51	0.06	-	Case, 2002
22	Monserrat Is.	?	19	1.71	0.10	-	Case, 2002
23	Santa Catalina Is.	?	10	1.62	0.09	-	Case, 2002
28	Zion	1967	19	4.105		Hurricane, UT	Tinkle et. al., 1970
29	Colorado Nat. Mon.	1965	135	3.2		-	Tinkle et. al., 1970
32	Lytle	1967	21	3.571		Santa Clara, UT	Tinkle et. al., 1970
33	Delta	1967	21	3.619		-	Tinkle et. al., 1970
34	Grantsville	1967, 1971, 1972	110	4.47		-	Parker and Pianka, 1975
35	Darwin Falls	1988	11	3.545	0.69	-	Sinervo & Wilson, unpublished data
36	Daylight Pass	1967	21	4.333		-	Tinkle et. al., 1970
37	Lovelock	1988	7	5.143	1.21	-	Sinervo & Wilson, unpublished data
39	Burns	2004	46	4.2	1.17	-	Zani, 2005
40	Horseridge	1988	14	3.786	0.97	Deschutes River, OR	Sinervo & Wilson, unpublished data
41	Vantage	1988	13	4.000	1.00	-	Sinervo & Wilson, unpublished data