

Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation

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Edited by David B. Wake, University of California, Berkeley, CA, and approved January 11, 2010 (received for review August 21, 2009)

Polymorphism may play an important role in speciation because new species could originate from the distinctive morphs observed in polymorphic populations. However, much remains to be understood about the process by which morphs found new species. To detail the steps of this mode of speciation, we studied the geographic variation and evolutionary history of a throat color polymorphism that distinguishes the “rock-paper-scissors” mating strategies of the side-blotched lizard, *Uta stansburiana*. We found that the polymorphism is geographically widespread and has been maintained for millions of years. However, there are many populations with reduced numbers of throat color morphs. Phylogenetic reconstruction showed that the polymorphism is ancestral, but it has been independently lost eight times, often giving rise to morphologically distinct subspecies/species. Changes to the polymorphism likely involved selection because the allele for one particular male strategy, the “sneaker” morph, has been lost in all cases. Polymorphism loss was associated with accelerated evolution of male size, female size, and sexual dimorphism, which suggests that polymorphism loss can promote rapid divergence among populations and aid species formation.

lizard | morph | phylogeny | rock-paper-scissors | *Uta stansburiana*

Polymorphic forms within a population could be the starting material for new species (1–4). Tests of how polymorphisms diversify into new lineages have been relatively rare (2, 5, 6), despite increasing recognition that polymorphisms, such as alternative mating strategies, are common within species (7–9). Here we investigate the processes by which a morph in a polymorphic population may diverge to found a new species.

Competition within a population can generate morphs possessing alternative adaptations, which may become as phenotypically distinct as separate species and thus primed for speciation (2, 3). West-Eberhard theorized how morphs may promote speciation (2, 10) and suggested that selection in new environments may favor a particular morph, destabilizing the dynamics maintaining the polymorphism. If a population loses a morph, this can lead to rapid phenotypic divergence in the remaining morph(s) (2). One cause of rapid divergence is that genetic evolution may be constrained in polymorphic populations, because alleles that increase the fitness of a single morph but decrease the fitness of other morphs may be unable to spread. Loss of a morph allows these alleles to spread, which can result in rapid phenotypic evolution in the direction of specialization on the remaining morph phenotypes, a process called *character release* (2). Another reason for rapid evolution coincident with morph loss is that colonization of a new ecological environment may select against a particular morph and also favor novel phenotypes in the remaining morphs. In addition, loss of a morph changes the competitive environment, because the fitness of morphs can depend on the frequency of other types in the population (8). Rapid evolution may occur when morph phenotypes beneficial for competition with a particular morph adapt to a new selective environment where that morph is absent (discussed in ref. 11). Rapid evolution resulting from any of these causes can promote reproductive isolation among populations with different numbers of morphs by accelerating the formation of Dobzhansky-Muller

incompatibilities (i.e., deleterious epistatic interactions that arise as a byproduct of genetic divergence) or by generating assortative mating due to mismatches between male traits and female preferences in different populations. The final result of this process is a new species that originated from a morph that evolved in a polymorphic population, but is now modified in a new environment.

We studied the geographic variation and evolutionary history of the side-blotched lizard, *Uta stansburiana*, to investigate the process by which polymorphic populations may diverge to form new, phenotypically distinct lineages. Both male and female *U. stansburiana* exhibit different strategies that are signaled by different throat colors. Orange-throated males control large territories with many females because of their greater mass, aggression, and testosterone levels (12). Blue-throated males mate-guard females by cooperative defense of territory (13). Yellow-throated males mimic females and sneak onto other males' territories to obtain mates (8, 14). These male mating types are maintained by negative frequency-dependent selection, with orange being most fit when blue is common, yellow being most fit when orange is common, and blue being most fit when yellow is common—a biological “rock-paper-scissors” game (8). Females exhibit different life history strategies, with orange-throated females (r-strategists) producing large numbers of small progeny and yellow-throated females (K-strategists) producing fewer but higher-quality offspring (15).

We investigated the following hypotheses that originate from West-Eberhard's theoretical development and that test whether speciation [in the sense of the formation of new evolutionary lineages (16)] is associated with changes in the number of polymorphic strategies. (i) If morphs fix and persist to become species, monomorphic lineages should resemble morphs found in closely related polymorphic lineages. (ii) If polymorphisms promote diversification, then polymorphic lineages should be ancestral and monomorphic lineages should be derived. (iii) If selection results in the loss of morphs, then there will be nonrandom fixation of morphs adapted to a particular environment. (iv) If morph loss promotes phenotypic divergence, then rapid evolution will accompany shifts in morph number. We call this process *morphic speciation*, because it relates to how a morph that originated as part of a polymorphism can go on to found a new species.

Author contributions: A.C. and B.S. designed research; A.C., A.R.D., S.R.K., and B.S. performed research; A.C. analyzed data; and A.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0909480107/DCSupplemental.

Results

We studied the geographic variation of *U. stansburiana* to investigate the prevalence of the polymorphism across different environments. Strategies were assessed by scoring the frequencies of alleles at the OBY locus (for orange, blue, and yellow), which governs the morph phenotypes of *U. stansburiana* (15, 17). The OBY locus is likely a single gene with three alleles, or two tightly linked genes, as determined by field pedigree data (15), laboratory breeding experiments (18), gene mapping studies (17), and theoretical models (19). OBY locus alleles have codominant effects on throat color expression, but *o* and *y* are dominant to *b* in their effects on male strategy ($O = oo, ob, oy$; $B = bb$; $Y = yy, by$). In females, *o* alleles have a dominant effect in generating the r-strategy (oo, ob, oy) vs. K-strategy ($Y = yy, by, bb$) life history types (15, 18).

Geographic surveys of the throat color polymorphism showed that the polymorphism is widely maintained in both males and females. All three color alleles were found in most populations of the two southern subspecies, *U. stansburiana elegans* and *U. s. stejnegeri* (Fig. 1). In total, the OBY polymorphism was found in 23 of our 41 sampled populations. However, many populations were missing some of the color alleles. Only orange and blue alleles were

found in four isolated populations within the range of *U. s. elegans* (Fig. 1: populations 8, 11, 19, and 20), one of which is recognized as a distinct species, *Uta squamata* (ref. 20). In addition, northern lineages were not polymorphic. The subspecies *U. s. stansburiana* was fixed for orange throats. A clade in western Utah (gray area, Fig. 1) was polymorphic for all three types in southern Utah and Nevada but was fixed for orange in the north. The subspecies *U. s. uniformis*, and independent populations in California (populations 4 and 5), were fixed for the phenotype of an *ob* heterozygote (a blue throat bordered by orange pigment; Fig. 2). However, both locations lacked evidence for the independent segregation of *o* and *b* alleles that is present in trimorphic populations (18) and dimorphic populations (*oo, ob*, and *bb* phenotypes were observed; Fig. 2), which suggests a genetic change in throat color expression in these locations. Regardless of the genetics, *U. s. uniformis* and the other monomorphic/dimorphic populations, subspecies, and species all resemble morphs found in polymorphic lineages (Fig. 2).

We tested whether monomorphic/dimorphic lineages are derived from polymorphic lineages by constructing an intraspecific phylogeny and mapping on the presence/absence of the OBY alleles (Fig. 3 and Fig. S1). The most parsimonious character mapping recon-

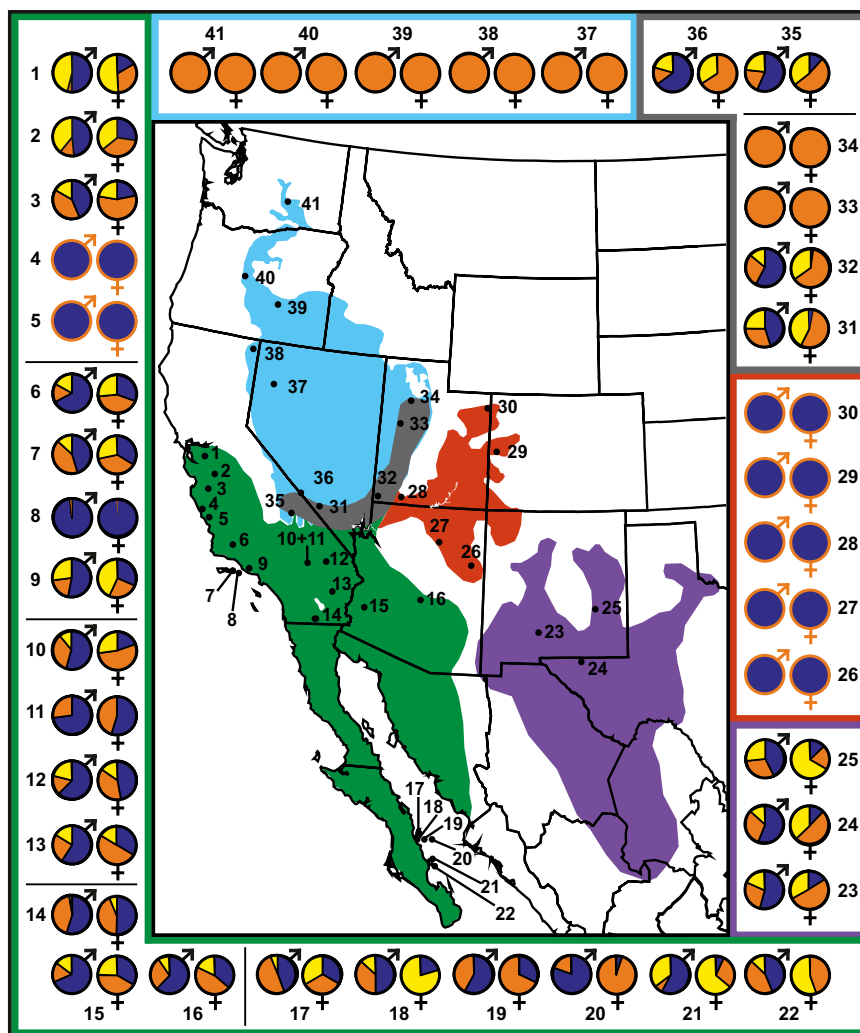


Fig. 1. Geographic variation in the throat color polymorphism of *U. stansburiana*. Pie charts in the male/female symbols show proportions of orange, blue, and yellow alleles in each sex observed across sampling trips. Monomorphic populations fixed for blue throats surrounded by orange are denoted by orange male/female symbols filled with blue. Ranges of subspecies are differently colored: green = *U. s. elegans*; purple = *U. s. stejnegeri*; light blue = *U. s. stansburiana*; red = *U. s. uniformis*; gray = intergrade zone. Population 20 represents a different species (*Uta squamata*) derived from *U. stansburiana*. Phylogeographic clades within subspecies are separated by black lines.

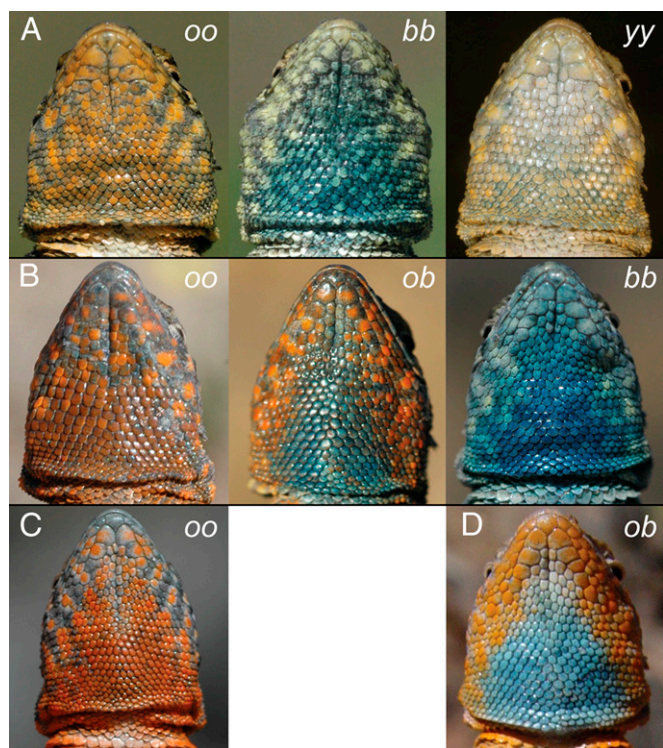


Fig. 2. Throat colors of males in different populations of *U. stansburiana*. (A) Homozygous individuals from trimorphic population 2. (B) All genotypes observed in dimorphic population 11. (C) Individual from monomorphic orange population 38. (D) Individual from monomorphic *ob* population 29.

structured basal branches within the tree as trimorphic and had reductions in the numbers of throat color alleles appearing at the tips of the tree. In addition, a closely related outgroup species (*Urosaurus ornatus*) is polymorphic for orange, blue, and yellow throats (21), which further supports a trimorphic ancestral state reconstruction

for *Uta*. Assuming a molecular clock of 1% to 2% divergence per million years (*Materials and Methods*), the common ancestor of *Uta* occurred 5–9 million years ago, and *Uta* and *Urosaurus* shared a common ancestor 14–27 million years ago. Thus, the OBY polymorphism has been maintained for millions of years but has recently destabilized in many locations.

Widespread convergent evolution has occurred during the evolution of the mating system, because in all cases the yellow allele has been lost. Most losses were in geographically separate clades with high bootstrap support, indicating that they have occurred independently (Fig. 3). For pairs of losses occurring within a geographic clade, independent evolution was assessed by constraining losses to be monophyletic and performing a Shimodaira-Hasegawa test to evaluate significant departure from the unconstrained tree (22). Losses in the Mexico clade were independent because monophyly was rejected ($P = 0.004$). Monophyly of *U. s. uniformis* populations sharing the same throat color could not be rejected ($P = 0.60$), suggesting a single loss event. In total there have been eight independent losses of the yellow allele.

Genetic drift is not a likely explanation for the biased loss of the yellow allele. Yellow is not a rare allele, as shown by the average allele frequencies at our focal population at Los Baños, California (1993–2005: $o = 0.170, b = 0.430, y = 0.401$) and across all trimorphic populations ($o = 0.346, b = 0.375, y = 0.279$). On the basis of the average frequency of yellow, the probability of eight independent losses occurring by random chance is low [Los Baños data: $P = (1 - 0.401)^8 = 0.017$; trimorphic populations: $P = 0.073$]. However, even a common allele may be prone to drift if it exhibits high variability in its frequency. This does not explain the loss of the yellow allele either, because the orange allele has the highest coefficient of variation at Los Baños (1993–2005: $o = 0.33, b = 0.14, y = 0.18$). Thus, losses in the polymorphism are best explained by selection.

The multiple independent losses of morphs that we observed allowed us to test the hypothesis that changes in numbers of morphs is associated with rapid phenotypic evolution. We studied the rates of evolution for male body size, female body size, and sexual size dimorphism (SSD). For all three traits, a model with different rates of phenotypic evolution for trimorphic and dimorphic/monomorphic populations provided a better fit to the data [i.e., had a lower second-

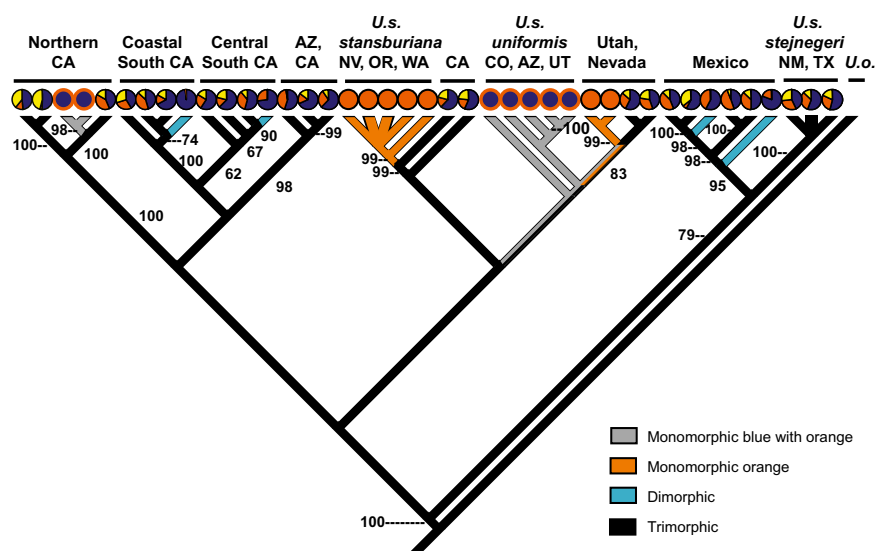


Fig. 3. Evolutionary history of the alternative mating strategy polymorphism of *U. stansburiana*. Colored branches on the maximum likelihood tree reconstruct transitions in the throat color polymorphism by parsimony. Branches shown with uncertainty in the reconstruction (i.e., multiple colors) are resolved as trimorphic if all *U. s. uniformis* populations are constrained to be monophyletic. Pie charts show male allele frequencies. Geographic regions correspond to groups of pie charts in Fig. 1. Monophyletic subspecies are labeled. The outgroup is *Urosaurus ornatus* (*U.o.*). Numbers adjacent to branches show bootstrap support values.

order Akaike information criterion (AIC_c) value] than a model with a single rate of phenotypic evolution across all populations (Fig. 4A). In all cases, dimorphic/monomorphic populations had higher rates of phenotypic evolution than trimorphic populations (Fig. 4B). Increased rates of evolution in dimorphic/monomorphic populations ranged from 9-fold higher for SSD to 66-fold higher for female size.

The direction of change in body size and SSD differed among the three categories of morph loss. Three of four dimorphic populations had increased male size and SSD relative to closely related trimorphic populations (*SI Text* and *Table S1*). The two independent sets of monomorphic *ob* populations both had smaller males than trimorphic relatives, and one set also had smaller females (*Table S2*). In contrast, one set of monomorphic orange populations had significantly larger females, and male size had not changed; the other set of orange populations had no significant changes. Monomorphic populations generally had reduced SSD relative to polymorphic populations (11). Thus, the direction of phenotypic changes associated with morph loss are contingent upon what morphs were lost.

Discussion

The geographic variation and evolutionary history of the OBY polymorphism is consistent with patterns expected from the theory of morphic speciation. First, we observed multiple dimorphic and monomorphic populations that resemble the morphs found in trimorphic populations. This resemblance, combined with the geographic isolation or northern distribution of monomorphic/dimorphic populations, suggests that rock-paper-scissors cycles of morphs have destabilized in many locations as *U. stansburiana* spread into new habitats, and this resulted in the loss and fixation of certain morphs. This result is not forgone, because polymorphism loss could proceed in directions not consistent with morphic speciation, such as by losing all throat colors or by a new throat color type

evolving that excluded all others. Prior evidence for morphic speciation has largely consisted of monomorphic species resembling a morph found in a closely related polymorphic species (2, 6, 10). We also found that morph loss was coincident with species formation, because two of the four subspecies we sampled were monomorphic and the one insular species sampled was dimorphic. Additionally, we observed all stages of divergence expected under morphic speciation, from populations to morphologically distinct subspecies/species exhibiting morph loss. This geographic variation may thus illustrate different time points of the process by which morphs are lost and go on to found separate subspecies/species.

Our phylogenetic analysis revealed that the OBY polymorphism has been maintained for millions of years and is the ancestral state for the species. This suggests that rock-paper-scissors dynamics can maintain genetic diversity for millions of years, even across speciation events. Trans-species polymorphism, such as self-incompatibility or MHC alleles, are considered to be rare (23); our study suggests that further investigations of negative frequency-dependent mating systems may reveal more of these kinds of polymorphism. Long-term maintenance of the polymorphism is relevant for the process of speciation because it allows sufficient time for morph phenotypes to diverge from one another into markedly distinct variants. For example, alternative mating strategies often have different behaviors, colors, sizes, and weapons (7–9). Polymorphic species thus contain abundant variation, which can facilitate the colonization of new habitats and diversification into new lineages.

Repeated losses of the yellow allele suggest that although rock-paper-scissors strategies form an evolutionarily stable state within many populations (8), over larger spatial and temporal scales the yellow sneaker male strategy (paper) may be beaten by blue mate-guarders (scissors) and orange territory usurpers (rock). This demonstrates that morphs may be lost from highly stable systems maintained by frequency-dependent selection, a result that is consistent with other studies of polymorphism (24, 25). Repeated loss of particular morphs suggests that selection acts on the phenotypic variation observed among morphs when new habitats are colonized and when new species are formed. Thus, divergence among morphs within populations can be a precursor for later diversification.

We found strong support for the hypothesis that rapid phenotypic evolution may occur when morphs are lost. In some cases, this rapid evolution has led to the evolution of new phenotypes, because whereas all polymorphic populations have male-biased SSD, some monomorphic populations have no SSD, and one monomorphic population has evolved female-biased SSD (11). This indicates that morph loss is important for phenotypic diversification.

Patterns of body size evolution after morph loss suggest that selection pressures differ among trimorphic, dimorphic, and monomorphic populations. Males in dimorphic populations may have increased in size relative to males in trimorphic populations because of the benefits of large size in male–male competition for territories (26). With the loss of nonterritorial yellow males, all males in dimorphic populations would be engaged in territorial defense, increasing the intensity of selection on male size. In contrast, males from monomorphic *ob* populations are generally smaller than males from trimorphic populations, which was unexpected because these males have throat colors associated with aggressive, territorial male morphs in our focal trimorphic study population (12). However, if all males in a population had the same aggressive strategy, fights would be especially costly, which would lead to selection for reduced aggression and relaxed selection for large male body size. Note that changes in male size among populations are not an artifact of the loss or increased frequency of a large-bodied morph, because although male morphs differ in mass (12), they do not differ in length (*SI Text*), which was the measure of body size that we used. Female size has also changed in some monomorphic populations, which could be due to selection on female life history. Changes in

A Trait	Single-Rate AIC_c	Multi-Rate AIC_c	Δ_i
SSD	-90.1	-108.3	18.27
M size	284.6	241.4	43.20
F size	292.5	229.0	63.52

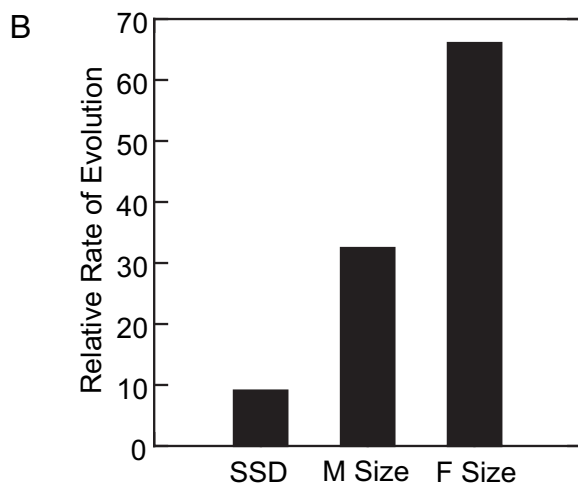


Fig. 4. Rates of evolution of SSD, male (M) body size, and female (F) body size. (A) Comparisons between a single-rate model of phenotypic evolution across the tree and a multi-rate model, which had one rate of evolution for trimorphic populations and another rate for monomorphic and dimorphic populations. Δ_i , differential AIC_c . Values of $\Delta_i > 10$ indicate essentially no support for the model with the higher AIC_c . (B) Relative rates of evolution are the rates of evolution in monomorphic and dimorphic populations divided by the rates of evolution in trimorphic populations.

clutch size are positively correlated with changes in female size in monomorphic populations, which suggests that fecundity selection has affected body size evolution in these populations (11). Detailed studies are needed in dimorphic and monomorphic populations to determine what strategies males and females adopt, understand the selective forces in these populations, and provide data on the possible causes of morph loss.

Rapid evolution arising from the loss or fixation of a morph could promote the formation of reproductively isolated species. Prezygotic isolation can result from females preferring particular male body sizes, which can lead to assortative mating (27). Females at our focal trimorphic study population at Los Baños prefer large males (28) and should thus assortatively mate with males from trimorphic populations over the smaller males in monomorphic *ob* populations. Similarly, if females from dimorphic populations also prefer large males, they should assortatively mate with males from their own population, which are larger than males in trimorphic populations. Thus, the rapid evolution of male size when morphs are lost could lead to a discordance with female size preferences, resulting in assortative mating among populations. In addition, processes that speed up genetic divergence among populations can lead to postzygotic isolation by promoting the accumulation of Dobzhansky-Muller incompatibilities, which progressively develop as the number of divergent loci increases (29). Body size is likely not to be the only trait that has changed with morph loss, because the morphs of *U. stansburiana* differ in many heritable traits, including behavior, hormone levels, clutch size, egg mass, and immunocompetence (3, 8, 12, 15). These phenotypic differences result from the competitive interactions among morphs, which select for adaptive combinations of throat color and trait alleles (3, 17, 18). Therefore, the loss of a morph could generate a large number of incompatibilities, as the multiple traits comprising morph phenotypes adapt to the new mating system.

Morph speciation offers insight into the process of speciation distinct from more traditional geographic modes of speciation (29, 30). Although allopatric/parapatric distributions likely facilitate changes in morph social structure, morph speciation suggests that phenotypic divergence relevant to speciation can originate before geographic isolation. Morph speciation also explains why monomorphic lineages resembling polymorphic types repeatedly evolve and why rapid phenotypic evolution may occur during speciation. Alternative mating types may be especially prone to morphic speciation given their involvement in reproductive traits. Morphic speciation offers new ways to understand the process of speciation and to link microevolutionary divergence within populations to macroevolutionary patterns of diversification.

Materials and Methods

Scoring Mating Strategy Genotypes. Three alleles at the OBY locus govern the distinct morph phenotypes of male and female *U. stansburiana* (15, 17, 18). Throat color genotypes of each lizard were visually scored immediately after capture. Genotypes were assigned according to the intensity and location of the orange, blue, and yellow coloration that varies across the throat of the lizard. Methods for scoring throat color genotypes are described in ref. 18 and are detailed in ref. 31. The University of California, Santa Cruz Institutional Animal Care and Use Committee approved our methods of capturing animals.

A challenge faced when scoring the presence/absence of OBY genotypes across different populations was that throat colors develop and fade depending on the reproductive state of the lizards. The following steps were taken to ensure that the presence/absence of alleles was scored accurately. First, the majority of populations were visited at least twice at different times of the year (either within or between years) to control for the temporal changes of breeding colors (Table S3). Second, throat color alleles were deemed to be accurately scored only when the lizards were reproductive. Reproductive state was assessed by abdominally palpating female lizards to verify that they either had developing follicles or oviductal eggs. Another indication that the lizards were reproductive was the presence of bright blue dots of pigment on the backs of male lizards, which develop at the beginning of the breeding season when throat colors can be reliably scored and then fade to white as the breeding season ends. Third, populations scored as missing an

OBY allele were either resampled across years, or large samples were collected within a year (Table S3). This controlled for the possibility that an allele was missed because of sampling error if it was at low frequency in a particular year. Fourth, a minimum sample size of 30 lizards was obtained for most populations. Thirty lizards yield a sample of 60 alleles, which provides a 95% probability of detecting an allele if it is at only 5% frequency in the population.

Phylogenetics. Two mtDNA genes, cytochrome *b* (1,150 bp), and ATPase 6 (666 bp) were sequenced according to methods given in ref. 11. A maximum likelihood tree was constructed in PAUP* 4.0b10 (32) with the TIM+I+G model selected by Modeltest 3.7 (33) as described in ref. 11. The topology of the maximum likelihood tree did not differ substantially from a tree constructed using Bayesian methods (11). Branch support was assessed by 1,000 bootstrap replicates. Note that mtDNA only gives a maternal evolutionary history, and the history of nuclear genes may differ. However, it is likely that our mtDNA phylogeny reflects the history of other loci, because major phylogenetic breaks are consistent with both geography and previous subspecies designations based on morphological characters (11, 34, 35).

Ancestral states were reconstructed by parsimony in Mesquite 1.12 (36). A reconstruction of ancestral states using the maximum likelihood Mk1 model does not differ significantly from the parsimony results, and also reconstructs eight independent losses of the yellow allele (Fig. S1). Shimodaira-Hasegawa tests (22) were implemented in PAUP* and were conducted with all of the tested topologies included together with the maximum likelihood tree when calculating significance, as recommended by Goldman et al. (37).

Estimating the Age of the OBY Polymorphism. We used the program BEAST v.1.4.8 (38) to estimate the time to the most recent common ancestor (TMRCA) for *Uta*. This provides a minimum estimate of how long the OBY polymorphism has been maintained because our analyses indicated that the OBY polymorphism is the ancestral state for *U. stansburiana* (see above). The OBY polymorphism is also likely to be the ancestral state for the common ancestor of *Uta* and *Urosaurus* if we make the parsimonious assumption that the polymorphic orange, blue, and yellow throat colors in *Urosaurus* (21) are not due to convergent evolution. Therefore, we also estimated a TMRCA for *Uta* + *Urosaurus*. Fossils to calibrate a molecular clock were not available, but mtDNA genes typically evolve at a rate of 1% to 2% per million years in other lizards (39). We used 2% divergence per million years to generate a lower bound for the TMRCA and 1% divergence per million years to generate an upper bound. Although using such a range in rates leads to considerable uncertainty in our estimates, our goal was simply to provide a rough estimate of the amount of time the OBY polymorphism has been maintained. In our BEAST analyses we used a strict molecular clock, constant population size, and the general time reversible + gamma + invariant sites model with four rate categories. We ran our Markov chain Monte Carlo analyses for 30 million generations and logged every 1,000 steps. We reran each analysis to verify that it was not stuck on a local optimum. We used Tracer v1.4 to determine convergence, measure the effective sample size of each parameter (all were above 600), and calculate the mean TMRCA.

Tests of Evolutionary Rates. The snout-vent length (SVL) of each lizard was measured to the nearest half millimeter to provide a measure of body size. Many populations were sampled multiple times to gather data on the OBY polymorphism, but we restricted our population estimates of mean male SVL, mean female SVL, and SSD to the sampling trip with the largest sample size to avoid problems of averaging body size across different time points of growth in the different samples. For one population (no. 24), data from the sampling trip with a smaller sample size were used because these data were collected in a time of year more comparable with the other populations. We only included animals above the minimum size for reproduction as determined by the smallest size at which breeding coloration or evidence of developing ovarian follicles was exhibited. SSD was calculated by the two-step ratio method (40), in which the size difference between males (M) and females (F) is calculated as follows: If $M \geq F$, $SSD = M/F$, and if $F \geq M$, $SSD = 2 - F/M$. Data on body size and SSD for each population are given in ref. 11.

We used the program Brownie (41) to test whether populations that have lost morphs had accelerated rates of evolution. Brownie requires a tree with branch lengths proportional to time. Therefore, we constructed a maximum likelihood tree constrained to obey a molecular clock in PAUP* 4.0b10 (32). The resulting tree differed in two ways from the optimal tree found in an unconstrained analysis, both involving groupings with little or no bootstrap support. For consistency, we used Mesquite to rearrange the tree topology to match the topology of the optimal tree (Fig. 3). Additionally, all *U. s. uniformis* populations were constrained to be monophyletic, as was suggested by their similar throat colors and the Shimodaira-Hasegawa test results described in

Results. Brownie also required a tree with no zero-length branches, so we set any zero-length branches to 0.0001 substitutions per site, a value just less than the shortest branch observed on the tree.

In Brownie, we reconstructed the history of the polymorphism, with trimorphic and dimorphic/monomorphic populations coded as two discrete characters (no-loss vs. loss). We then used the noncensored rate test to compare rates of evolution in the two types of populations. The noncensored test calculates the likelihood of two models of trait evolution, one in which there were different rates of phenotypic evolution for each discrete state and the other with a single rate of evolution across the tree (41, 42). The AIC_c was then used to assess whether the multi-rate or single-rate model of evolution had more support (41). The best model was the one with the smallest AIC_c value (43). The number of free parameters (K) was two for the single-rate model and three for the multi-rate model. We calculated Δ_i , which was the difference between the model with the lowest AIC_c value and the other model. Values of $\Delta_i > 10$ indicated that the model with the higher AIC_c had essentially no support rel-

ative to the model with the lower AIC_c (43). We used the rates of evolution from the multi-rate model to compute relative rates of evolution, which were calculated by dividing the rates of evolution in monomorphic and dimorphic populations by the rates of evolution in trimorphic populations.

ACKNOWLEDGMENTS. We thank all the parks/reserves (Table S3) that helped us obtain geographic samples (all permits were to A.C. except the California state permit, to B.S.); Island Conservation, Grupo de Ecología y Conservación de Islas, W. Frick, and P. Heady for assistance in sampling in Mexico; D. Mulcahy for providing a range map of the subspecies of *U. stansburiana*; and A. Thanukos, W. Cover, A. D'Amore, K. Dlugosch, G. Pogson, J. Thompson, B. Lyon, and two anonymous reviewers for comments on the manuscript. This research was supported by a National Science Foundation dissertation improvement grant, a Gaige Fund award, a Sigma Xi grant, and a Mildred E. Mathias grant (to A.C.); a National Geographic grant (to A.C. and B.S.); NSF Grant DEB 0317182 (to B.S. and S.R.K.); and multiple National Science Foundation grants (to B.S. and colleagues).

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