Rise and Fall of a Hybrid Zone: Implications for the Roles of Aggression, Mate Choice, and Secondary Succession


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Abstract

Hybridization can be an important evolutionary force by generating new species and influencing evolution of parental species in multiple ways, including introgression and the consequences of hybrid vigor. Determining the ecological processes underlying evolution in hybrid zones is difficult however because it requires examining changes in both genotypic frequencies over time and corresponding ecological information, data that are rarely collected together. Here, we describe genetic and ecological aspects of a hybrid zone between the Eastern Fence Lizard, Sceloporus undulatus, and the Florida Scrub Lizard, Sceloporus woodi, occurring over at least 23 generations. The hybrid zone, discovered greater than 35 years ago using morphological characters, originally consisted of nearly even proportions of parental species and hybrids. Now, using genetic markers (species-diagnostic mtDNA sites and 6 nDNA microsatellite loci across a total of n = 117 individuals), we confirm not only that hybridization occurred but also that subsequent backcrossing has resulted in highly introgressed hybrids, with many hybrids containing mitochondrial DNA from one species on a nuclear DNA background of the other. Ecological aspects explaining these shifts in genetic composition include female mate choice, changes in habitat associated with secondary succession, and, most strongly, a hierarchy of male territorial advantage—ecological mechanisms likely to be involved in the emergence and disappearance of many animal hybrid zones. Our results suggest that genetic assimilation is not a significant threat to either species and that rather transient hybrid zones such as this may serve to increase genetic diversity and are candidates for causing genetic discordance in phylogeographic analyses.

Key words: introgression, metapopulation dynamics, phylogeographic discordance, Sceloporus, territoriality, transgressive segregation

Hybridization is an important evolutionary process that can generate new species and alter the evolutionary trajectories of parental species. Although genetic composition of hybrid zones is often assessed, the ecological processes underlying any observed genetic changes or the time the processes take for those changes to occur rarely are known. Evolutionary outcomes of hybridization occur through multiple pathways that include introgression and consequences of ecological processes such as hybrid vigor (Rieseberg and Carney 1998; Edmands 1999; Johansen-Morris and Latta 2006). Introgression is an influx of genes from one species into another via hybridization and subsequent backcrossing of hybrids with one of the parent species (Futuyma 1998). When introgression occurs, each generation is less able to replace...
itself— with genotypically similar individuals, potentially leading to genetic assimilation and extirpation or complete extinction of one or both of the parental species (Levin et al. 1996; Rhymer and Simberloff 1996; Rieseberg and Carney 1998). Hybrid vigor occurs when hybrids have increased fitness relative to the parental species. Hybrid vigor can decrease population growth rates of parental species through competition (Slattery et al. 2008) and potentially lead to extirpation or extinction of the parental species (Huxel 1999; Rosenfeld et al. 2004). Conversely, hybrid vigor can result in a stable situation (no threat to the parental species) if the increased hybrid fitness occurs only in the hybrid zone and may even foster hybrid speciation (Gross and Rieseberg 2005; Melo et al. 2009). Determining both the genetic and ecological processes occurring in hybrid zones is necessary to understand, as well as predict, evolutionary outcomes. Here, we relate ecological data associated with a focal population with the change in genetic composition of this population over a 35-year period. Knowing the ecology of the species and hybrids involved, such as mating preferences and dominance hierarchies, along with the change in genetic composition over time, will not only increase our understanding of ecological dynamics behind gene flow but also possibly elucidate a chronological aspect of those ecological dynamics that might be missed if the current genetic composition was assessed alone. Studies such as this are therefore necessary to ultimately understand the ecological mechanisms resulting in evolutionary change.

Hybrid zones between the Eastern Fence Lizard, Sceloporus undulatus, and the Florida Scrub Lizard, Sceloporus woodi, were previously described within ecotones between species-specific habitats (sandhill and scrub, respectively) at the Ocala National Forest, Marion County, Florida, United States (Jackson 1973). The habitat specificity exhibited by each of these species can result in well-defined and narrow hybrid zones existing where the sandhill and scrub habitats are adjacent (Jackson 1972, 1973). Indeed, the phenetic transitions in the S. undulatus–S. woodi hybrid zones studied to date have occurred within 400 m (Jackson 1972, 1973). These phenetic transitions were based on linear discriminant analyses of morphological characters, which defined a distinct hybrid class between S. undulatus and S. woodi as individuals exhibiting intermediate scale counts relative to both parental species (Jackson 1973). In one particular hybrid zone markedly defined by a stark juxtaposition due to management of sandhill and scrub habitat (Alexander Springs, FL; Jackson 1973) parental species were found in nearly equal numbers, and individuals with intermediate morphology occurred in a narrow zone between parental populations—mostly in the sandhill habitat of S. undulatus or in highly disturbed sandhill habitat where S. woodi also was found (Jackson 1973). The ongoing habitat management at this site has maintained the general structure of the juxtaposed habitats, resulting in a current environment similar to that examined 35 years prior (see Figures 1 and 2).

Individuals from the Alexander Springs hybrid zone (a subset of those used in this study, thus most from later than F1 generations) exhibit hybrid vigor (Robbins et al. 2010), and the parental species exhibit asymmetrical mate choice (Jackson 1973). Hybrid males possess traits that are extreme relative to males of the parental species (Robbins et al. 2010). Extreme traits in hybrids often occur through epistasis and the segregation of complementary alleles, which is termed transgressive segregation (Rieseberg et al. 1999). These hybrids exhibit two transgressive traits, larger head-width and greater aggression, with the increased aggression associated with an advantage in agonistic encounters between males (Robbins et al. 2010). This hybrid advantage should result in a greater ability to both defend and invade territories and could, therefore, provide hybrid males a greater number of mating opportunities than males of either parental species (Abell 1997; Haenel et al. 2003a, 2003b; Hews 1990). More importantly, the aggression and territorial advantage follows a hierarchy of hybrid > S. undulatus > S. woodi, with S. woodi males losing every agonistic encounter with both hybrids and S. undulatus (Robbins et al. 2010). The territorial advantage of S. undulatus males may decrease mating success of male S. woodi in parapatric habitat, allowing female S. woodi to mate with male S. undulatus in greater numbers than female S. undulatus with male S. woodi. Such interactions would facilitate a biased introgression of S. woodi mtDNA into the hybrid population (Figure 3c). After original hybridization events, furthermore, mating frequencies of males with female lizards, regardless of genotype, should parallel the hybrid > S. undulatus > S. woodi hierarchy, thus facilitating a biased backcrossing toward S. undulatus. If male aggression was the major ecological factor influencing the current genetic composition of the hybrid zone, we should observe mtDNA biased toward S. woodi, but nuclear markers biased toward S. undulatus (Figure 3e).

Mate choice also can play a role in determining direction and degree of backcrossing in hybrid zones. Neither hybrid nor parental species males discriminate between females of either parental species, suggesting that male mate choice is not a factor (Jackson 1972, 1973). Female mate choice, however, may play a role. Sceloporus woodi-like (i.e., based solely on morphological characters) females from the hybrid zone (i.e., likely hybrids) do not discriminate between males of either parental species, but S. undulatus-like females from the hybrid zone do tend to associate with S. undulatus males more often (1.6 times) than with S. woodi males (Jackson 1972, 1973), which would result in a bias in backcrossing toward S. undulatus. Females of both parental species, however, prefer male S. woodi over male S. undulatus (1.6–2× more often; Jackson 1972, 1973), which would result in a ratio of mtDNA biased toward S. undulatus (Jackson 1972, 1973). If female mate choice was the major ecological factor influencing the current genetic composition of the hybrid zone, we should observe mtDNA biased toward S. undulatus and nuclear markers slightly biased toward S. undulatus (Figure 3a).

Lastly, S. woodi generally exist as metapopulations (Hokit et al. 1999; Hokit et al. 2001) with subpopulations often found in early successional, open scrub habitat (Greenberg et al. 1994; Hokit et al. 2001). When Jackson (1972) surveyed the Alexander Springs region in 1971, 2 recently clear-cut areas existed where S. woodi was abundant (Figure 2A). When we resampled the area in 2005–2006, the clear-cut sections had...
regrown (i.e., secondary succession occurred; Figure 2B). Densities of both sexes of *S. woodi* would likely have declined as available habitat reduced. Thus, after an initial period of hybridization (assuming no mating preferences), bias in parental population densities would favor backcrossing to *S. undulatus* because it was the most frequently encountered mate (Figure 3b). If secondary succession was the major ecological factor influencing the current genetic composition of the hybrid zone, we should observe no bias in mtDNA, but nuclear markers biased toward *S. undulatus*. Thus, this hybrid system provides an opportunity to examine how transgressive aggression and territoriality, mate choice, and time shape mating dynamics in a hybrid zone.

Although morphological identification of hybrids appeared relatively straightforward, with putative hybrids possessing intermediate morphological traits (Jackson 1973), morphology can sometimes provide subtle to nonexistent indications that hybridization has occurred especially in later generation backcrossed individuals. Furthermore, morphological traits are not necessarily intermediate in hybrids (Rieseberg et al. 1999; Robbins et al. 2010). Therefore, using only morphological characters to define hybrids may give a limited perspective on the pattern and degree of hybridization, especially under high rates of backcrossing. Distinguishing highly backcrossed individuals from parental species can still be difficult even using genetic analyses such as Bayesian clustering analysis of microsatellite loci data (Sanz et al. 2009; Vaha and Primmer 2006). Combining microsatellite and mitochondrial loci in one analysis, however, can provide a more sensitive assay (e.g., Arias et al. 2008; Wilson et al. 2009). We employed the morphological characters previously used by Jackson (see for details 1972, 1973) to assess the accuracy of morphological classifications by comparing them to their genetic classifications using individuals that we scored both morphologically and genetically. We used this integrative approach on individuals from the *S. undulatus–S. woodi* hybrid zone in Alexander Springs, Florida (Jackson 1973; Robbins et al. 2010) to determine if hybridization has occurred and, if so, to assess the direction and degree of introgression. We used the known ecology of these populations to assess the relative influence of male aggression, mate choice, and secondary succession on the evolution of these local populations based on the changes in genetic composition.
Materials and Methods

Study Animals

*Sceloporus woodi* lives in open scrub habitats on remnant Pliocene and Pleistocene sand ridges in central Florida (Jackson 1972) consisting primarily of sparse sand pines, oak shrubs, and extensive bare ground (Myers and Ewel 1990). *Sceloporus woodi* occurs in disjunct, genetically divergent populations along the Florida ridge (Branch et al. 2003; Clark et al. 1999; McCoy et al. 2004), and, although generally considered rare, it can be locally abundant (McCoy and Mushinsky 1992). *Sceloporus undulatus* is common throughout the southeastern United States (Conant 1975) and abundant in sandhill habitats of central Florida, which consist of long-leaf pines, turkey oaks, and ground cover of wiregrass and fallen pine needles (Myers and Ewel 1990). Fossil remains of *Sceloporus* spp. (Myers and Ewel 1990) and genetic variation among *S. woodi* populations (72% divergence in some cases; Clark et al. 1999) suggest that *S. undulatus* and *S. woodi* have been distinct and in Florida for more than 1,000,000 years. The

Figure 2. Maps of the hybrid zone reflecting where each lizard was captured. (A) The hybrid zone in 1971 (redrawn from Jackson 1973) with species identity based on morphology. Scrub habitat is shaded with sandhill habitat elsewhere. (B) The hybrid zone in 2006 (drawn based on satellite image from Google Earth and Lake County; see Supplementary Material Figure S1) with species identity based on cytonuclear DNA. The general juxtaposition of scrub and sandhill habitat is similar to that depicted in 1971. White circles represent *Sceloporus undulatus*, black circles *Sceloporus woodi* and grey diamonds hybrids. The sampling area searched in its entirety in 2006 is bounded by the double line.
most recent phylogenetic hypotheses indicate a monophyletic \textit{S. woodi} clade nested within the \textit{S. undulatus} species complex (Leache 2009; Leache and Reeder 2002).

**Collection of Lizards and DNA**

Lizards were captured in the field with a noose and taken to the laboratory for morphological measurements (see Robbins et al. 2010 for husbandry methods). Tissue samples consisting of 10-mm clips of tail were taken from each lizard and stored at $-80^\circ$C until analysis. Each lizard was marked with a unique combination of toe-clips (Wächter 1992) to avoid resampling, and individuals were ultimately released at the capture site. Lizards ($N = 117$) and associated Global Positioning System coordinates were collected from their respective habitats between September 2005 and August 2006. \textit{Sceloporus undulatus} ($N = 32$) were collected in sandhill habitat ($N 29^\circ02'18" W 81^\circ33'35"$), \textit{S. woodi} ($N = 34$) were collected in open sand-pine scrub habitat ($N 29^\circ06'29" W 81^\circ48'34"$; as described in Myers and Ewel 1990), and putative hybrids ($N = 51$) were collected along an ecotonal region that was previously described as a hybrid zone based on morphological characters (Jackson 1973) in the Ocala National Forest, 3.2 km southwest of Alexander Springs, Florida ($N 29^\circ03'58" W 81^\circ35'00"$; Figure 1). The hybrid zone was approximately 3 km long and 1 to 2 km wide (Figure 2). The identification and collection of both parental species were based on morphology (parental species are easily distinguishable; Conant 1975) and geographic location. Discriminant analyses of the 10 morphological characters used by Jackson (see for details 1972; 1973) resulted in a clear split between the parental species (a subsample from reference sites of $N = 31$ females, 24 males) with no overlap. Putative hybrids ($N = 50$; 1 lizard was released without morphological data collected) were treated as unknown with regard to group identity. Discriminant functions were subsequently used, however, to calculate $Z$-scores and classify unknown individuals as \textit{S. undulatus}, \textit{S. woodi}, or hybrids based

**Figure 3.** Diagrams depicting predominant matings between parental species and hybrids based on associated behavior and ecology: (a) female mate choice. The dashed line indicates mating suggested by female mate choice but was likely minimized by the hierarchy in male aggression causing historically low availability of \textit{Sceloporus woodi} males where \textit{Sceloporus undulatus} males existed; (b) secondary succession, which is succession after a disturbance (i.e., clear cutting) that reduces species diversity, in this case, excluding \textit{S. woodi}; and (c) male aggression, which in this case would exclude \textit{S. woodi} males. +mtDNA indicates the predominant origin of mitochondrial DNA in the hybrid population. The current genetic composition of the hybrid zone suggests that male aggression was the major ecological factor influencing the mating dynamics of the hybrid zone, with secondary succession likely increasing influence with time.
on the morphological characteristics. Classifications based on morphology were compared with those based on genetics. This allowed us to assess how the composition of the hybrid zone has shifted based on morphological classifications and assess the accuracy of Jackson's methodology of classifying individuals as hybrids.

**Genetic Data Analysis**

Total cell DNA was extracted from tissue samples using NucleoSpin® Tissue Kits (BD Biosciences, Palo Alto, CA) or standard phenol-chloroform methods. A 307-bp fragment of the mitochondrial cytochrome b gene was amplified and sequenced from 6 putatively pure (non-hybridized) *S. woodi* and 8 putatively pure *S. undulatus* individuals using previously developed primers (L14990 and H15149; Kocher et al. 1989). Sequences were aligned and edited manually, when needed, using SEQUENCER 6.1.0 (Gene Codes, Ann Arbor, MI). The only intraspecific variation observed was a single transition (2 individuals) in the *S. woodi* sequences. A large number of interspecific polymorphisms were detected, however, consisting of 27 transitions and 7 transversions resulting in 30 synonymous and 4 nonsynonymous substitutions. With small intra- but large interspecific variation, we were able to identify species-diagnostic restriction endonuclease recognition sites. *Sceloporus undulatus* possesses 1 Ban II restriction site that is not seen in *S. woodi*, and *S. woodi* has 1 Bst BI site that is not seen in *S. undulatus*. For population screening, individuals were amplified with the same primers and the product digested with each of the enzymes separately and electrophoresed in a 2% agarose gel treated with GelStar nucleic stain (Cambrex Bio Science Rockland Inc., Rockland, MA). An amplicon cutting with Ban II but not Bst BI was scored as being *S. undulatus*-like mtDNA where fragments with a Bst BI but not Ban II site were *S. woodi*-like mtDNA. No individuals had or lacked both restriction endonuclease sites.

Nuclear DNA from *S. undulatus*, *S. woodi*, and putative hybrids also was examined for genetic variation. Six microsatellite loci (SWA1, SWA4, SWA7, SWB1, SWB6, and SWB10) were amplified by polymerase chain reaction following the recommendations in Ernst et al. (2004). We tested each locus for conformation to Hardy–Weinberg equilibrium in intraspecific populations. Genetic diversity was estimated using allelic richness (Kalinowski 2005). The Δk method of Evanno et al. (2005) was used to select the most likely number of groups without a priori sample identification. For each individual, and the 95% critical regions (CR) were used to assign individuals to groups with 0.00 ≤ q < 0.70 being *S. undulatus*, q ≤ 0.30 being *S. woodi*, and all other values being hybrids. The primary data underlying these analyses have been deposited with Dryad in fulfillment of data archiving guidelines (Baker 2013).

**Results**

Thirty-three of 34 individuals from the *S. woodi* geographic region had *S. woodi* mtDNA (97%, Table 1), and 30 of 32 individuals from the *S. undulatus* geographic region had *S. undulatus* mtDNA (94%, Table 1). Both mtDNA types were found in individuals from the putative hybrid zone (Table 1). Only 1 microsatellite locus, SWB6 in *S. undulatus*, departed significantly from Hardy–Weinberg equilibrium, indicating a possible null allele (Table 2). At this locus, *S. undulatus* and the hybrid zone shared 4 alleles, while *S. woodi* had 2 alleles, which also occurred in *S. undulatus* and the hybrid zone. Because

<table>
<thead>
<tr>
<th>Sample assignmenta</th>
<th>Sample source</th>
<th>S. undulatus</th>
<th>S. woodi</th>
<th>Hybrid zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial</td>
<td>Nuclear</td>
<td>30 (94)</td>
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<td>19 (37)</td>
</tr>
<tr>
<td><em>Sceloporus undulatus</em></td>
<td><em>Sceloporus undulatus</em></td>
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<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td><em>Sceloporus undulatus</em></td>
<td><em>Sceloporus woodi</em></td>
<td>2 (6)</td>
<td>0</td>
<td>30 (59)</td>
</tr>
<tr>
<td><em>Sceloporus woodi</em></td>
<td><em>Sceloporus undulatus</em></td>
<td>0</td>
<td>33 (97)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>34</td>
<td>51</td>
</tr>
</tbody>
</table>

aDefined as *S. undulatus* or *S. woodi* when nDNA q-values are ≥ 0.70 or ≤ 0.30, respectively, and by mtDNA restriction profile. Here, the putative F₁ hybrid from the hybrid zone with a *S. undulatus* mtDNA haplotype is grouped with *S. undulatus* nuclear types despite its q-value = 0.622.
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this locus had such a low number of alleles and correcting for a potential null allele in *S. undulatus* would possibly bias *q*-values of potential hybrids by forcing a circular argument if the hybrid zone was also treated as having a null allele, we opted to perform STRUCTURE with and without the locus to ensure no bias. The mean and private allelic richness were relatively low in *S. undulatus*. Allelic richness was intermediate in putative hybrids for most loci (Table 2). The mean observed heterozygosity over all loci also was relatively low in *S. undulatus* and generally intermediate in the putative hybrids. The inbreeding coefficients were similar for the putative hybrids and *S. undulatus* (Table 2); however, no consistent pattern was present among loci. Thus, the putative hybrids appear to have alleles from both species, with generally intermediate diversity, but genetically are more like *S. undulatus* (Figure 4). Significant genetic differentiation was observed among samples, with the global *F*_{ST} value (*F*_{ST} = 0.17) and the *F*_{ST} values at all 6 loci statistically different from zero.

**Table 2** Summary statistics, allelic richness (*Ar*), private allelic richness (*pAr*), observed (*H*_{O}) and expected (*H*_{E}) heterozygosities, and inbreeding coefficient (*f*) for individuals from the hybrid (HZ), pure *Sceloporus undulatus* (SU), and pure *Sceloporus woodi* (SW) zones

<table>
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<tr>
<th>Statistic</th>
<th>Sample</th>
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<th>SWA4</th>
<th>SWA7</th>
<th>SWB1</th>
<th>SWB6</th>
<th>SWB10</th>
<th>Overall</th>
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<td><em>Ar</em></td>
<td>HZ</td>
<td>7.25</td>
<td>3.78</td>
<td>7.55</td>
<td>4.89</td>
<td>3.96</td>
<td>8.85</td>
<td>6.05</td>
<td></td>
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<tr>
<td></td>
<td>SU</td>
<td>5.51</td>
<td>2.65</td>
<td>5.88</td>
<td>3.54</td>
<td>4.00</td>
<td>7.43</td>
<td>4.83</td>
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<tr>
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<td>SW</td>
<td>6.26</td>
<td>4.59</td>
<td>7.45</td>
<td>8.95</td>
<td>2.00</td>
<td>9.94</td>
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<tr>
<td></td>
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<td>—</td>
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<tr>
<td><em>pAr</em></td>
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<td>2.22</td>
<td>0.14</td>
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<td>0.19</td>
<td>0.00</td>
<td>0.76</td>
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<td></td>
<td>SU</td>
<td>0.96</td>
<td>0.38</td>
<td>0.003</td>
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<tr>
<td><em>H</em>_{O}</td>
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<td>0.84</td>
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<tr>
<td><em>f</em></td>
<td>HZ</td>
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<td>0.62</td>
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<tr>
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<td><em>F</em>_{ST}</td>
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<td>0.03</td>
<td>0.10</td>
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</table>

Summary of *F*_{ST} estimates are for each locus, and overall samples and all loci. Bolded numbers are statistically significant at *P* < 0.05.

**Figure 4.** *q*-values with 95% critical regions identified by Bayesian clustering with structure for all individuals using microsatellite data. Individual points are binned by geographic sample (hybrid zone and both putatively pure parental populations) and have been color coded by the mtDNA species assignment (white circle = *Sceloporus undulatus*; black circle = *Sceloporus woodi*). Values greater than 0.70 indicate assignment to *S. undulatus*, those less than 0.30 indicate assignment to *S. woodi*. 

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(P < 0.0001, Table 2). Pairwise estimates of $F_{ST}$ identified significant differentiation ($P < 0.05$) between all pairwise comparisons except for *S. undulatus* and the putative hybrids (data not shown).

Based on microsatellite loci, Bayesian clustering with STRUCTURE indicated the presence of 2 genetic groups ($\Delta K$ maximized at $k = 2$). All individuals from each of the pure *S. undulatus* and *S. woodi* geographic samples were assigned to the expected genetic group (Figure 4, Table 1). Only 6 *S. woodi* (18%) and 3 *S. undulatus* (9%) had 95% CR for the $q$-values outside of the pure species ranges ($q < 0.30$ and $> 0.70$, respectively). Putative hybrids were predominantly assigned to *S. undulatus* (48 of 51). Two individuals from the hybrid zone (4%) were assigned to *S. woodi* ($q = 0.005$ and 0.007), and 1 individual was intermediate ($q = 0.622, 95% \text{ CR} = 0 - 1$) and thus genotypically consistent with an F1 hybrid. Eleven individuals (22%) exhibited relatively greater uncertainty, with 95% CRs spanning outside of the 70% assignment to pure *S. undulatus* (Figure 4), suggesting the presence of early filial hybrids. Individual assignments did not change with or without locus SWB6.

Taken together, the nuclear and mtDNA data characterize the hybrid zone as consisting almost entirely of pure *S. undulatus* (37%) and hybrids backcrossed to *S. undulatus* (59%; Table 1). The preponderance of putative hybrids have $q$-values for nuclear DNA indicative of pure *S. undulatus* status (i.e., $q \geq 0.70$), suggesting that most hybrids are second- (or later) generation backcrosses to *S. undulatus*. That most of the putative hybrids have *S. woodi* mtDNA, indicates that most of the F1 hybridization was likely between *S. undulatus* males and *S. woodi* females. Interestingly, the 1 likely F1 hybrid found ($q = 0.622, 95% \text{ CR} = 0 - 1$) had *S. undulatus* mtDNA consistent with mating between a *S. undulatus* female and a *S. woodi* male. Even so, our mtDNA data suggest that most hybridization events occurred between *S. undulatus* males and *S. woodi* females, and our nuclear DNA data indicate that backcrossing occurred more frequently with *S. undulatus* than with *S. woodi* (Figure 4).

The current composition of the hybrid zone based on morphological classifications is 78% *S. undulatus*, 8% *S. woodi*, and 14% hybrids. The composition based on our morphological assessments is similar to our nuclear data (excluding mtDNA data), which shows 73% *S. undulatus*, 4% *S. woodi*, and 23% hybrids. Thus, we consider the morphological classifications both in this study and Jackson's (1973) reasonably accurate with regard to genetic classifications based on nuclear DNA, especially with the underlying high degree of backcrossing associated with the hybrid zone. Furthermore, the 1 individual classified by microsatellites as a likely F1 hybrid also was assigned to the hybrid group based on Jackson's method. To make a broader assessment, we compared hybrid assignment based on morphology to that based on relaxed cytogenetic criteria, which assigned hybrid classification to individuals with *S. woodi* mtDNA and/or CR of $q$-values that spanned outside the 70% threshold used to assign individuals to pure *S. undulatus*. Four of the seven (57%) individuals classified as hybrids by morphology also were hybrids based on these relaxed genetic classifications.

**Discussion**

We found evidence of hybridization that is mostly, but not entirely, constrained to the area where habitats specific to each species are juxtaposed. Hybrid vigor, in this case manifested through male aggression, appears to be a major ecological factor influencing the mating dynamics between these species, with secondary succession likely increasing in influence as it occurs. Specifically, our data indicate that hybridization has occurred in the region and subsequent backcrossing to *S. undulatus* has been extensive. Within the Alexander Springs hybrid zone, many of the putatively pure nuclear DNA *S. undulatus* individuals (i.e., $q \geq 0.70$) have *S. woodi* mtDNA haplotypes (Figure 4, Table 1) unequivocally indicating that they are of hybrid origin. The near absence of clear F1 hybrids and the preponderance of latter generation backcrosses, however, indicate that hybridization is an historical event. It is important to note that 1 individual from the hybrid zone is most likely an F1 hybrid (*S. woodi* mtDNA and $q = 0.622$; see Figure 2B), so hybridization probably is still occurring although the frequency may have decreased over time. Given the relatively large 95% CR in the $q$-values for some individuals in the hybrid zone, 22% of the individuals could be F1 hybrids. Interestingly, most of them (73%) had *S. woodi* mtDNA haplotypes. If these truly are F1 hybrids, then we would conclude that hybridization is occurring and has been for quite some time. Two individuals that could not be unequivocally differentiated from *S. woodi* (i.e., *S. woodi* mtDNA and $q = 0.005$ and 0.007) and 19 from *S. undulatus* also occurred in the hybrid zone. These could have been pure parental individuals collected in the hybrid zone. Pure parental-like cytogenetic genotypes are, however, expected to result from backcrossing and hybrid–hybrid matings and the frequencies of each type are consistent with backcrossing primarily to *S. undulatus*. Thus, there is potential for underestimating the number of hybrids. With regard to the parental populations, 3 individuals (2 from the *S. undulatus* source population and 1 from the *S. woodi* source population) also were found to have mixed cytogenetic genotypes, indicating rare hybridization within these putatively pure parental regions or dispersal of hybrid individuals out of a hybrid zone.

Our results are consistent with Jackson’s (1973) morphological analysis of the hybrid zone in showing that hybridization has occurred. Unlike Jackson (1973), however, our genetic data show a considerable degree of asymmetrical backcrossing toward *S. undulatus*. Jackson (1973) found nearly even proportions of *S. undulatus* (34%), *S. woodi* (35%), and hybrids (31%) of varying degrees spread evenly across the morphological range between parental species. Our nuclear data (similar to our morphological data) show a shift from Jackson's even proportions to one skewed toward *S. undulatus* with 73% *S. undulatus*, 4% *S. woodi*, and 23% hybrids. Our cytogenetic data show a greater shift toward hybrids with 37% *S. undulatus*, 4% *S. woodi*, and 59% hybrids. Highly backcrossed individuals likely are misclassified as nonhybrids in our assay of nuclear DNA. If this also is true for the morphological characters assessed by Jackson (1973), then the hybrid zone may have contained a greater proportion of
hybrids 35 years ago than previously reported (Jackson 1973). Nevertheless, our classifications based on morphology alone are directly comparable to Jackson’s classifications, and our analysis shows a shift in hybrid zone composition similar to the nuclear genetic data.

The more sensitive combined cytonuclear data from the hybrid zone revealed, specifically, a bias toward *S. woodi* in mtDNA markers (a lower limit of 59%) and a bias toward *S. undulatus* in nuclear markers (94%). If all individuals sampled from the hybrid zone are of hybrid origin the bias toward *S. woodi* mtDNA would be 63% (32 of 51; Table 1). If only individuals with contrasting mt and nuclear DNA are hybrids, the bias would be 100% (30 of 30; Table 1). Putative F1 hybrids based on nuclear markers suggest a bias toward *S. woodi* mtDNA of 73% (see results section). Thus, we conclude that the lower limit of the bias toward *S. woodi* mtDNA in hybrids is 59%, but the actual bias is likely higher. Some level of immigration and/or asymmetrical mating has likely occurred over time in order to prevent the mismatches between cytoplasmic and nuclear markers from decaying (Asmussen et al. 1989). Four, nonexclusive ecological processes could explain these biases: differential fitness among genotypic classes of hybrids, mate choice, temporal changes in relative densities of the parental species, and class-specific hierarchy in male agonistic encounters (Fig. 3).

The genetic composition of the hybrid zone may be a component of differential success among hybrids. It is possible that pure hybrid lineages suffer from hybrid breakdown (e.g., Ellison et al. 2008; Johansen-Morris and Latta 2006; Sait et al. 2007) and backcrosses to *S. undulatus* have higher fitness compared with backcrosses to *S. woodi* (i.e., Bieri and Kawecki 2003). Such a scenario could result in the bias in successful backcrossing toward *S. undulatus*. Unfortunately, we have no data to address differential fitness among the various crossing classes, but differential fitness is not necessary to explain the current genetic composition of the hybrid zone (see below).

Although mate choice can play a role in determining the direction and degree of introgression and backcrossing in hybrid zones, the data to date do not support a major role in this *Sceloporus* hybrid zone (Jackson 1972; Jackson 1973). The tendency for *S. undulatus*-like hybrid females to associate, and thus potentially mate, with *S. undulatus* males more often than *S. woodi* males may facilitate the bias in backcrossing toward *S. undulatus*, but the effect of this biased associating is likely overshadowed, or at least confounded, with the effects of secondary succession explained below (Figure 3a). Furthermore, females of both parental species prefer male *S. woodi* over male *S. undulatus* (1.6–2× more often; Jackson 1972, 1973), which would result in a ratio of mtDNA in the hybrid zone in contrast to what our genetic analyses detected and suggests historically that male *S. woodi* may have been less available (for mating) than male *S. undulatus*.

Temporal changes in relative densities of the parental species resulting in a bias toward matings between hybrids and *S. undulatus* may explain the bias toward *S. undulatus* nuclear markers. Because *S. woodi* is an early successional species, the secondary succession occurring since 1971 in the clear-cut (where many *S. woodi* were found 35 years ago, see Fig. 2; Jackson 1972, 1973) likely caused densities of both sexes of *S. woodi* to decline as availability of early successional habitat reduced. Indeed, we found only 2 putative pure *S. woodi* in the hybrid zone. Such changes in relative densities of the parental species, along with the asymmetrical female mate choice in hybrids, may explain the preponderance of *S. undulatus* nuclear markers in the hybrid zone, but they do not, however, explain the preponderance of *S. woodi* mtDNA in the hybrid zone.

Differences in male aggression can explain the preponderance of *S. woodi* mtDNA observed in the hybrid zone and may facilitate the bias in backcrossing toward *S. undulatus*. Hybrid males have an advantage in male agonistic encounters because they show greater aggression than males of both parental species (Robbins et al. 2010). That advantage follows a hierarchy of hybrid > *S. undulatus* > *S. woodi*, with *S. woodi* males losing every agonistic encounter with both hybrids and *S. undulatus*. Because advantages in defending or intruding on territories generally lead to greater mating opportunities (Abell 1997; Haenel et al. 2003a, 2003b; Hews 1990), female *S. woodi* should have mated with male *S. undulatus* in greater numbers than female *S. undulatus* with male *S. woodi*. Such interactions would facilitate a biased introgression of *S. woodi* mtDNA into the hybrid population (Figure 3c). Additionally, hybrid males winning 67% of the agonistic encounters with *S. undulatus* and 100% of those with *S. woodi* (Robbins et al. 2010) would facilitate the biased backcrossing toward *S. undulatus*. Male aggression could thus explain both the preponderance of *S. woodi* mtDNA and the biased backcrossing toward *S. undulatus* (Figure 3c). Indeed, it is the most likely ecological factor assessed that could explain the bias toward *S. woodi* mtDNA in the hybrid zone.

Our cytonuclear data also suggest that genetic introgression may not be a significant threat to either species. The hybrid zone is relatively well defined, and despite *S. undulatus* being common in the general area, only 2 *S. undulatus*-like hybrids were found in suitable habitat outside of the hybrid zone. Additionally, only 1 *S. woodi*-like hybrid was found in suitable habitat outside the hybrid zone. We suggest that transient hybrid zones such as this one, more likely, are positive forces in maintaining or increasing the genetic diversity of co-occurring closely related species (e.g., Rieseberg et al. 2003).

It is likely that hybridization has occurred in this area for, at least, 35 years or 23–47 generations, assuming survival and fecundity of hybrids is between that of *S. undulatus* (with a generation time of approximately 1.5 years; Crenshaw 1955) and *S. woodi* (with a generation time of 0.75 years, based on female age at reproduction and longevity; McCoy et al. 2004). The major hybridization event, however, was likely historical. This conclusion is consistent with our finding very few F1 or early backcrossed individuals. Furthermore, hybridization events in general between these 2 species are likely often ephemeral, occurring when *S. woodi* moves into early successional or disturbed habitat that is adjacent to *S. undulatus* populations, and then abating as habitat succession occurs and the densities of *S. woodi* decline. The genetic composition
of these hybrid zones would then fluctuate over time, beginning with a nonbiased composition of nuclear markers, and mtDNA biased toward \textit{S. woodi} (because the influence of male aggression) but resulting in many \textit{S. undulatus}-like (nuclear) individuals with \textit{S. woodi} mtDNA (because the influences of male aggression and secondary succession). The dynamics we have found are likely associated with the emergence and disappearance of many hybrid zones. The transient nature of such hybrid zones also would influence assessment of genetic relatedness between species if representative samples were taken from introgressed populations. We suggest the hypothesis that ephemeral hybrid zones may underlie the discordance found in phylogeographic analyses of “species” complexes, including patterns of discordance found within the \textit{Sceloporus} species complex of North America (Leache 2009). Studies looking more closely at hybrid–hybrid mating preferences, differential success of genotypic classes, and ecological correlations between habitat structure and habitat choice could add further insights into the hybrid zone dynamics between these species and elucidate other subtleties involved in pathways of evolution.

Supplementary Material
Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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