

NEWS AND VIEWS

PERSPECTIVE

The evolution of species recognition signals

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'Sibling species', an old term that has fallen out of use, refers to closely related species that are so similar that it is hard to tell them apart. The existence of such species raises the obvious question: How do the animals themselves tell one another apart? And indeed, this is an active area of research (Tibbetts & Dale 2007; Uy *et al.* 2009). Usually, the species differ in one or more traits (i.e. species recognition signals) detectable with the sensory modalities upon which they rely (e.g. raptors use visual signals, frogs use sound and electric fish use different patterns of electric discharge).

A more general question concerns how such differences evolve. Over the last decade, it has become increasingly evident that mating signals can evolve under simultaneous selection for two functions (Fleishman *et al.* 2009): (i) eliciting attention (i.e. detectability); and (ii) species identification (i.e. distinguishing conspecifics from non-conspecifics). Historically, species recognition has attracted a significant amount of research from evolutionary biologists based on the assumption that if hybrids suffer reduced fitness or cannot be produced at all, then natural selection should favour individuals bearing traits that prevent such matings. This idea—confusingly termed either reinforcement or reproductive character displacement—had a rocky time in the evolutionary literature for many years, though now it is widely accepted (Servedio & Noor 2003; Rundle and Nosil, 2005; Pfennig & Pfennig 2009).

Near the dawn of the era of molecular ecology, one of the first studies to employ molecular tools to study the evolution of species recognition signals was Webster & Burns' (1973) study of the evolution of dewlap colour in *Anolis* lizards. Anoles possess a retractable flap of skin under the throat, termed as dewlap, that is used in court-

ship, aggressive interactions and even encounters with predators (reviewed in Losos 2009). Anoles can be found in communities of as many as 15 species, and sympatric species never have identical dewlaps, leading to the hypothesis that the dewlap is used in species identification (Rand & Williams 1970).

Webster and Burns studied a highly unusual pattern of dewlap distribution in the Hispaniolan bark anole, *Anolis brevirostris*, along a transect on the western coast of Haiti (Fig. 1). Starting in the south, the lizards have a white dewlap. Then, abruptly the dewlaps become intensely orange; moving northwards, the intensity and size of the orange spot diminishes until it has almost disappeared, whereupon again there is an abrupt transition back to intense orange coloration that characterizes the northernmost populations.

Using the tools of the day, Webster and Burns employed starch-gel electrophoresis to examine six geographically varying protein loci. Analysis of these data yielded three important discoveries. First, the populations sorted into three groups: the white-dewlapped forms in the south, the orange-dewlapped forms in the north and a third, intervening form that exhibited clinal variation in the proportion of white vs. orange in the dewlap. Second, at the point of contact between the groups in both the north and the south, adjacent populations did not share alleles at several loci. Third, within the middle, clinally varying group, populations showed little genetic differentiation despite the differences in dewlap colour among populations.

Webster and Burns concluded that they were dealing not with a single species, but three—subsequently, the middle populations were described as *A. caudalis* and the northern ones as *A. websteri*. More importantly, what had seemingly been an incoherent pattern of geographic variation in dewlap colour variation now had a clear explanation. The apposition of orange vs. white at both ends of *A. caudalis*'s range is most parsimoniously explained as the result of selection for differences in species recognition signals in sympatry. The fact that *A. caudalis* maintains the clinal variation in the face of possibly strong ongoing gene flow, as evidenced by the lack of genetic differentiation among populations, was interpreted as powerful evidence for ongoing natural selection favouring dewlap colour differences at the contact zones with the other species.

Given this provocative pattern and the great interest in evolutionary reinforcement, it is surprising that this example has not been subject to further investigation as molecular tools have developed over the past four decades. Undoubtedly, the transect's occurrence in Haiti, a notoriously difficult place for fieldwork, has been a factor. Finally, however, this case study has come under further scrutiny. On a trip in Haiti that was no doubt a story in itself, Lambert *et al.* revisited Webster and Burns' transect

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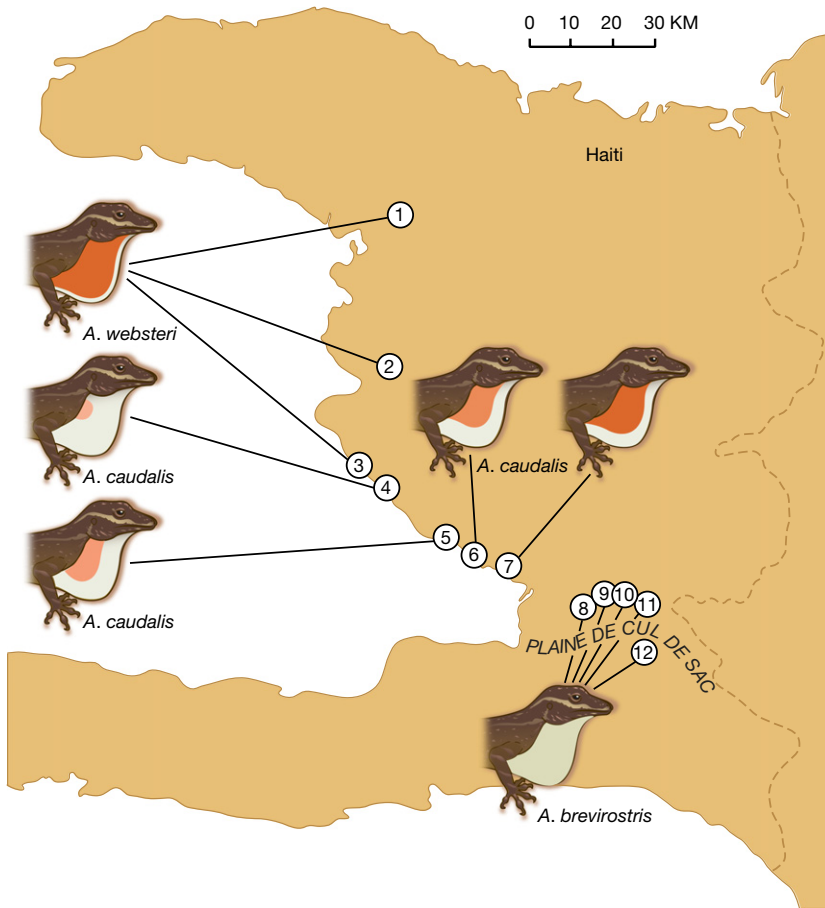


Fig. 1 The distribution of dewlap colours in the *Anolis brevirostris* species complex along a transect in Haiti. Figure from Losos (2009), modified from Webster & Burns (1973).

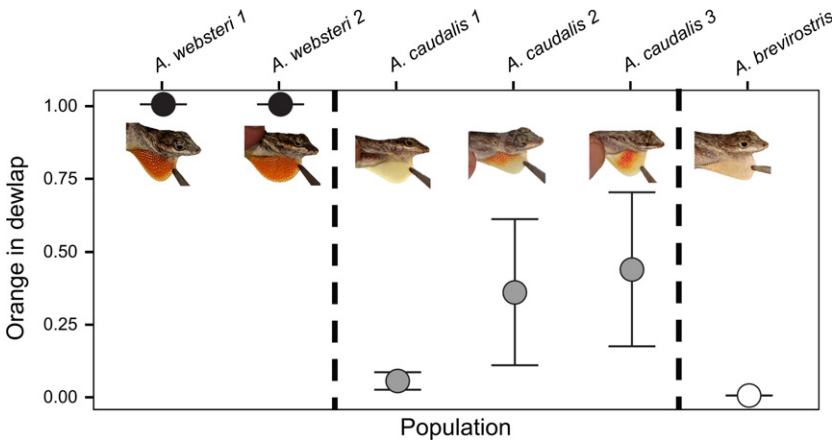


Fig. 2 Lambert *et al.*'s quantitative analysis confirms the pattern of dewlap coloration suggested by Webster and Burns.

and report in this issue of *Molecular Ecology* the results of their phylogenetic and phenotypic analyses.

Examining variation at mitochondrial and nuclear loci, Lambert *et al.* have demonstrated that Webster and Burns pretty much got it exactly right. Chalk one up for old school electrophoresis! Not only do the three species each fall out as monophyletic, but, as with the allozymes, *A. caudalis* exhibits little interpopulation genetic differentiation, in contrast to the deep genetic structure apparent

among populations in the other two species. Moreover, phenotypic examination of dewlap coloration reaffirmed the patterns of clinal variation within *A. caudalis* and the abrupt shifts in coloration between sympatric species at either end of its range (Fig. 2).

Lambert *et al.*'s study not only completely corroborates Webster and Burns' conclusions, but adds several important new perspectives on this case study. First, since the time of Webster and Burns, a new view has taken hold

about the importance of dewlap colour. A number of recent studies have indicated that variation may reflect adaptation to the light environment—to be effective at communication, dewlaps must be detectable given available light spectra and the background, thus favouring the evolution of colours most detectable in a species' or population's light environment (Leal & Fleishman 2004; Fleishman *et al.* 2009). This hypothesis would predict that the environment changes at the points at which the two species meet, which Lambert *et al.* discount with GIS environmental data. Lambert *et al.* also note that a more direct approach would be measurement of the light environments experienced by displaying lizards across the transect. Indeed an interesting caveat of this study system is that the dewlap of *A. caudalis* may represent a compromise between maintaining detectability in its light environment while being sufficiently distinct from sympatric congeners (see Fleishman *et al.* 2009). In this scenario, this three-species system is ripe for further research addressing the relationship between signal detection and discrimination that occurs when multiple species are found sympatrically, a trade-off that to date has been mostly overlooked in studies addressing dewlap evolution and mating signals in general.

Second, Webster and Burns stated that they could not polarize evolutionary change in *A. caudalis*'s dewlap colour. Was it ancestrally orange, with the pale coloration in the north the derived state, or vice versa? Implicit in their discussion was the idea that the three species likely formed a clade. However, Lambert *et al.*'s results surprisingly show this not to be the case. Although *A. caudalis* is sandwiched geographically between *A. websteri* and *A. breviostris*, its closest relative is actually *A. marron* (Fig. 3 in Lambert *et al.* 2013), which occurs some distance away on the southwest coast of Haiti. How these two taxa came to their geographic situation, separated by land occupied by the clade's sister taxon, *A. breviostris*, is unknown. More importantly, *A. marron* has a dewlap that appears more similar to that of *A. breviostris* and northern *A. caudalis* than to that of *A. websteri*, which suggests that the orange coloration of southern populations of *A. caudalis* is the derived state from an initially *breviostris*-like ancestral condition.

Finally, Lambert *et al.* point out the striking stasis in the distribution of the species and of their phenotypes despite the tremendous environmental changes that have occurred both globally and locally in Haiti since the early 1970s. Like many taxa, anoles have been demonstrated to evolve rapidly in response to changing conditions, so much so that stasis—rather than rapid change—is now the unexpected finding requiring special explanation. In this case, the fact that trunk anoles seem to thrive around human-modified surroundings must be part of the explanation, although one might have thought that Haiti's notoriously high rate of deforestation must be challenging for a trunk-living anole.

As always, good research highlights further questions that need investigation. In this case, what goes on at the contact zones is still a mystery. The deep genetic divergence implies

that there is no genetic introgression, but why not? Are the dewlap differences sufficient to prevent intermating or is postzygotic isolation also involved? In either case, one would expect natural selection to still be operating strongly, weeding out the inappropriate alleles that may be arriving from elsewhere in the cline, but that assumes that gene flow is actually ongoing, a hypothesis suggested, but not demonstrated, by the low levels of differentiation within *A. caudalis*. Focused studies measuring natural selection and gene flow are needed to round out the story.

Preston Webster was a rising star in the nascent field of molecular systematics when he died in a car accident in 1975. Had he lived, it seems likely that he would have followed up his electrophoretic work with newer tools as they appeared. In his absence, it took 40 years, but Lambert *et al.* have re-established Haitian trunk anoles as a fascinating case study of the evolution of reproductive isolation.

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The authors jointly wrote the paper.

doi: 10.1111/mec.12377