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Comparative tests of the role of dewlap size in *Anolis* lizard speciation

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Phenotypic traits may be linked to speciation in two distinct ways: character values may influence the rate of speciation or diversification in the trait may be associated with speciation events. Traits involved in signal transmission, such as the dewlap of *Anolis* lizards, are often involved in the speciation process. The dewlap is an important visual signal with roles in species recognition and sexual selection, and dewlaps vary among species in relative size as well as colour and pattern. We compile a dataset of relative dewlap size digitized from photographs of 184 anole species from across the genus' geographical range. We use phylogenetic comparative methods to test two hypotheses: that larger dewlaps are associated with higher speciation rates, and that relative dewlap area diversifies according to a speciational model of evolution. We find no evidence of trait-dependent speciation, indicating that larger signals do not enhance any role the dewlap has in promoting speciation. Instead, we find a signal of mixed speciational and gradual trait evolution, with a particularly strong signal of speciational change in the dewlaps of mainland lineages. This indicates that dewlap size diversifies in association with the speciation process, suggesting that divergent selection may play a role in the macroevolution of this signalling trait.

1. Introduction

Species' traits can play at least two distinct roles in the process of speciation. First, species with particular values of a discrete or continuous trait may be more likely to undergo speciation, resulting in heterogeneity in net diversification rates across clades. Traits can influence speciation by facilitating any form of reproductive isolation, including geographical and behavioural isolation. Traits including sexual dichromatism in cichlid fish [1], asexuality in plants [2], flightlessness in beetles [3], and herbivory in mammals [4] have been linked to increased net diversification (speciation minus extinction) rates. An alternative to speciation being promoted by particular trait values, is speciational trait evolution, where traits diversify in concert with the speciation process. Rapid trait evolution concurrent with speciation may occur during the formation of geographically isolated populations, or because traits are frequently subject to divergent natural or sexual selection during speciation. If traits evolve by rapid speciational evolution rather than by the gradual accumulation of change over time the extent of trait divergence in a lineage should be a function of the frequency of speciation in the lineage's history [5,6]. These two processes—trait-dependent speciation and speciational trait

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evolution—can both be investigated using phylogenetic comparative methods, and present alternative but not mutually exclusive hypotheses linking traits to speciation.

Traits related to signal transmission are often involved in reproductive isolation, and are therefore good candidates to demonstrate either trait-dependent speciation or speciational trait evolution. The evolution of a novel mode of transmission may open a new 'signal space' that allows signal diversification in previously unexplored dimensions. Signalling innovations such as electric signalling in mormyrid fish [7] and a derived melanosome morphology in African starlings [8] have been associated with increased speciation rates. An elevated speciation rate may result if the signalling trait increases the strength of sexual selection, facilitating the evolution of assortative mating and improved species recognition. Signal evolution may also occur during the speciation process, resulting in a speciational pattern of evolution. In *Carduelis* finches, a speciational model best explains the evolution of ornamental carotenoid-derived colouration believed to be under sexual selection by female choice [9], and speciational evolution was detected in traits related to male courtship behaviour (but not in other traits) in *Timema* walking stick insects [10]. Speciational evolution of signals should occur if speciation involves divergent sexual selection acting on the trait, potentially in conjunction with divergent natural selection. The process of sensory drive has the potential to link signal evolution to ecological divergence to promote speciation owing to correlated adaptation of signal transmission and reception to different sensory environments [11,12].

The Neotropical lizard genus *Anolis* is a promising clade in which to identify traits associated with speciation. Anoles in the Caribbean and Central and South America have adaptively radiated into approximately 400 species occupying diverse niches associated with different structural microhabitats and climates. Species also vary in signalling traits, notably an extensible fold of skin below the chin called the dewlap, which is present in both sexes of most species but generally more prominent in males [13]. Species' dewlaps vary in relative size, colour, and the presence and absence of pattern elements such as stripes and marginal bands [14]. Dewlap extension is used along with behaviours such as push-ups and head bobs during courtship and territorial encounters, and dewlap appearance contributes to species recognition [15]. Dewlap differentiation sometimes occurs as species adapt to different light environments, such as open versus closed habitats [16], suggesting the potential for sensory drive [11]. The dewlap could therefore function as a signalling innovation that increases speciation rate or could undergo divergence during the speciation process.

Testing hypotheses about the dewlap's role in the *Anolis* radiation is complicated by the fact that the dewlap originated in an ancestor of all extant anoles, and has been completely lost only once. The small number of transitions between dewlap presence and absence severely limits statistical power to test for either trait-dependent speciation [17] or transitions associated with speciation events [18]. Dewlap-like structures have evolved in other Iguanian lizard clades, including *Polychrus*, *Draco*, and *Sitana* + *Otocryptis* [19], but sister clade comparisons provide mixed evidence for increased species richness in clades with dewlaps [20] and the extent to which these features play comparable roles in species recognition and communication in each clade has not been confirmed. However, even if almost all species in a clade possess a dewlap, it is possible that the size of the dewlap influences its effectiveness as a signal. Therefore, we can design comparative tests for both trait-dependent speciation and speciational trait evolution based on relative dewlap size rather than dewlap presence or absence. Losos [20] noted that some clades in which the dewlap is absent (sister species *A. bartschi* and *A. vermiculatus*) or reduced (e.g. *A. hendersoni*, *A. ophiolepis*, and *A. agassizi*) are relatively species-poor. If this represents a general pattern, one explanation is that the role of the dewlap in sexual selection or species recognition is enhanced if it is large relative to the size of the lizard [13], perhaps because it increases visibility of the signal or because it provides a larger 'canvas' on which colour and pattern differentiation can occur. An alternative scenario is that as a major component of the dewlap signal, relative dewlap size itself may be subject to divergent sexual selection. For example, if different environments favour different signal sizes, dewlap size could diverge in concert with speciation that involves adaptation to different macrohabitats, and thus show a signal of speciational evolution. In what follows, we describe a dataset for male dewlap size in *Anolis*, and test whether dewlap size either promotes trait-dependent speciation or conforms to a pattern of speciational trait evolution across the anole radiation.

2. Material and methods

(a) Phylogeny

We used the most complete molecular phylogeny currently available for *Anolis*, from Gamble *et al.* [21]. Their analysis of approximately 1 500 base pairs of mitochondrial DNA for 216 *Anolis* species used Bayesian Markov chain Monte Carlo implemented in BEAST [22] to simultaneously estimate topology and relative branching times. For our main analyses, we used the maximum clade credibility (MCC) chronogram of 10 000 post-burn-in trees from four independent chains from [21], using median node ages. We also subsampled a set of 100 trees from the posterior distribution to partly account for uncertainty in the estimation of the phylogeny. For all analyses, we pruned trees to include only the 184 species in our dewlap dataset, and rescaled crown ages to 50 Ma, consistent with most recent estimates [23,24].

(b) Image analysis and size-correction

We compiled a total of 1 101 images of adult male anoles with fully extended dewlaps. A total of 254 species were represented in the photographs, 184 of which were included in the phylogenetic tree and could therefore be used in the following analyses. Digital photos were taken by multiple investigators throughout the geographical range of anoles, and were supplemented with photos of anoles with extended dewlaps from a field guide [25] and from the CaribHerp database (www.caribherp.org). We digitally measured the area of the extended dewlap and the head length from snout to ear, establishing the scale based either on an object of known size or an estimate of the lizard's body size (figure 1; see the electronic supplementary material).

We used head length to represent lizard size, to allow inclusion of individuals without snout–vent length (SVL) data. To calculate the size-adjusted dewlap area, we first used phylogenetic generalized least-squares with the MCC tree to carry out a phylogenetic regression of species mean log dewlap area against log head length. These and all subsequent analyses were carried out in the R environment [26]. We used the function 'gls' to fit the phylogenetic regression while modelling the appropriate level of phylogenetic signal in the residuals by optimizing the λ parameter

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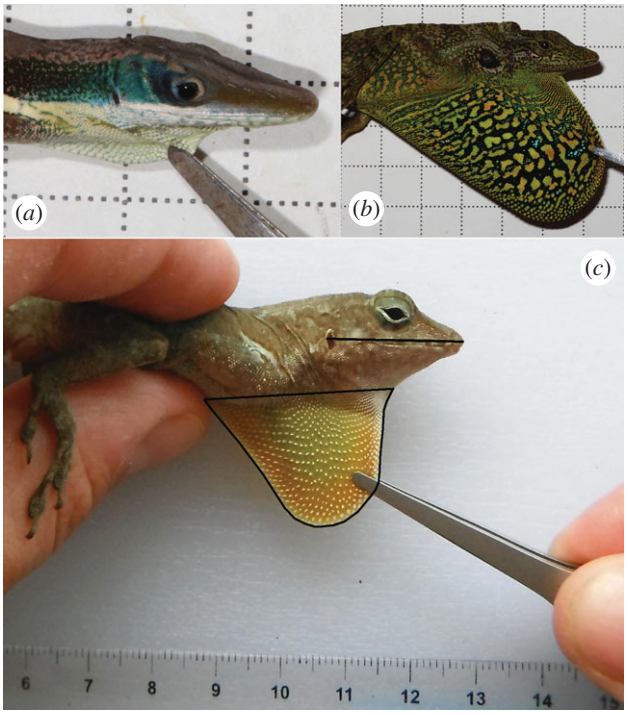

Figure 1. (*a*,*b*) Examples of small- and large-dewlapped anoles, *A. hendersoni* from Haiti and *A. aequatorialis* from Ecuador (photos by D.L. Mahler). (*c*) Illustration of the measurements of head length and dewlap area (photo of *A. cristatellus* from Puerto Rico by T. Ingram). (Online version in colour.)

(hereafter 'λ_{sig}' to distinguish it from the speciation rate λ) [27], which ranges from 0 (no phylogenetic signal) to approximately 1 (strong signal as expected under random walk Brownian motion (BM)). We used the regression coefficients to calculate residual log dewlap area for all individuals, then calculated the mean relative dewlap area and intraspecific variability for each species. We focused on relative dewlap size as the focal trait in subsequent analyses, but results were generally similar if we used absolute dewlap area (see electronic supplementary material).

(c) Geography

Anoles have a broad geographical distribution, and we anticipated that regions might differ in the relationship between relative dewlap area and speciation. Speciation in anoles varies with geographical location, with *in situ* speciation on the mainland and on large but not small islands [28,29]. Morphological and ecological studies have indicated that island and mainland anoles appear to be on different evolutionary trajectories, occupying distinct regions of morphospace and exhibiting different relationships between morphology and habitat use [20,30,31]. These differences are hypothesized to reflect differences in anole density or predator regime, either of which could influence the evolution of signals such as dewlaps, so we decided to partition our analyses by island versus mainland. To do this, we identified major lineages centred on either island or mainland habitats (figure 2). We designated the root of the tree and the Dactyloa clade as 'mainland' with a transition to 'island' at the crown of the clade comprising all other anoles. Within Dactyloa, we set a transition to 'island' for the Lesser Antillean *roquet* series. We set a transition to 'mainland' for the majority of the clade Norops (excluding the Cuban *sagrei* series) [32], and a transition back to 'island' for the Jamaican *grahami* series that is nested in the 'mainland' Norops clade in the Gamble *et al.* tree [21] (though it is sister to mainland Norops in other studies [32,33], so its presence in the Caribbean does not necessarily imply a back-colonization from the mainland). We note that a small number of other species have changed geographical states (e.g. *A. carolinensis* has colonized mainland North America), but our simple partitioning allows geography to be consistently incorporated into both of our analyses.

(d) Trait-dependent speciation

Recent developments in phylogenetic comparative methods allow the inference of trait-dependent diversification (speciation and extinction) while accounting for the effect of this process on ancestral character estimation and tree shape [34,35]. We used quantitative state speciation and extinction (QuaSSE) in the R package 'diversitree' [36] to test whether the speciation rate λ changes as a function of relative dewlap area. Trait-dependent extinction rates (μ) are also detectable in principle, but such models do not follow from our biologically motivated hypotheses, and require very large trees to achieve reasonable statistical power [36]. QuaSSE simultaneously models trait evolution and diversification, estimating maximum-likelihood parameters for a birth–death model of lineage diversification where λ can vary as an arbitrary function of a continuous trait evolving under BM with rate σ^2.

We fit four QuaSSE models to test for trait-dependent speciation with and without the inclusion of geographical state. The first two were a constant speciation rate model, and a linear model in which λ varies linearly with relative dewlap area. We explored a more complex sigmoid function, but as it performed more poorly than the linear model and was very slow to converge for some trees, we did not include it as a candidate model. The other two models were geographically informed 'split' QuaSSE analyses, allowing us to test whether the speciation rate or its relationship with relative dewlap area differ between states, and accounting for any trait differences between major subclades that can otherwise lead to spurious inference of trait-dependent speciation [35]. We fitted a split-constant model with no trait-dependent speciation but with separate speciation rates for 'island' and 'mainland' states, and a split-linear model with both intercepts and slopes for the relationship between λ and relative dewlap area allowed to differ between states. For all four models, we assumed that the extinction rate μ and the Brownian diffusion rate σ^2 were independent of relative dewlap size and geographical state, that Brownian evolution was non-directional (no 'drift' term), and that sampling was independent of relative dewlap size. To account for geographical variation in species sampling, we inputted the fraction of species sampled in each geographical partition of the tree (26/83 Dactyloa, 108/151 primary island group, 38/150 mainland Norops, 7/7 *grahami* series, 5/8 *roquet* series; [37]). We inputted estimated standard deviations for each species to account for intraspecific variation in relative dewlap size.

We fitted the constant, linear, split-constant, and split-linear models for the MCC tree and for each of 100 trees from the posterior distribution. We evaluated support for each model, using the Akaike information criterion ($AIC = 2k - 2 \ln L$), which balances model fit and complexity (lower values indicating better performance), and Akaike weights, which measure the proportional support for each model out of the set of models considered [38].

(e) Speciational trait evolution

We estimated the extent to which anole dewlaps diversify via gradual or speciational evolution. Several methods can compare the fit of a gradual (BM) model to models in which some or all of trait change occurs at speciation events (nodes in a phylogenetic tree) [5,6,39,40], though no currently available models calculate likelihoods that can be compared directly with trait-dependent speciation models. We use the 'ψ' model [6] owing to its intuitive parameters and simple methods to accommodate missing species. It models gradual evolution using the Brownian rate parameter σ_a^2 and speciational evolution as step change in both daughter species, drawn from a Gaussian distribution with variance σ_c^2. The ψ parameter indicates the proportion of the total

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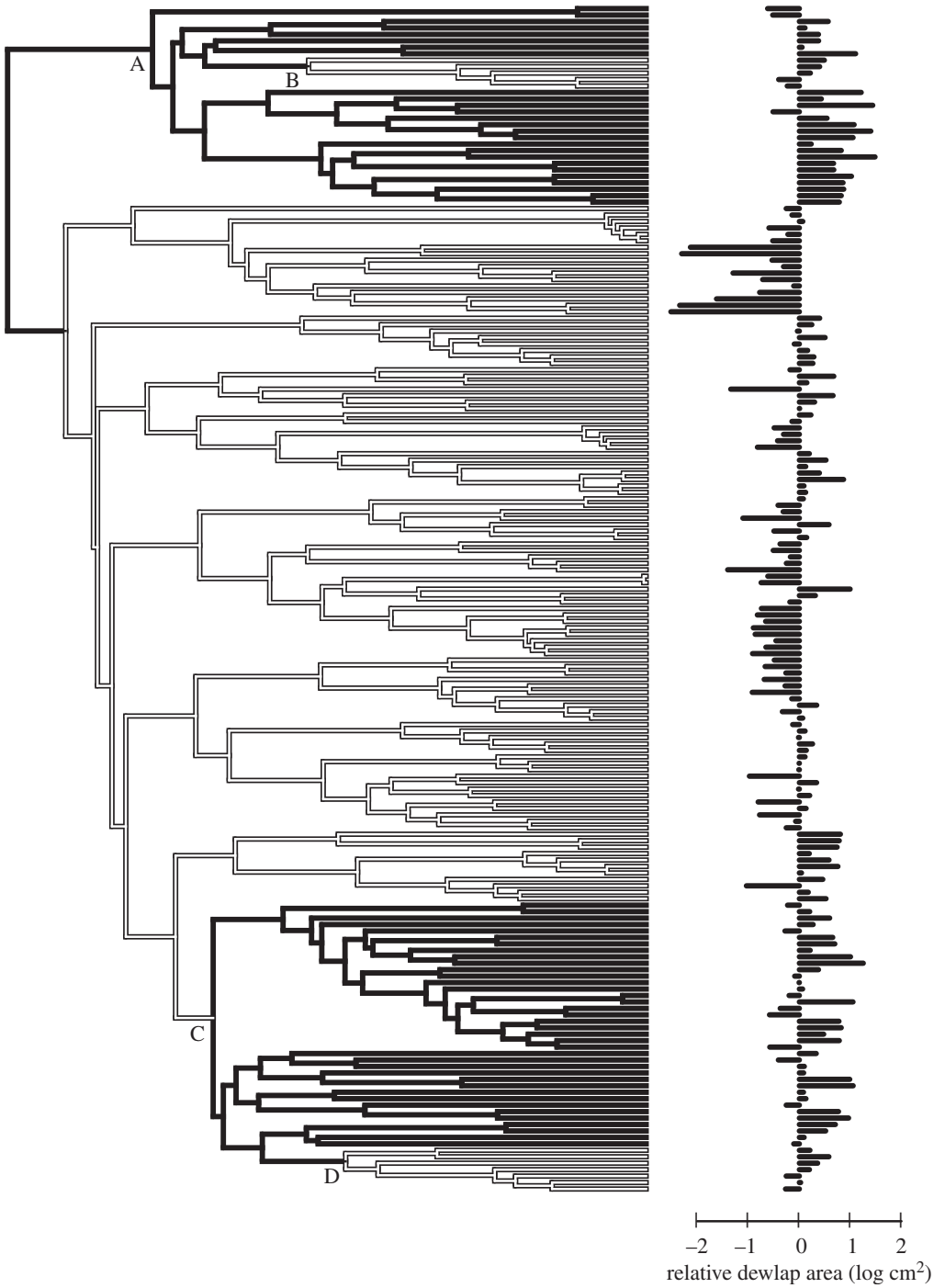

Figure 2. MCC tree used in the analyses, with the relative dewlap area of species indicated by barplots to the right. Geography is indicated by branch colours ('island', white; 'mainland', black) and by letters denoting notable subclades (A, Dactyloa; B, *roquet* series, C, Norops excluding *grahami* series; D, *grahami* series).

rate of evolution ($\sigma_t^2 = \sigma_a^2 + 2\lambda\sigma_c^2$) attributable to speciational change ($\psi = 2\lambda\sigma_c^2/\sigma_t^2$). To allow for geographical variation in the mode of trait evolution, we introduced a new 'multi-ψ' model that allows the contribution of speciation ψ to vary across branches of a tree (details in the electronic supplementary material).

We fitted six candidate models of relative dewlap area evolution. We fitted the BM model of strictly gradual evolution, the ψ model of mixed gradual and speciational evolution, and a single-optimum Ornstein–Uhlenbeck (OU) model that combines stochastic diffusion with a pull towards a central 'optimum' value. The OU model is another alternative to BM that produces some patterns superficially similar to speciational evolution (e.g. close relatives can be relatively dissimilar compared with more distant relatives), so including it reduces the risk of spurious inference of speciational evolution. We also fitted three models that estimated different parameters for island and for mainland lineages in the tree. We fitted a multi-rate BM model (multi-BM) [41] with the phytools function 'brownie.lite'. We fitted the new multi-ψ model in which σ_t^2 is constant but ψ varies between states. Finally, we fitted a multiple-optimum OU model (multi-OU) implemented in OUwie [42] that allows the optimum dewlap area to vary between geographical states, whereas σ^2 and the strength of attraction to the optimum are constant. All six models account for intraspecific variability using species-specific standard errors.

When fitting the ψ and multi-ψ models, we retained the positions of nodes that were removed when 32 species lacking dewlap data were pruned from the 216 species tree. We estimated λ and μ from branching times using the function 'bd' in the R package 'laser' [43]. The maximum-likelihood estimate of μ was zero, but we also estimated λ and μ conditional on a much higher extinction fraction ($\mu/\lambda = 0.5$) to assess sensitivity to either extinction or unsampled species that result in many

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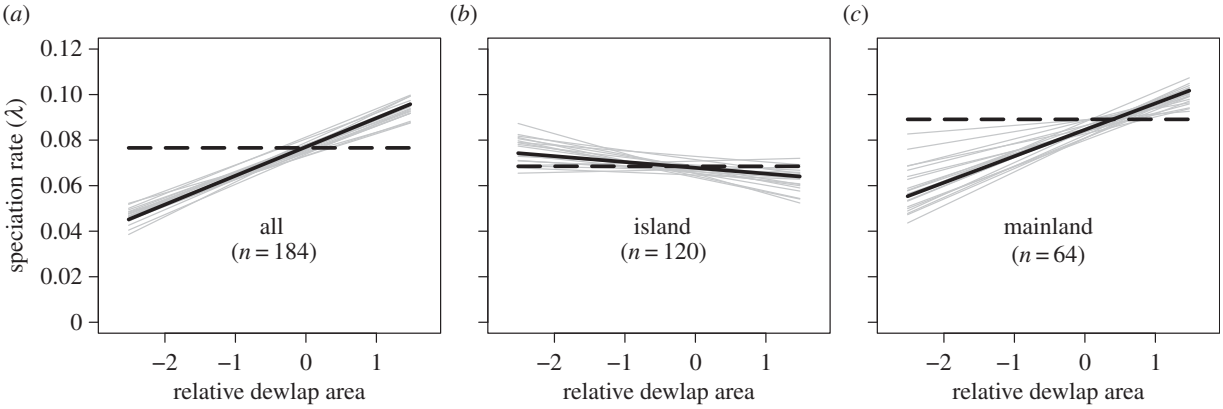

Figure 3. Results of QuaSSE modelling of trait-dependent speciation for (*a*) the entire tree and with partitions for (*b*) 'island' and (*c*) 'mainland' geographical states. Maximum-likelihood estimates are shown for the constant (thick dashed line) and linear (thick solid line) models, and grey lines illustrate linear model fits for 20 trees from the posterior distribution.

Table 1. Results of model fitting to test for trait-dependent speciation in anoles using the MCC tree. For models with separate parameter estimates for each geographical state, these are given as (mainland, island). λ_0 is the intercept (or estimate of a constant speciation rate) and β is the estimated slope of the relationship between λ and relative dewlap area.

	λ_0	β	μ	σ^2	k	log L	ΔAIC_c	weight
constant	0.077	—	<0.001	0.010	3	−742.33	2.88	0.145
linear	0.077	0.013	<0.001	0.010	4	−741.37	2.95	0.140
split-constant	(0.089, 0.069)	—	<0.001	0.011	4	−739.89	0.00	0.614
split-linear	(0.085, 0.068)	(0.012, −0.003)	<0.001	0.010	6	−739.70	3.62	0.101

missing nodes in the reconstructed tree. We then fitted the ψ and multi-ψ models using 10 realizations of the number of hidden speciation events on each branch (see electronic supplementary material) for each of the 100 trees from the posterior distribution. We obtained maximum-likelihood parameter estimates, and measured the relative support for the BM, ψ, OU, multi-BM, multi-ψ, and multi-OU models using AIC_c (AIC corrected for finite sample size, taken to be the number of taxa) and Akaike weights.

3. Results

A strong relationship existed between species mean log dewlap area and log head length (electronic supplementary material, figure S1). The phylogenetic signal in the residuals of the phylogenetic regression was $\lambda_{sig} = 0.83$, and the model with maximum-likelihood λ_{sig} strongly outperformed models assuming either BM ($\lambda_{sig} = 1$, $\Delta AIC = 28.91$) or no phylogenetic signal ($\lambda_{sig} = 0$, $\Delta AIC = 100.04$).

Relative dewlap area varied from −2.51 to 1.48 on a log scale, with a slight negative skew. The smallest measures belonged to two pairs of sister species: *A. bartschi* and *A. vermiculatus*, which lack dewlaps, and *A. hendersoni* and *A. dolichocephalus*. The latter two species have elongated snouts [44], so our use of head length to represent size slightly underestimates their relative dewlap area, but the difference is likely to be small given that head length and SVL are strongly correlated. The mean within-species standard deviation was 0.24, and species identity accounted for 86% of the total variance in individual relative dewlap area. Measurement error associated with positioning lizards during photography, measuring traits from the photographs, and incorporating images from CaribHerp, was low (see the electronic supplementary material).

(a) Trait-dependent speciation

Our hypothesis that relative dewlap area would be positively correlated with speciation rate across *Anolis* was not supported (table 1 and figure 3). The relationship was slightly positive (slope 0.013), but the constant-rate model was weakly preferred by AIC. When we split the QuaSSE analysis by geography, there were slight tendencies towards a flat or weakly negative slope in island lineages and a flat or weakly positive slope in mainland lineages. Speciation rates were generally higher in the mainland lineages, but again there was no support for trait-dependent speciation. For the MCC tree, the split-constant model had the lowest AIC, followed by the constant ($\Delta AIC = 2.88$), linear ($\Delta AIC = 2.95$), and split-linear ($\Delta AIC = 3.62$) models, and had the highest Akaike weight for all 100 trees from the posterior distribution (range 0.51–0.81).

Given the lack of strong support for any model, we carried out *post hoc* pseudo-power analyses to infer our ability to detect a biologically relevant effect. We simulated trees and trait data under a range of slope parameters, mimicking our sampling by randomly pruning 399 taxon trees to 184 taxa, and counted the frequency with which AIC favoured the linear model over the constant model. With a true slope of at least |0.0225| (in units species Myr^{-1} per log unit change in relative dewlap area), the true linear model was preferred for more than 80% of datasets.

(b) Speciational trait evolution

Relative dewlap area was best explained by a model of mixed speciational and gradual evolution (table 2 and figure 4). With maximum-likelihood speciation ($\lambda = 0.053$) and extinction rates ($\mu = 0$), the ψ model outperformed the BM and

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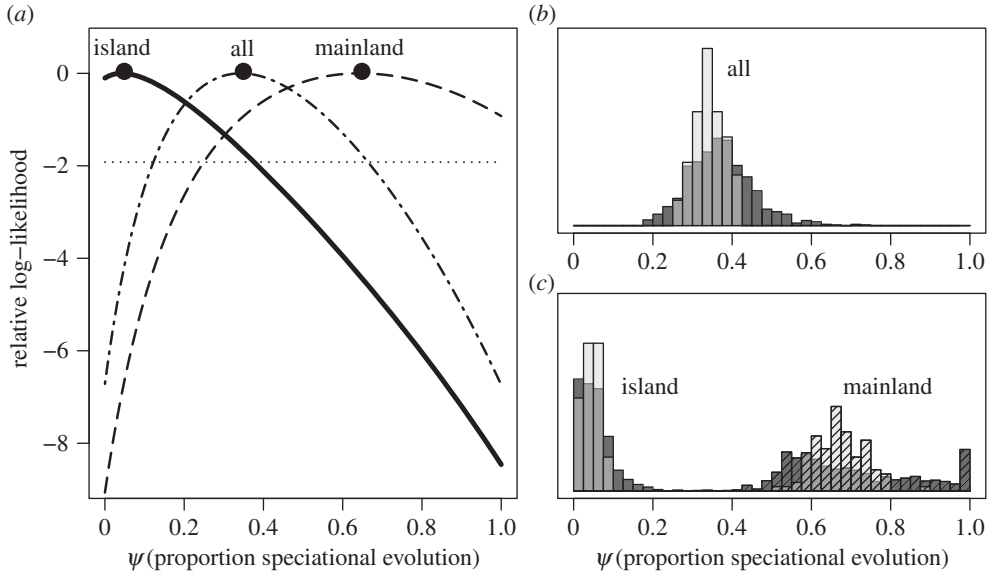

Figure 4. Results of speciational trait evolution analysis with and without geographical information. (*a*) Likelihood surfaces and maximum-likelihood estimates for ψ inferred using the MCC tree for all species, and separately for 'island' and 'mainland' species. (*b*) Distribution of maximum-likelihood estimates of ψ across 100 trees from the posterior distribution across all species, both with (dark grey) and without (light grey) a higher rate of extinction enforced. (*c*) Distributions of ψ estimates for 'island' (no shading lines) and 'mainland' (shading lines), with colour scheme as in (*b*).

Table 2. Results of model fitting to compare speciational evolution models to BM and OU models using the MCC tree. For models with separate parameter estimates for each geographical state, these are given as (mainland, island). For OU models θ is the inferred optimum, α is the strength of the pull to the optimum, and the ancestral state at the root of the tree is assumed to be stationary around θ (for BM and ψ models the ancestral state is estimated but not shown).

	σ^2	ψ	θ	α	k	log$L$	ΔAIC_c	weight
BM	0.0128	—	—	—	2	−155.86	12.56	0.001
ψ	0.0107	0.344	—	—	3	−150.12	3.15	0.140
OU	0.0185	—	0.0183	0.0181	3	−152.04	6.99	0.021
multi-BM	(0.0164, 0.0105)	—	—	—	3	−154.18	11.28	0.002
multi-ψ	0.0110	(0.646, 0.034)	—	—	4	−147.50	0.00	0.676
multi-OU	0.0212	—	(0.398, −0.201)	0.0273	4	−148.95	2.90	0.159

OU models based on AIC_c for the MCC tree and for 95 of 100 trees from the posterior distribution. The maximum-likelihood estimate of ψ was 0.34 (range 0.27–0.42 across trees).

When we allowed different ψ values for the two geographical states, we found a strong speciational signal in 'mainland' lineages ($\psi = 0.64$, range across trees 0.52–0.88) and a weak or absent signal in 'island' lineages ($\psi = 0.043$, range across trees 0–0.09). The multi-ψ model was strongly supported by AIC_c over the single-ψ, BM, multi-BM, OU, and multi-OU models (table 1), and was favoured for 82 of 100 trees with a mean relative support by Akaike weight of 0.62 (range 0.18–0.94). Simulations indicated that with a reasonably large sample size, the multi-ψ model can estimate values of ψ for different clades with reasonable accuracy, and is typically favoured by AIC_c when it is the true model (electronic supplementary material, figures S2 and S3). The only other model to ever be favoured was the multi-OU model, which indicated a higher optimum relative dewlap area in mainland than in island lineages (figure 2).

Assuming a much higher extinction fraction ($\mu/\lambda = 0.5$, $\lambda = 0.065$, $\mu = 0.032$) and sampling multiple realizations of the number of hidden speciation events per branch increased the variance of estimates of ψ (figure 4) and somewhat weakened the support of the multi-ψ model. With higher extinction and 10 samples of hidden speciation events drawn per tree, the multi-ψ model was preferred for 56.7% of comparisons, compared with 42.0% for the multi-OU model and 1.3% for the single-ψ model.

4. Discussion

We did not find evidence that dewlap size influences speciation rate, whereas we did find that diversification in dewlap size appears to be associated with speciation events, particularly in mainland anoles.

In contrast to our hypothesis that larger dewlaps would promote a higher speciation rate, we found no support for trait-dependent speciation. This result also conflicts with the observation that some lineages with reduced dewlaps are relatively species-poor [20], and this does not appear to be a general phenomenon. The dewlap is one of two key traits that are thought to have sparked the adaptive radiation of anoles, along with expanded subdigital toepads [20]. While this may

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still be the case, our results suggest that any effect of the presence of a dewlap on speciation rate is independent of dewlap size. Divergence in the hue of a monochromatic dewlap can be sufficient for species recognition [15], so the mere presence of a dewlap may allow it to serve its role in species recognition and communication. Furthermore, dewlap extension is one of a number of display behaviours that can vary among species [45], so species recognition is not entirely dependent on dewlap differences. Any increase in signal information content allowed by a larger dewlap [46] may therefore have little if any effect on factors promoting speciation.

Methods for inferring trait-dependent speciation are subject to several caveats. These analyses are prone to phylogenetic pseudo-replication, where the presence of large subclades that differ in both average trait values and diversity obscures a true relationship—or lack thereof—between the trait and speciation rate [35,47,48]. Mainland anole lineages generally had larger dewlaps, but the presence of both large- and small-dewlapped species in most subclades (figure 2) should reduce the vulnerability of our analyses to phylogenetic pseudo-replication. Statistical power can also be limited in these analyses, but our simulations indicated that we had power to detect a biologically interesting relationship if one existed. Finally, the test for trait-dependent speciation did not take into account our finding that a mixed gradual and speciational model explains relative dewlap area evolution better than the gradual BM model of trait evolution used by QuaSSE. State changes during speciation have been incorporated into categorical state-dependent diversification models [18,49] but are not yet available for continuous trait-dependent diversification models. As the two methods calculate likelihoods differently, it remains a challenge to directly compare models of trait-dependent speciation and speciational trait evolution, or to model both processes simultaneously.

Our second hypothesis, that relative dewlap area would show a signal of speciational evolution, was supported, though with some unexpected geographical variation. When a single speciational evolution model was fitted to the entire tree, speciational change was estimated to account for approximately one-third of the total evolutionary rate. This signal was much stronger in mainland clades; when we split the analysis by geography, speciational evolution accounted for approximately two-thirds of the rate in mainland anoles while there was little speciational signal in island lineages. Aside from the geographically split speciational model, the only model to receive any support was the multi-OU model of different optimum dewlap sizes in mainland and island anoles. This model was consistent with a general tendency for mainland anoles to have larger dewlaps, albeit with substantial variation around the optima. Parameter estimates imply a very slow approach to the optima, with a phylogenetic half-life (time to evolve halfway to a new optimum $= \ln[2]/\alpha$) of 25.4 Myr, half the age of the root of the tree. Current methods do not allow both speciational evolution and adaptation to optima to be fit simultaneously, but such models may be needed to fully characterize the evolution of the dewlap in anoles.

The signal of speciational evolution could result from a number of processes that are not mutually exclusive. *Anolis* species living in sympatry consistently differ in one or more of dewlap colour, pattern, and relative size [14,46]. This does not imply that this divergence occurred during speciation, but does indicate that dewlap similarity may hinder species coexistence. If anole speciation is primarily or exclusively allopatric [20], only populations that happened to have diverged in dewlap attributes, or that are able to evolve differences through reproductive character displacement [50], may be able to remain distinct upon secondary contact. Alternatively, dewlap divergence could occur as adaptation to distinct habitats drives speciation, without the need to invoke direct interactions between the incipient species. Adaptation to different climatic or vegetation macrohabitats can increase the likelihood or rate of speciation of allopatric populations [51]. The effectiveness of different dewlap attributes varies between light environments [16], and dewlap size varies with climate across Hispaniolan populations of *A. distichus* [52]. A dewlap with either larger area or greater colour contrast may maximize visibility in low-light environments such as forest interiors, so environmental adaptation has the potential to contribute to dewlap size divergence.

At present, we can only speculate as to why the speciational signal was much stronger in mainland than island anoles, as we lack detailed studies of the speciation process in multiple regions. Mainland anole species have relatively large geographical ranges [53] which may increase the opportunity for populations to become isolated in different environments that may favour different signalling traits, and potentially to come into secondary contact. Island anole speciation also likely involves adaptation to different macrohabitats [51], but if more speciation events involve dispersal between islands, then the probability of secondary contact may be reduced. The other obvious differences between island and mainland anoles are the more diverse range of predators and the lower population density of anoles on the mainland. Either of these factors could increase the importance of having a signal that is optimal for a particular light environment, by increasing the cost of failure to attract the attention of the intended conspecific recipient. More detailed studies of the geography of speciation and the strength of sexual selection in island and mainland species will be necessary to identify the factors responsible for speciational evolution being more pronounced in mainland anoles.

The fit of a speciational model of trait evolution does not necessarily mean that any particular process is involved in speciation, and this method also comes with caveats. The sampling of hidden nodes and estimation of ψ can be affected if λ and μ are poorly estimated or vary greatly among lineages. Specifically, Rabosky [54] found that a speciational model can be supported if rates of speciation and BM trait evolution covary positively across the tree. If rapidly speciating lineages also evolve greater trait diversity, then we expect an association between the number of nodes and the extent of trait divergence similar to the signal of speciational trait evolution. This would imply that some factor causes rapidly speciating lineages to also diversify more rapidly in relative dewlap area, even if trait divergence is not directly linked to speciation events. Rates of speciation and trait evolution have been found to positively covary across clades of bony fishes [55], but as we currently lack likelihood methods for fitting such rate covariation models to full trees and comparing their fit with speciational models, it remains a challenge to identify the timescale at which diversification and trait change are linked. Finally, while we used the largest molecular phylogeny currently available for *Anolis*, more than half of the species still lack sequence data, particularly in undersampled parts of South America and Cuba. The ψ model attempts to model 'hidden' speciation events, but incomplete sampling may still

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be an issue if taxonomic practices differ between regions or if species sampling is not independent of dewlap characteristics. Improved sampling of mainland anoles in particular will be necessary to corroborate the signal of speciational evolution with a more robust dataset.

This study suggests that even if the evolution of the dewlap promoted anole diversification, subsequent changes in the size of this signalling trait have not left a signal of trait-dependent speciation. In contrast, diversification of dewlap size appears to have become linked to speciation, at least in mainland anoles, with divergence in dewlap size occurring at some stage of the speciation process. Confirming whether relative dewlap size evolution can be explained by divergent sexual selection will require demonstration of sufficient genetic variation (as has been found for dewlap colour [56]) and measurements of selection in populations that are either adapting to distinct light environments or that have come into secondary contact [50]. The observed mix of positive, neutral, and negative relationships between male dewlap size and measures of fitness or performance [57,58] hints at variation in the form of selection, and studies of the form of selection at different stages of speciation could prove illuminating. Overall, our results show evidence that dewlap size evolution is linked to speciation, but not through any effect on the speciation rate of anoles.

Data accessibility. Data available from the Dryad Digital Respository: http://dx.doi.org/10.5061/dryad.9vr0c [59].

Competing interests. We declare we have no competing interests.

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