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Analysis of an evolutionary species–area relationship

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Large islands typically have more species than comparable smaller islands. Ecological theories, the most influential being the equilibrium theory of island biogeography[1], explain the species–area relationship as the outcome of the effect of area on immigration and extinction rates. However, these theories do not apply to taxa on land masses, including continents and large islands, that generate most of their species *in situ*. In this case, species–area relationships should be driven by higher speciation rates in larger areas[2–6], a theory that has never been quantitatively tested. Here we show that *Anolis* lizards on Caribbean islands meet several expectations of the evolutionary theory. Within-island speciation exceeds immigration as a source of new species on all islands larger than 3,000 km², whereas speciation is rare on smaller islands. Above this threshold island size, the rate of species proliferation increases with island area, a process that results principally from the positive effects of area on speciation rate. Also as expected, the slope of the species–area relationship jumps sharply above the threshold. Although *Anolis* lizards have been present on large Caribbean islands for over 30 million years, there are indications that the current number of species still falls below the speciation–extinction equilibrium.

The 143 species of Caribbean island *Anolis* lizards are ideal for a test of evolutionary theories of diversity. The group has been there since at least the middle Oligocene and has radiated extensively within the archipelago[7–9]. Current species richness on islands is related to area, and both immigration and speciation have contributed[10,11]. We used a phylogeny for Caribbean *Anolis* species based on mitochondrial DNA[9] to estimate the number of immigration and speciation events on islands. By comparing these quantities with island area, we can test three predictions that derive from an evolutionary theory of species–area relationships: (1) a threshold island size should exist, above which speciation surpasses immigration as a source of new species; (2) above the threshold size, recorded speciation events per unit time on the large islands should increase with island area; (3) the slope of the species–area

relationship should become steeper above the threshold. Because speciation is potentially a slow process, we also tested whether the number of *Anolis* species on large islands has reached speciation–extinction equilibrium.

Counts of reconstructed immigration and speciation events on a phylogeny underestimate the true number of events because lineages that have not survived to the present go unrecorded[12]. However, if the rate of extinction of a lineage on an island does not depend on whether it immigrated or arose *in situ*, then the ratio of counts, when plotted against area, should correctly indicate the threshold island size above which local speciation exceeds immigration from outside as a source of new species. Figure 1 reveals that speciation is the dominant source of new species on islands greater than 3,000 km^2 (hereafter, 'large' islands). For example, the ten species on Puerto Rico (8,959 km^2) are derived from three ancestral lineages that experienced seven *in situ* speciation events, and the seven species on Jamaica (11,425 km^2) are derived from two colonizing species. Similarly, 42 of the 57 species on Cuba (114,524 km^2) belong to two of the clades that have radiated on Cuba; at least 12 of the 15 other Cuban species are also the result of within-island speciation. In contrast, on small islands in the Caribbean, multi-species islands always harbour species that are distantly related and thus must have immigrated from elsewhere; of 143 islands smaller than 3,000 km^2 (hereafter, 'small' islands), no within-island speciation events were counted. On these smaller islands, speciation either does not occur, or its rate is too low to offset extinction. These results indicate an area threshold for speciation, in accord with the first prediction. Existence of such a threshold has been posited before in other taxa[4,13], but not previously quantified or documented using phylogenetic (sister species) criteria.

To test the second prediction we compared the number of recorded speciation events per unit time with island area using just the large islands. The number of recorded events by itself gives a biased estimate of speciation rate because some islands had more ancestral lineages than others (resulting from more immigration events or from more lineages present when islands fragmented) and
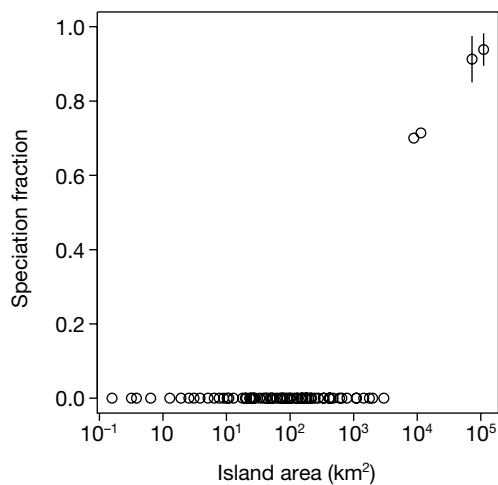
because it does not account for the different time periods that these lizards have been present on different islands[9,14,15]. We used computer simulation to account for these two confounding factors. We simulated speciation using the phylogeny for Caribbean anoles in which branch lengths were scaled to relative time units. The result is clear: the recorded number of speciation events is positively related to island area (Fig. 2), suggesting that speciation rate itself scales positively with area.

Extinction rate decreases with island area in other taxa[13,16–18] and might alone produce an apparent pattern of increased speciation rate on large islands even if the true rate of speciation is invariant. However, a declining extinction rate with increasing area is unlikely to be a sufficient explanation for the pattern in Fig. 2. First, estimates of speciation and extinction rates over all the large islands suggest that the effects of extinction are small (see below). Second, a strong relationship exists between island area and the number of species in 'superspecies complexes'. Superspecies complexes are groups of closely related allopatric or parapatric species that differ little in morphology or ecology and are regarded as an early stage in allopatric speciation[19,20]. If the rate of speciation were comparable among islands, we would expect to see a similar proportion of the species on each island belonging to superspecies complexes, but this is not the case. More than half (56%) of the species on Cuba are members of superspecies complexes, and Hispaniola's proportion is nearly that high (45%). By contrast, no superspecies complexes are present on the two smaller islands. This result implies that at least one mode of speciation—allopatric speciation—is continuous and frequent on the largest islands, but rare on the smaller large islands. A third line of evidence supporting the presence of higher speciation rates on larger islands comes from changes in the slope of species–area relationships, discussed next.

The third prediction is that the slope of the evolutionary species–area relationship should be higher than that resulting when immigration is the sole source of new species. This prediction stems from the idea that speciation rate should increase with island area, in contrast to the weak expected effect of island area on immigration rate[5,13,18]. An initially high rate of species accumulation on large islands is expected to lead to an even greater rate of speciation there because speciation is multiplicative and its rate per unit time rises
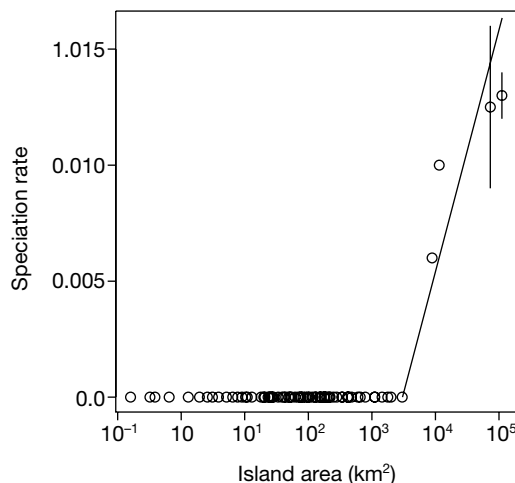

Figure 1 Recorded speciation events on islands, as a proportion of the total number of speciation and immigration events, in relation to island area. 'Immigration' probably includes some between-island speciation events resulting from island fragmentation (vicariance). Vertical bars represent the range of estimates resulting from different phylogenetic geographic reconstructions of the occurrence of ancestral taxa (see Methods). No ambiguity exists in the reconstructions for Puerto Rico and Jamaica; hence, each estimate is a single point. The increase in the speciation fraction with area is significant, according to a logistic regression ($\chi^2 = 26.8$, degrees of freedom, d.f. = 1, $P < 0.0001$; tested using the midpoints of vertical ranges).

Figure 2 Speciation rate on large islands in relation to island area. Error bars as in Fig. 1. Regardless of which estimates are used, a relationship exists between island area (log-transformed) and speciation rate; the line shown here is based on an analysis using the midpoints of the vertical ranges ($F_{1,3} = 49.16$, $P = 0.006$), but any of the reconstructed values yields significant results. Analyses assumed a speciation threshold (*x* intercept) of 3,000 km^2 (compare Fig. 1) and no extinction. Branch lengths in the phylogeny were not calibrated against time, so there are no units for the *y* axis.

with the number of lineages present. The expected result is a steep species–area relationship when islands of different size are compared. Such a transition in slope is observed among the Caribbean islands (Fig. 3). A two-slope regression fits the species–area data far better than a single slope ($F_{2,143} = 34.12$, $P < 0.0001$), with the maximum likelihood breakpoint occurring near the island size at which speciation begins to exceed immigration as the source of new species (Fig. 1).

Several of the arguments above rest on the assumption that speciation exceeds extinction on the large Caribbean islands. We estimated overall rates of speciation and extinction by fitting the branching phylogeny for species on large islands to a constant birth–death process[12]. Because our tree was incomplete, we used only the first 33 branching events (34 lineages), counted up from the root, which corresponded to approximately the first half of the total time span of the radiation. We assume that almost no branches are missing from this early period. This assumption is justified because the species selected to estimate the phylogeny were not sampled randomly, but rather were chosen to represent each of the major taxonomic series (clades) present in the archipelago. We therefore expect the vast majority of missing branches in the tree to occur after the first 33 branching events, towards the present time. To fit the birth–death process we also made use of the information provided by the present-day number of species descended from each of the initial 34 lineages. The best fit was a model in which species number increased exponentially through time and extinction was absent (Table 1). Confidence limits show that the data are also consistent with a non-zero (positive) extinction rate, but this rate does not approach that for speciation. The result suggests that
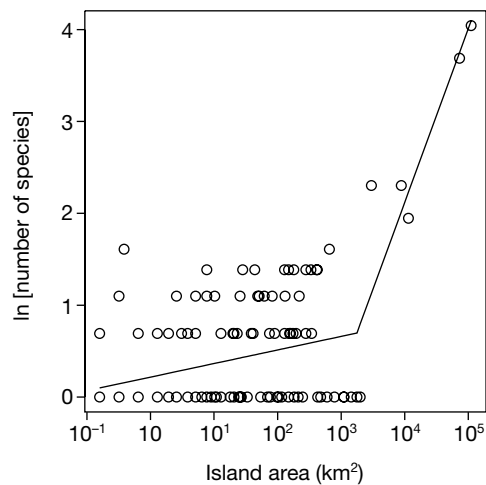
after more than 30 million years of adaptive radiation by *Anolis* on Caribbean islands, species numbers are still increasing and fall below the speciation–extinction equilibrium.

Our results support the central tenet of the evolutionary theory of species–area relationships, that speciation rate increases with area. What mechanisms underlie this effect in *Anolis*? One likely explanation is that the opportunity for geographic isolation increases with island area. Hispaniola and Cuba are dissected by mountain ranges and many species are endemic to a single range. The largest islands have also been broken up into multiple smaller islands at times of higher sea level[21,22]. It is also possible that the diversity of habitats increases with area[23], providing more opportunities for speciation by divergent natural selection[24]. A relationship between area and habitat diversity is one aspect of ecological theories of species–area relationships, and it may likewise play a role in evolutionary theories of species diversity. However, an explanation based on a link between habitat diversity and speciation rate may be insufficient by itself here because some of the larger of the small islands (such as Guadeloupe, with one *Anolis* species, and Martinique, also with one *Anolis* species) are old and topographically and climatically diverse, yet have not experienced any speciation at all. Furthermore, among the Greater Antilles, Puerto Rico is a very old island with great vegetational and topographic diversity, yet it has experienced many fewer speciation events than Hispaniola and Cuba.

MacArthur and Wilson[1] offered Caribbean island reptiles as a primary example of the positive species–area relationship, but their ecological theory does not fully account for this relationship if within-island speciation is the source of many new species. We have shown that *Anolis* lizards of large islands require an evolutionary theory of diversity instead. This extension to ecological theories of island diversity is unlikely to be unique. For example, intra-island speciation is an important source of new species in many Hawaiian taxa including the drosophilids[25] as well as in reptiles on isolated Pacific islands[26]. Lizard taxa on large islands elsewhere in the world (for example, Sri Lanka and Taiwan) have not experienced evolutionary radiations comparable to that of the Caribbean anoles, but their diversities might nevertheless follow similar patterns. Consequently, studies of speciation in relation to area would be well worth pursuing in other taxa and regions, as they would reveal the spatial context of speciation and the diversity of processes leading to the positive species–area relationship. □

Figure 3 The species–area relationship fitted with a regression model having a breakpoint and two slopes. Slopes b_1 and b_2 were estimated using a nonlinear regression package that fitted the equation $\log(y) = a + b_1 \log(x) + b_2[\log(x) - c]\delta$ to the data, where δ is an indicator variable equalling 1 when $x > c$ and 0 otherwise, a is the intercept and c is the breakpoint. Estimated slopes were $b_1 = 0.06$ (± 0.02, standard error of the mean, s.e.m.) and $b_2 = 0.76$ (± 0.09, s.e.m.).

Methods

Phylogeny

The phylogeny of *Anolis* is based on 1,455 base pairs of mitochondrial DNA for 53 species, 48 of which occur in the Caribbean (see Fig. 12a in ref. 9). To estimate the timing of divergence events on this phylogeny, we calculated branch lengths using maximum likelihood with the constraint that the total branch length from each species to the root of the phylogeny is equal, using DNAMLK in PHYLIP[27]. To determine the validity of these branch lengths, we also calculated maximum likelihood branch lengths without the constraint that all species be equally distant from the root, using DNAML in PHYLIP. Values for branch lengths calculated in these two ways were highly correlated ($r = 0.94$), which indicates that the DNA data provides a reasonable estimate of the relative timing of divergence events. In addition, we examined residuals of the regression of constrained branch lengths on unconstrained branch lengths to determine whether there was any island bias resulting from the assumption of equal distance to the root (that is, did this constraint cause branches for taxa on some Greater Antillean islands to be shortened or lengthened relative to branches for taxa on other Greater Antillean islands?). No evidence for a bias was found (analysis of variance, $P > 0.84$), regardless of whether branches whose geographic assignment was equivocal, as discussed below, were assigned to either Cuba or Hispaniola.

Geographic inference of within-island speciation

Our data base[10,11] included species occurrences on 147 Caribbean islands ranging in size from 0.15 to more than 100,000 km^2 (our database does not include species introduced by humans or islands that were connected to mainland America when sea levels were lower during the last ice age). To count the number of speciation events on an island, we assumed that the presence of sister taxa on an island resulted from speciation *in situ*. We consider the alternative that an island was colonized several times, followed by the extinction of ancestral species on other islands, to be far less likely. We also used information on

Table 1 Estimated parameters of a birth and death process fit to the phylogeny of *Anolis* lizards on the four largest Caribbean islands

Parameters	Maximum likelihood estimate	Likelihood limits
Speciation – extinction	12.47	(9.44, 15.04)
Extinction/speciation	0.00	(0, 0.38)

Branches leading to other islands were pruned from the tree of ref. 9 before calculating. Two parameters were estimated using maximum likelihood[12]. The first is the difference between the rates of speciation (birth) and extinction (death). The second is the ratio of extinction and speciation. The maximum likelihood estimates indicate that extinction is rare and speciation is the dominant process. Likelihood limits are parameter ranges whose log-likelihood is within 2 units of the maximum (equivalent to 95% confidence intervals).

membership in species groups to assign speciation events to islands. Previous researchers assigned all Caribbean species to species groups (series); recent phylogenetic work[9] confirms that the species groups are almost invariably monophyletic. Therefore, if all species within a species group occur on a single island, then all speciation events within that species group were assumed to have occurred on that island, even those involving species not included in the phylogeny. In a few cases all members of a species group occur on a single island, with the exception of obviously recent dispersers to other islands (for example, all 15 species of the *sagrei* series occur on Cuba, with the exception of some populations of *A. sagrei* that occur on other islands); in these cases, we considered all species on the primary island to have arisen by within-island speciation. The phylogenetic affinities of two rare Hispaniolan species are unknown and these species were not included in our analysis.

On all landbridge islands near the Greater Antilles and the Bahamas, co-occurring species are always more closely related to species on other islands and thus do not provide evidence for within-island speciation. The one equivocal case involves two members of the *equestris* series that occur on Santa Maria, off the northern coast of Cuba. Whether these species are sister taxa remains to be determined. Again with one exception, all species on small nonlandbridge (oceanic) islands also belong to different species groups. The one exception is the two species on the Lesser Antillean island of St Vincent, whose sister taxa status is controversial[28]. In addition, sympatric species on islands in the northern Lesser Antilles belong to the same species group (the *bimaculatus* series), but each island is occupied by one species from each of the two distinct subclades within the series[29]. Because the *bimaculatus* series is monophyletic, it is conceivable that these two subclades initially arose by within-island speciation on one island, but we consider the alternative of allopatric differentiation on different islands to be more plausible. In summary, within-island speciation occurs very rarely or not at all on small islands. We do not consider any of the three possible exceptions as representing strong cases for within-island speciation. However, even if we had included them in our analyses, they would not have altered our conclusions.

Estimation of rate of speciation and extinction

We used computer simulation to test our prediction that number of recorded speciation events on large islands should correlate with area. To carry out the simulation, we used parsimony to infer inter-island immigration events and to determine the relative date on which new lineages immigrated to islands. In the most parsimonious reconstruction, Hispaniola was the ancestral locality for much of the anole radiation from which lineages on Cuba were derived independently several times. To examine the robustness of our analyses, we included the slightly less parsimonious (14 versus 15 steps) alternative possibility that Cuba was ancestral and that Hispaniola had been occupied independently by seven different lineages[30]. Each simulation run began at the time, as indicated by the phylogeny, that the first species to a given island was recorded. In each time interval, all species present on an island had a probability, *r*, of speciating, thus increasing the number of species on the island by one. Additional species were added to the island at the times at which new lineages appeared (presumably by immigration or possibly by vicariance as island blocks collided[21,22]), again as indicated by the phylogeny. In this way, the effect of the addition of new lineages to an island was incorporated into speciation rate estimates. Five hundred simulation runs were conducted and the mean number of species produced was calculated. Simulation trials were conducted iteratively, changing the value of *r*, until the mean number of species produced converged on the actual number of species that occurs on that island.

We used a modification of the likelihood method of ref. 12 to fit the *Anolis* phylogeny to a birth and death process. Our likelihood for birth and death parameters was the product of two parts. The first part is equation (17) of ref. 12 and is the probability density of the 32 observed waiting times between successive branching events of the phylogeny from the first branching event near the root to the 33rd branching event near the half-way point. The second part of the likelihood is based on equation (11) of ref. 12 and is the product of the probabilities that each lineage *i* living after the 33rd branching event has exactly k_i species at the present time, where k_i is its observed number of descendants, $k_i > 0$, and $i = 1, 2, \ldots, 34$. For each lineage *i* this probability is $(1 - \eta_t)\eta_t^{k_i - 1}$ where *t* is the time between the 33rd branching event and the present time, $\eta_t = (\exp(rt) - 1)/(\exp(rt) - a)$, $r = $ (speciation − extinction) and $a = $ extinction/speciation.

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