Title: Evidence for ecological processes driving speciation among endemic lizards of Madagascar

Running title: Speciation among endemic lizards of Madagascar

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Author contributions

L.A.N and R.G.P conceived of and designed the project. L.A.N and C.J.R compiled the species locality and systematic data. L.A.N performed the analyses. L.A.N, R.G.P and C.J.R wrote the paper.

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Data Accessibility Statement

Species occurrence data are available through the Dryad Digital Repository database (**https://doi.org/10.5061/dryad.rbnzs7hcb**). Custom R scripts for reproducing the paper's RTR analysis are available at: **https://github.com/LauraANunes/NunesPearson2017**

Abstract

Although genetic patterns produced by population isolation during speciation are well documented, the biogeographic and ecological processes that trigger speciation remain poorly understood. Alternative hypotheses for the biogeography and ecology of speciation include geographic isolation combined with niche conservation (soft allopatry), or parapatric distribution on an environmental gradient with niche divergence (ecological speciation). Here we utilize species' distributions, environmental data and two null models (Random Translation and Rotation, RTR, and the Background Similarity Test, BST) to test these alternative hypotheses among 28 sister pairs of micro-endemic lizards in Madagascar. Our results demonstrate strong bimodal peaks along a niche divergence-conservation spectrum, with at least 25 out of 28 sister pairs exhibiting either niche conservation or divergence, and the remaining pairs showing weak ecological signals. Yet despite these significant results, we do not find strong associations of niche conservation with allopatric distributions, or niche divergence with parapatric distributions. Our findings thus provide strong evidence of a role for ecological processes driving speciation, rather than the classic expectation of speciation through geographic isolation, but demonstrate that the link between ecological speciation and parapatry is complex and requires further analysis of a broader taxonomic sample to fully resolve.

Keywords: speciation, biogeography, niche conservatism, allopatry

Introduction

Speciation is the process by which populations evolve to become distinct species. The segregation of ancestral species' distributions during speciation is most commonly described as a geographic separation (Doebeli and Dieckmann 2003; Fig. 1a). Examples of this 'hard allopatry' mode of speciation include single island endemics that became isolated via jump dispersal to new islands (Ricklefs and Bermingham 2007). However, alternative hypotheses for the biogeography of speciation bring into question the ubiquity of hard allopatry. One alternative hypothesis, termed 'soft allopatry' (Pyron and Burbrink 2010), is that populations may become geographically isolated when environmental disturbances, such as changes in climatic conditions, cause an ancestral population to split its distribution (vicariance) because it is unable to persist in intervening ecological conditions (Wiens 2004) (Fig. 1b). An example of soft allopatry is observed in eastern North American salamanders, where sister pairs occupy ecologically similar habitats in Appalachian montane regions but do not overlap geographically because of unsuitable climates in intervening lowlands (Kozak and Wiens 2006). A third hypothesis, termed 'ecological speciation,' describes a process that takes place without immediate disruption to gene flow (Schluter 2001; Rundle and Nosil 2005; Nosil 2008) (Fig. 1c). Ecological speciation has been proven to be possible both theoretically (Fisher 1930) and experimentally (Doebeli and Dieckmann 2003; Berner et al. 2009), and speciation with gene flow has been observed in cave salamanders (Niemiller et al. 2008) and in the flora of Lord Howe Island (Papadopulos et al. 2014). Ecological speciation typically occurs along environmental gradients (ecotones), which results in adjacent and overlapping geographic ranges between descendent species (Caro et al. 2013), in contrast to the non-overlapping geographic ranges of allopatric descendent species (Graham et al. 2004; Anacker and Strauss 2014).

Inferring speciation mechanisms from current geographic distributions is challenging because of the likelihood of post-speciation range shifts (Losos and Glor 2003). Another approach is to infer speciation mechanisms through the ecological processes associated with each sister pair, which may be retained despite post-speciation range shifts (Losos and Glor 2003).

Given that soft allopatry results from the inability of ancestral species to survive in novel ecological conditions, this speciation mechanism is associated with low ecological differentiation between descendent species, termed phylogenetic niche conservatism (PNC, Fig. 1e; Peterson et al. 1999; Wiens 2004). PNC is defined as the substantial retention of ecological traits through time; thus, species within a sister pair will have similar ecological niches (Wiens and Graham 2005; Losos 2008). In contrast, substantial ecological differentiation between descent species, termed phylogenetic niche divergence (PND, Fig 1f), is expected under ecological speciation because the process is driven by ecological differentiation (Warren et al. 2008; Jezkova and Wiens 2018). PND is defined as the differentiation of ecological traits through time, which results in species within sister pairs having distinct ecological niches (Pyron et al. 2015; Nunes and Pearson 2017). While soft allopatry and ecological speciation are mediated by intrinsic environmental processes, hard allopatry takes place primarily through a process of isolation due to discrete geographic features which limit population dispersal between potentially favorable habitats (Pyron and Burbrink 2010). In this case, ecological differentiation between sister pairs will not be due to environmental conditions selected by the species but rather be related to the ecological conditions available in the landscape, or else represent the random independent evolution of niches subsequent to isolation. As a result, we would not expect to find evidence of either PNC or PND in cases of hard allopatry (Fig. 1d).

Several previous studies have tested for associations between speciation processes and PNC/PND by combining species distribution data, fine-scale global climate data and statistical null models (Warren et al. 2008; McCormack et al. 2009; Nunes and Pearson 2017; Jezkova and Wiens 2018). Methods focus on testing sister pairs, which are useful for assessing speciation processes because their recent divergence results in less time for range and niche shifts to have occurred (Losos and Glor 2003; Anacker and Strauss 2014). To test for evidence of PNC/PND, we apply two approaches: the Random Translation and Rotation null model (RTR; Nunes and Pearson 2017) and the Background Similarity Test (BST; Warren et al. 2008). These approaches provide complementary ways to assess whether the observed niche overlap between two closely related species is higher or lower than by chance. The RTR model has several features that make it suitable for this test, including: i) it preserves the range size and shape of each species; ii) it maintains the underlying spatial autocorrelation of the climatic data while decoupling the link between distribution and environment (Cardillo et al. 2019); iii) it avoids reciprocal testing between two species, which may lead to conflicting estimates of speciation mechanisms (Warren et al. 2008; Jezkova and Wiens 2018); and iv) it does not require the fitting of ecological niche models which may be unreliable for species with small number of occurrences (Elith and Graham 2009; Proosdij et al. 2016; Nunes and Pearson 2017; Soultan and Safi 2017). BST complements the RTR method in that it incorporates local environmental heterogeneity between species occurrences and their surrounding landscapes (Warren et al. 2008) and thus compares the ecological suitability of each species within species-specific accessible areas (Barve 2011). Both tests enable the testing of three distinct categorical outcomes: ecological niche overlap may be significantly higher than random (PNC), lower than random (PND), or not significant; and they can contextualize the observed niche overlap along a continuous niche divergence-conservation spectrum.

We use the RTR and BST null tests to infer three possible outcomes: (1) when observed niche overlap is not statistically distinct from the null expectation, the sister pair shows neither PNC or PND, which is consistent with geographic drivers of hard allopatry (Fig. 1a,d) or may reflect limitations of the data and/or methods (see Discussion); (2) when niche overlap is statistically higher than the null expectation, the sister pair demonstrates PNC and is associated with ecological drivers of soft allopatry (Fig. 1b,e); and (3) when niche overlap is statistically lower than the null expectation, the sister pair demonstrates PND and is associated with divergence due to ecological speciation (Fig. 1c,f). Using a metric for the degree of conservatism (where low degrees of niche conservatism reflect niche divergence), we expect that pairs that speciated through soft allopatry would cluster toward the top end of this distribution (i.e., statistical signal close to or significant for PNC) and pairs that speciated through ecological speciation would cluster toward the bottom end of the distribution (i.e., statistical signal close to or significant for PND). To account for the potential accumulation of ecological differences and range shifts through time (Culumber and Tobler 2016), we also tested for associations between speciation age and a suite of biogeographic traits within our sampled taxa.

Malagasy lizards stand out as suitable candidates for testing speciation processes and local endemism because: i) ectothermic organisms are more sensitive to climatic conditions (Hu et al. 2016) and are therefore more susceptible to ecological-mediated processes; ii) they are relatively young, with radiations emerging during the Cenozoic period (Yoder and Nowak 2006) within a relatively stable and isolated tropical environment and therefore have less potential for post-speciation range and ecological shifts compared to sister pairs of older radiations (Blair et al. 2013); iii) almost resolved phylogenetic trees are available (Townsend et al. 2009; Pyron et al. 2013; Zheng and Wiens 2016) with high sister pair richness (Raxworthy et al. 2008); and iv) there is a high number of range restricted species, which indicates low dispersal abilities (Brown et al. 1996; Lester et al. 2007) and therefore high potential for ranges to be stable through time.

Madagascar is an ideal setting for studying evolutionary processes because it has been isolated from the mainland since the Mesozoic and therefore harbors multiple monophyletic lineages and unique patterns of local endemism (Wilme 2006; Raxworthy et al. 2008; Vences et al. 2009; Brown et al. 2014). Allopatric speciation has been proposed to be the dominant speciation mechanism driving micro-endemism in Madagascar (Wilme 2006), yet cross-taxon analyses question this and point to multiple potential mechanisms (Pearson and Raxworthy 2009).

Methods

We identified sister taxa within monophyletic lineages of Malagasy lizards from phylogenetic studies (Townsend et al. 2009; Zheng and Wiens 2016). These monophyletic lineages included the chameleons (*Brookesia*, *Calumma* and *Furcifer*), geckos (*Blaesodactylus*, *Geckolepsis*, *Paroedura*, *Phelsuma* and *Uroplatus*), iguanas (*Oplurus*), plated lizards (*Tracheloptychus* and *Zonosaurus*) and skinks (*Amphiglossus*, *Madascincus*, *Pygomeles* and *Voeltzkowia*). We collected distribution data for 68 lizard sister pairs that are endemic to Madagascar from the Global Biodiversity Information Facility (GBIF.org), VertNet (Constable et al. 2010) and available literature (Pearson and Raxworthy 2009; Brown et al. 2014; Jezkova and Wiens 2018) and Raxworthy (pers.comns.) and corrected for locality errors by review of the occurrence dataset with reference data (IUCN Red List) and expert knowledge (Raxworthy). We removed sister pairs that occupied more than 50% of the total area of the island to focus our analysis on sister pairs that exhibit a localized distribution pattern and allow sufficient geographic space for random sampling within the island (Nunes and Pearson 2017). Using range-restricted taxa also provides higher confidence in species delimitations because broadly ranged reptiles may actually contain cryptic complexes of species (e.g., Florio and Raxworthy 2016; Ruane et al. 2018) and therefore will confound analysis of speciation processes (Raxworthy et al. 2007). A total of 28 lizard species pairs were kept (Supporting Information Table 1). Data on divergence dating of speciation was obtained from Zheng and Wiens (2016). This study was selected due to the use of a large genetic data set and primary fossil calibrations (Title and Rabosky 2016). The inclusion of our sampled taxa within this phylogenetic tree was based on 12 (of 52 total) gene markers used to construct the supermatrix phylogeny, with similar data coverage across all sister pairs (see Supporting Information Table 6).

Species occurrence data and global climate data provide insight into the climatic conditions that species are known to survive (i.e., species realized climatic niche *sensu* Soberón, 2007). Despite being an incomplete representation of a species' fundamental ecological niche (Saupe et al. 2018; Owens et al. 2020), occurrence data are the most available data to generate ecological models for a variety of species (Guillera-Arroita et al. 2015; Warren et al. 2020). By comparing the similarity between the realized climatic niches of sister species and their geographic mode of speciation, we analyze speciation in the context of ecological niche differentiation across monophyletic phylogenies.

We conducted the analyses at a spatial resolution of 2.5 arc minutes (~4.5km grid cells) to account for potential errors in locality measurements which could lead to errors in realized climatic condition (Graham et al. 2007). To construct the realized climatic niche of each of the sister taxa, we extracted climatic data on temperature and precipitation from the locality data (WorldClim v2, Fick and Hijmans 2017). We selected seven climatic variables that are associated with the physiology and life history requirements of reptiles (Pearson et al. 2014): temperature seasonality, maximum temperature of warmest month, mean temperature of wettest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of driest month and precipitation seasonality.

To characterize the biogeographic distributions and distinguish between allopatric and parapatric pairs, we drew minimum convex polygons (MCPs) and calculated the minimum distance (isolation distance) and the amount of overlap between polygons of sister taxa. The isolation distance was measured as the minimum distance between occurrences of each sister species using the spDistN1 function from the ‘sp’ R package (Pebesma and Bivand 2005). The range overlap of each species was calculated by dividing the total area of intersecting sister pairs MCP by the area of the smaller ranged species (Anacker and Strauss 2014). Sister taxa with non-overlapping MCPs were classified as having allopatric distributions, while those with overlapping MCPs were classified as having parapatric distributions. Using minimum convex polygons to describe biogeographic patterns in complex landscapes presents potential caveats, such as inflating the amount of range overlap between two geographic distributions. As a result, we found 4 pairs with apparent substantial range overlap, which could indicate sympatric speciation (Table 1 and Table S1). However, we did not consider these pairs to be strong candidates for sympatric speciation because they do not have any observed sympatry in the field (Raxworthy pers. comns.) and some (i.e., *B. superciliaris* – *B. therezieni*) were distributed along an elevational gradient. Therefore, these pairs were considered to be parapatric. We calculated the relative occurrence area of each sister pair (ROA; Jiménez-Valverde et al. 2008; Lobo et al. 2008) , which is a measure of the degree of endemism of each sister pair, with decreasing ROA values indicating increasing endemism (e.g., a ROA of 0.3 suggest that the sister pair occupied 30% of the island of Madagascar). The ROA of each sister pair was measured as the ratio between the area occupied by each sister pair relative to the extent of the island of Madagascar by dividing the combined area occupied by each polygon of a sister pair by the total area of the island of Madagascar.

For the RTR approach, we quantified the amount of niche overlap using the MO metric (Nunes and Pearson 2017), which averages the overlap between the climatic conditions occupied by each species of a sister pair. This approach retains the biological meaning of the climatic variables (instead of transforming the climatic variables into PCA axis, Broennimann et al. 2012) and avoids assumptions of climatic suitability obtained from species distribution models (Warren et al. 2008; Jezkova and Wiens 2018). We then used a null model to test the degree of niche conservatism (the RTR null biogeographic test, Nunes and Pearson 2017). The RTR null biogeographic model tests whether the observed niche overlap is likely to be higher or lower than expected by random, given the available climate of a region of interest. Given that several wide-ranging endemic reptiles occupy almost the entire island of Madagascar, we constrained our region of interest to the whole island thus including areas within the dispersal range of the broader taxa. We considered cases where observed niche overlap was within the top or bottom 2.5% of the null distributions as cases of significant PNC or PND respectively.

To further assess our hypotheses, we also applied the BST in environmental space using ordination statistics (Warren et al. 2008; Broennimann et al. 2012). Unlike the RTR model, BST requires a reciprocal approach to assess whether the observed niche overlap is lower or higher than the simulated niche overlaps (derived from comparison of the observed niche of one species against the simulated niche from the background of the other species). Therefore, we conducted two BST analyses for each sister pair (one for each reciprocal test). The environmental space was calibrated using the same climatic variables as in the RTR model and the background climate of each species was calculated based on a minimum convex polygon around the occurrence data for each species including a 5km buffer zone (Hu et al. 2016) to reflect the low dispersal ability of these range-restricted species. Species occurrence densities were projected onto a gridded PCA environment with a resolution of 100x100 cells and niche overlap was calculated using Schoener's *D* statistic (Warren et al. 2008). Statistical significance of niche overlap was tested using a two-sided background similarity test (i.e., either lower or higher than by chance) against 100 niche simulations per species background (Broennimann et al. 2012; Hu et al. 2016).

We used these approaches to test three possible outcomes: ecological niche overlap may be significantly higher than random (PNC), lower than random (PND), or not significant. We also define the degree of niche conservation as the cumulative frequency distribution of the observed niche overlap within the null distribution, thus generating a continuous measure of ecological niche conservatism from 0 to 1, where values closer or equal to 1 represent high degree of niche conservatism and values closer or equal to 0 suggest low degree of niche conservatism. We analyzed the tendency for sister pairs to cluster at either ends of the PND-PNC spectrum (i.e., suggesting either ecological or soft allopatric speciation but not hard allopatry) using Hartingan's dip test for unimodal distributions from the 'diptest' R package (Maechler 2016). A significant deviation from a unimodal distribution indicates a multimodal distribution. We also calculated the location of the two modes and the antimode of the bimodal distribution using the 'locmode' function from the 'multimode' R package (Ameijeiras-Alonso et al. 2021). We hypothesized that sister pairs centered around the upper and lower modes would suggest potential ecological processes during speciation (soft allopatry and ecological speciation respectively) while sister pairs clustered around the antimode would suggest either no ecological processes during speciation (and therefore a hard allopatry mode of speciation) or that any ecological signal has been lost over time or is undetectable using our methods (see Discussion). To test for differences in observed niche overlap and degree of niche conservation between sister pairs exhibiting allopatric and parapatric distributions, we conducted a permutation test. In this permutation test, observed differences between the medians of the two speciation groups are calculated (with the highest median being subtracted by the lowest median). This observed difference was then tested against mean differences generated from randomly allocated sister pairs to either group but keeping the sample size of each group equal to the original dataset. We conducted generalized linear models to test for significant relationships correlations between speciation age, range overlap and degree of niche conservatism with other biogeographic traits. The analyses were carried out in R (R Core Team 2020).

Results

Both the RTR and BST tests point to a bimodal distribution in the distribution of degree of conservatism across our sample of sister pairs (RTR Hartingan's $D = 0.11062$ and $P < 0.05$, Fig. 2; BST Hartingan's $D = 0.98391$, 0.12963 and $P < 0.05$, Fig. S2, Fig. S4;). These results suggest a tendency for sister pairs to tend towards either PNC or PND, thus supporting a role for ecological processes in driving speciation for the majority of our sample taxa. With RTR, we found that among sister pairs clustered around the upper mode of the distribution (i.e., suggesting high degree of niche conservatism), 8 pairs have allopatric distributions and 5 have parapatric distributions and around the lower mode (low degree of niche conservatism), 6 have allopatric distributions and 8 have parapatric distributions. With BST, the upper modes consisted of 6 allopatric pairs and 9 parapatric pairs, and the lower modes consistent of 7 allopatric pairs and 4 parapatric pairs (Figs. S2, S4). Among pairs with distributions that are currently parapatric, the RTR model showed one pair with significant PND (as expected) but one pair showed significant PNC, which is the opposite of our expectation for parapatric pairs (Fig. 1c,f). Among allopatric pairs, the RTR model showed two pairs with significant PNC (as expected) and three with significant PND, which is the opposite of our expectation for allopatric pairs (Fig. 1b,e). BST showed 0-1 allopatric pair with significant PNC and 2 parapatric pairs with significant PND (as expected) but 5 allopatric pairs showed significant PND and 2-3 parapatric pairs showed significant PNC. BST results were reciprocal between all pairs (i.e., no pairs had signals for both PNC and PND, Supporting Information Table 2). We also found pairs with consistent ecological signals between the RTR and BST tests (Table S2). Specifically, PND signals were consistent in *U. pietschmanni- U. alluaudi* and *P. dubia – P. ravenala* and PNC signals were consistent in *L. guibei – L. miops*. Only one pair, *C. boettgeri - C. nasutum*, had PND signal with the RTR test but PNC signals with the BST test (Table S2).

For both the RTR model and the BST test we also found that observed niche overlap is higher in parapatric pairs than allopatric pairs ($P < 0.05$; Fig. 3a, Fig. S3a; Fig S5a), which is expected given that parapatric pairs have partially overlapping geographic ranges and therefore are more likely to experience similar climatic conditions (climate overlap increases with geographic range overlap; Supporting Information Table 3); however, when observed niche overlap was tested against the null RTR model, we found that allopatric pairs tend to have niches that are more similar than expected by chance (median = 0.663), whereas parapatric pairs have niches that are more divergent (median = 0.281; Fig. 3b). The opposite was found when observed niche overlap was assessed with BST, with allopatric pairs tending to have niches more divergent than expected by chance (median = 0.26 and 0.32) than parapatric pairs (median = 0.69 and 0.73; Fig. S3b, Fig. S5b). For all sets of analysis, these findings do not confirm our expectation that pairs with allopatric distributions tend toward niche conservatism, and pairs with parapatric distributions tend toward niche divergence ($P = 0.223$; Fig. 3b and $P = 0.964$ and 0.865; Fig. S3b; Fig. S5b), but they suggest a complex role for ecological processes and point to a need for further analysis of a larger number of sister pairs. We also found no significant relationship between estimated divergence date for speciation (based on Zheng and Wiens, 2016) and any of the other variables given in Table 1 (Supporting Information Table 3-5). These results suggest that post-speciation range shifts (which otherwise should be more pronounced in older sister pairs) were not contributing a major source of signal in our analyses.

Discussion

Several studies of reptiles support either speciation with PNC or PND (Wollenberg Valero et al. 2019), which aligns with mixed evidence for PNC in other taxa (Peterson 2011). Examples of mixed evidence among reptiles include a study of 11 Caribbean Anolis lizards that found no evidence that niche conservatism was more common in closely related species than in distantly related species (Knouft et al. 2006) and a survey of 49 allopatric squamate reptiles that found niche divergence to be 3 times more likely than niche conservatism (Jezkova and Wiens 2018).

Our findings provide a first systematic assessment of speciation processes for a sample of lizard sister taxa in Madagascar using two complementary null model approaches. Overall, both approaches suggest tendencies for sister pairs to cluster at either end of the niche divergence-conservation spectrum, thus providing robust support for ecologically-mediated speciation, including both soft allopatry and ecological speciation (Fig. 2). These results highlight a strong influence of ecological processes on speciation events of Malagasy reptiles. Various mechanisms have been proposed to explain diversification patterns in Madagascar including forest contractions (Raxworthy and Nussbaum 1995), riverine barriers (Pastorini et al. 2003), watersheds (Wilme 2006), topography (Townsend et al. 2009) and selection along ecotones (Pearson and Raxworthy 2009). However, individual mechanisms have weak support for explaining speciation in multiple taxa, such as the influence of riverine barriers on the diversification of reed frogs (Gehring et al. 2012) and lemurs (Blair et al. 2013) while evidence of both watershed barriers and ecotone selection were found among reptiles and lemurs (Pearson and Raxworthy 2009). In this study, we also found evidence of multiple speciation processes among range-restricted reptiles which further supports the notion that not a single mechanism may fully explain diversification patterns in amphibians and reptiles of Madagascar (Brown et al. 2014). By testing for multiple speciation hypothesis, including both ecological (i.e., soft allopatry and ecological speciation) and non-ecological processes (i.e., hard allopatry), our study also provides novel insights into the high prevalence of ecological niche divergence and conservatism among closely-related lineages.

Neither test showed a significant difference in the tendency for niche conservatism between allopatric and parapatric sister pairs (Fig 3b; Fig. S3b; Fig. S5b). Higher degree of niche conservatism among pairs with allopatric distributions would support the hypothesis of limited ecological divergence during soft allopatric speciation (Peterson et al. 1999; Graham et al. 2004) and thus a role for PNC in promoting soft allopatric speciation (Wiens 2004). However, the lack of statistically significant differences in the degree of niche conservatism between the two groups concurs with findings of non-statistical niche divergence among allopatric pairs of squamate reptiles worldwide (Jezkova and Wiens 2018). Building on from our study, potential future work could apply these analyses to older lineages to further test for the consistency of these patterns across deeper nodes of this phylogeny, with the caveat that older lineages allow for longer times for post-speciation shifts to take place, and also recognizing the challenges of estimating niches for ancestral lineages. Future work could also use additional Bayesian phylogenetic methods to further test the robustness of the patterns observed from the supermatrix phylogeny of Zheng and Wiens (2016).

Sister pairs that cluster around the antimode of the niche divergence-conservation spectrum suggest a potential hard allopatric model of speciation with no role of ecological processes. One of the pairs whose ecological signal was closely centered around the antimode in the RTR test, *P. quadriocellata* – *P. antanosy*, is a strong candidate for hard allopatry, given its lack of sympatry in the field and specialization to distinct palm tree species (Raxworthy pers. obs.), which would be an example of jump dispersal and consequent geographic isolation due to specialization of habitat but not of climatic niche preferences. However, there are several alternative explanations for why an ecological signal may not be detected. For example: i) our tests may not have included the relevant climatic variables or non-climatic traits such as soil microhabitats (Blair et al. 2013; Wollenberg Valero et al. 2019); ii) the climatic baseline we used may not be representative of the climatic conditions during speciation (Roubicek et al. 2010); iii) ecological processes may have operated at a finer spatial resolution than we are able to capture (Hurlbert and Jetz 2007); iv) speciation events were not related to either environmental conditions or geographic barriers (for example, speciation may have been driven by sexual selection (Lande 1982) or socially-mediated factors (Wollenberg Valero et al. 2019)); or v) low statistical power due to model inadequacy, low sample sizes or, in the case of the BST, a narrow buffer distance which could lead to conservative estimates of species dispersal ability. Furthermore, non-biogeographical factors could also lead to non-overlapping species distributions and thus confound analysis of ecological niche evolution. For example, one possible explanation for the maintenance of non-overlapping ranges through time could be biotic interactions, such as interspecific territoriality between closely-related species rather than geographic or climatic barriers (Freeman et al. 2019; Cowen et al. 2020). Secondly, human activities have resulted in differences between current reptile distributions and pre-human distributions in Madagascar, as a result of anthropogenic habitat modification (Raxworthy 2003). Given that there are these many possible reasons for not finding an ecological signal in our analyses, it is thus especially noteworthy that we *did* find ecological signal, suggesting that ecological processes played a role during speciation and that those signals remain detectable.

Both methods that we used to measure niche overlap are sensitive to the choice of background area (for a comparison see Brown and Carnaval 2019). Increasing the background area generates random niches that may be more dissimilar to observed niches, resulting in higher likelihood of type I errors (false rejection of the null hypothesis, Nunes and Pearson 2017). However, the methods use distinct approaches for sampling background climates: the RTR test samples climates within the largest contiguous geographical area available to the studied taxa without dispersal constraints (i.e., the entire island of Madagascar) whereas the BST samples the climatic space that is accessible to the species based on a dispersal buffer. Given these differences, the two approaches may be expected to result in different outcomes for the same sister pair (Nunes and Pearson 2017) yet, remarkably, while we found some sister pairs with different ecological signals between methods, overall patterns were consistent across methods, particularly the significant bimodal curves that resulted from the clustering of sister pairs in opposite ends of the niche divergence-conservation spectrum.

Although we aimed to minimize the influence of post-speciation range shifts by our selection of recently-diverged and range-restricted sister taxa, evolving in a relatively more stable tropical environment; and we found no evidence for the effect of speciation age on any biogeographic traits (Table S5); the potential for range shifts to confound these types of analyses remains (Losos and Glor 2003). For instance, extirpations may have resulted in distribution contractions and thus post-speciation loss of a contact zone in the *U. pietschmanni- U. alluaudi* pair, both species of which are considered to be biogeographic relicts (Raxworthy et al. 2008). In contrast, range expansion and secondary contact after speciation might explain the current parapatric distributions and unexpected PNC signal in the pair *P. androyensis – P. picta*. To more definitively pinpoint post-speciation range shift events and historical gene flow, these non-concordant pairs should be targets for future genetic work to look for evidence of gene flow during speciation (Martin et al. 2013; Sousa and Hey 2013; Seehausen et al. 2014) and for post-speciation population expansion (Gehring et al. 2013; Rakotoarisoa et al. 2013a,b; Gehara et al. 2017).

In conclusion, despite there being several factors that will confound our ability to identify ecological signals, and there being a broad set of circumstances under which ecological speciation could occur, our analyses find evidence of a tendency for sister pairs to cluster at either end of the niche divergence-conservation spectrum. This supports a strong role for ecological processes – both soft allopatry and ecological speciation – in driving speciation and points to multiple mechanisms as explanations for the origin of local endemism and high species diversity in Madagascar.

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Competing interests

The authors declare no competing interests.

Additional information

Supporting information includes Table 1 showing full set of results for 28 pairs, including scaled CI from RTR model, Table 2 showing the full set of results for the background similarity test, Table 3-5 showing results for generalized linear models for range overlap, degree of niche conservation and speciation age, Table 6-7 showing gene data used for phylogenetic placement of 56 sampled taxa from Zheng and Wiens (2016), Figures S1-S5 showing the analysis based on the background similarity test and a spreadsheet with occurrences for all pairs used in this analysis.

Figure Legends

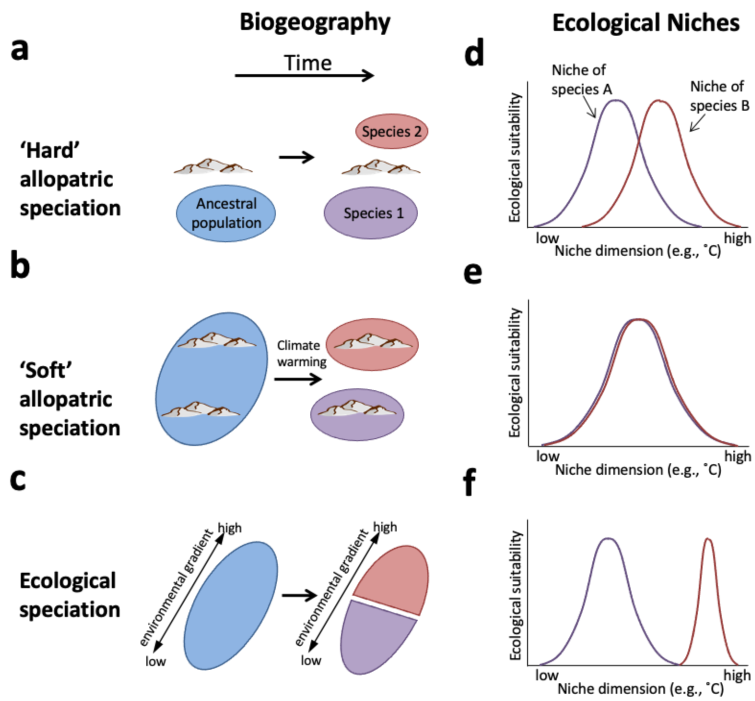

Fig. 1: Alternative mechanisms of speciation illustrated in terms of biogeography and realized ecological niches. a, Hard allopatry occurs when a population disperses to a new area that has been isolated by a physical barrier (e.g., a mountain range) which leads to the isolation of populations from the same ancestral species, resulting in descendent species with non-overlapping geographic ranges or gene flow. **b**, Soft allopatry occurs when an environmental disturbance (e.g., climate warming) causes incipient species to become geographically and genetically isolated due to failure to adapt to novel intervening conditions. **c**, Ecological speciation occurs when populations adapt to novel environmental features that are distinct from their close relatives, at first with gene flow but this ceases with time. **d,** In hard allopatry, there may be some ecological niche differences among sister species, but this differentiation is associated with differences in the availability in ecological features within landscapes (or random independent evolution) rather than due to species selecting particular features, resulting in no significant signal of either niche conservation or divergence (i.e., non-ecological signal). **e**, In soft allopatry, lack of adaptation to novel conditions by descendent species results in ecological niches that are conserved over evolutionary timescales. **f**, In ecological speciation, species adapt to ecological features that are distinct from their ancestors and close relatives, leading to ecological niche differentiation between two descendent species.

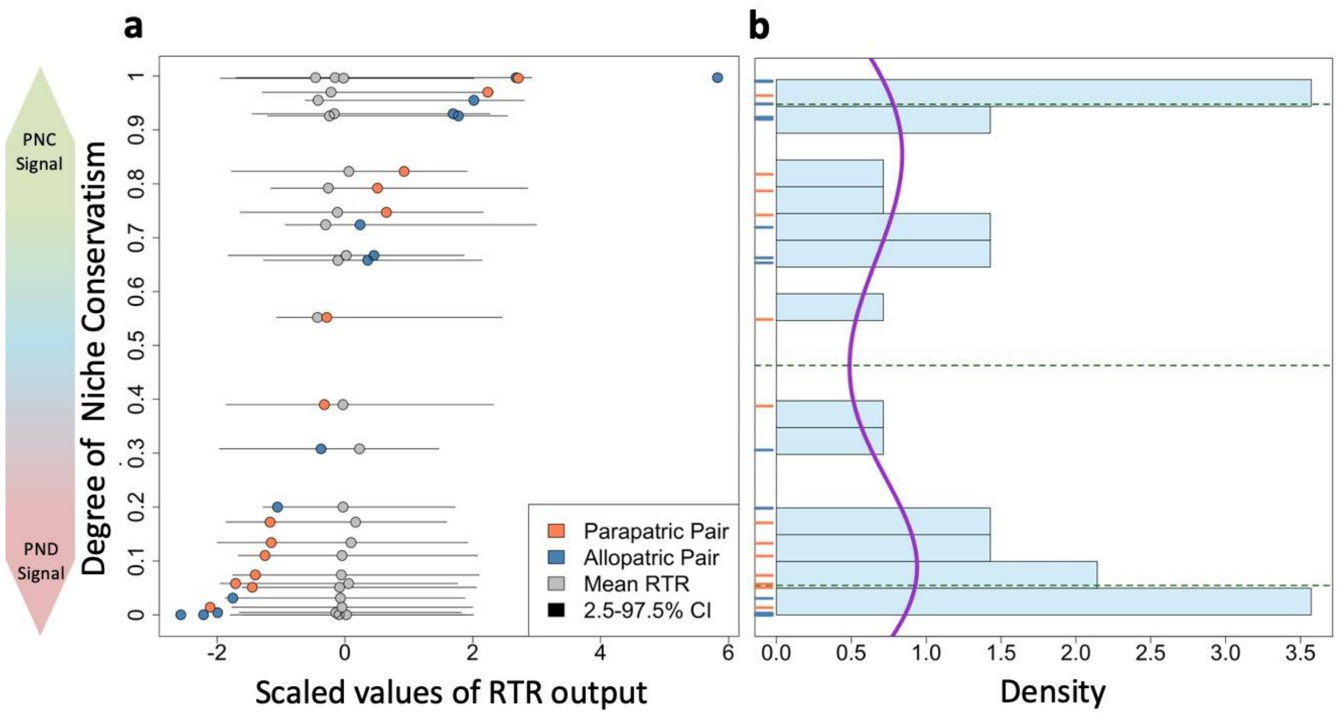

Fig. 2: Bimodal distribution for the degree of niche conservatism for 28 sister pairs of endemic Malagasy lizards. a, Degree of niche conservatism against scaled values of RTR outputs, ordered by decreasing scaled niche overlap values for each sister pair. To illustrate the results for all sister pairs under one common scale, each set of RTR outputs were scaled using their corresponding means and standard deviations using the scale function in R. The degree of niche conservatism of each sister pair is measured as the proportion of niche overlap replicates that are lower than the observed niche overlap (circles), so that higher values reflect a higher degree of phylogenetic niche conservatism (PNC) and lower values are associated with a lower degree of phylogenetic niche conservatism (PND). Cases where the observed niche overlap does not overlap with the 2.5-97.5% intervals of the replicate distributions have a significant ecological signal for either PNC (higher than 97.5%; 3 pairs) or PND (lower than 2.5%, 4 pairs). **b**, Density histogram showing the degree of niche conservatism for 28 pairs fitted with a kernel density line curve (purple). Hartingan's dip test supports a bimodal distribution (D = 0.11062, P < 0.005). Tick marks represent individual allopatric (blue) and parapatric (orange) sister pairs and dotted lines represent the upper and lower modes (0.954 and 0.055 respectively) and the antimode (0.466) of the distribution. Full table of results in Supplementary Table 1.

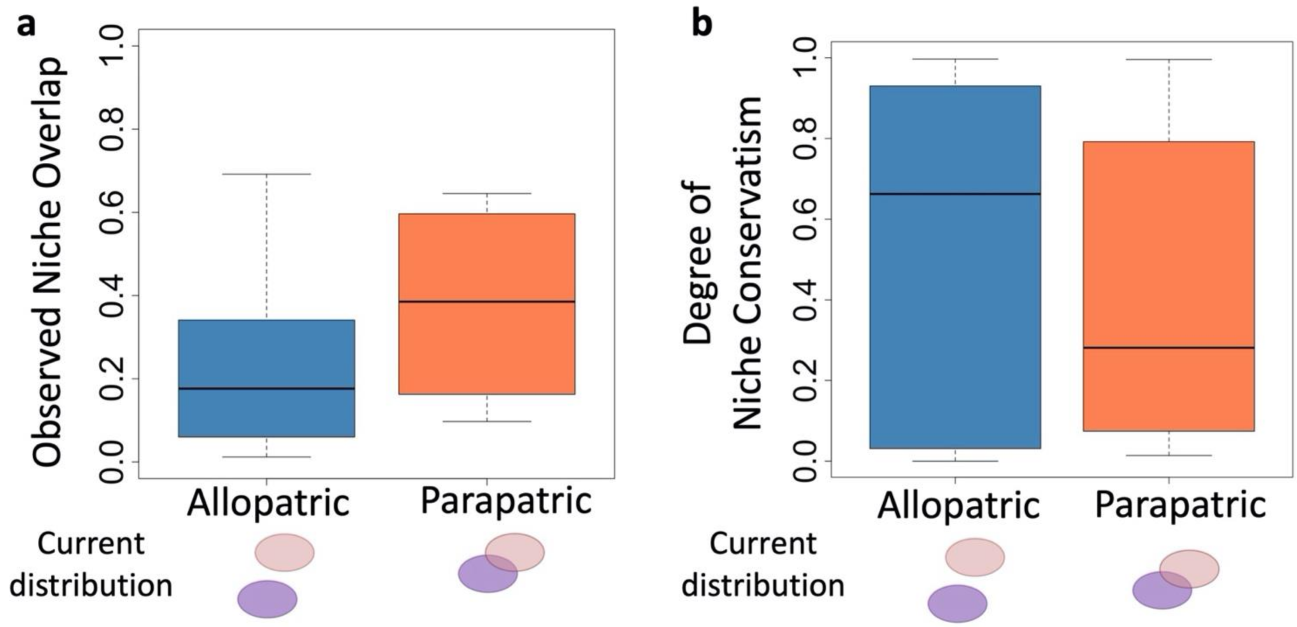

Fig. 3: Comparison of observed niche overlap and degree of niche conservatism between allopatric (n=14) and parapatric sister pairs (n=14) of endemic Malagasy lizards. a, Allopatric sister pairs have significantly lower observed niche overlap compared to parapatric sister pairs (*P* = 0.055). **b**, The degree of niche conservatism in allopatric pairs tends to be higher than in parapatric pairs but differences are not statistically significantly (*P* = 0.223).

Table 1. Geographic range, biogeographic pattern, niche overlap and degree of niche conservatism among 28 sister pairs of endemic lizards of Madagascar, ranked by genus and degree of niche conservatism based on the RTR model. Data on age of speciation was obtained from Zheng and Wiens (2016). Relative occurrence area refers to the proportion of the total island that is occupied by the minimum convex polygon of the sister pair. Results on niche overlap and degree of niche conservatism based on the 'background similarity test' can be found in the Supporting Information Table 2.

Genus	Species #1	Species #2	Age of Speciation (MYBP)	Relative occurrence area	Isolation distance (km)	Range overlap	Biogeography	Climatic niche overlap	Degree of Niche Conservatism
Amphiglossus	*A. mandokava*	*A. tanysoma*	7.88	0.067	0.000	0.018	Parapatry	0.132	0.074
Amphiglossus	*A. punctatus*	*A. frontoparietalis*	9.64	0.153	0.000	0.819	Parapatry	0.645	0.058
Brookesia	*B. dentata*	*B. exarmata*	27.55	0.013	290.840	0.000	Allopatry	0.134	0.955
Brookesia	*B. minima*	*B. tuberculata*	32.92	0.012	96.134	0.000	Allopatry	0.466	0.930
Brookesia	*B. thieli*	*B. vadoni*	10.47	0.130	0.000	0.034	Parapatry	0.617	0.823
Brookesia	*B. therezieni*	*B. superciliaris*	19.04	0.174	0.000	0.983	Parapatry	0.460	0.134
Brookesia	*B. betschi*	*B. lineata*	11.53	0.008	0.000	0.153	Parapatry	0.162	0.110
Calumma	*C. hilleniusi*	*C. guibei*	13.21	0.034	613.034	0.000	Allopatry	0.164	0.997
Calumma	*C. furcifer*	*C. gastrotaenia*	16.48	0.093	0.000	0.091	Parapatry	0.418	0.747
Calumma	*C. tsaratananense*	*C. brevicorne*	4.48	0.097	39.492	0.000	Allopatry	0.189	0.658
Calumma	*C. boettgeri*	*C. nasutum*	4.32	0.283	5.500	0.000	Allopatry	0.312	0.004
Furcifer	*F. willsii*	*F. petteri*	19.41	0.358	141.509	0.000	Allopatry	0.409	0.308
Furcifer	*F. labordi*	*F. antimena*	4.31	0.107	0.000	0.244	Parapatry	0.210	0.051
Lygodactylus	*L. guibei*	*L. miops*	19.62	0.255	0.000	0.546	Parapatry	0.597	0.996
Lygodactylus	*L. pictus*	*L. mirabilis*	25.71	0.024	60.206	0.000	Allopatry	0.040	0.724
Madascincus	*M. stumpffi*	*M. polleni*	4.76	0.343	304.155	0.000	Allopatry	0.303	0.926
Oplurus	*O. fierinensis*	*O. grandidieri*	8.78	0.068	185.458	0.000	Allopatry	0.051	0.000
Paroedura	*P. androyensis*	*P. picta*	43.75	0.220	0.000	0.796	Parapatry	0.604	0.390
Paroedura	*P. oviceps*	*P. karstophila*	27.98	0.039	0.000	0.085	Parapatry	0.118	0.014
Phelsuma	*P. quadriocellata*	*P. antanosy*	16.14	0.185	0.000	0.548	Parapatry	0.174	0.552
Phelsuma	*P. dubia*	*P. ravenala*	10.73	0.376	274.942	0.000	Allopatry	0.012	0.031
Tracheloptychus	*T. madagascariensis*	*T. petersi*	17.04	0.097	0.000	0.047	Parapatry	0.353	0.970
Trachylepis	*T. dumasi*	*T. aureopunctata*	7.55	0.284	0.000	0.804	Parapatry	0.571	0.172
Uroplatus	*U. fimbriatus*	*U. giganteus*	14.14	0.246	35.756	0.000	Allopatry	0.692	0.997
Uroplatus	*U. malahelo*	*U. guentheri*	56.83	0.366	276.172	0.000	Allopatry	0.123	0.200
Uroplatus	*U. pietschmanni*	*U. alluaudi*	41.43	0.052	428.676	0.000	Allopatry	0.060	0.000
Voeltzkowia	*V. rubrocaudata*	*V. lineata*	0.01	0.165	34.615	0.000	Allopatry	0.341	0.667
Zonosaurus	*Z. trilineatus*	*Z. quadrilineatus*	4.57	0.046	0.000	0.122	Parapatry	0.097	0.792