



Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot

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27% closer, respectively, to queen sounds than to those of workers (Fig. 2B) [mean normalized euclidean distances between individual butterflies' and ants' sounds are as follows: pupa-queen $2.47 \pm (\text{SE}) 0.10$, pupa-worker 3.03 ± 0.15 , $t = -3.14$, $\text{df } 87$, $\text{distance}_{\text{pupa-queens}} < \text{distance}_{\text{pupa-workers}}$, $P < 0.001$; larva-queen 2.52 ± 0.11 , larva-worker 3.21 ± 0.12 , $t = -4.32$, $\text{df } 237$, $\text{distance}_{\text{larva-queens}} < \text{distance}_{\text{larva-workers}}$, $P < 0.001$]. The distributions in Fig. 2 also satisfy the concept that the perfect mimic should have maximal overlap with queen acoustics and minimal overlap with those of workers.

Playing recordings of *Maculinea* pupal calls to the same naïve cultures of *Myrmica schencki* workers resulted in enhanced benevolent responses similar to those elicited by queen ant sounds. We found no significant differences toward *Maculinea* pupal and *Myrmica* queen calls in any of the four behaviors scored, and pupal calls elicited six times more instances of royal on-guard attendance than occurred when worker sounds were played (Fig. 3 and table S1) ($P < 0.001$). Recordings of *M. rebeli* larvae induced lower worker responses and, despite eliciting 2.3 times more on-guard attendances than worker calls, did not differ significantly from responses toward worker sounds (Fig. 3 and table S1). We did not play *Maculinea* calls to queen ants but predict that they would provoke rivalry similar to that observed when live *Maculinea* pupae were artificially enclosed with *Myrmica schencki* queens (11) (fig. S1).

We suggest that regional host specificity in *Maculinea* populations is mediated first through chemical mimicry (6, 22); but once the intruder is admitted and accepted as a member of a host society, it mimics adult ant acoustics (particularly queens) to advance its seniority toward the

highest attainable position in the colony's hierarchy. Selection for accurate acoustical mimicry may have been stronger in pupae, which lack the main secretory organs of *M. rebeli* larvae and offer only weak rewards to tending workers.

The young stages of other *Maculinea* species make similar pulsed sounds to *M. rebeli* (12): All differ substantially from those of other studied Lycaenidae, most of which are commensals or mutualists or have no known relationship with ants (12, 23–27). None of the latter mimics the acoustics of associated ants in obvious ways, although the sound of one strongly mutualistic species attracts workers (23–26). Thus, the use of acoustics to signal superior status to ants is unlikely to be a basal trait in the Lycaenidae, although we might expect it in *Phengaris*, the sister genus to *Maculinea*.

Beyond the Lycaenidae, ~10,000 species of ant social parasites may exist (5), particularly among other Lepidoptera, Coleoptera, Diptera, and inquiline ants (1, 6). If acoustics plays the role that we suggest in reinforcing an ant's hierarchical status, it seems likely that this cue has evolved in other social parasites to infiltrate and exploit their societies.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 and S2
Table S1
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Audio S1 to S4

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Stability Predicts Genetic Diversity in the Brazilian Atlantic Forest Hotspot

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Biodiversity hotspots, representing regions with high species endemism and conservation threat, have been mapped globally. Yet, biodiversity distribution data from within hotspots are too sparse for effective conservation in the face of rapid environmental change. Using frogs as indicators, ecological niche models under paleoclimates, and simultaneous Bayesian analyses of multispecies molecular data, we compare alternative hypotheses of assemblage-scale response to late Quaternary climate change. This reveals a hotspot within the Brazilian Atlantic forest hotspot. We show that the southern Atlantic forest was climatically unstable relative to the central region, which served as a large climatic refugium for neotropical species in the late Pleistocene. This sets new priorities for conservation in Brazil and establishes a validated approach to biodiversity prediction in other understudied, species-rich regions.

Late Quaternary climate fluctuations helped to shape present-day diversity in temperate and boreal systems (1), providing a general context for understanding current patterns of endemism. In the tropics, Pleistocene

refugia models have been dismissed because of conflicting evidence (2, 3) or circularity in identifying putative refugia (4), but historical processes must be invoked to explain regions of high endemism (5, 6). Recent studies from sub-

tropical biomes have usefully employed post hoc palaeoclimate models of species and habitats to provide insights about processes shaping genetic and species diversity (5, 7). Building on them, we first map the palaeodistribution of endemic species to identify temporally stable (refugial) and unstable (recently colonized) regions for species occurrence, which are then validated with multispecies molecular data. Going beyond the traditional species-by-species approach, the molecular analyses contrast the fit of assemblage-level data to the spatially explicit demographic scenarios suggested by the climate-based models.

We apply this approach to one of the world's most species-rich, yet notoriously endangered and understudied ecosystems: the Brazilian Atlantic

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rainforest. Originally extending for 1,300,000 km² along the Brazilian coast and reaching into Paraguay and Argentina, this biome has been reduced to less than 8% of its range (8). Today's fragments harbor one of the largest percentages of endemic species in the world, with many species and even genera of vertebrates still being described (8, 9). Our ultimate goal is to pinpoint regions for inventory work and habitat protection before we lose a substantial fraction of described and undocumented diversity. The approach differs from previous methods by directly modeling historical processes, as opposed to observed biodiversity patterns (10), with the aim of informing conservation.

We use molecular genetic data from multiple, largely codistributed species to test whether spatial modeling of species-specific Late Quaternary refugia sheds light on historical processes and hence improves prediction of genetic endemism and diversity in tropical Brazil (11). We focus on three common species of tree frogs that are widely distributed along the Brazilian Atlantic forest: *Hypsiboas albomarginatus*, *H. semilineatus*, and *H. faber*. Given their life history traits, amphibians are useful indicators of environmental changes through time (12). Whereas *H. albomarginatus* and *H. semilineatus* occur in low and mid altitudes and are mostly restricted to the evergreen or semideciduous components of the Atlantic Forest in eastern Brazil, *H. faber* has a broader altitudinal range and also inhabits mixed and deciduous areas, occupying interior and coastal sites in the Atlantic Forest south to Paraguay and Argentina (figs. S1 and S2) (13). The comparative phylogeographic approach is a powerful test of assemblage-scale responses to former environmental change and thereby provides a means for critical assessment of the scenarios produced by modeling of species' distributions under palaeoclimates (7).

The palaeomodeling method intersects predicted species' distributions under current conditions and climatic extremes of the Late Quaternary (6000 years before present, or 6 kybp, and 21 kybp) to predict areas of stability (regions in which species are predicted to occupy irrespective of time period) and unstable areas (7, 14). Because the stability maps raise specific hypotheses about regional differences in persistence and hence diversity, they lead to phylogeographic predictions for both individual species and assemblages (co-distributed taxa; Fig. 1). Field sampling is driven by the model predictions to cover both predicted refugia and unstable (recently colonized) areas, particularly emphasizing previously undersampled areas. If the approach correctly predicts current patterns of biodiversity at the regional scale, species should consistently show (i) higher genetic diversity within and among populations in refugia relative to unstable areas, because of long-term persistence and population structure; (ii) genetic signature of population expansion in unstable areas, reflecting multispecies colonization from adjacent refugial regions after the Last Glacial Maximum

(LGM, 21 kybp); (iii) absence of genetic patterns of isolation-by-distance in unstable areas, given that colonization has been too recent to permit restoration of equilibrium between migration and genetic drift (15); and (iv) strong phylogeographic structure between refugia, reflecting assemblage-wide, long-term population persistence in isolated areas.

Distribution models developed under current climatic conditions accurately predict distributions of each of the target species along the Atlantic rainforest domain [area-under-the-curve (AUC) values (16) 0.968, 0.989, and 0.994; maximum Kappa (17) 0.81, 0.925, and 0.94 in *H. albomarginatus*, *H. faber*, and *H. semilineatus*, respectively (fig. S2)]. Stability maps, depicting the intersection of distribution models for each taxon under current, 6 kybp, and 21 kybp climates, predict for all species a large central refugium throughout the Late Quaternary ("Bahia refugium") (Fig. 2). A second, much smaller refugium is predicted in the northeasternmost portion of the forest ("Pernambuco refugium"). In *H. faber*, a third, southeastern refugium of intermediate size is also predicted ("São Paulo refugium"). This is not surprising, given that this species occupies a broader environmental niche. In contrast to the central and northern regions, populations south of the Bahia or São Paulo refugia appear much less stable, despite the more extensive (preclearing) range of the forest in southern and southeastern Brazil. We hypothesize that these areas received a significant influx of migrants from adjacent, large refugial populations after the LGM. These palaeomodel results are congruent with the fossil pollen record, which documents a replacement of forests by grasslands in the southern Atlantic forest during the LGM (14, 18) and suggests the occurrence of small forest refugia in the southernmost range of

the putative Bahia refugium (19). The results also agree generally with forest models published previously (14), although the central refugium extends farther south in the frog-based models. Such differences are expected because the forest and its associated species may differ slightly in their climatic tolerances and realized niches. In *H. albomarginatus* and *H. faber*, the extension of the predicted São Paulo refugium westward into the neighboring Cerrado biome reflects model overprediction (fig. S2) (14).

Models of habitat stability through fluctuating climates correctly predict patterns of phylogeography in the Brazilian Atlantic rainforest (Fig. 2 and figs. S3 to S5). In all species, high levels of divergence and population structure are observed across refugia (Tamura-Nei corrected distances (20): 4 to 7% between Bahia and Pernambuco refugia, 1% between the nearby Bahia and São Paulo refugia in *H. faber*). Similarly, in all taxa there are multiple, divergent clades within the Bahia region, agreeing with model-based predictions of a large refugium in this area. In *H. faber*, divergent clades are also represented in the São Paulo region, matching predictions of a mid-sized refugium in this area. All taxa show low genetic diversity across the southernmost range of the forest, an area predicted to be less stable by the palaeomodels. Furthermore, mitochondrial DNA (mtDNA) lineages found in this region are shared with adjacent refugia (one in *H. albomarginatus* and *H. semilineatus*, two in *H. faber*).

Metrics of genetic diversity confirm the above patterns (Table 1). In *H. albomarginatus* and *H. semilineatus*, genetic diversity (21) is an order of magnitude larger in the central (Bahia) refugium relative to the less stable (southern) portion of the forest. Diversity of *H. faber* in this southern area is higher than the other species because of the

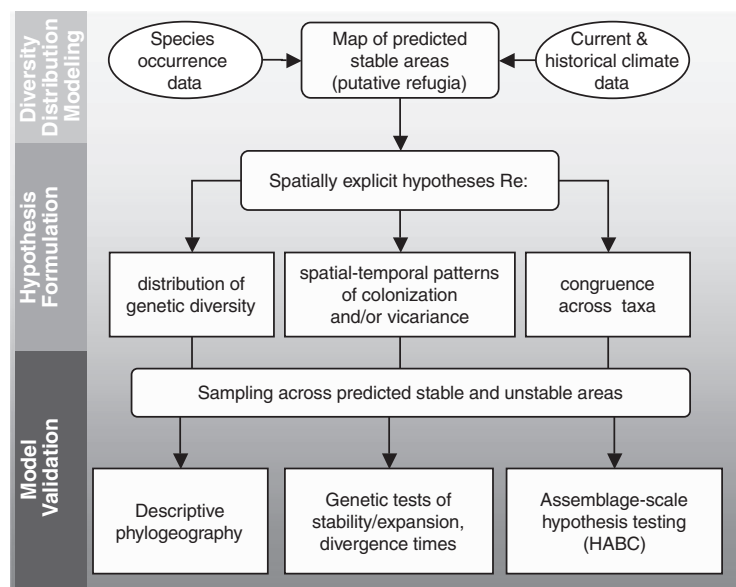


Fig. 1. Proposed method of biodiversity prediction. Three stages are involved: biodiversity distribution modeling (top), model-based hypothesis formulation (middle), hypothesis testing and model validation (bottom).

presence of two lineages that co-occur in the adjacent refugia. In all species, average net nucleotide differences across localities (22) reflects high geographic structure within refugia (2.6 to

6.2% divergence). In contrast, sites located outside (south of) the refugia are genetically more similar to each other, although to a lesser extent in *H. faber* (0.1 to 1.6%). Signatures of

population expansion (23) are found in the unstable area for *H. albomarginatus* and *H. faber*, as well as in the Bahia refugia area for *H. faber* and *H. semilineatus*. The lack of signature of

Fig. 2. Genetic diversity in putative refugial (stable) versus unstable areas in the Brazilian Atlantic rainforest. **(Top)** Species-specific stability maps; modeled refugia in black. **(A)** *H. albomarginatus*, **(B)** *H. semilineatus*, **(C)** *H. faber*. Note the absence of large stable regions in the southern portion of the forest (south of the Bahia and São Paulo refugia) relative to the central and northern areas. Asterisks denote refugia inferred beyond the current ranges of the target species. Symbols indicate localities sampled for molecular analysis. Scale bar, 400 km. **(Bottom)** The 50% majority-rule consensus Bayesian phylogenetic trees, rooted with sequences from the other two congeneric species studied (root not shown). Thick internodes denote clades with posterior probability greater than 90%. Percentages indicate Tamura-Nei corrected distances between clades (20).

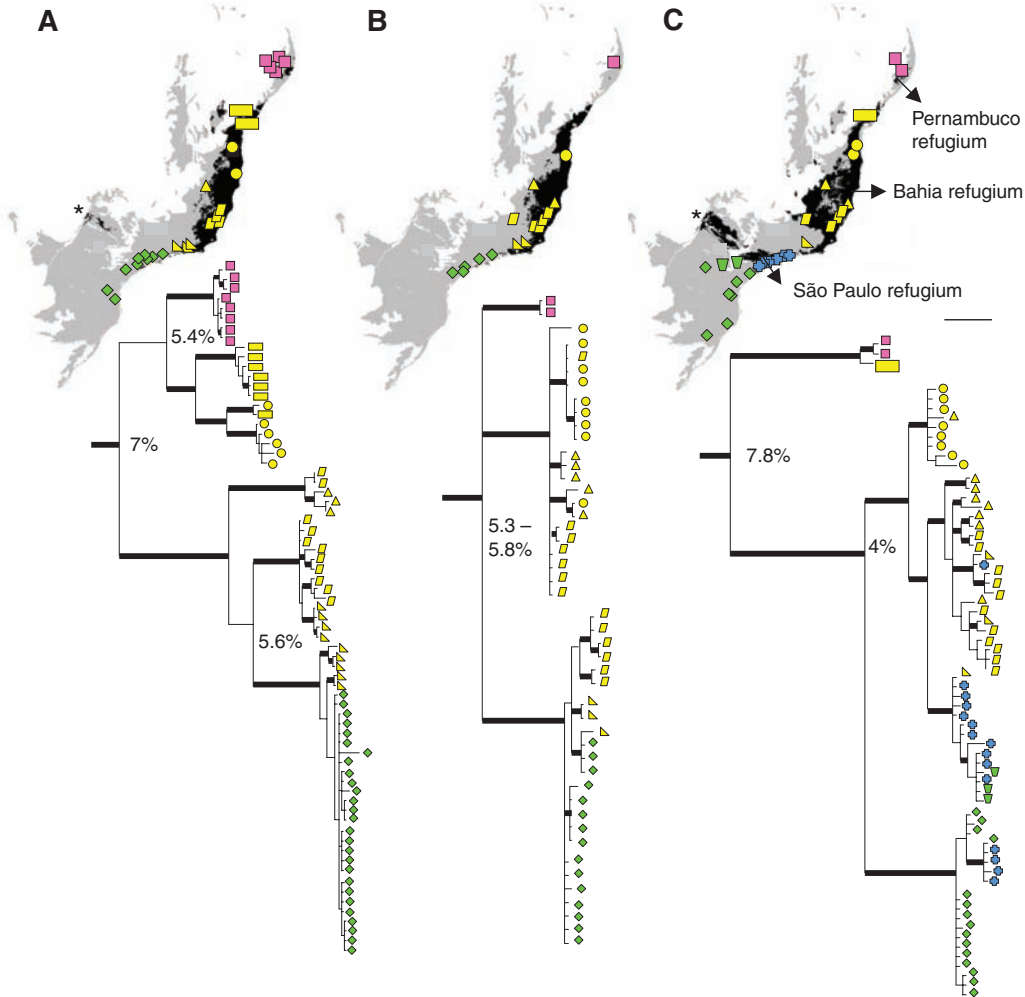


Table 1. Population genetic summary metrics used in model validation. *n*, Sample size; *S*, number of segregating sites. The diversity parameter θ and mean D_a across localities are given per base pair (bp). *Hs* test (23) is used to detect population expansion. BA, Bahia; SP, São Paulo refugia. Because predicted refugia were often larger than predicted unstable (recently colonized) areas, *n*, *S*,

θ , and average D_a values of the former were obtained not only from the total number of samples, but also from all possible combinations of spatially contiguous localities distributed within the geographic extension of the unstable area. Parentheses encompass minimum and maximum values from subsamples. *P* values in bold highlight statistical significance at 0.05 probability level.

Species	Area	<i>n</i> (min.; max.)	<i>S</i> (min.; max.)	θ (min.; max.)	Mean D_a (min.; max.)	<i>Hs</i> (<i>P</i> value)	Mantel's corr. coef. (<i>P</i> value)
<i>H. albomarginatus</i> (970 bp)	Stable (BA)	36 (13; 23)	207 (81; 155)	0.076 (0.034; 0.072)	0.062 (0.020; 0.082)	-20.546 (0.141)	0.499 (0.001)
	Unstable (south of BA)	27	22	0.003	0.001	-11.498 (0.004)	-0.140 (0.580)
<i>H. semilineatus</i> (718 bp)	Stable (BA)	28 (6; 13)	71 (14; 58)	0.031 (0.009; 0.034)	0.036 (0.007; 0.041)	-17.778 (0.029)	0.054 (0.460)
	Unstable (south of BA)	15	9	0.003	0.004	0.114 (0.357)	0.436 (0.248)
<i>H. faber</i> (771 bp)	Stable (BA)	28 (13; 23)	94 (42; 80)	0.018 (0.012; 0.022)	0.026 (0.001; 0.044)	-38.111 (0.003)	0.803 (0.0003)
	Stable (SP)	15	48	0.023	0.028	-5.981 (0.115)	0.305 (0.221)
	Unstable (south of SP)	18	40	0.015	0.016	-13.255 (0.014)	0.0001 (0.456)

population expansion in the southernmost localities of *H. semilineatus* may reflect low statistical power because of the exceptionally low levels of diversity observed in this species. As predicted, isolation by distance is not observed in unstable regions, but is detected within refugial areas for *H. albomarginatus* and *H. faber*.

The hierarchical approximate Bayesian computation (HABC) method (24) allows us to use data from all three species at once to test for assemblage-wide responses to Late Quaternary climate change. These analyses support both model-driven hypotheses of (i) simultaneous, multispecies colonization of unstable areas from adjacent refugial populations since the LGM, as opposed to long-term persistence of populations in unstable areas, and (ii) assemblage-scale, long-term persistence of populations in isolated refugial areas, as opposed to post-LGM colonization of refugial regions.

To test for assemblage-wide colonization of predicted unstable areas, we group mtDNA sequences from the southernmost refugial sites [population 1 (Fig. 3A)] and from localities in unstable areas south of the refugium [population 2 (Fig. 3A)] to contrast two alternative historical models across the three codistributed species, while allowing the taxon-specific demographic parameters to vary. In H_1 , the long-term persistence model, two contemporary populations split from an ancestral population prior to the LGM (120,000 to 1.2 million years before present, or Mybp, Fig. 3A). In H_2 , the recent colonization model, population 2 is modeled as being colonized from refugial population 1 subsequent to

the LGM (0 to 20 kybp; Fig. 3A). The results indicate that all three species colonized the southern (unstable) areas after the LGM ($Z_2 = 3$, the number of species evolved under H_2), even when allowing for postisolation migration (Fig. 3, B and C). When Bayes factor is used (25), there is strong support for recent colonization in all three species ($Z_2 = 3$) under the no-migration model [$B(Z_2 = 3, Z_2 < 3) = 35.16$], and moderate support under a postisolation migration model [$B(Z_2 = 3, Z_2 < 3) = 5.70$].

Using the same framework to test for long-term persistence of refugial populations, we compare mtDNA sequences between the predicted Pernambuco refugium [population 1 (Fig. 3A)] and adjacent (northern) populations from the Bahia refugium [population 2 (Fig. 3A)] to contrast alternative historical models H_1 and H_2 . In this case, the HABC results infer long-term persistence of populations in isolated refugia for all three species ($Z_2 = 0$, i.e., $Z_1 = 3$), even when allowing for postisolation migration (Fig. 3, D and E). Using Bayes factor (25), we also detect evidence for stability in both areas under the no-migration model [$B(Z_2 = 0, Z_2 > 0) = 4.89$], as well as under a postisolation migration model [$B(Z_2 = 0, Z_2 > 0) = 4.84$].

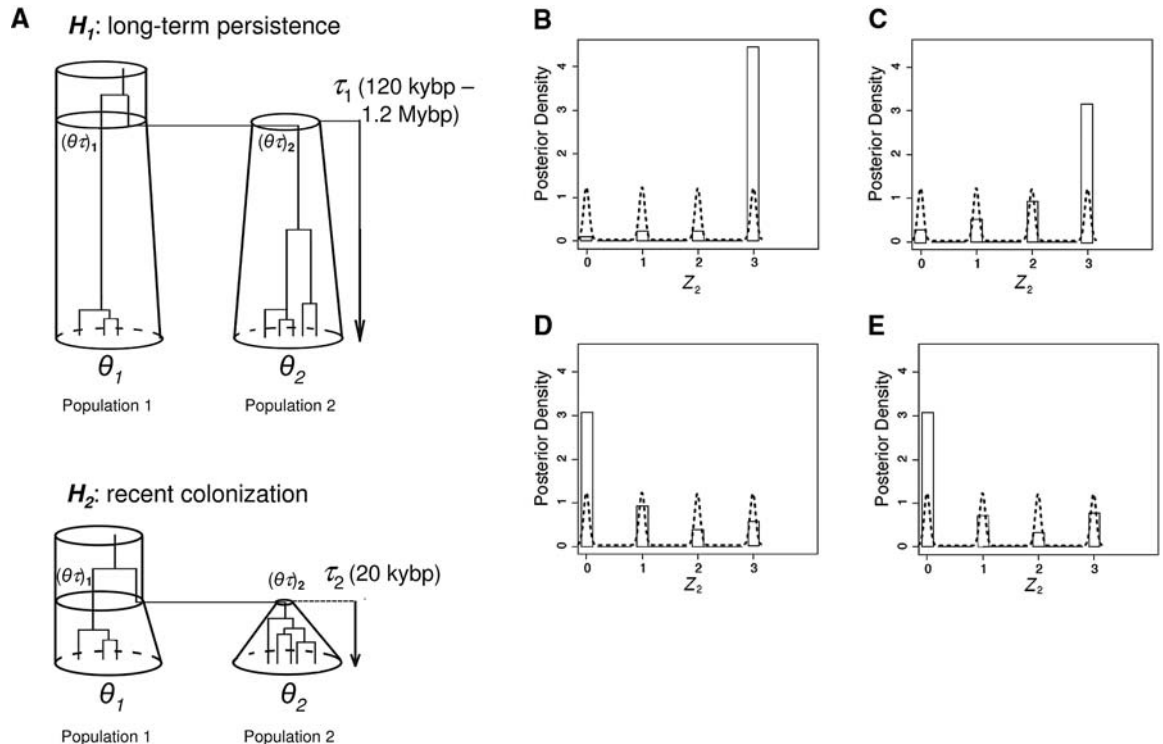
Relative to nuclear loci, mtDNA data are more variable and readily collected and often provide key insights into biological response to environmental modification (1). Although single-locus inference can be imprecise in the face of coalescent variance and the possibility of selection (26), our method benefits from a multitaxon approach, while explicitly accounting for the

stochasticity of a single-locus coalescent across taxa. Combining data sets from several codistributed groups into a single hierarchical Bayesian analysis allowed us to estimate congruence across species, while borrowing strength from the full comparative phylogeographic sample (24). This can translate into higher analytical power and be more informative than qualitative comparisons of species-specific analyses. By capturing the historical signal that emerges from larger, combined multispecies molecular data sets, HABC will offer the possibility of looking at patterns of historical community assembly in codistributed nonmodel organisms for which barcode-type DNA sequence information (e.g., mtDNA data) can be feasibly collected.

Collectively, the results identify the central region as a hotspot within the Atlantic rain-forest hotspot and a refuge for biodiversity during climatic extremes of the Late Pleistocene. This is not to say that southern areas entirely lacked forested habitats in the late Pleistocene: The existence of species and genera endemic to the southern forests (27), as well as some palaeoecological and genetic evidence (28), offer evidence to the contrary. Rather, the phylogeographically validated palaeomodels presented here show that the central region had much higher stability relative to the south. Forest lizards (14, 29) and birds (30) also show high diversity in the central portion of the biome relative to southern areas, and provide evidence for population expansion in southern regions. This reassures us that the processes uncovered by the amphibian data may be generalized to and help to explain patterns of

Fig. 3. HABC analyses.

(A) Simulated models H_1 (long-term persistence) and H_2 (recent colonization). In both cases, each species was modeled as two contemporary populations with mutation-drift parameters θ_1 and θ_2 that split from an ancestral population at a time τ in the past. Ancestral population sizes are represented by $(\theta_1)_1$ and $(\theta_1)_2$; ybp, years before present. (B to E) Hyperposterior (bars) and hyperprior (dashed) densities of Z_2 (number of species evolved under H_2) given data from three codistributed frog species. (B) and (C) Models of refugial sites (population 1) and unstable, southern areas (population 2). (D) and (E) Models of Pernambuco refugium (population 1) and Bahia refugium (population 2). (B) and (D) Postisolation migration not included in model; (C) and (E) postisolation migration included in model.



diversity in other, much more distantly related groups of Atlantic forest endemics.

Because collection efforts, molecular studies, and conservation priorities have been heavily biased toward southern and southeastern Brazil (8, 9, 31), we predict that genetic diversity and narrow endemism in the central corridor of the biome have been substantially underestimated. This is serious, given the higher rate of deforestation in this region relative to the more extensive forests in São Paulo and southern Brazil (9, 31). Not only could much unique diversity be lost, but ongoing habitat destruction could quickly erase the signature of the historical processes that led to it, preventing a full understanding of the mechanisms underlying local endemism and, therefore, impeding more effective conservation measures.

At a broader level, the congruence between model-based demographic hypotheses and joint, multispecies analyses of mtDNA diversity shows that palaeoclimatic niche models and assemblage-scale molecular genetic analyses can be used to forecast spatial patterns of diversity in poorly explored, highly threatened ecosystems. In a world of ever-accelerating environmental changes, this approach can help to guide research and conservation in other global hotspots or similarly complex tropical ecosystems.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5915/785/DC1
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Orc1 Controls Centriole and Centrosome Copy Number in Human Cells

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Centrosomes, each containing a pair of centrioles, organize microtubules in animal cells, particularly during mitosis. DNA and centrosomes are normally duplicated once before cell division to maintain optimal genome integrity. We report a new role for the Orc1 protein, a subunit of the origin recognition complex (ORC) that is a key component of the DNA replication licensing machinery, in controlling centriole and centrosome copy number in human cells, independent of its role in DNA replication. Cyclin A promotes Orc1 localization to centrosomes where Orc1 prevents Cyclin E-dependent reduplication of both centrioles and centrosomes in a single cell division cycle. The data suggest that Orc1 is a regulator of centriole and centrosome reduplication as well as the initiation of DNA replication.

The assembly of a bipolar, microtubule spindle during mitosis is essential for accurate chromosome segregation. In animal cells, spindle formation is organized by centrosomes, organelles that contain a pair of centrioles surrounded by pericentriolar material (PCM) that need to be duplicated exactly once every cell division cycle, in coordination with DNA replication to maintain genome stability (1). Licensing DNA for replication is a critical regulatory

step involving the origin recognition complex (ORC), the first component for assembly of a pre-replicative complex (pre-RC) at each origin (2). Accumulated evidence supports roles for ORC subunits in addition to licensing DNA replication (3). In particular, human Orc2 subunit localizes to centrosomes, and depletion of Orc2 and Orc3 causes centrosome amplification in mitosis (4).

Several regulators of the DNA licensing machinery have been reported to be involved

in the control of both DNA and centriole duplication (5). Both Cyclin E and Cyclin A, as well as Cdk2 activity, are well-known positive regulators of DNA replication and also promote centrosome duplication (or reduplication) (6–11). Depletion of the DNA replication licensing inhibitor Geminin causes reduplication of both DNA and centrosomes in human cells (12).

In a screen using small interfering RNA (siRNA) for human ORC proteins with roles in centrosome biology, we found that depletion of the largest human ORC subunit, HsOrc1, leads to excess centrosomes (fig. S1 and Fig. 1A). Orc1 siRNA-treated U2OS cells were analyzed for centrosome defects by dual-color indirect immunofluorescence (IF) using antibodies to centrin 2 (stains centrioles) and antibodies to γ -tubulin (stains centrosomes). Seventy-two hours after siRNA treatment, 39.77 $\pm$ 3.5% of cells transfected with Orc1-1 siRNA and 25.53 $\pm$ 0.3% of

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