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Phylogenetic Conservatism of Extinctions in Marine Bivalves

Kaustuv Roy, *et al.*

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(10 to 70 Tg/year) and top-down (140 to 910 Tg/year) estimates of global SOA production (*30*). Nevertheless, IEPOX is expected to undergo hundreds of collisions with aerosol surfaces before reacting with OH, and its detection in the atmosphere (fig. S8) suggests that a complex suite of conditions likely controls its uptake to aerosols (e.g., the pH and chemical composition of aerosol). Furthermore, *i*SOA formation may depend on the unquantified differences in the yields and uptake characteristics of the β- and δ-IEPOX. Quantitative understanding of these complex interactions is required to assess the effect of this chemistry on the overall SOA abundance and its associated impacts [e.g., cloud condensation nuclei (*31*)].

The efficient formation of dihydroxyepoxides, a previously unknown class of gas-phase compounds, addresses many of the issues currently being debated about isoprene chemistry. Because their formation is accompanied by the reformation of OH, this chemistry contributes to the remarkable stability of HO_x in remote regions of the troposphere subjected to high isoprene emissions. The formation of IEPOX also provides a gas-phase precursor for the *i*SOA formation. Further investigation of the multiphase chemistry of IEPOX is needed to elucidate the complex interaction between emissions from the biosphere and atmospheric composition (*32*, *33*). In particular, the development of a proper chemical description of these interactions is essential for assessing the sensitivity of this chemistry to changes in isoprene emissions caused by environmental changes (e.g., climate change and deforestation) and to the further development of anthropogenic activities and the accompanying NO_x emissions in these regions.

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Phylogenetic Conservatism of Extinctions in Marine Bivalves

Kaustuv Roy,[1]* **Gene Hunt,**[2] **David Jablonski**[3]

Evolutionary histories of species and lineages can influence their vulnerabilities to extinction, but the importance of this effect remains poorly explored for extinctions in the geologic past. When analyzed using a standardized taxonomy within a phylogenetic framework, extinction rates of marine bivalves estimated from the fossil record for the last ~200 million years show conservatism at multiple levels of evolutionary divergence, both within individual families and among related families. The strength of such phylogenetic clustering varies over time and is influenced by earlier extinction history, especially by the demise of volatile taxa in the end-Cretaceous mass extinction. Analyses of the evolutionary roles of ancient extinctions and predictive models of vulnerability of taxa to future natural and anthropogenic stressors should take phylogenetic relationships and extinction history into account.

Groups of organisms differ in their vulnerability to extinction (*1*–*5*), but the nature and magnitude of that variation is still poorly quantified. Extinction risk of species and lineages is determined by a variety of ecological and life history traits (*2*), as well as emergent properties such as geographic range (*5*–*8*). Many of these extinction-related traits are phylogenetically conserved, suggesting that extinctions should be phylogenetically clustered: Taxa in some clades should be consistently more extinction-prone than others (*3*, *9*, *10*). Consistent with this idea, current extinction risk and documented anthropogenic extinctions are nonrandomly distributed among vertebrate lineages (*9*, *11*–*15*), but whether such phylogenetic selectivity holds in general, including for extinctions in the geologic past, remains poorly known. In this study, we used the Mesozoic-Cenozoic fossil record of marine bivalves, in conjunction with molecular phylogenies, to test for phylogenetic clustering of extinctions within and among bivalve families and how this clustering varies over time.

The fossil record of marine bivalves preserves a rich history of past extinctions, and although this record is not free of taphonomic biases, such biases are increasingly well understood (*16*, *17*). We used a taxonomically standardized database

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[1] Section of Ecology, Behavior and Evolution, University of California San Diego, La Jolla, CA 92093–0116, USA. [2] Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013–7012, USA. [3] Department of Geophysical Sciences, University of Chicago, 5734 South Ellis Avenue, Chicago, IL 60637, USA.

*To whom correspondence should be addressed. E-mail: kroy@ucsd.edu

of stratigraphic ranges of marine bivalves (*18*) to calculate background extinction rates (i.e., for times other than the massive end-Cretaceous event) of 1678 genera and subgenera (hereafter termed genera) over the last ~200 million years (Jurassic to Pleistocene). Genera are the preferred units of large-scale paleontological analyses because, relative to species, their taxonomy is better standardized and more stable, and their fossil record is far more complete and more robust to taphonomic biases (*19*, *20*). Furthermore, comparative analyses indicate that morphologically defined molluscan genera generally reflect the topologies of molecular phylogenies (*21*). Taxonomic standardization is clearly a prerequisite for any quantitative analysis of extinction rates, and the data we used were subjected to extensive revisions and standardization (*18*, *19*, *22*). We used the family level for analyses of phylogenetic clustering of extinction rates because families provide the necessary balance between adequate sample size and phylogenetic resolution. In general, families of marine bivalves have proven to be robust taxonomic units, and recent molecular phylogenies suggest that none of the major families of marine bivalves are blatantly polyphyletic (*23*). Although some bivalve families may prove to be paraphyletic when a more complete molecular phylogeny of the group is available, for our analyses, paraphyly is more likely to add noise than to produce artifactual trends.

If extinctions of bivalve genera were random with respect to family membership, then an index of taxonomic clustering for individual extinction events [R_{CL} (*18*)] should not differ systematically from zero across a time series of such events. Positive values of R_{CL} indicate more (and negative values less) clustering than random extinction (*18*). Of the 26 standard time intervals in our data (*18*) for which R_{CL} could be reliably calculated, 21 (81%) have positive values (Fig. 1), a result that is highly unlikely under a model of phylogenetically random extinctions ($P = 0.002$, exact binomial test). When R_{CL} for individual time intervals is compared with the null distribution for that interval (*18*), 8 out of the 26 intervals show significantly positive clustering (i.e., the observed R_{CL} is at least as great as the upper 95% confidence limit), and no interval is significantly less clustered than random (Fig. 1). These eight intervals include the end-Cretaceous event, the only major mass extinction in our data. A ninth interval, the Campanian, is marginally significant. Thus extinctions of marine bivalves over the last 200 million years show a general pattern of phylogenetic conservatism within families, both during background and mass extinctions, but the strength of such clustering varies over time, and not all extinctions show significant clustering.

Our data also reveal that extinction magnitude is not correlated with phylogenetic clustering. The highest R_{CL} value is associated with the end-Cretaceous mass extinction (Fig. 2), but many high-extinction intervals, such as the Late Eocene, lack strong clustering (table S1), and the overall relation between phylogenetic clustering and extinction intensity is not significant (Spearman rank correlation, $r_s = 0.20$, $P = 0.34$). Extinction rates declined significantly over time, but this decline was caused by culling of volatile clades rather than by a decrease in extinction intensity within individual clades. Overall, rates before the end-Cretaceous extinction (excluding the Maastrichtian stage, which ends with the mass extinction) are higher than in the Cenozoic (median for Mesozoic stages = 0.087, median for Cenozoic stages = 0.029; Wilcoxon rank sum test, $W = 157$, $P = 0.054$). For families well-represented in both the Mesozoic and the Cenozoic, extinction rates do not differ significantly before and after the end-Cretaceous event (Wilcoxon paired signed rank test, $V = 61$, $P = 0.74$, $n = 16$ families), and families show high rank-order agreement between Mesozoic and Cenozoic background rates of extinction (Fig. 3) (Spearman rank correlation, $r_s = 0.75$, $P = 0.0008$). The lower Cenozoic extinction rates instead result from preferential losses during the end-Cretaceous extinction in families with inherently high extinction rates (Fig. 4), so that Cenozoic bivalve diversity was dominated by the more extinction-resistant families. The three families with the highest Mesozoic background extinction rates went globally extinct at the end of the Cretaceous, and other high-rate Mesozoic clades (e.g., trigoniids and arcticids, both with background rates >0.15, at least twice the Mesozoic median) (Fig. 4) were severely hit and have since remained minor components of the bivalve fauna. The only family for which background extinction rates were much lower in the Cenozoic than the Mesozoic is the Veneridae, which also suffered major losses at the end of the Cretaceous. Thus, the end-Cretaceous extinction had a filtering effect on lineage-specific extinction rates, removing the most volatile families but not systematically altering within-family extinction rates.

Extinction rates analyzed in conjunction with recently published molecular phylogenies of living bivalve families (*18*) also indicate phylogenetic conservatism at deeper levels in the bivalve tree. Extinction rates of closely related families are significantly more similar to each other than is expected by chance (Fig. 5) [$P = 0.014$ using a permutation test (*18*); the maximum-likelihood estimate of λ, an index of phylogenetic dependence (*24*), for within-family extinction rate is 0.84, a value within the range typically found for ecological and morphological traits (*24*) and significantly different from zero, $P < 0.0004$; see (*18*)]. The phylogenetic signal remains significant ($P = 0.049$) under an alternative model of character change (*18*). Thus, the taxonomic and phylogenetic analyses together suggest that extinction rates in bivalves are conserved at multiple levels of evolutionary divergence, within individual families as well as among related families.

Stratigraphic ranges of taxa can be distorted by preservational and sampling biases (*17*). Our analyses hinged on differences between clades rather than differences between temporal bins, so the primary concern is not temporal variation in the quality of the fossil record (*25*, *26*), but systematic differences in preservation potential across bivalve lineages. Extinction-rate estimates are robust to differences in shell composition (*16*), but other variables known to influence preservation, such as shell size, thickness, and preferred habitats, may be important (*17*). To evaluate the robustness of our results to preservational biases, we repeated all analyses after omitting families identified by Valentine *et al.* (*17*) as having low

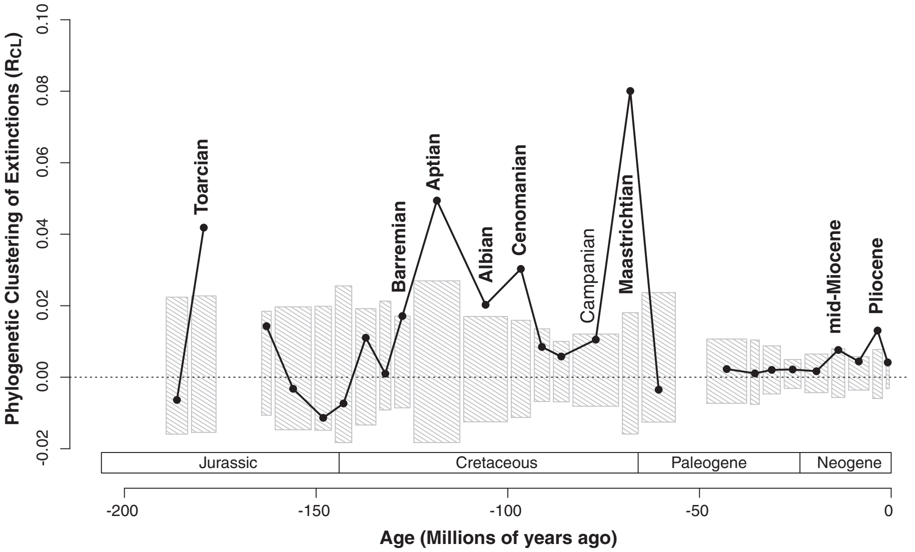

Fig. 1. Temporal trend in phylogenetic clustering of extinctions (R_{CL}). Shaded bars represent 95% confidence intervals around the expected value of R_{CL} (*18*). The intervals showing statistically significant phylogenetic clustering of extinctions are labeled in bold; an additional interval, the Campanian, is marginally significant. Only intervals with enough extinctions to analyze (eight or more) are plotted. The dotted line indicates the value expected if extinctions were not phylogenetically clustered.

preservation potential. The results were qualitatively unchanged (fig. S2). Another potential concern is that the observed differences in extinction rates between the Mesozoic and Cenozoic reflect taxonomic oversplitting of some Cretaceous groups relative to others. However, the families with disproportionate extinction represent a large and ecologically diverse assemblage (table S2), and multiple lines of evidence (*18*) suggest that the Mesozoic-Cenozoic difference is unlikely to simply reflect taxonomic practices. Phylogenetic clustering also does not appear to be driven only by extinctions associated with major environmental changes. For example, the overall signal remains highly significant, even when the Toarcian, Aptian, and Cenomanian stages, all of which include oceanic anoxic events (*27*), are excluded from the analysis, along with the Maastrichtian mass extinction (17 out of 22 extinctions with $R_{\mathrm{CL}} > 0$, $P = 0.017$, exact binomial test).

Taken together, our results show that extinction rates of marine bivalve genera tend to be phylogenetically conserved, but the strength of this effect varies over time and can be substantially and permanently changed by a mass extinction. Lineage-level clustering of extinction rates, as seen here, is expected to follow from phylogenetic conservatism of traits that correlate with extinction vulnerability (*28*), making some lineages more prone to extinction than others (*2*, *9*, *10*). As extinction-prone taxa are winnowed out, both the rate of extinction and the associated phylogenetic clustering are expected to decrease. The stronger phylogenetic clustering seen for the Cretaceous extinctions is likely to reflect the prominence during this time of clades with volatile dynamics (table S2). The demise of these high-rate taxa at the end of the Cretaceous shifted the overall distribution to lower values and also reduced the range of variation of within-family extinction rates (Fig. 4). Such hardening of the biota over evolutionary time has been hypothesized before (*29*–*31*). Though we cannot reject the alternative hypothesis that the decline in extinction rates from Mesozoic to Cenozoic is due to a systematic decrease in extinction forcing mechanisms, we see no reason to assume that forcing mechanisms became less intense, especially given that the Cenozoic is characterized by dramatic shifts in climate, occurring on multiple temporal scales (*32*). The Mesozoic-Cenozoic differences also do not reflect differences in statistical power, because each of these eras has similar average numbers of extinctions per time interval (table S1).

Other factors such as the nature of the extinction mechanism can also contribute to the observed variations in phylogenetic clustering. Different kinds of environmental stresses are likely to cause extinctions that are selective with respect to different traits, and we might expect phylogenetic clustering of extinctions to track, in part, the degree to which the relevant traits are conserved over phylogeny. Extinction triggers that disproportionately affect specific regions or environments might also contribute to clustered extinctions in families with restricted distributions. However, virtually all bivalve families are geographically and environmentally widespread (*33*), and such spatial effects are weak in the end-Cretaceous extinction (*5*, *6*). Information on environmental drivers of past extinctions and their spatial heterogeneity is currently insufficient to permit more detailed exploration of these factors. Irrespective of the underlying causes, the influence of previous extinctions on both the magnitude of extinction rates and the pattern of phylogenetic conservatism suggests that attempts to understand the biological basis for differential extinction vulnerabilities of clades should take into account their past history of extinctions. These results also corroborate a peculiarity of the end-Cretaceous mass extinction (and perhaps of major extinctions in general), where the phylogenetic pattern of extinction is consistent with preceding intervals, as shown here, but the functional or ecological selectivity is not (*5*, *29*, *34*).

Phylogenetic nonrandomness and the temporal decline in extinction rates documented here are potentially problematic for calculating speciation and diversification rates from molecular phylogenies because they violate the assumption that extinction rates are stochastically constant over time (*35*, *36*), although recent work has started to

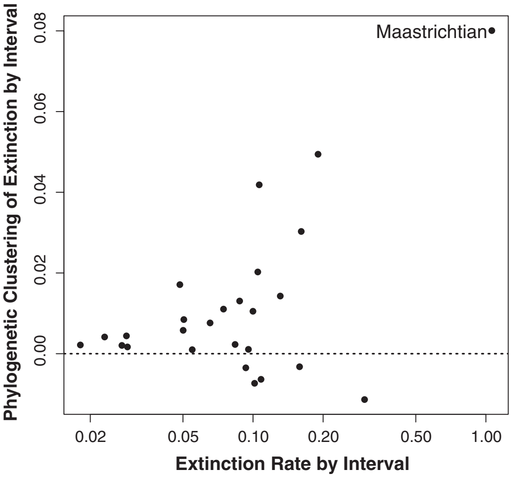

Fig. 2. Phylogenetic clustering of extinctions (R_{CL}) as a function of extinction rate, for each time interval in Fig. 1. The relation is not significant. The dotted line indicates the value expected if extinctions were not phylogenetically clustered.

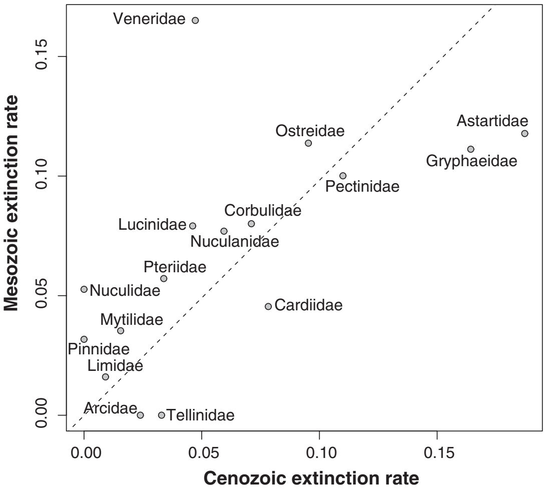

Fig. 3. Mesozoic versus Cenozoic background extinction rates for families well represented in both intervals. The dashed line represents equality in rates between the two eras ($y = x$).

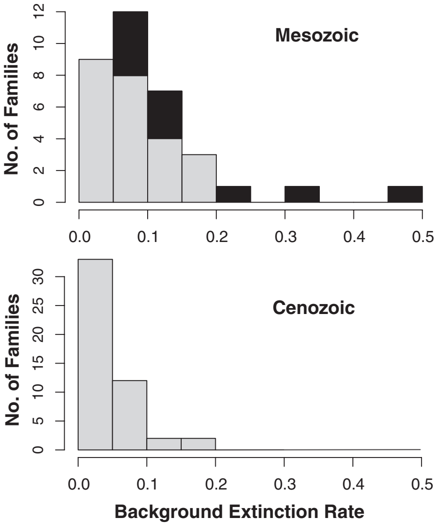

Fig. 4. Distribution of background extinction rates by family during the Mesozoic and Cenozoic eras. Black bars indicate families that went extinct during the end-Cretaceous mass extinction; gray bars indicate surviving families.

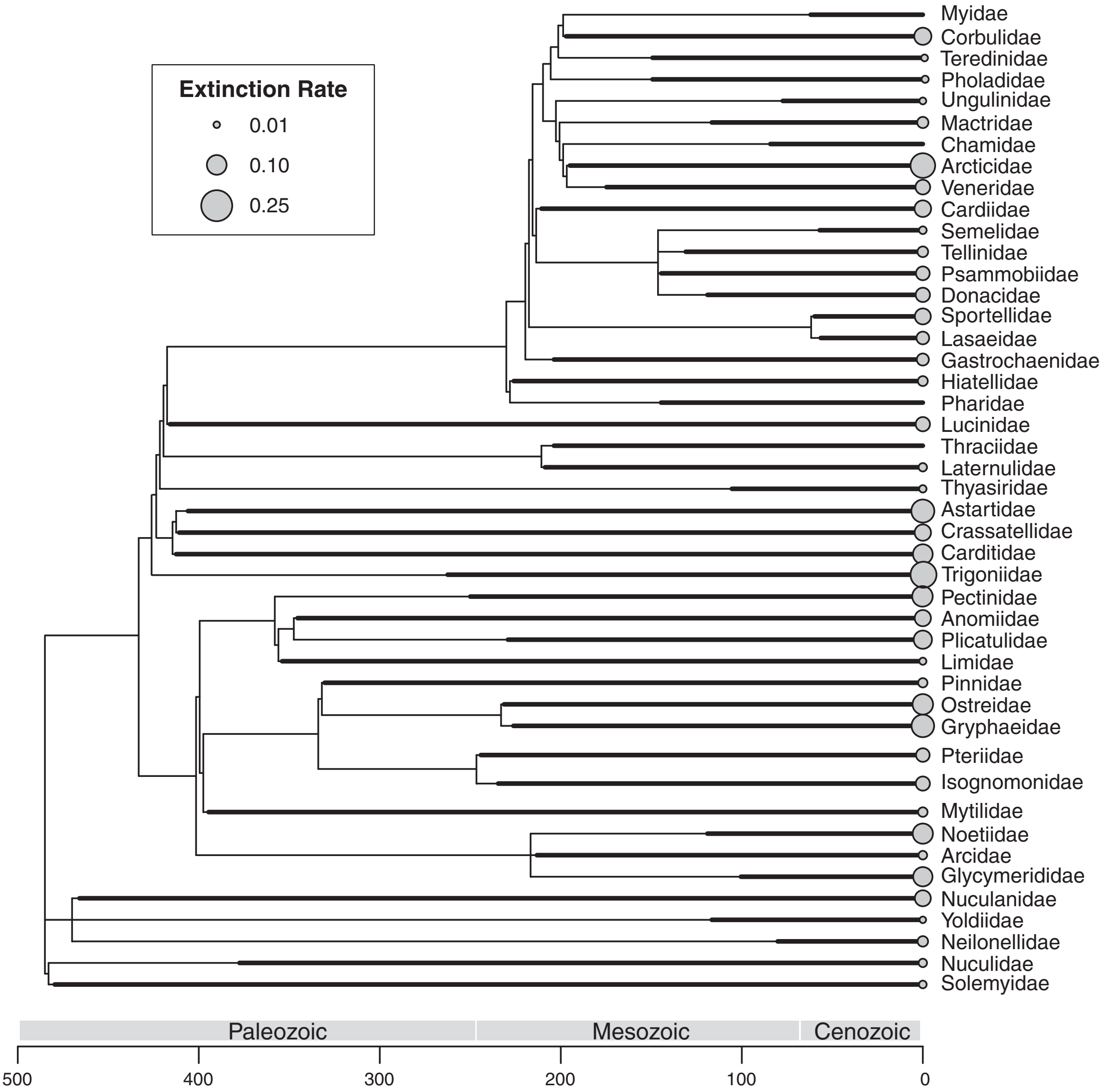

Fig. 5. Mean extinction rates for living families of bivalves mapped on the composite phylogeny used in this study (*18*). Only families for which background extinction rates could be reliably estimated (*18*) are shown. The dark bar along each branch denotes the known stratigraphic range of that taxon, omitting extinct stem groups. The size of the symbol for each family is proportional to its extinction rate. Extinction rates of closely related families are significantly more similar to each other than is expected by chance.

consider less restrictive models (*37*, *38*). On the other hand, if lineages or biotas tend to harden over time, perhaps through the filter of mass extinctions, then the assumption of stochastically constant extinction rates may be more reasonable for later histories of lineages once the more volatile taxa have been winnowed. Caution is needed, however, because such hardening might operate at multiple levels, ranging from the well-documented decline in background extinction rates through the Phanerozoic attributed to the culling of the more volatile Paleozoic fauna in favor of the more resistant Modern fauna (*39*, *40*) to the species-level selectivities seen during the marine extinction pulse near the onset of Pleistocene glacial cycles (*41*). Finally, our results combined with previous studies (*9*, *12*, *14*, *28*) imply that evolutionary histories of individual lineages are important determinants of extinction vulnerabilities of their constituent taxa, under both natural and anthropogenic forcing. This commonality suggests that more detailed studies of phylogenetic selectivity of extinctions in the geological past, and the traits involved, should provide useful insights about the consequences of extinctions unfolding today. For example, if phylogenetic clustering is a general rule, then anthropogenic extinctions are likely to eliminate substantially more evolutionary history in the near future than models based on random extinctions of the same intensity would predict (*15*).

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Genetic Properties of the Maize Nested Association Mapping Population

Michael D. McMullen,[1,2]|| Stephen Kresovich,[3]|| Hector Sanchez Villeda,[2]* Peter Bradbury,[1,3] Huihui Li,[4,5,3] Qi Sun,[6] Sherry Flint-Garcia,[1,2] Jeffry Thornsberry,[7] Charlotte Acharya,[3] Christopher Bottoms,[2] Patrick Brown,[3] Chris Browne,[1] Magen Eller,[1] Kate Guill,[1] Carlos Harjes,[3]† Dallas Kroon,[3] Nick Lepak,[1] Sharon E. Mitchell,[3] Brooke Peterson,[1] Gael Pressoir,[3]‡ Susan Romero,[1] Marco Oropeza Rosas,[8]¶ Stella Salvo,[1] Heather Yates,[3] Mark Hanson,[9] Elizabeth Jones,[10] Stephen Smith,[10] Jeffrey C. Glaubitz,[11] Major Goodman,[8] Doreen Ware,[1,12] James B. Holland,[1,8]|| Edward S. Buckler[1,3,13]||

Maize genetic diversity has been used to understand the molecular basis of phenotypic variation and to improve agricultural efficiency and sustainability. We crossed 25 diverse inbred maize lines to the B73 reference line, capturing a total of 136,000 recombination events. Variation for recombination frequencies was observed among families, influenced by local (cis) genetic variation. We identified evidence for numerous minor single-locus effects but little two-locus linkage disequilibrium or segregation distortion, which indicated a limited role for genes with large effects and epistatic interactions on fitness. We observed excess residual heterozygosity in pericentromeric regions, which suggested that selection in inbred lines has been less efficient in these regions because of reduced recombination frequency. This implies that pericentromeric regions may contribute disproportionally to heterosis.

The majority of phenotypic variation in natural populations and agricultural plants and animals is determined by quantitative genetic traits (*1*). Maize (*Zea mays* L.) exhibits extensive molecular and phenotypic variation (*2*–*4*). Understanding the genetic basis of quantitative traits in maize is essential to predictive crop improvement. However, only slow progress has been made in identifying the genes controlling quantitative agronomic traits because of limitations in the scope of allelic diversity and resolution in available genetic mapping resources. Linkage mapping generally focuses on the construction and analysis of large families from two inbred lines to detect quantitative trait loci (QTLs) (*5*). However, resolution of these QTLs can be poor because of the limited number of recombination events that occur during population development. Association analysis takes advantage of historic recombination from deep coalescent history as linkage disequilibrium (LD) generally decays within 2 kb (*1*, *6*). However, because of the number of single-nucleotide polymorphisms (SNPs) required and the confounding effects of population structure, whole-genome association analysis can be difficult in maize (*4*).

To provide a genetic resource for quantitative trait analysis in maize, we have created the nested association mapping (NAM) population. NAM was constructed to enable high power and high resolution through joint linkage-association analysis, by capturing the best features of previous approaches (*7*, *8*). The genetic structure of the NAM population is a reference design of 25 families of 200 recombinant inbred lines (RILs) per family (fig. S1). The inbred B73 was chosen as the reference inbred line because of its use for the public physical map (*9*) and for the Maize Sequencing Project (www.maizesequence.org). The other 25 parents [named the 25 diverse lines (25DL)] maximize the genetic diversity of the RIL families (*8*, *10*), independent of any specific phenotype. The lines were chosen to represent the diversity of maize—more than half are tropical in origin, nine are temperate lines, two are sweet corn lines (representing Northern Flint), and one is a popcorn inbred line (fig. S2).

The NAM genetic map is a composite map created with 4699 RILs combined across the 25 families, representing 1106 loci, with an average marker density of one marker every 1.3 centimorgans (cM) (fig. S3 and table S1). The proportion of SNP loci from the composite map polymorphic in an individual family ranged from 63 to 74%. Among RILs, 48.7% of all marker genotypes were inherited from B73, 47.6% were inherited from the 25DL parent, and 3.6% were heterozygous, which suggests that they were broadly representative of the parents and fall within the expected range for S5 generation RILs. The NAM population captured ~136,000 crossover events, corresponding, on average, to three crossover events per gene. This allows genetic factors to be mapped to very specific regions of the

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[1] United States Department of Agriculture–Agriculture Research Service (USDA–ARS). [2] Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA. [3] Institute for Genomic Diversity, Cornell University, Ithaca, NY 14853, USA. [4] School of Mathematical Science, Beijing Normal University, Beijing 100875, China. [5] Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China. [6] Computational Biology Service Unit, Cornell University, Ithaca, NY 14853, USA. [7] Northwest Missouri State University, Maryville, MO 64468, USA. [8] Department of Crop Science, North Carolina State University, Raleigh, NC 27695, USA. [9] Illumina Inc., San Diego, CA 92121, USA. [10] Pioneer Hi-Bred, Johnston, IA 50131, USA. [11] Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, USA. [12] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA. [13] Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853, USA.

*Present address: International Maize and Wheat Improvement Center (CIMMYT), kilometer 45, Carretera Mex-Veracruz, El Batan, Texcoco, Mexico.

†Present address: Monsanto, Leesburg, GA 31763, USA.

‡Present address: Fondation CHIBAS, 30 Rue Pacot, Port-au-Prince, Haiti.

¶Present address: Delta Pine/Monsanto, Post Office Box 194, Scott, MS 38772, USA.

||To whom correspondence should be addressed. E-mail: mcmullenm@missouri.edu (M.D.M.); sk20@cornell.edu (S.K.); james_holland@ncsu.edu (J.B.H.); esb33@cornell.edu (E.S.B.)