

Holocene shifts in the assembly of plant and animal communities implicate human impacts

S. Kathleen Lyons¹, Kathryn L. Amatangelo², Anna K. Behrensmeyer¹, Antoine Bercovici¹, Jessica L. Blois³, Matt Davis^{1,4}, William A. DiMichele¹, Andrew Du⁵, Jussi T. Eronen⁶, J. Tyler Faith⁷, Gary R. Graves^{8,9}, Nathan Jud^{10,11}, Conrad Labandeira^{1,12,13}, Cindy V. Looy¹⁴, Brian McGill¹⁵, Joshua H. Miller¹⁶, David Patterson⁵, Silvia Pineda-Munoz¹⁷, Richard Potts¹⁸, Brett Riddle¹⁹, Rebecca Terry²⁰, Anikó Tóth¹, Werner Ulrich²¹, Amelia Villaseñor⁵, Scott Wing¹, Heidi Anderson²², John Anderson²², Donald Waller²³ & Nicholas J. Gotelli²⁴

Understanding how ecological communities are organized and how they change through time is critical to predicting the effects of climate change¹. Recent work documenting the co-occurrence structure of modern communities found that most significant species pairs co-occur less frequently than would be expected by chance^{2,3}. However, little is known about how co-occurrence structure changes through time. Here we evaluate changes in plant and animal community organization over geological time by quantifying the co-occurrence structure of 359,896 unique taxon pairs in 80 assemblages spanning the past 300 million years. Co-occurrences of most taxon pairs were statistically random, but a significant fraction were spatially aggregated or segregated. Aggregated pairs dominated from the Carboniferous period (307 million years ago) to the early Holocene epoch (11,700 years before present), when there was a pronounced shift to more segregated pairs, a trend that continues in modern assemblages. The shift began during the Holocene and coincided with increasing human population size^{4,5} and the spread of agriculture in North America^{6,7}. Before the shift, an average of 64% of significant pairs were aggregated; after the shift, the average dropped to 37%. The organization of modern and late Holocene plant and animal assemblages differs fundamentally from that of assemblages over the past 300 million years that predate the large-scale impacts of humans. Our results suggest that the rules governing the assembly of communities have recently been changed by human activity.

How are plant and animal communities organized, and does their structure change through time? This question has dominated many decades of research on community assembly rules and is critical to charting the future of biodiversity¹. Whereas most studies have described overall community structure with simple indices such as species richness⁸ and average co-occurrence³, some analyses categorize individual species pairs in assemblages as random, aggregated, or segregated^{2,9}. Segregated species pairs may be generated by processes such as negative species interactions, distinct habitat preferences, and dispersal limitation. Aggregated species pairs may be generated by processes such as positive species interactions, shared habitat preferences, and concordant dispersal². Recent meta-analyses document an

excess of segregated species pairs in modern communities: most significant species pairs co-occur less frequently than would be expected by chance^{2,10}. The relative dominance of segregated versus aggregated species pairs suggests an important role for biotic interactions such as competition and predation, habitat selectivity, and dispersal limitation in structuring modern communities.

Do the patterns of species segregation that characterize modern assemblages also hold in the fossil record, or is the present different? If there was a change, when did the modern condition arise? There are many examples from the fossil record of times of major reorganization in ecological communities, such as a shift in the complexity of marine invertebrate communities after the end-Permian mass extinction¹¹. But even during the lengthy periods between mass extinctions, the nature of species interactions may change. For example, the diversity and intensity of insect herbivory increased during a warming trend from the Late Palaeocene to the Eocene¹². Moreover, many late Pleistocene plant and animal assemblages that contain some extant species have no modern analogues^{13,14}. Such results hint that general patterns of species associations observed in contemporary assemblages could have been quite different in the past.

Here we ask whether non-random species associations of plant and mammal assemblages over the past 300 million years (Myr) are dominated by segregated or aggregated species pairs. This novel analysis is designed to compare statistical patterns of taxon associations for fossil and modern data using a consistent set of methodologies. We analysed 80 well-sampled fossil and recent assemblages: 38 for mammals and 42 for plants (see Supplementary Information, Extended Data Fig. 1 and Extended Data Table 1). Each data set contained information on taxon presence and absence across multiple localities in a particular time period (Extended Data Fig. 1 and Extended Data Table 1). Ages of plant data sets range from 307 million years ago (Ma) to the present and are from North America and Africa. Mammal data sets range in age from 21.4 Ma to the present and are from North America, Eurasia, and Africa. We compared each data set to a 'null' assemblage generated by randomization, scored each taxon pair as random, aggregated, or segregated, and used an empirical Bayes approach to control for the rate of false positive discoveries¹⁵; see Methods). Finally we

¹Department of Paleobiology, National Museum of Natural History, Smithsonian Institution, Washington DC 20013, USA. ²Department of Environmental Science and Biology, The College at Brockport – SUNY, Brockport, New York 14420, USA. ³School of Natural Sciences, University of California, Merced, 5200 North Lake Road, Merced, California 95343, USA. ⁴Department of Geology and Geophysics, Yale University, New Haven, Connecticut 06520, USA. ⁵Hominid Paleobiology Doctoral Program, Center for the Advanced Study of Hominid Paleobiology, Department of Anthropology, George Washington University, Washington DC 20052, USA. ⁶Department of Geosciences and Geography, University of Helsinki, PO Box 64, 00014 University of Helsinki, Finland. ⁷School of Social Science, The University of Queensland, Brisbane, Queensland 4072, Australia. ⁸Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, Washington DC 20013, USA. ⁹Center for Macroecology, Evolution and Climate, University of Copenhagen, Copenhagen 2100, Denmark. ¹⁰Biological Sciences Graduate Program, University of Maryland, College Park, Maryland 20742, USA. ¹¹Florida Museum of Natural History, University of Florida, Gainesville, Florida 32611, USA. ¹²Department of Entomology, University of Maryland College Park, College Park, Maryland 20742, USA. ¹³Key Lab of Insect Evolution and Environmental Changes, Capital Normal University, Beijing 100048, China. ¹⁴Department of Integrative Biology and Museum of Paleontology, University of California Berkeley, Berkeley, California 94720, USA. ¹⁵School Biology and Ecology & Sustainability Solutions Initiative, University of Maine, Orono, Maine 04469, USA. ¹⁶Department of Geology, University of Cincinnati, Cincinnati, Ohio 45221, USA. ¹⁷Department of Biological Sciences, Macquarie University, Sydney, New South Wales 2109, Australia. ¹⁸Department of Anthropology, Human Origins Program, National Museum of Natural History, Smithsonian Institution, Washington DC 20013, USA. ¹⁹School of Life Sciences, University of Nevada-Las Vegas, Las Vegas, Nevada 89154, USA. ²⁰Department of Integrative Biology, Oregon State University, Corvallis, Oregon 97331, USA. ²¹Chair of Ecology and Biogeography, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland. ²²Evolutionary Studies Institute, University of the Witwatersrand, Jorissen Street, Braamfontein, Johannesburg 2001, South Africa. ²³Department of Botany, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA. ²⁴Department of Biology, University of Vermont, Burlington, Vermont 05405, USA.

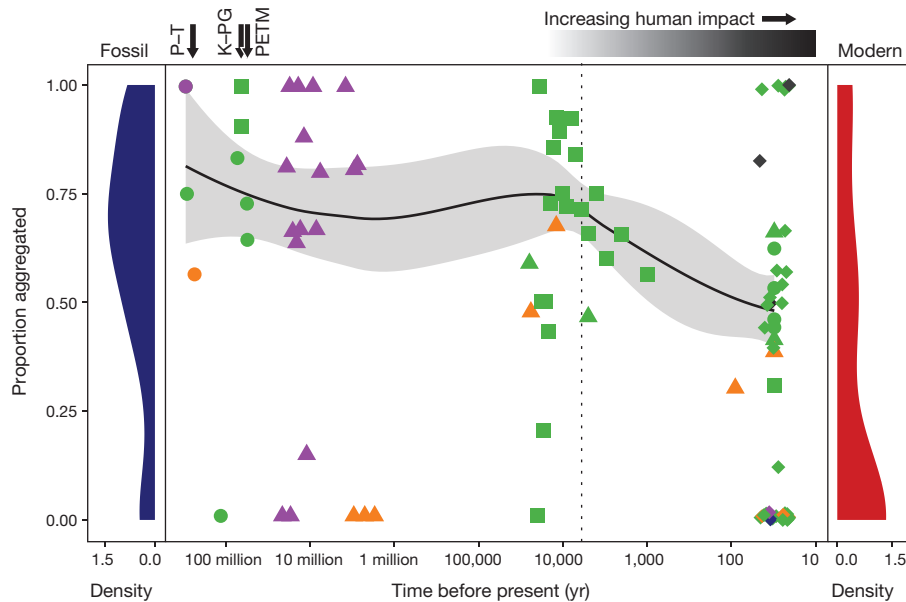


Figure 1 | Proportion of aggregated pairs over the past 300 Myr.

Weighted Loess curve with shaded 95% confidence intervals illustrates reduction in the proportion of aggregated species pairs in the Holocene (log scale). Dotted vertical line at 5,998 years delineates the linear model breakpoint in the trend (Methods and Extended Data Fig. 2). Non-random species pairs of 'Fossil' data (blue density profile) are predominantly aggregated, whereas 'Modern' data (red density profile) are predominantly segregated. Colours indicate continent: North America (green), Eurasia (purple), Australia (dark grey), South America (dark blue), Africa

synthesized our results with those from a meta-analysis of 39 modern communities that used the same methodology^{2,10}.

For all fossil data sets, most taxon pairs were random (87–100% of possible pairs; Extended Data Table 1), which is also typical for modern assemblages². This result reflects the statistically conservative nature of the tests used to identify significantly associated pairs, and the fact that most taxon pairs in a diverse, well-sampled assemblage interact weakly, or not at all. In 62 of 80 assemblages analysed here, a subset of taxon pairs showed significant associations that are stronger than can be explained by the null model, even after controlling for the false discovery rate (Fig. 1). Unlike modern mainland assemblages, most significant associations in the fossil record are aggregated, positive associations (Fig. 1). This pattern is consistent across the past 300 Myr for the diverse fossil assemblages in this study, which encompass mammals, plant macrofossils, and pollen from multiple continents and time slices.

However, beginning in the Holocene, there was a significant temporal trend towards a greater proportion of segregated species pairs, which is consistent with the results for modern assemblages. A breakpoint analysis indicates that the shift began approximately 6,000 years ago (Extended Data Fig. 2). Confidence intervals of the breakpoint are large owing to a lack of appropriate data sets between 20,000 and 1 million years ago. Therefore, it is difficult to pinpoint the exact time of the shift, but a closer examination of the data suggests that placing it within the Holocene is reasonable. Before the breakpoint, on average 64% of significant pairs were aggregated (median = 73%). After the breakpoint, the average dropped to 37% (median = 42%). This trend is not driven by the modern data and persists when only fossil data are analysed (Extended Data Fig. 3).

Why are species associations so different in fossil versus modern assemblages? We first tested and eliminated five potential 'artefact' hypotheses that are related to sampling issues (see Methods for details). (1) Collection modes were discounted because they were heterogeneous both for the modern and for fossil assemblages, and because the decrease in aggregated pairs was strong in fossil pollen

(orange). Point shapes indicate type of data: pollen (square), mammals (triangle), macroplants (circle). Data on terrestrial communities from ref. 2 are diamonds. All fossil and modern data are from mainland sites; no island sites were included. Time values of modern data points were assigned a single age (see Supplementary Information data sets), but are jittered for visual representation. P–T, Permo–Triassic transition; K–Pg, Cretaceous–Palaeogene transition; PETM, Palaeocene–Eocene thermal maximum.

and mammal assemblages that spanned the shift. Moreover, sampling methodology was consistent within an assemblage type across periods that encompass the change (Extended Data Fig. 4). (2) Scale was discounted because there was no relationship between the spatial or temporal extent and grain of each data set and the percentage of aggregated pairs (Fig. 2 and Extended Data Fig. 5). (3) Taphonomic bias was discounted because the null model algorithm preserved the marginal totals of the data matrix in each randomized assemblage, controlling for simple taphonomic biases that could generate heterogeneity in the number of species per site or the number of occupied sites per species. (4) Taxonomic resolution was discounted because parallel analyses at the genus and species levels did not produce systematic changes in the proportions of aggregated pairs (Extended Data Table 2). (5) Increased sampling of rare species in modern data sets was discounted because segregated pairs tend to form in species with intermediate occupancy, whereas aggregated pairs form both in common and in rare species in modern and fossil data sets. All of these mechanisms can potentially affect assemblage structure in fossil and modern data sets. However, our analyses suggest that these mechanisms cannot account for the prominent decrease in aggregated species pairs that began during the Holocene (Fig. 1).

The failure of sampling issues to account for the temporal change in the percentage of non-randomly associated taxon pairs suggests that a mechanistic explanation is required. We consider two hypotheses that invoke a systematic change in either abiotic or biotic factors as drivers of co-occurrence patterns.

One of the most obvious differences between the present interval and the past 300 Myr of geological history represented by these fossil assemblages is the increasing variability of climate towards the present, associated with the glacial–interglacial cycles of the Quaternary period¹⁶. This is not to say that there were no periods of high climate variability before the ice ages, but that our data do not regularly sample times of high climate variability in deep time. If climate variability is responsible for the shift in the frequency of aggregated species pairs, there should be a negative relationship between climate variability

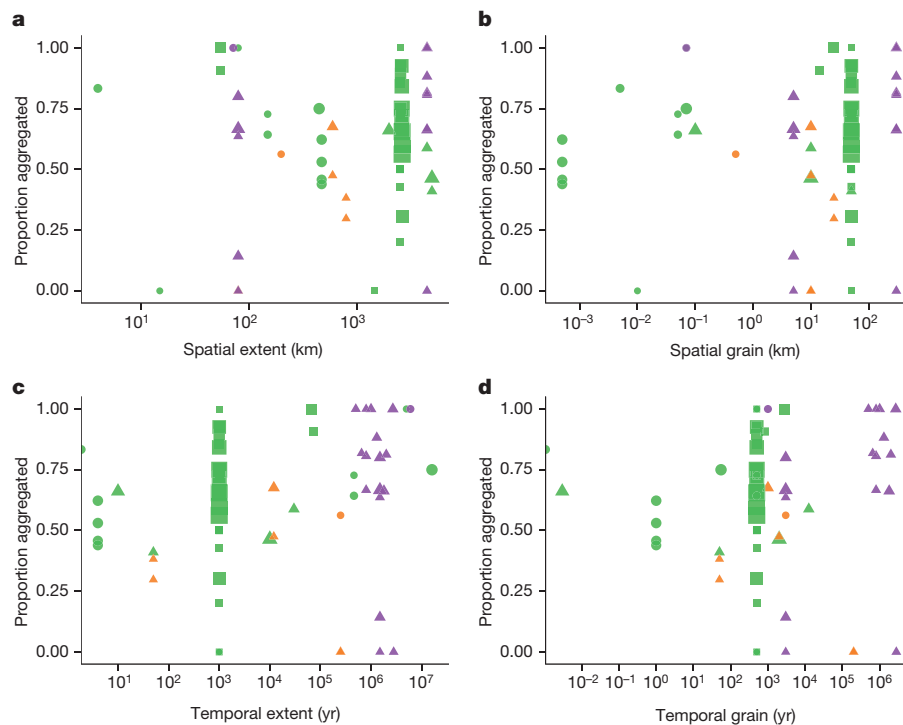


Figure 2 | Relationship between scale and proportion of aggregated pairs. The proportion of significant pairs that are aggregated does not depend on the temporal or spatial scale of data. Each point represents a single data set. **a, b**, Aggregated pairs versus spatial extent (longest linear distance between any two sites in a data set; **a**) or spatial grain (estimated radius of collection area that fossil specimens would have been transported

to the depositional environment in a typical locality; **b**). **c, d**, Proportion of aggregated pairs versus temporal extent (duration from the oldest to youngest locality in a data set; **c**) or temporal grain (typical amount of time-averaging of localities in a data set; **d**). Colours and shapes as in Fig. 1. Note the logarithmic scale of the x axes. Modern data from ref. 2 are excluded from this analysis.

and the percentage of aggregations. We quantified climate variability within the temporal extent of each data set for the past 65 Myr, using climate data from ice¹⁷ and deep sea¹⁶ cores that were standardized to a common scale (Methods). We found no relationship between the proportion of aggregated pairs and the standard deviation of climate within the sampled time slice (Extended Data Figs 6 and 7), or the standard deviation of the first differences of climate within the sampled time slice (Fig. 3a). Collectively, these results suggest that the increasing variability in climate in the Quaternary cannot explain the decreased frequency of aggregation.

An alternative explanation is that the mid- to late Holocene is unusual in the evolutionary history of terrestrial ecosystems, and that biotic drivers (as opposed to climate) now are different from what they have typically been over the past 300 Myr. First, we asked whether there was a significant shift in the proportions of aggregated versus segregated pairs across critical geological intervals that spanned periods of mass extinctions or major climatic change during the past 300 Myr (Extended Data Table 3 and Extended Data Fig. 4). We found a significant decrease in the percentage of positive associations only in data sets that spanned the Pleistocene–Holocene transition (11,700 years ago). With the exception of large-bodied mammals in North America and Africa (Extended Data Fig. 4b), aggregated species pairs decreased in all data sets through the Pleistocene–Holocene transition. In contrast, there was no significant change in the percentage of aggregations across the three other critical intervals that were encompassed by these data: the Permo–Triassic transition (252 Ma), the Cretaceous–Palaeogene transition (66 Ma), and the Palaeocene–Eocene thermal maximum (56 Ma). These intervals include the Cretaceous–Palaeogene mass extinction, responsible for the loss of the non-avian dinosaurs¹⁸, and the Permo–Triassic extinction, the largest mass extinction ever recorded¹⁸. Even the Palaeocene–Eocene thermal maximum, a period of major climatic change in which global temperatures increased ~ 5 – 8°C in a few millennia¹⁹, did not coincide with a change in the relative proportions of aggregated versus segregated pairs.

It is difficult to pinpoint the exact mechanism responsible for the uniqueness of the present time interval. However, our analyses provide some clues about possible cause. Data that encompass the shift towards the modern pattern are almost exclusively North American (Fig. 1 and Extended Data Fig. 4). The statistical confidence interval bracketing the breakpoint at 6,000 years ago encompasses the beginning of agriculture in North America around 8,000 years ago⁶ and the increase in human populations during the Holocene^{4,5}. The trend towards greater segregations in North American pollen (Fig. 1 and Extended Data Fig. 4), with particularly strong shifts occurring in the past 2,000 years²⁰, is also consistent with the history of agriculture in North America. Cultivation of multiple species of domesticated plants began approximately 3,800 years ago^{6,7}, with evidence for more general dependency on agriculture in North America beginning 1,300 years ago^{6,7}. Estimates of global land area under cultivation increase rapidly starting 6,000 years ago and are as high as 4×10^8 hectares (1 hectare = 10^4 m^2) by 2,000 years ago^{4,5}.

Possible drivers by which increasing human impacts led to an increase in segregated pairs include (1) increases in hunting and domestication of particular species^{21,22}, (2) changes in land use^{4,5}, (3) increases in the frequency of fire⁴, (4) increases in habitat fragmentation and dispersal barriers^{23,24}, and (5) deliberate and accidental spread of species beyond their native geographical ranges^{25–27}. We note that modern island assemblages (which we excluded from our comparisons with fossil assemblages) are more segregated than modern mainland assemblages (Fig. 3b), which is consistent with the hypothesis that habitat fragmentation and dispersal limitation have increased segregated pairs. Possibly all of the processes listed play a role. Although their combined effects on taxon pairs are difficult to predict, the relative importance of factors structuring species co-occurrence appears to have changed through the Holocene. Future work comparing the co-occurrence structure of fossil and modern communities should allow us to better understand how this alteration will play out in the future. Regardless of the precise mechanisms,

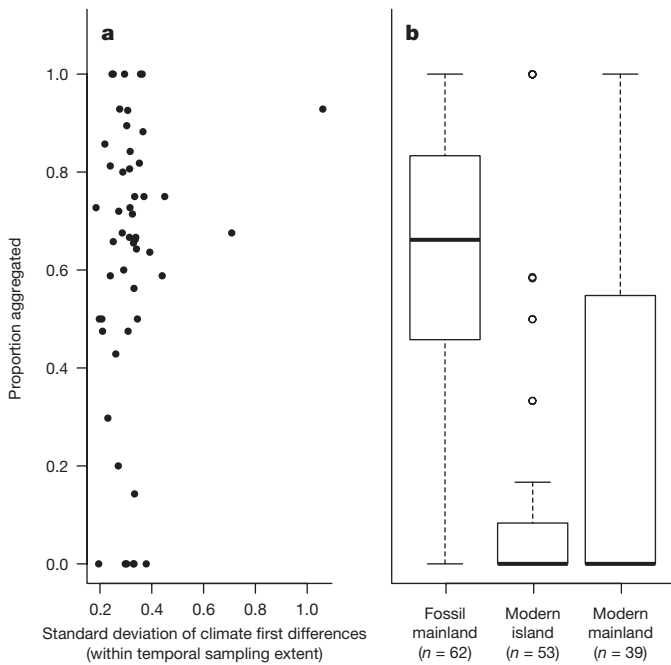


Figure 3 | Tests of possible mechanisms for decreasing spatial aggregation through time. **a**, Climate variability within the temporal extents of the fossil data sets is uncorrelated with the proportion of aggregated species pairs. Climate variability is measured as the standard deviation of the first differences in climate across the interval (see also Extended Data Fig. 6). **b**, Box plots show the proportion of aggregated species pairs for fossil data, modern islands and modern mainland assemblages. Dashed lines indicate maximum and minimum values, circles are outliers. Island assemblages, with more limited capacity for dispersal, have the smallest and least variable fraction of aggregated pairs. Mainland fossil assemblages are significantly more aggregated than mainland modern assemblages. Note, these island data were excluded from other analyses.

humans appear to be agents of disturbance on a large scale and have been so for longer than is often recognized.

Our results suggest that assemblage co-occurrence patterns remained relatively consistent for 300 Myr but have changed over the Holocene as the impact of humans has dramatically increased. Across shallower time intervals, other studies have documented that local and regional species composition has changed substantially over recent decades^{28,29} and millennia³⁰. The rules governing community assembly, at least as implicated by co-occurrence patterns, seem to have changed during the Holocene and continue to change with the increasing influence of human activity. The co-occurrence structure of modern and recent plant and animal assemblages thus appears to be unique in the evolutionary history of terrestrial ecosystems, an important perspective for assessing challenges to these ecosystems in the face of present and future human impacts.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 27 August; accepted 13 November 2015.

Published online 16 December 2015.

1. Botkin, D. B. *et al.* Forecasting the effects of global warming on biodiversity. *Bioscience* **57**, 227–236 (2007).
2. Gotelli, N. J. & Ulrich, W. The empirical Bayes approach as a tool to identify non-random species associations. *Oecologia* **162**, 463–477 (2010).
3. Gotelli, N. J. & McCabe, D. J. Species co-occurrence: a meta-analysis of J.M. Diamond's assembly rules model. *Ecology* **83**, 2091–2096 (2002).
4. Marlon, J. R. *et al.* Global biomass burning: a synthesis and review of Holocene paleofire records and their controls. *Quat. Sci. Rev.* **65**, 5–25 (2013).
5. Riddiman, W. F. & Ellis, E. C. Effect of per-capita land use changes on Holocene forest clearance and CO₂ emissions. *Quat. Sci. Rev.* **28**, 3011–3015 (2009).

6. Zeder, M. A. & Smith, B. D. A conversation on agricultural origins: talking past each other in a crowded room. *Curr. Anthropol.* **50**, 681–691 (2009).
7. Smith, B. D. & Yarnell, R. A. Initial formation of an indigenous crop complex in eastern North America at 3800 B.P. *Proc. Natl Acad. Sci. USA* **106**, 6561–6566 (2009).
8. Connor, E. F. & McCoy, E. D. The statistics and biology of the species-area relationship. *Am. Nat.* **113**, 791–833 (1979).
9. Connor, E. F., Collins, M. D. & Simberloff, D. The checkered history of checkerboard distributions. *Ecology* **94**, 2403–2414 (2013).
10. Ulrich, W. & Gotelli, N. J. Null model analysis of species associations using abundance data. *Ecology* **91**, 3384–3397 (2010).
11. Wagner, P. J., Kosnik, M. A. & Lidgard, S. Abundance distributions imply elevated complexity of post-Paleozoic marine ecosystems. *Science* **314**, 1289–1292 (2006).
12. Wilf, P. & Labandeira, C. C. Response of plant-insect associations to Paleocene-Eocene warming. *Science* **284**, 2153–2156 (1999).
13. Lyons, S. K. A quantitative model for assessing community dynamics of Pleistocene mammals. *Am. Nat.* **165**, E168–E185 (2005).
14. Jackson, S. T. & Williams, J. W. Modern analogs in Quaternary paleoecology: here today, gone yesterday, gone tomorrow? *Annu. Rev. Earth Planet. Sci.* **32**, 495–537 (2004).
15. Ulrich, W. *Pairs* – a FORTRAN program for studying pair wise species associations in ecological matrices. <http://www.keib.umk.pl/wp-content/uploads/2013/04/PairsManual.pdf> (2008).
16. Zachos, J. C., Dickens, G. R. & Zeebe, R. E. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* **451**, 279–283 (2008).
17. Augustin, L. *et al.*; EPICA community members. Eight glacial cycles from an Antarctic ice core. *Nature* **429**, 623–628 (2004).
18. Bambach, R. K. Phanerozoic biodiversity mass extinctions. *Annu. Rev. Earth Planet. Sci.* **34**, 127–155 (2006).
19. Bowen, G. J. *et al.* Two massive, rapid releases of carbon during the onset of the Palaeocene–Eocene thermal maximum. *Nature Geosci.* **8**, 44–47 (2015).
20. Blois, J. L. *et al.* A framework for evaluating the influence of climate, dispersal limitation, and biotic interactions using fossil pollen associations across the late Quaternary. *Ecography* **37**, 1095–1108 (2014).
21. Delcourt, P. A. & Delcourt, H. R. *Prehistoric Native Americans and Ecological Change: Human Ecosystems in Eastern North America since the Pleistocene*. (Cambridge Univ. Press, 2004).
22. Barnosky, A. D. Colloquium paper: megafauna biomass tradeoff as a driver of Quaternary and future extinctions. *Proc. Natl Acad. Sci. USA* **105** (suppl. 1), 11543–11548 (2008).
23. Haddad, N. M. *et al.* Habitat fragmentation and its lasting impact on Earth's ecosystems. *Sci. Adv.* **1**, e1500052 (2015).
24. Williams, M. *Deforesting the Earth: From Prehistory To Global Crisis* (Univ. Chicago Press, 2003).
25. Grayson, D. K. The archaeological record of human impacts on animal populations. *J. World Prehist.* **15**, 1–68 (2001).
26. van Kleunen, M. *et al.* Global exchange and accumulation of non-native plants. *Nature* **525**, 100–103 (2015).
27. Capinha, C., Essl, F., Seebens, H., Moser, D. & Pereira, H. M. The dispersal of alien species redefines biogeography in the Anthropocene. *Science* **348**, 1248–1251 (2015).
28. Dornelas, M. *et al.* Assemblage time series reveal biodiversity change but not systematic loss. *Science* **344**, 296–299 (2014).
29. Tóth, A. B., Lyons, S. K. & Behrensmeyer, A. K. A century of change in Kenya's mammal communities: increased richness and decreased uniqueness in six protected areas. *PLoS ONE* **9**, e93092 (2014).
30. Thompson, J. R., Carpenter, D. N., Cogbill, C. V. & Foster, D. R. Four centuries of change in northeastern United States forests. *PLoS ONE* **8**, e72540 (2013).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank G. Dietl for comments that improved the manuscript. Support for this research was provided by a National Museum of Natural History Program grant to the Evolution of Terrestrial Ecosystems Program (ETE) and NSF-DEB 1257625. This is ETE publication 338.

Author Contributions All authors provided input into the final manuscript. S.K.L. helped design the study, contributed and analysed data, and wrote the paper. K.L.A., J.L.B., J.T.F., and W.U. were involved in the study design, and contributed and analysed data. A.T. was involved in the study design, contributed and analysed data, and created Supplementary Fig. 1. A.K.B., A.B., W.A.D., J.T.E., N.J., C.L., C.V.L., S.W., and B.R. were involved in the study design and contributed data. H.A., J.A., and D.W. contributed data. M.D. and J.H.M. were involved in the study design, analysed data, created figures and wrote text for the supplementary information. A.D. was involved in the study design and analysed data. G.R.G., B.M., D.P., S.P.-M., R.T., R.P., and A.V. were involved in the study design. N.J.G. designed the study and helped write the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.K.L. (lyonss2@si.edu).

METHODS

No statistical methods were used to predetermine sample size.

Detection of non-random species pairs. The data for each analysis consist of a binary presence–absence matrix in which each row is a taxon and each column is a sample. The entries represent the presence (1) or absence (0) of a particular taxon in a particular sample. Within this matrix, each of the $S(S-1)/2$ unique species pairs is tested and classified as random, aggregated, or segregated. The tests were performed with the PAIRS version 1.0 software application^{15,31}. The methodology is described fully in ref. 2 and is briefly described here.

The analysis begins by calculating a scaled C score³²: $C_{ij} = (R_i - D)(R_j - D)/R_i R_j$, where C_{ij} is the C score for species pair i and j , R_i is the row total (the number of species occurrences) for species i , R_j is the row total for species j , and D is the number of shared sites in which both species are present. For each species pair, this index ranges from 0.0 (aggregation: maximal co-occurrence of both species) to 1.0 (segregation: minimal co-occurrence of both species). PAIRS calculates the C score for each pair of species and assigns it to a histogram bin. There are 20 bins that range from 0 to 1 in 0.05 intervals, plus a bin for 0.0 (perfectly aggregated pairs) and a bin for 1.0 (perfectly segregated pairs).

We next estimate the P value associated with each species pair by a randomization test. The data matrix is first randomized by reshuffling all matrix elements, with the restriction that the row and column sums of the original matrix are preserved. This ‘fixed-fixed’ algorithm has been subject to extensive benchmark testing with artificial random and structured matrices^{2,33,34}. Compared with a variety of other null model algorithms, the fixed-fixed algorithm most effectively screens against type I errors (incorrectly rejecting the null hypothesis for a random matrix), but is somewhat conservative³³.

An alternative algorithm ‘fixed-equiprobable’ retains row sums (species occurrence frequencies), but allows column totals (species richness per site) to vary freely. The fixed-equiprobable algorithm also has good statistical properties, and is appropriate for modern data sets in which sampling effort has been standardized, such as quadrat samples of fixed area. However, this algorithm is not appropriate for fossil data because the number of species detected per site in fossil assemblages is determined primarily by sampling effort of the collector and by site-specific taphonomic biases in preservation.

For these reasons, we have used only the fixed-fixed model, both for the analysis of fossil assemblages and for comparison with modern assemblages. Details of the randomization are discussed further in refs 2, 35. Using 1,000 randomizations, we create a simple P value (two-tailed test) for each species pair, which leads to a classification of each species pair as aggregated, random, or segregated.

However, with a total of $S(S-1)/2$ unique pairs in a matrix of S species, retaining all of the significant pairs ($P < 0.05$) would generate a potentially large number of false positive results. This problem has frequently arisen in the analysis of micro-arrays, genomic surveys, and other examples of ‘big data’³⁶. The PAIRS analysis relies on an empirical Bayes approach by creating a histogram of C score values based on the pairs generated in each null assemblage. To screen out false positives, we calculated the average number of species pairs in each bin of the histogram. Next, we determined the observed number of pairs from the empirical assemblage in each bin, ordered by their P values from the simulation. We retained only those pairs that were above the mean number for each bin and were statistically significant on the basis of the simple P value criterion. This double screen effectively eliminates many of the false-positives that can arise in random data sets².

Weighted Loess regression. A Loess smoothing line was created with the `stat_smooth` function in the R package `ggplot2` version 1.0.0 (ref. 37) using default parameters. For Loess fitting, the fit at point x is made using points in the neighbourhood of x (closest 75% of total points), with tricubic weighting (proportional to $(1 - (\text{distance}/\text{maximum distance}))^3$). Points were additionally weighted by the number of sites in each matrix and 95% confidence intervals were generated using a t -based approximation.

Analysis of climate variability. To examine the how climate variability impacts the percentage of aggregated species pairs, we used climate proxy data from ice¹⁷ and deep sea cores¹⁶, which collectively encompass the past 65 Myr of the assembled data sets. The European Project for Ice Coring in Antarctica (EPICA) data were used preferentially when there was temporal overlap between proxy data sets. Climate data were mean centred and standardized before pooling into a single data time series. We then sampled the climate data across the ‘temporal extents’ (Extended Data Table 1) of the individual Evolution of Terrestrial Ecosystems Program (ETE) data sets to test if there were relationships between the percentage of aggregated species pairs and climate variability. Climate variability was calculated in two ways: (1) as the standard deviation of climate within the temporal extent of each data set and (2) as the standard deviation of the first differences (changes in climate from available time-step to time-step within the temporal extent of a data set) of climate. We used standard deviation because it helps address

issues with changes in data density over time. Estimated rates of change are sensitive to the time span over which they are measured and more closely spaced data would shift apparent rates of change. Approaches using standard deviation are less sensitive to this issue. We also compared climate variability with age (years before present) of ETE data sets to test for Phanerozoic-scale trends in climate variability sampled by ETE data sets.

Breakpoint analysis. We used a maximum likelihood approach, available in the R package ‘segmented’ version 1.1, to estimate the breakpoint time at which the sharper decline in aggregated species pairs began. This analysis used an initial linear model of the proportion of aggregated pairs as a function of community age (\log_{10} of years before present) to generate a best-fitting number of breakpoints, with separate regression lines fit to each segment. A bootstrap of 1,000 replicates was used to estimate uncertainty in the model parameters (including uncertainty in the time of the breakpoint).

Tests of artefacts. Collection modes. We thought that differences in the way fossil and modern data are collected might be responsible for the observed difference in the relative proportions of aggregated versus segregated species pairs in modern communities^{2,10} and fossil communities. There are two reasons why collection modes are not likely to be responsible for this difference. First, fossil collections are heterogeneous by nature. Different collecting methods are used for different taxonomic groups (for example, bulk sampling, surface sampling, cores). Moreover, even within a taxonomic group, the type of depositional environment imposes different kinds of bias (for example, cave sites versus open pits for Pleistocene mammals). Second, we see a switch from species pairs that are dominated by aggregations to those dominated by segregations in our data sets that span the Pleistocene–Holocene transition (Extended Data Fig. 4 and Extended Data Table 1). In particular, mammal assemblages show a switch from $>50\%$ aggregations in the Pleistocene to $<50\%$ aggregations in the Holocene. The data encompassing this switch are all fossil localities and there are similar biases in both time slices. Although there is variation in the pollen assemblages, a weighted regression that takes into account the sampling in each time slice shows a significant decrease through time ($P = 0.04$, $R^2 = 0.15$). This trend of increasing percentage of segregated pairs begins approximately 14,000 years ago and continues across the Holocene with the switch occurring in the final 1,000 year time slice²⁰. The fact that these data were all collected using the same sampling techniques suggests that sampling cannot account for this pattern.

Issues of scale. It is generally assumed that fossil data are biased. Although the type of bias is not the same for all taxonomic groups, most fossil assemblages contain some degree of temporal or spatial averaging³⁸. That is, they represent accumulations of species that can occur over hundreds or thousands of years and may mix species that did not exist at the locality at the same time³⁹. The fossil data sets in this analysis include assemblages that range from no time-averaging (for example, fossil leaves preserved in volcanic event beds) to those that are time-averaged over thousands or hundreds of thousands of years (for example, some mammal assemblages). In addition, some data sets could not be resolved to time bins of less than a million years. Spatial averaging is less of an issue in these data sets, but individual samples are drawn from areas with diameters ranging from a few metres to more than 300 km (Supplementary Table 1).

If issues of scale are contributing to the pattern found here, there should be a relationship between the proportion of significant pairs that are aggregated and the spatial or temporal scale of the data. We evaluated this by estimating the spatial or temporal grain and extent of each data set included in the analyses (Extended Data Table 1) and determining if there was a significant relationship with the percentage of aggregations. The spatial grain is the estimated radius of collection area over which fossil specimens would have been transported to the depositional environment in a typical locality. The temporal grain is the typical amount of time-averaging of localities in a data set. Spatial extent is the longest linear distance between any two sites in a data set and temporal extent is the duration from the oldest to youngest locality in a data set.

We found no relationship between the scale of the data sets and the proportion of significant pairs that were aggregated versus segregated (Fig. 2 and Extended Data Fig. 5). Regression analyses were not significant and explained very little of the variation in the data (Extended Data Fig. 6). The pattern of segregated versus aggregated pairs was not different in fossil versus modern assemblages because of biases related to the scale of fossil data.

Taphonomic bias. How can taphonomy and palaeoenvironment affect species frequencies (richness) and spatial representation? The fossil record contains buried assemblages of species that were derived from living communities at different times in the past. Species representation (presence or absence) in individual fossil assemblages is a critical attribute of our data sets, therefore we need to consider how this variable might be biased relative to original associations of species in communities. Taphonomic processes operate during the transition of dead remains into preserved samples and thus control the biological information that passes

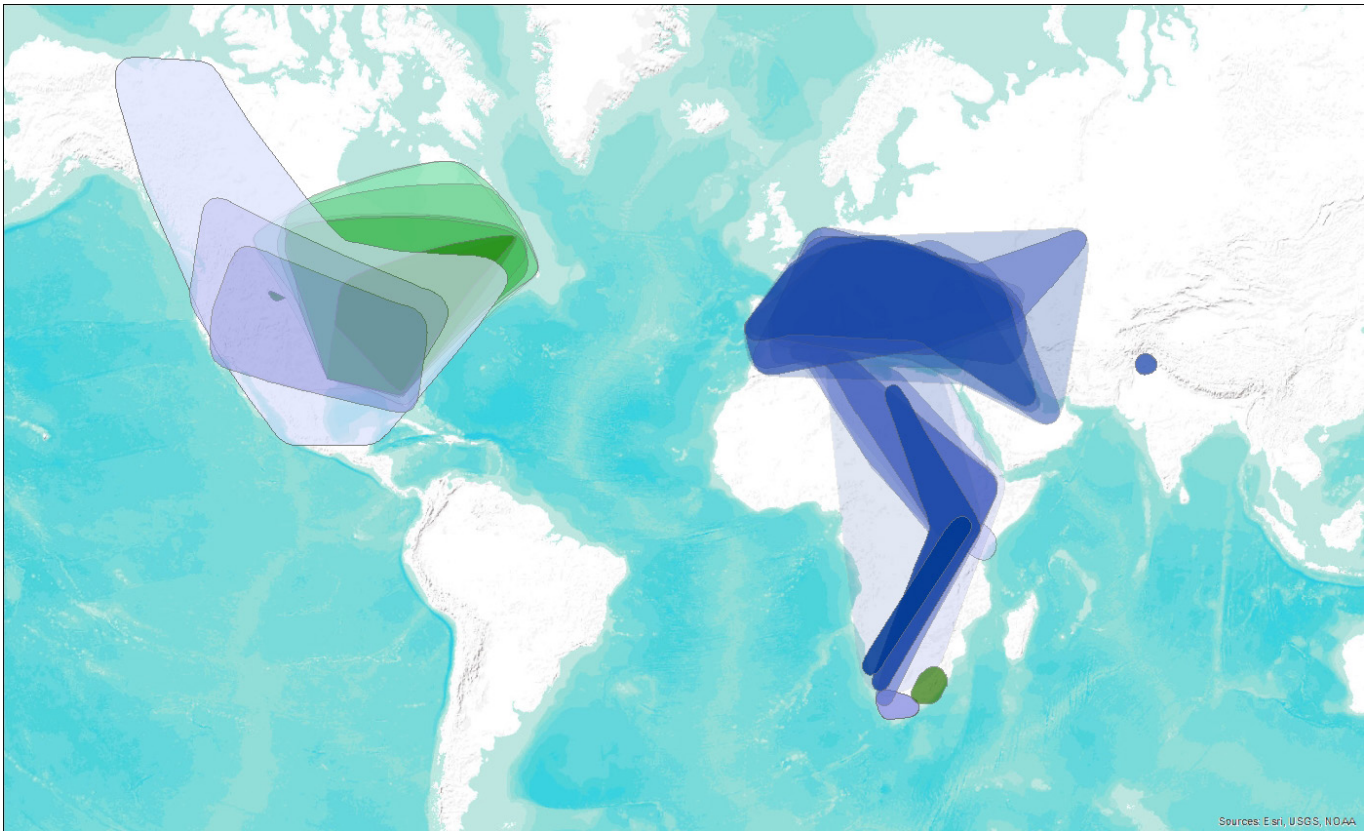
from the living community into the fossil record³⁹. These processes act as filters that can alter species representation in fossil samples in a variety of ways: (1) selective preservation of organisms with particular body compositions and sizes, for example organisms with and without mineralized skeletons, larger versus smaller individuals; (2) variable preservation of organisms depending on their population abundance, spatial distribution and life habits, for example aquatic versus terrestrial; (3) post-mortem or depositional mixing of species that did not live together (time-averaging), or separation of species that did (selective transport or destruction). Additionally, some types of environment are better represented in the depositional record than others, such as wetlands versus dry land surfaces. All of these add up to potential biases that could affect biological signals and the proportions of random versus significant species pairs, or the proportions of segregated versus aggregated pairs, in our analyses.

However, the particular null model algorithm used effectively controls for major sources of taphonomic bias in the data set. This 'fixed-fixed' algorithm³³ creates null assemblages that have the same species richness per sample, and the same number of occurrences per species, as the original data. Thus, if there are preservation biases that generate heterogeneity in the total number of fossil species per sample, or biases in the number of specimens per species, these are effectively controlled for in the analysis. Significant patterns of species aggregation are those measured beyond the effects of sampling heterogeneity in the occurrences of species or the number of species per sample. Similar sampling effects are controlled for in the modern data, which can also exhibit variation in the commonness or rarity of species and in the number of species per sample.

Taxonomic resolution of the data. Fossils are not always resolvable to the species level and are frequently analysed at the genus level. This may have the effect of increasing geographical ranges and overlap between taxa, and may contribute to the dominance of aggregated pairs found in this study. To test whether this was the case, we analysed 18 of the data sets at the species and genus level (16 mammal and 2 plant data sets). If taxonomic resolution is driving the pattern, we expect to see an increase in the proportion of aggregated pairs when species are lumped into genera. We found that six of the data sets showed the expected increase. However, nine showed a decrease and three showed no change (Extended Data Table 2). Interestingly, one of the modern data sets on small mammals from the Great Basin had genetic information that indicated that some were cryptic species. When the analysis was re-run with the cryptic species identified, there was an increase in the proportion of significantly aggregated pairs (from 50% to 61%). This is in the opposite direction that we would expect if lumping species into genera artificially increased aggregated pairs. Taken together, these results suggest that the differences between species associations over the past 300 Myr and the present are not driven by the taxonomic resolution of fossil data.

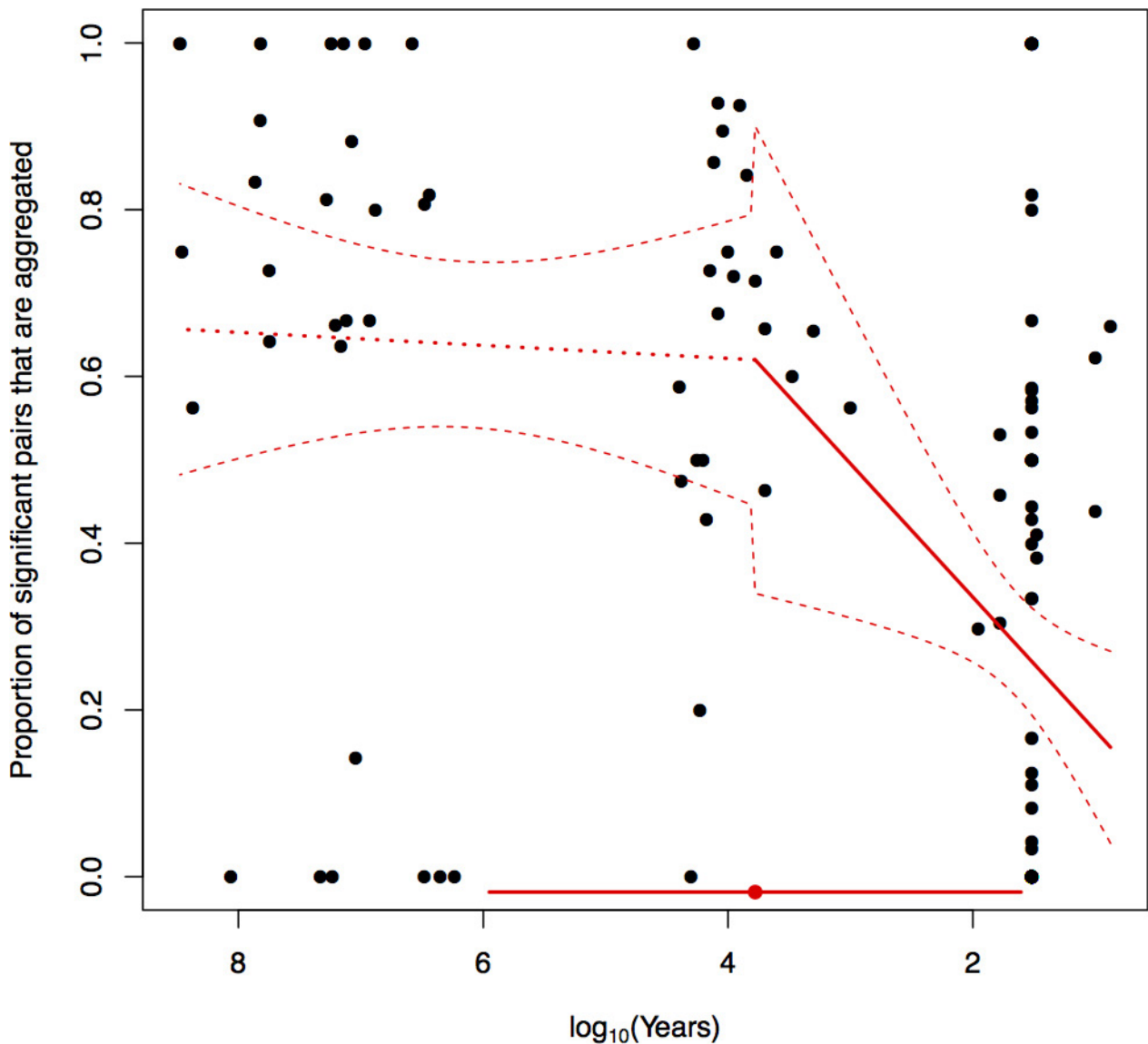
Sampling of abundant and rare species in fossil and modern data. The results of null model analyses of abundance versus presence-absence data are compared in ref. 10. The two kinds of analysis give qualitatively comparable results, although the abundance analyses are somewhat more powerful in detecting non-randomness. It is generally assumed that fossil deposits miss the rarest species in a community because preservation potential increases with abundance; more individuals means more opportunities for fossilization events. If rare species are more likely to form segregated pairs, we would expect to see more segregations in modern data sets because they should sample more of the rare species than comparable fossil data sets. Within fossil data sets, we would expect to see more segregated pairs in data sets with better sampling and more rare species. We evaluated this using a data visualization technique. We present the results of our analyses as a series of pairwise species by species matrices and order species by occupancy (see Supplementary Information: data sets). Occupancy decreases as one moves to the right on the x axis and up on the y axis. Species with the highest occupancy are close to the origin. The pairwise associations are colour-coded: grey for random pairs, blue for aggregated pairs, and red for segregated pairs. If increasing sampling of rare species is responsible for the pattern we document, then we would expect to see a preponderance of red, segregated pairs in the upper, right-hand corner of the species by species matrices. In particular, this should show up in data sets with better sampling and those that encompass the shift from more aggregated to more segregated pairs (for example, Pleistocene-Holocene mammals and pollen, modern mammals in Kenya, and modern plants in Wisconsin). This is not the pattern that we see. In fact, we find that segregated pairs tend to form with species of intermediate occupancy and that aggregated pairs form both with common species and with rare species. Differences in the sampling of rare species between fossil and modern data sets cannot account for the shift in species associations over time.

31. Ulrich, W. Pairs – a FORTRAN program for studying pair-wise species associations in ecological matrices. <http://www.keib.umk.pl/pairs/?lang=en>, (2010).
32. Stone, L. & Roberts, A. The checkerboard score and species distributions. *Oecologia* **85**, 74–79 (1990).
33. Gotelli, N. J. Null model analysis of species co-occurrence patterns. *Ecology* **81**, 2606–2621 (2000).
34. Ulrich, W. & Gotelli, N. J. Null model analysis of species nestedness patterns. *Ecology* **88**, 1824–1831 (2007).
35. Gotelli, N. J. & Ulrich, W. Statistical challenges in null model analysis. *Oikos* **121**, 171–180 (2012).
36. Efron, B. Bayesians, frequentists, and scientists. *J. Am. Stat. Assoc.* **100**, 1–5 (2005).
37. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis* (Springer, 2009).
38. Kidwell, S. M. & Holland, S. M. The quality of the fossil record: implications for evolutionary analyses. *Annu. Rev. Ecol. Syst.* **33**, 561–588 (2002).
39. Behrensmeier, A. K., Kidwell, S. M. & Gastaldo, R. A. Taphonomy and paleobiology. *Paleobiology* **26**, 103–147 (2000).



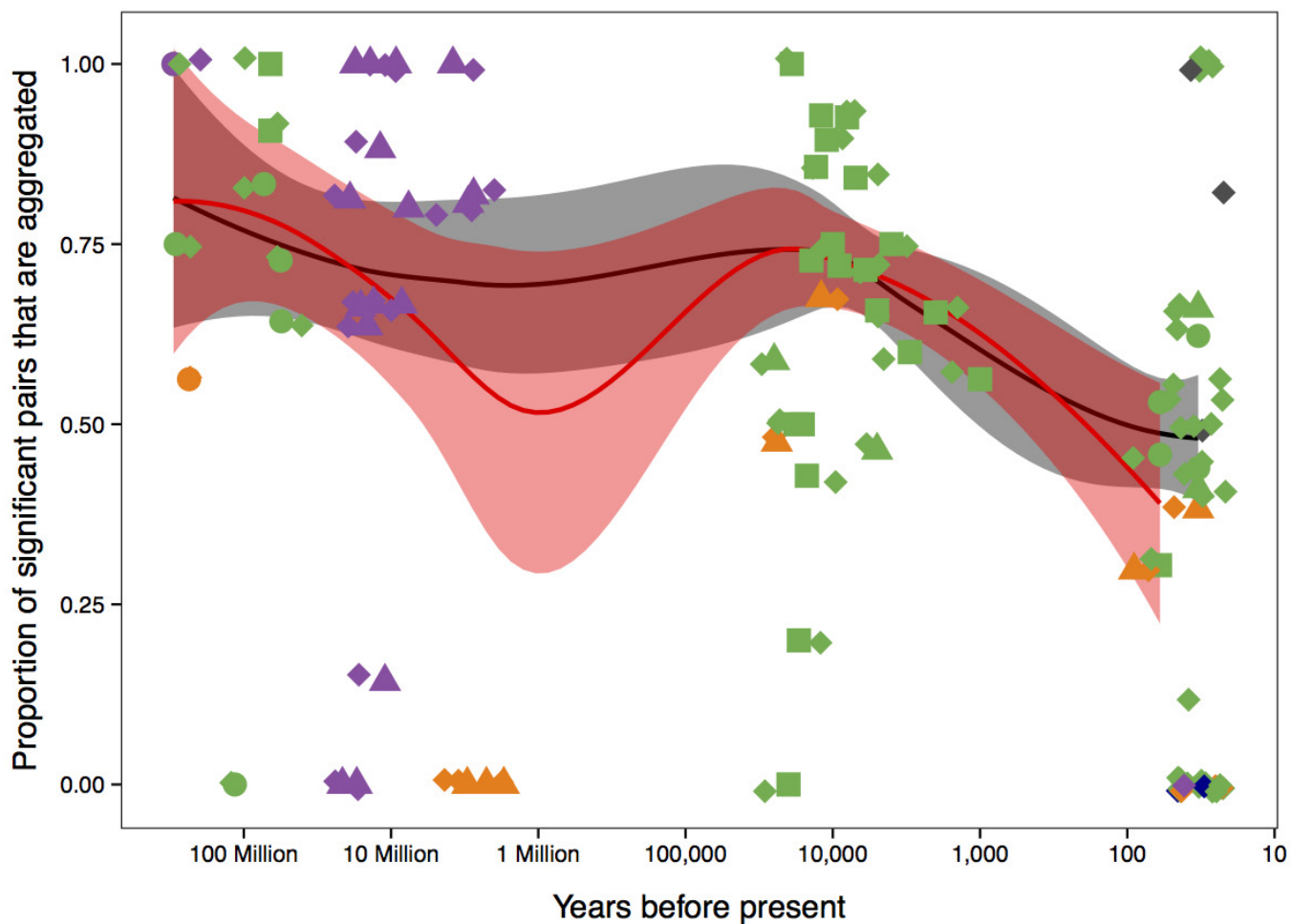
Extended Data Figure 1 | Map showing distribution of fossil data sets. Polygons enclose the localities for each fossil data set included in our analyses. Mammals are in blue, plants are in green. Dark colours represent data sets that are older. This map was created using ArcGIS software by Esri and can be found at <http://www.arcgis.com/home/item.html?id=>

[c61ad8ab017d49e1a82f580ee1298931](http://www.arcgis.com/home/item.html?id=c61ad8ab017d49e1a82f580ee1298931). ArcGIS and ArcMap are the intellectual property of Esri and are used herein under license. Copyright © Esri. All rights reserved. For more information about Esri software, please visit <http://www.esri.com>.



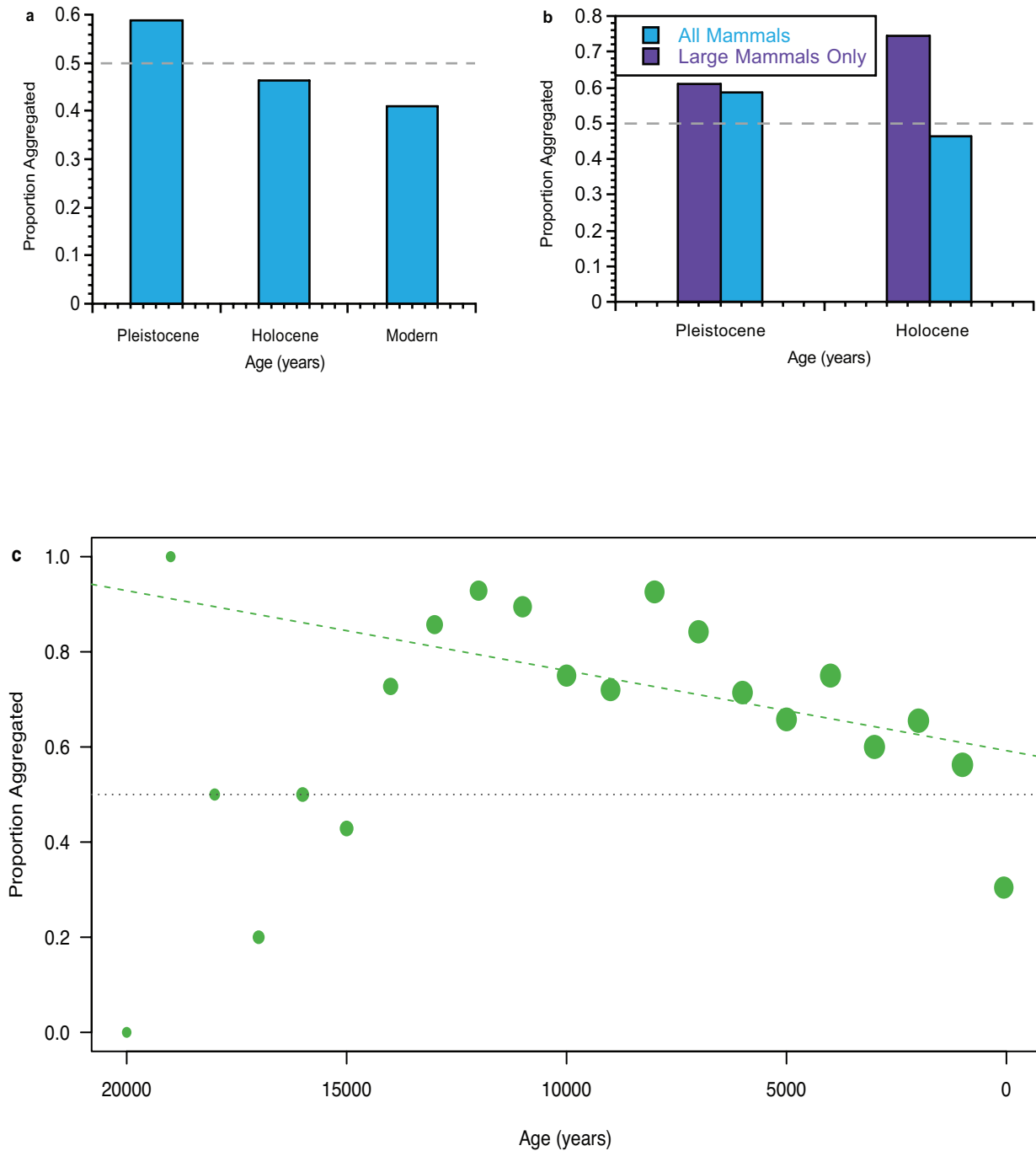
Extended Data Figure 2 | Breakpoint analysis of the composite data. The analysis was performed on all data including the islands (see Fig. 3 main text), showing the mean estimate (red point; $10^{3.778}$ years) and 95% confidence interval (error bar at base of plot; $10^{1.606}$, $10^{5.951}$ years) of the initiation of reduced percentage of aggregated species pairs, as well as

the mean and confidence intervals around the change in slope of the two resulting linear models. The breakpoint analysis was run using all the data resolved to the best possible dates to allow for the greatest amount of power in detecting where the switch occurred. However, the results were similar when island data were excluded.



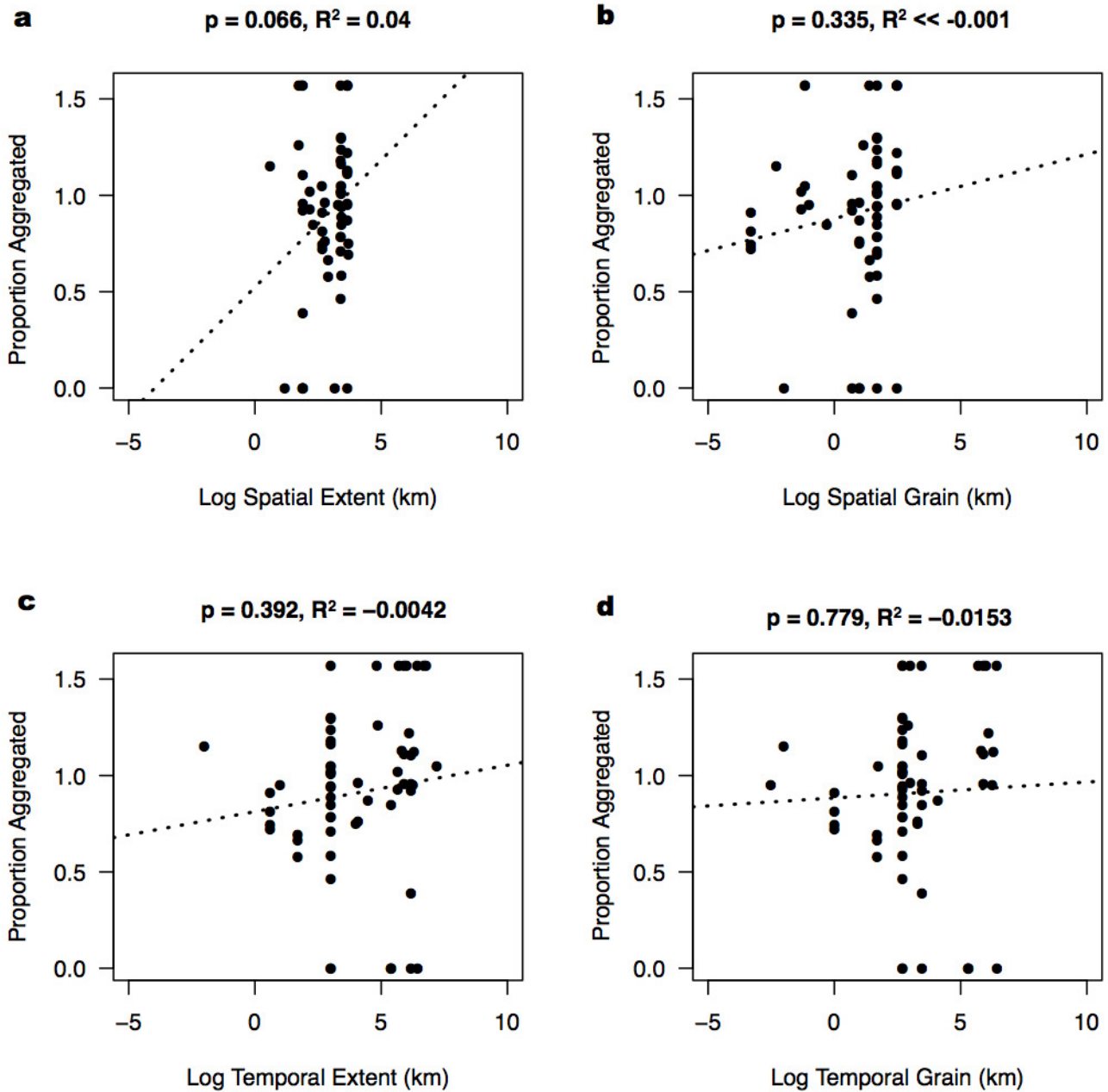
Extended Data Figure 3 | Weighted Loess curve with and without modern data. Loess curve weighted by number of sites with shaded 95% confidence intervals illustrates the reduction in the proportion of aggregated species pairs towards the present. Data are analysed with (black line and shading) and without (red line and shading) the modern data. Colours indicate continent: North America (green), Eurasia (purple),

Australia (dark grey), South America (dark blue), Africa (orange). Point shapes indicate type of data: pollen (square), mammals (triangle), macroplants (circle). Data on terrestrial communities from ref. 2 are dark diamonds. Only mainland assemblages were included in the calculation for the weighted Loess curve and the density plots here and in Fig. 1.



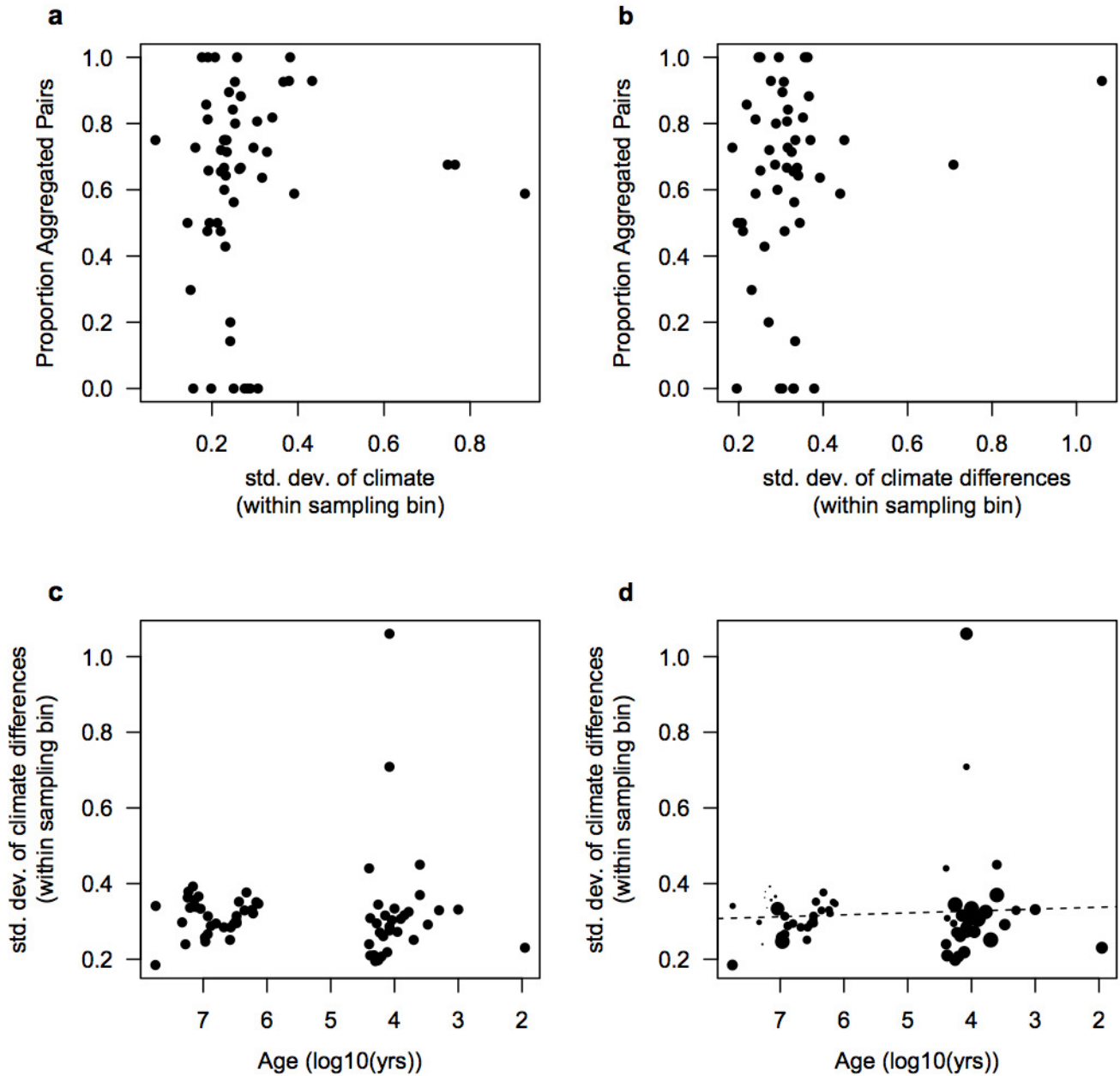
Extended Data Figure 4 | Results of PAIRS analyses of two Pleistocene–Holocene fossil data sets. **a**, Mammal data for three periods: late Pleistocene (40,000–10,000 years ago), Holocene (10,000–500 years ago), and modern (present, literature survey data). Note the switch from >50% aggregated pairs to <50% aggregated pairs in the Pleistocene versus Holocene data sets. **b**, Results for large and small mammals separately. There is a significant difference ($P < 0.001$) between the Holocene and the Pleistocene for all mammals (blue bars) and for large mammals (purple bars) only ($P = 0.015$). However, the direction of the shift was different. For all mammals, there were fewer positive associations in the Holocene, whereas, for large mammals only, there were more positive associations in the Holocene. **c**, North American pollen data from the past 21,000 years

(modified from ref. 20). Data are from cores resolved into 1,000-year time slices. The size of the circle is related to the number of sites in the data set. The point at 0 represents a period from the present to 1,000 years ago, but is sampled from the top of the pollen cores using the same methodology as the older time slices. Note the trend of decreasing percentage of aggregations towards the present, especially in times with the largest numbers of sites (after 14,000 years). A weighted regression that takes into account the number of sites in each time slice is significant (dashed green line; $P = 0.04$, adjusted $R^2 = 0.15$). The final time slice at 0 records a shift from a dominance of aggregated pairs to a dominance of segregated pairs. The sampling methods and data structure are the same for all time slices. Grey dashed line is at 50% in each panel.



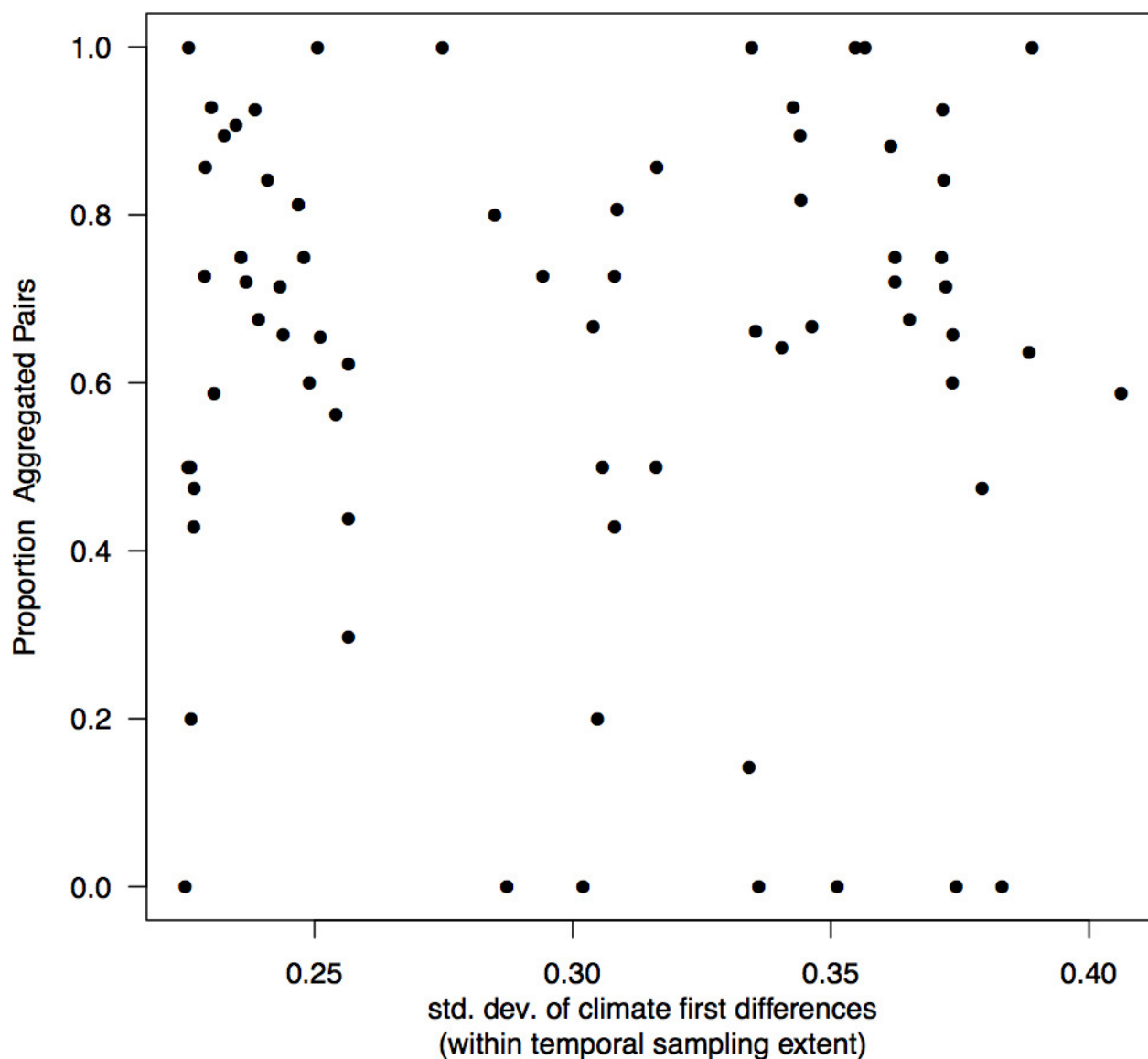
Extended Data Figure 5 | Relationship between the proportion of aggregated pairs and scale. The proportion of significant pairs that are aggregated is not the result of temporal or spatial scale of data. Arcsine transformation of the proportion of significant pairs that are aggregated

plotted as a function of spatial (a, b) or temporal (c, d) grain (b, d) or extent (a, c). Linear regressions are non-significant and adjusted R^2 values are extremely low.



Extended Data Figure 6 | Climate variability measured during the temporal extents of the fossil data sets. Proportion of significant pairs that are aggregated shows no relationship with climate variability within a time interval. **a, b**, Climate variability was quantified as the standard deviation of all climate proxy data for that time interval (**a**), or the standard deviation of the first differences in climate across the interval (**b**). **c, d**, Climate variability (standard deviation of first differences) and

age of data sets show no relationship (**c**), suggesting no trend in climate variability sampled by the fossil data sets across the Phanerozoic. There is a significant, but weak, positive relationship (**d**, dashed line) between climate variability and decreasing age of the data sets when the linear model is weighted by sample size of climate proxy data enveloped by the temporal window of the fossil data sets ($P=0.007$, adjusted $R^2=0.0998$).



Extended Data Figure 7 | Relationship between proportion of aggregated pairs and fixed-width time intervals. High-amplitude Pleistocene climate variability oscillating between glacial and interglacial cycles may have imposed its own novel pressures on floral and faunal communities. Furthermore, ecological impacts may lag behind climate episodes themselves, complicating efforts to quantify climatic links to changes in the proportion of aggregated species pairs over time. Thus, limiting our measure of climate variability to the temporal span of the data

sets themselves may potentially not account for important (and possibly ecologically significant) climatic variability from the previous millennia. To incorporate this possibility, we re-analysed the relationship between the proportion of aggregated species pairs and climate variability of each data set, but included climate across the preceding 100,000 years, 10,000 years (not shown), and 1,000 years (not shown). As in the more restrictive analysis (Fig. 3a), there is consistently no relationship between climate variability and the proportion of aggregated species pairs.

Extended Data Table 2 | Effect of taxonomic resolution

Dataset	% Aggregations for Species	% Aggregations for Genera	Direction of difference when lumping
WI Overstory Plants-1950	0.531	0.800	Increase
WI Overstory Plants-2000	0.622	0.773	Increase
Eurasia mammals MN02	0	0	No change
Eurasia mammals MN03	0.815	0.732	Decrease
Eurasia mammals MN04	1	0.75	Decrease
Eurasia mammals MN05	0.662	0.608	Decrease
Eurasia mammals MN06	1	0.75	Decrease
Eurasia mammals MN07	0.882	0.864	Increase
Eurasia mammals MN09	1	0.75	Decrease
Eurasia mammals MN10	0.667	0.857	Increase
Eurasia mammals MN11	NA	1	Increase
Eurasia mammals MN14	NA	NA	No change
Eurasia mammals MN15	1	1	No change
Eurasia mammals MN16	0.806	1	Increase
Eurasia mammals MN17	0.818	0.619	Decrease
Africa mammals - historical	0.298	0.028	Decrease
Africa mammals – modern	0.382	0.031	Decrease
Great Basin Rodents Cryptic	0.661	0.500	Decrease

Change in the proportion of significant pairs when data sets are analysed at the species and genus levels. If lower taxonomic resolution of fossil data sets is driving the pattern of increased aggregations in the fossil data, we would expect to see increases in the percentage of aggregations when data are analysed at the genus level. Instead, most data sets show a decrease in the percentage of aggregated pairs. Only 6 of the 18 data sets analysed at multiple taxonomic resolutions show the expected increase. Nine show a decrease and three show no change. One data set (Great Basin Rodents Cryptic) was analysed at the species level and then taxonomically resolved with genetic data to include cryptic species. For that data set only, '% aggregations for genera' corresponds to the data set with cryptic species lumped and '% aggregations for species' corresponds to the data set with cryptic species resolved.

Extended Data Table 3 | Proportion of aggregated pairs across critical intervals

Taxon	Event	Place	Trend in % Positive Associations	p value
Large Mammals	Pleistocene-Holocene Transition	South Africa	Increase	0.007
Mammals	Pleistocene-Holocene Transition	North America	Decrease	<0.001
Pollen	Pleistocene-Holocene Transition	North America	Decrease	0.0008
Plants	PETM	North America	No Change	0.556
Pollen	K-Pg	North America	No Change	0.853
Pollen	Permian Crises	Greenland	No significant pairs	na

Significance of change in positive versus negative associations across critical intervals.