UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
DOUTORADO EM BIOLOGIA ANIMAL
PROCESSO SELETIVO 2017-2018

Prova Teórica

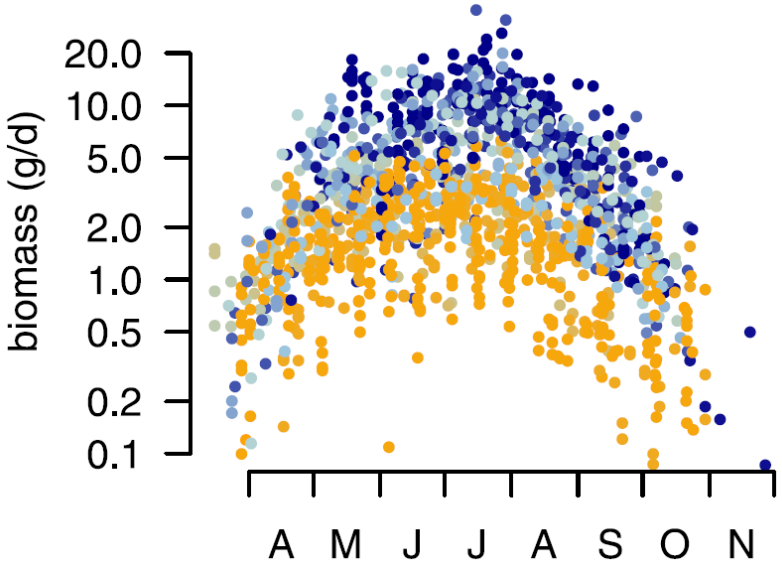
No. de Inscrição: _____ **13 de novembro de 2017**

PARTE 1 [6,0 pontos]

a) Escreva um resumo de no máximo de 200 palavras para cada um dos artigos científicos apresentados, de onde foram removidos o título e resumo originais (Anexos 1 a 3). Cada sentença do texto deve começar em uma linha diferente. [Valor: 1,5 pontos cada resumo]
b) Proponha um título criativo que melhor sintetize cada um dos artigos [Valor: 0,5 ponto cada título]

PARTE 2 [4,0 pontos]
Questões objetivas.

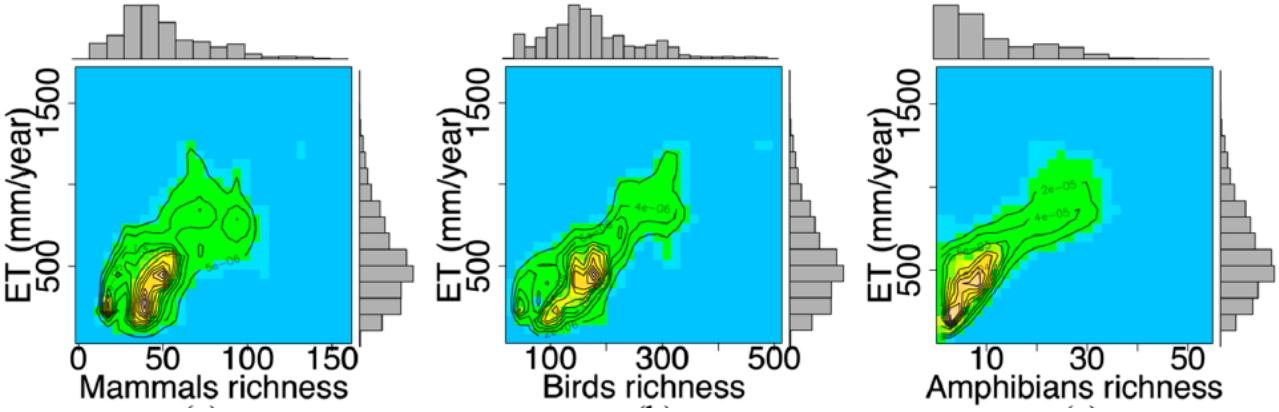
Questão 01. Qual a conclusão mais relevante que pode ser tirada dos dados exibidos pelo gráfico abaixo, de uma pesquisa conduzida em um país de clima temperado?



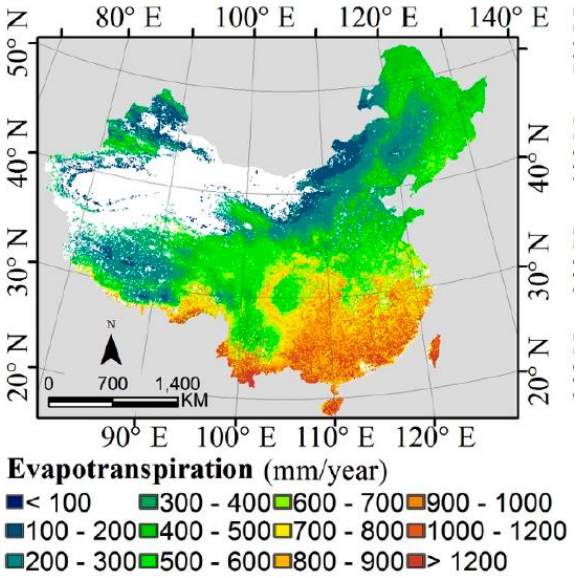
Seasonal distribution of insect biomass (gram per day) pooled over all traps and catches in each year (n = 1503). Color gradient ranges from 1989 (blue) to 2016 (orange).

A. Diminuição do número de espécies de insetos
B. Convergência da biomassa em direção ao mês de julho
C. Redução da atividade de vôo no início/final do ano
D. Declínio anual da biomassa de insetos
E. Perda de massa corporal média anualmente
F. Regularidade exponencial da diversidade ao longo do ano
G. Distribuição anual claramente parabólica ($ax^2 + bx + c$)

Questão 02. Analisando os resultados apresentados nas figuras abaixo, qual a conclusão correta sobre a diversidade de vertebrados na China?



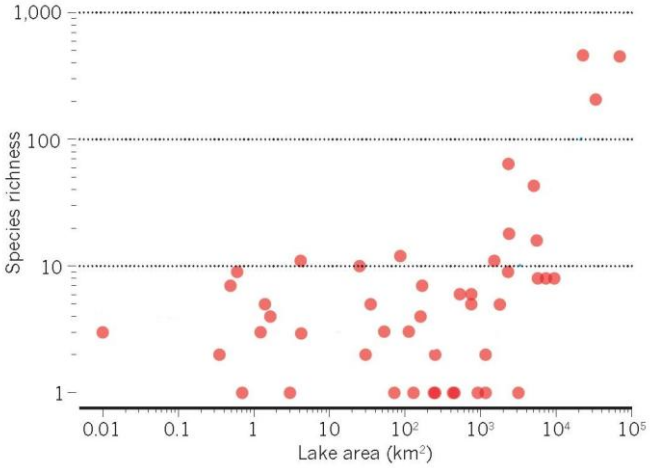
Frequency distributions of species richness based on field surveys versus long-term averages of evapotranspiration (ET) in China.



Average evapotranspiration in China.

A. Deve ser máxima no noroeste
B. Deve ser maior na faixa central de latitude
C. Deve ser máxima aproximadamente ente 19˚-25˚ N
D. Deve aumentar progressivamente do sul para o norte
E. Deve aumentar progressivamente do norte para o sul
F. Deve ser máxima no sudoeste
G. Deve ser inversamente proporcional à latitude

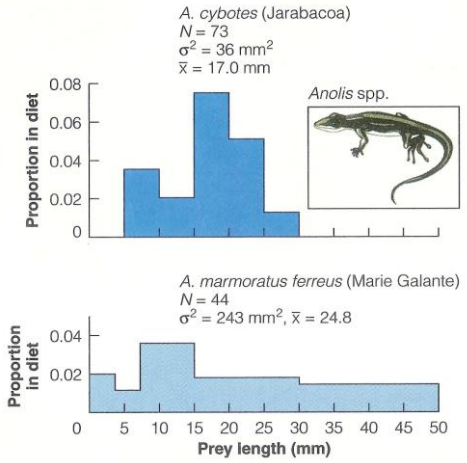
Questão 03. Analise o gráfico e marque a melhor interpretação para os resultados que apresenta:



Species-area relationship for cichlid fish in African lakes.

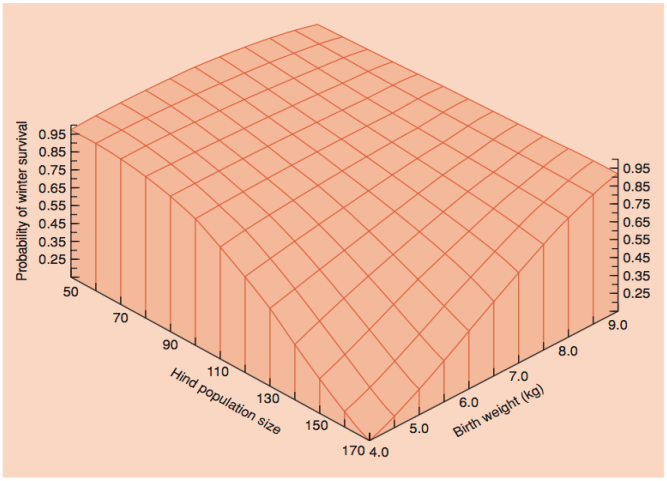
A. A maior diversidade de ciclídeos ocorre em lagos entre100 e 1000 km^2, aproximadamente
B. Há correlação exponencial entre o tamanho dos lagos e o número de espécies de ciclídeos
C. Não há correlação significativa entre o tamanho dos lagos e a diversidade de ciclídeos
D. Há correlação logarítimica entre o tamanho dos lagos e a diversidade total de ciclídeos
E. O tamanho corporal médio dos ciclídeos aumenta com o tamanho dos lagos
F. O efeito dos lagos na especiação de ciclídeos é nulo até 1000 km^2, mas intenso a partir daí
G. A razão entre o número de espécies e a área do lago é aproximadamente constante

Questão 04. Com base nos resultados apresentados abaixo, de um estudo com duas espécies de *Anolis*, de duas ilhas diferentes (Jarabacoa e Marie Galante), escolha a conclusão correta:



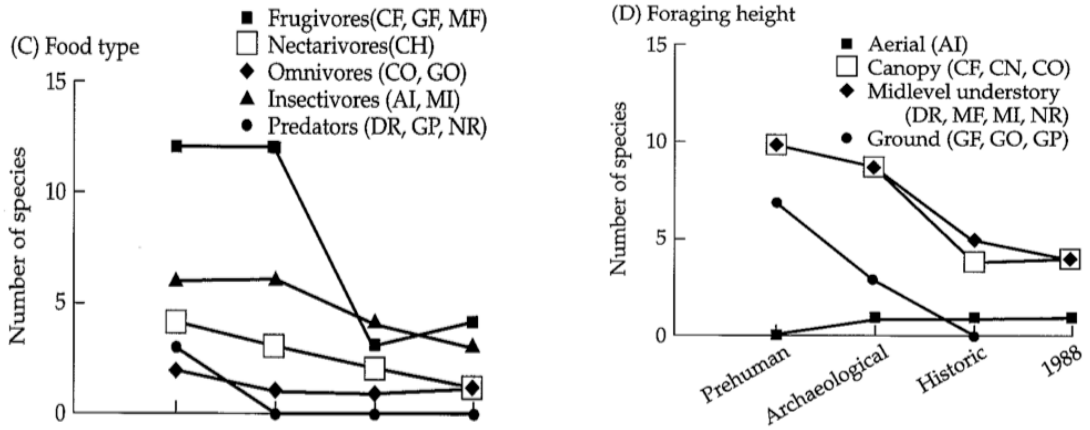
A. *Anolis cybotes* enfrenta competição mais acirrada do que *A. marmoratus*
B. *Anolis marmoratus* é dominante em relação a *A. cybotes*
C. *Anolis cybotes* é um predador menos eficiente que *A. marmoratus*
D. A área sob as curvas é igual, indicando que as duas espécies são predadores equivalentes
E. Marie Galante tem extensão territorial maior do que Jarabacoa
F. *Anolis marmoratus* ocupa um nicho mais restrito que *A. cybotes*
G. *Anolis cybotes* é mais voraz que *A. marmoratus*

Questão 05. Assinale a alternativa correta com base no gráfico abaixo:



A. A chance de sobrevivência de uma animal que nasce com 5 kg em uma população de 150 indivíduos é maior do que a chance de sobrevivência de um animal que nasce com 8 kg em uma população de 70 indivíduos.
B. A probabilidade de sobrevivência no inverno é diretamente proporcional ao tamanho populacional e inversamente proporcional ao peso do animal ao nascer.
C. Indivíduos que nascem maiores tem menor chance de sobreviver no inverno.
D. A probabilidade de sobrevivência no inverno é maior do que no verão.
E. Quanto maior o tamanho da população, menor o peso ao nascer.
F. A probabilidade de sobrevivência no inverno de quem nasce com 4 kg em uma população de 150 indivíduos é a mesma de quem nasce com 5,5 kg em uma população de 170 indivíduos.
G. O tamanho populacional depende do peso no inverno.

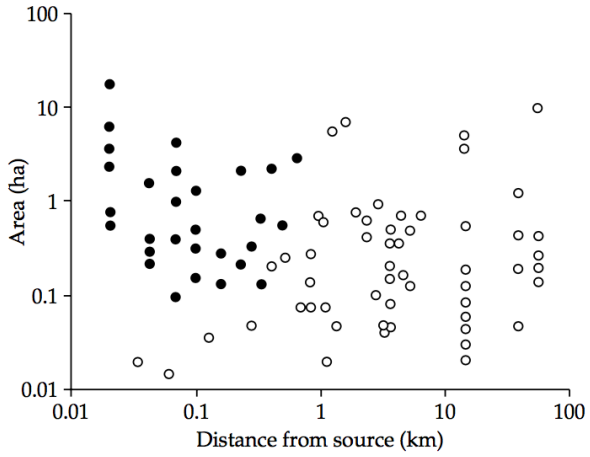
Questão 06. Escolha a alternativa ***falsa*** com base nos gráficos abaixo.



Richness of forest birds on an island through time according to diet (C) and (D) foraging preference.

A. A eliminação das espécies terrestres levou à extinção dos predadores.
B. Frugívoros e insetívoros não sofreram com as primeiras ocupações humanas.
C. Espécies frugívoras e terrestres foram as mais impactadas.
D. A riqueza diminuiu com o tempo em todos os grupos, exceto nas espécies aéreas.
E. Espécies da copa e do sub-bosque apresentaram tendências quase idênticas ao longo do tempo.
F. Onívoros foram os que apresentaram menor flutuação na riqueza durante todo o período.
G. Frugívoros foram beneficiados com a introdução recente de árvores frutíferas.

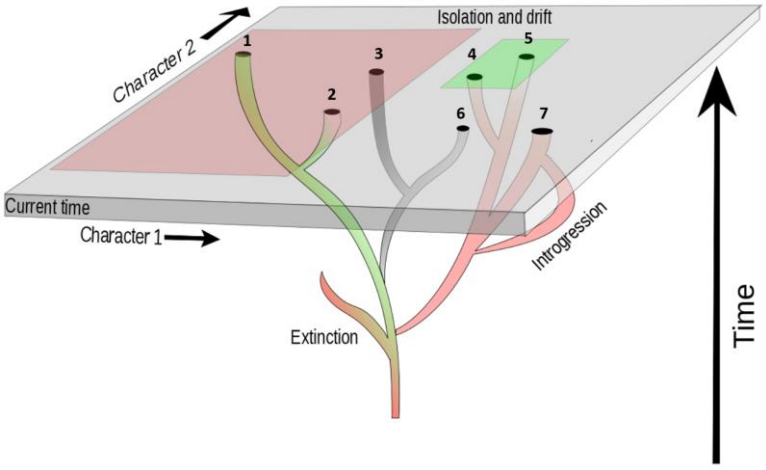
Questão 07. Escolha a alternativa correta com base no gráfico abaixo.



Occupancy of suitable habitat by the silver-studded blue butterfly (Plebejus argus) in North Wales in 1990. Filled circle = occupied patch; open circle = unoccupied patch.

A. A distância da fonte aumenta na medida em que a área aumenta.
B. A área é inversamente proporcional à distância percorrida pelas borboletas.
C. As borboletas ocupam fragmentos de mais de 0,1 ha, desde que eles estejam a pelo menos 1 km de distância da fonte.
D. O habitat ideal para as borboletas tem menos de 1 ha e está entre 0,1 e 1 km de distância da fonte.
E. A distância da fonte é mais importante do que a área para a ocupação das borboletas.
F. Borboletas ocupadas percorrem distâncias menores se estiverem em círculos abertos.
G. As borboletas ocupam fragmentos de mais de 0,1 ha, desde que com menos 1 km de distância da fonte.

Questão 08. Com base na figura, indique qual das alternativas abaixo está ***incorreta***.

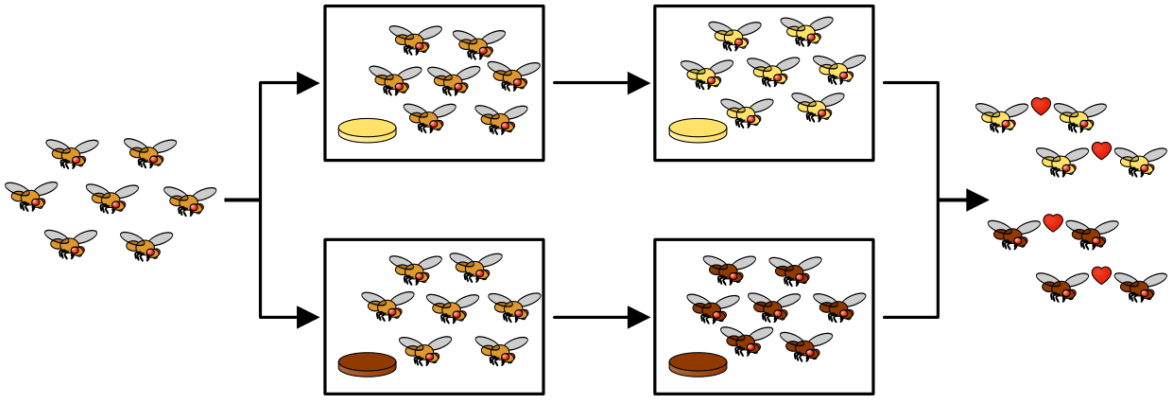


A comparison of phylogenetic and phenetic (character-based) concepts.

A. A fenética estabele a relação de organismos baseado em similaridades não fazendo distinção entre plesiomorfias e apomorfias.
B. Os agrupamentos fenéticos 1+2+3 e 4+5 não seriam recuperados no método filogenético.
C. O caráter 1 apresenta uma resolução maior que o caráter 2 na distinção de grupos fenéticos.

D. Os agrupamentos filogenéticos e fenéticos são distintos.
E. A figura deixa claro que a similaridade dos caracteres não reflete a filogenia.
F. A espécie 3 é mais parecida com a espécie 2, mas é filogeneticamente mais próxima da espécie 4.
G. A espécie 6 compartilha ancestral comum mais recente com a espécie 1 do que com a espécie 4.

Questão 09. Observe o esquema abaixo do famoso experimento de Dodd com populações de *Drosophila pseudoobscura*, criadas isoladas por várias gerações em meios com amido (amarelo) ou maltose (marrom) e marque a alternativa ***correta***:



A. O isolamento reprodutivo das populações levou ao isolamento geográfico entre as espécies.
B. O meio selecionou positivamente os animais homozigotos para a coloração.
C. O meio provocou mutação, aparecendo formas variantes na população com o passar do tempo e consequentemente o início de uma especiação parapátrica.
D. Estamos observando o início de uma especiação simpátrica.
E. No final do experimento, moscas criadas no amido preferem cruzar com moscas criadas na maltose e vice-versa.
F. Após longo tempo isoladas, ocorreu isolamento reprodutivo entre as populações do amido e da maltose.
G. Após muitas gerações, as moscas não conseguem diferenciar parceiros criados no mesmo meio ou em um meio diferente.

[continua...]

Questão 10. Qual alternativa melhor explica a figura abaixo?

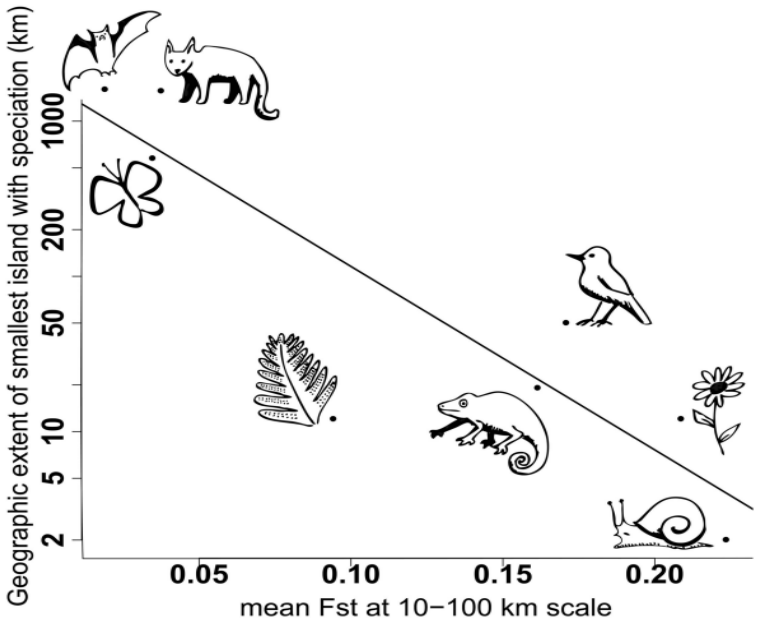


Figure 4: Minimum island size for speciation versus the average strength of gene flow when measured over geographic ranges of 10–100 km.

A. Quanto menor o táxon, maior o fluxo gênico
B. Táxons com menos fluxo gênico especiam em ilhas menores
C. Quanto maior a ilha, maiores os indivíduos
D. Quanto maior o fluxo gênico, maior a especiação
E. O tamanho da ilha não influencia na taxa de fluxo gênico
F. Animais apresentam mais especiação e fluxo gênico do que plantas
G. Especiação nas aves ocorre em ilhas menores do que o esperado dado seu fluxo gênico.

* * *

No. de Inscrição: _____

Questão:	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**
Resposta:										

Anexo 1

Gerardo Ceballos[a,1], Paul R. Ehrlich[b,1], and Rodolfo Dirzo[b]

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Contributed by Paul R. Ehrlich, May 23, 2017 (sent for review March 28, 2017; reviewed by Thomas E. Lovejoy and Peter H. Raven)

The loss of biological diversity is one of the most severe human-caused global environmental problems. Hundreds of species and myriad populations are being driven to extinction every year (1–8). From the perspective of geological time, Earth's richest biota ever is already well into a sixth mass extinction episode (9–14). Mass extinction episodes detected in the fossil record have been measured in terms of rates of global extinctions of species or higher taxa (e.g., ref. 9). For example, conservatively almost 200 species of vertebrates have gone extinct in the last 100 y. These represent the loss of about 2 species per year. Few realize, however, that if subjected to the estimated "background" or "normal" extinction rate prevailing in the last 2 million years, the 200 vertebrate species losses would have taken not a century, but up to 10,000 y to disappear, depending on the animal group analyzed (11). Considering the marine realm, specifically, only 15 animal species have been recorded as globally extinct (15), likely an underestimate, given the difficulty of accurately recording marine extinctions. Regarding global extinction of invertebrates, available information is limited and largely focused on threat level. For example, it is estimated that 42% of 3,623 terrestrial invertebrate species, and 25% of 1,306 species of marine invertebrates assessed on the International Union for Conservation of Nature (IUCN) Red List are classified as threatened with extinction (16). However, from the perspective of a human lifetime it is difficult to appreciate the current magnitude of species extinctions. A rate of two vertebrate species extinctions per year does not generate enough public concern, especially because many of those species were obscure and had limited ranges, such as the Catarina pupfish (*Megupsilon aporus*, extinct in 2014), a tiny fish from Mexico, or the Christmas Island pipistrelle (*Pipistrellus murrayi*, extinct in 2009), a bat that vanished from its namesake volcanic remnant.

Species extinctions are obviously very important in the long run, because such losses are irreversible and may have profound effects ranging from the depletion of Earth's inspirational and esthetic resources to deterioration of ecosystem function and services (e.g., refs. 17–20). The strong focus among scientists on species extinctions, however, conveys a common impression that Earth's biota is not dramatically threatened, or is just slowly entering an episode of major biodiversity loss that need not generate deep concern now (e.g., ref. 21, but see also refs. 9, 11, 22). Thus, there might be sufficient time to address the decay of biodiversity later, or to develop technologies for "deextinction"—the possibility of the latter being an especially dangerous misimpression (see ref. 23). Specifically, this approach has led to the neglect of two critical aspects of the present extinction episode: (*i*) the disappearance of populations, which essentially always precedes species extinctions, and (*ii*) the rapid decrease in numbers of individuals within some of the remaining populations. A detailed analysis of the loss of individuals and populations makes the problem much clearer and more worrisome, and highlights a whole set of parameters that are increasingly critical in considering the Anthropocene's biological extinction crisis.

Author contributions: G.C., P.R.E., and R.D. designed research; G.C. and P.R.E. performed research; G.C., P.R.E., and R.D. contributed new reagents/analytic tools; G.C. analyzed data; and G.C., P.R.E., and R.D. wrote the paper.

Reviewers: T.E.L., George Mason University; and P.H.R., Missouri Botanical Garden.

The authors declare no conflict of interest.

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In the last few decades, habitat loss, overexploitation, invasive organisms, pollution, toxification, and more recently climate disruption, as well as the interactions among these factors, have led to the catastrophic declines in both the numbers and sizes of populations of both common and rare vertebrate species (24–28). For example, several species of mammals that were relatively safe one or two decades ago are now endangered. In 2016, there were only 7,000 cheetahs in existence (29) and less than 5,000 Borneo and Sumatran orangutans (*Pongo pygmaeus* and *P. abelli*, respectively) (28). Populations of African lion (*Panthera leo*) dropped 43% since 1993 (30), pangolin (*Manis* spp.) populations have been decimated (31), and populations of giraffes dropped from around 115,000 individuals thought to be conspecific in 1985, to around 97,000 representing what is now recognized to be four species (*Giraffa giraffa*, *G. tippelskirchi*, *G. reticulata*, and *G. camelopardalis*) in 2015 (32).

An important antecedent to our work (25) used the number of genetic populations per unit area and then estimated potential loss on the basis of deforestation estimates and the species–area relationship (SAR). Given the recognized limitations of the use of SAR to estimate extinctions, our work provides an approach based on reduction of species range as a proxy of population extirpation. The most recent Living Planet Index (LPI) has estimated that wildlife abundance on the planet decreased by as much as 58% between 1970 and 2012 (4). The present study is different from LPI and other related publications in several ways, including that here we use all decreasing species of vertebrates according to IUCN, mapping and comparing absolute and relative numbers of species, and focusing on population losses. Previous estimates seem validated by the data we present here on the loss of local populations and the severe decrease in the population size of many others (see also refs. 3, 4, 6–8, 26). Here we examine the magnitude of losses of populations of land vertebrate species on a global system of 10,000-km^2 quadrats (*Methods*). Species vary from common to rare, so that our analysis, which includes all land vertebrate species (amphibians, birds, reptiles, and mammals) deemed as "decreasing" by IUCN, provides a better estimate of population losses than using exclusively IUCN data on species at risk. Obviously, common species decreasing are not ordinarily classified as species at risk. IUCN criteria provide quantitative thresholds for population size, trend, and range size, to determine decreasing species (28, 33). We also evaluate shrinking ranges and population declines for 177 species of mammals for which data are available on geographic range shrinkage from ~1900 to 2015. We specifically focus on local extinctions by addressing the following questions: (*i*) What are the numbers and geographic distributions of decreasing terrestrial vertebrate species (i.e., experiencing population losses)? (*ii*) What are the vertebrate groups and geographic regions that have the highest numbers and proportions of decreasing species? (*iii*) What is the scale of local population declines in mammals—a proxy for other vertebrates? By addressing these questions, we conclude that anthropogenic population extinctions amount to a massive erosion of the greatest biological diversity in the history of Earth and that population losses and declines are especially important, because it is populations of organisms that primarily supply the ecosystem services so critical to humanity at local and regional levels.

Results

Patterns of Variation in Population Loss Among Vertebrates. Considering all land vertebrates, our spatially explicit analyses indicate a massive pulse of population losses, with a global epidemic of species declines. Those analyses support the view that the decay of vertebrate animal life is widespread geographically, crosses phylogenetic lineages, and involves species ranging in abundance from common to rare (Figs. 1–4). The losses, however, are not uniform: some regions exhibit higher concentrations of species with local population extinctions than others, including a strong latitudinal signal corresponding to an intertropical peak (i.e., roughly between the Tropics of Cancer and Capricorn) of number of decreasing species, particularly strong in mammals and birds, which largely drive the overall land vertebrate pattern (Fig. 3, *Center*). Notably, some parts of the planet harbor low absolute numbers of vertebrate species undergoing decline (Figs. 2 and 3), such as those areas of low species richness located in hypercold (northernmost locations, particularly of the Western Hemisphere) and hyperarid (Saharan Africa and Central Asia) regions. However, it is instructive to examine their corresponding proportional numbers, an aspect we discuss in detail in another section below.

The number of decreasing species of all land vertebrates in each of the 10,000-km^2 quadrats over Earth's land surface ranges from a few to more than 365 (Fig. 2). As expected, large concentrations of decreasing vertebrate species occur in species-rich areas of moist tropical forests adjacent to mountainous regions, such as the Andes–Amazon region, the Congo basin-adjacent eastern African highlands, and the Himalayas–south Asian jungle belt. The distribution of the number of decreasing species considering vertebrate classes separately reveals notable differences. First, the maximum number of decreasing species in a 10,000-km^2 quadrat varies from a high value of 296 decreasing birds per quadrat, to a low maximum of 60 decreasing reptiles in a quadrat. Second, mammals and birds have relatively similar distribution patterns of

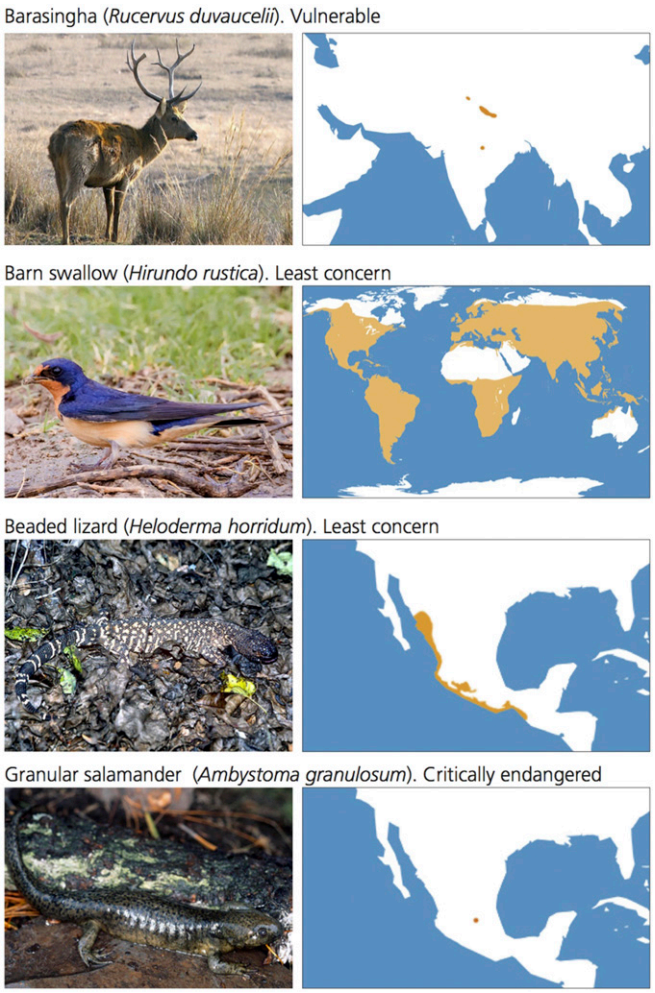

Fig. 1. Decreasing land vertebrates, as exemplified with these four species, include taxa with different conservation status (e.g., low concern, critically endangered), current geographic range (e.g., large, very restricted), and abundance (e.g., common, rare). The data on conservation status, current geographic range, and abundance are from IUCN (28). Barn swallow image courtesy of Daniel Garza Galindo (photographer).

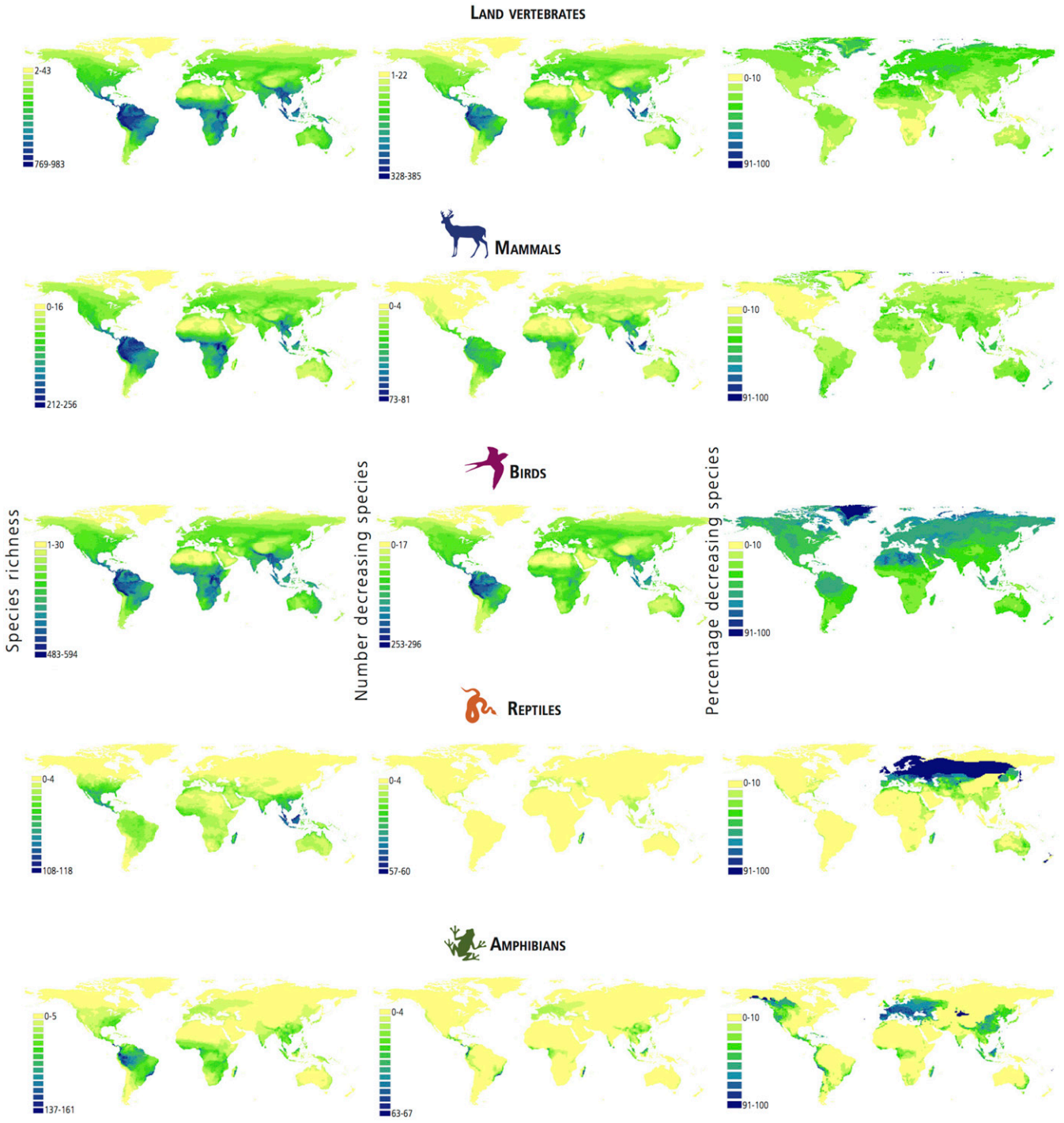

Fig. 2. Global distribution of terrestrial vertebrate species according to IUCN (28). (*Left*) Global distribution of species richness as indicated by number of species in each 10,000-km^2 quadrat. (*Center*) Absolute number of decreasing species per quadrat. (*Right*) Percentage of species that are suffering population losses in relation to total species richness per quadrat. The maps highlight that regions of known high species richness harbor large absolute numbers of species experiencing high levels of decline and population loss (particularly evident in the Amazon, the central African region, and south/southeast Asia), whereas the proportion of decreasing species per quadrat shows a strong high-latitude and Saharan Africa signal. In addition, there are several centers of population decline in both absolute and relative terms (Borneo, for example).

decreasing species, except that birds have more decreasing species in the temperate zones. Third, mammals and birds have patterns of decreasing species quite distinct from those of reptiles and amphibians (Figs. 2 and 3), given that the latter are rarer in the northern and southern temperate and subpolar regions (both are essentially absent from the Arctic and are missing from the Antarctic). Fourth, reptiles and amphibians clearly differ from each other in regions where decreasing species are concentrated. For example, there are more decreasing reptiles in the Eurasian and African continents, and more decreasing amphibians in the Americas.

There is also great variation in the total population size and geographic ranges among individual species. Although there is no

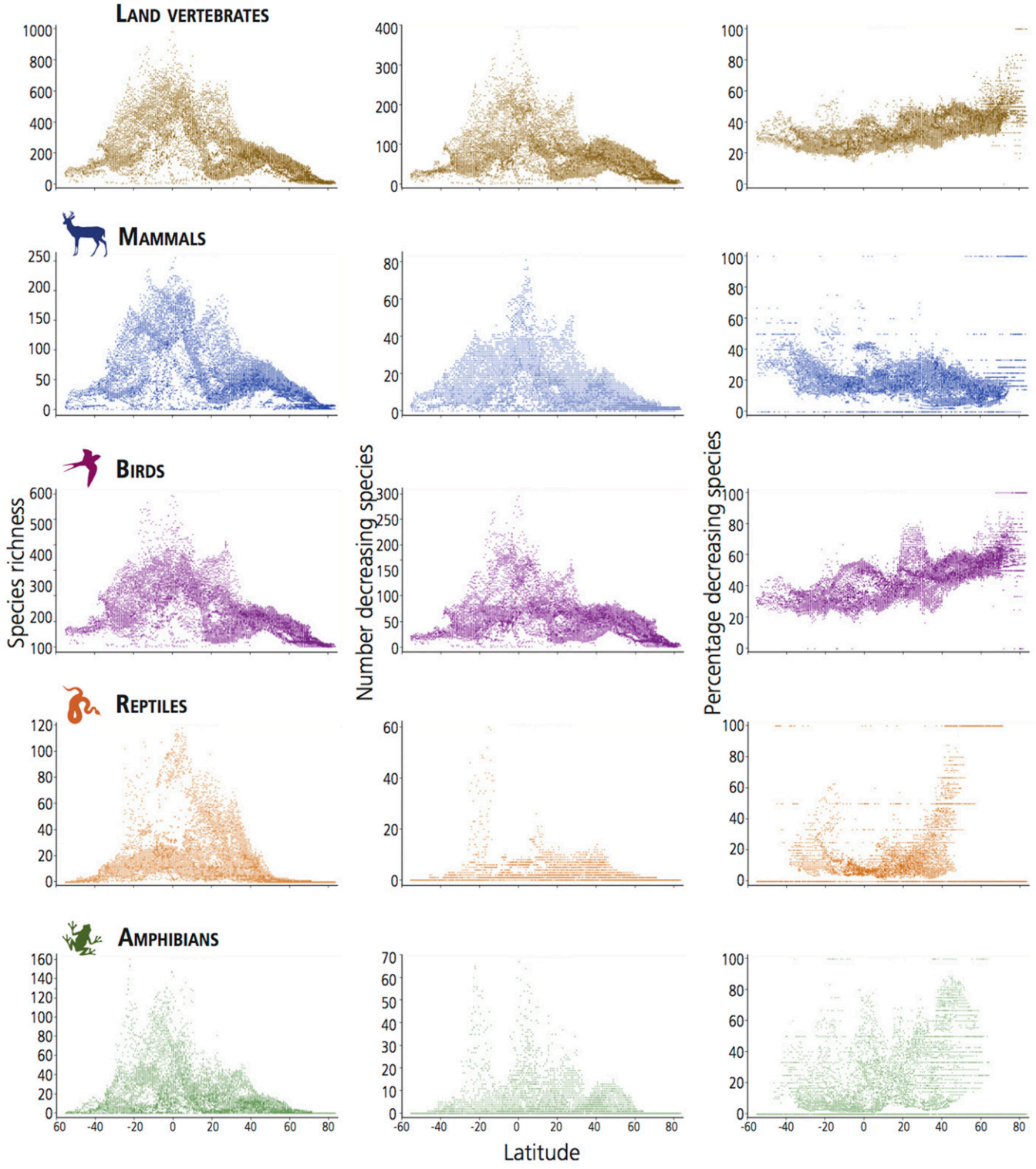

Fig. 3. Latitudinal distribution of species richness (*Left*), decreasing species (*Center*), and the percentage of species (*Right*) that are suffering population losses in relation to total species richness, in each 10,000-km^2 quadrat. Patterns of species richness in relation to latitude are similar in all vertebrates, although there are more species per quadrat in birds and mammals and, as expected, a scarcity of reptiles and amphibians at high latitudes. The patterns of number of species with decreasing populations indicate that regions with high species richness also have high numbers of decreasing species, but the percentage of decreasing species in relation to species richness shows contrasting patterns between mammals and birds compared with reptiles and amphibians. In mammals and birds, the percentage of decreasing species is relatively similar in regions with low and high species richness. In contrast, there are proportionally more decreasing species of reptiles and amphibians in regions with low species richness.

accurate information on population size for most taxa, whatever is available indicates that the total population size in species with decreasing populations varies from fewer than 100 individuals in critically endangered species such as the Hainan black-crested

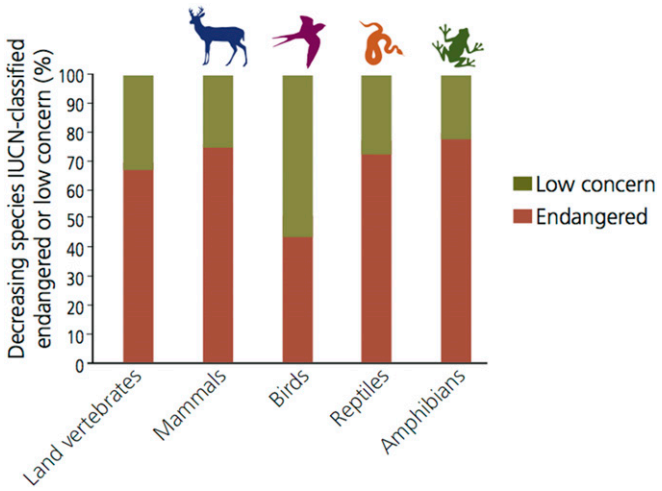

Fig. 4. The percentage of decreasing species classified by IUCN as "endangered" (including "critically endangered," "endangered," "vulnerable," and "near-threatened") or "low concern" (including "low concern" and "data-deficient") in terrestrial vertebrates. This figure emphasizes that even species that have not yet been classified as endangered (roughly 30% in the case of all vertebrates) are declining. This situation is exacerbated in the case of birds, for which close to 55% of the decreasing species are still classified as "low concern."

gibbon (*Nomascus hainanus*), to many millions of individuals in decreasing common species such as the barn swallow (*Hirundo rustica*). Similarly, the smallest ranges (i.e., <1 km^2) are seen in species such as the Carrizal seedeater (*Amaurospiza carrizalensis*) from Venezuela and Herrera's false coral snake (*Lampropeltis herrerae*) from Mexico, both denizens of tiny islands. The largest ranges are hundreds of thousands of square kilometers, as in the bush dog (*Speothos venaticus*) from South America and the common lizard (*Zootoca vivipara*) from Eurasia. The sum of the 10,000-km^2 quadrats representing the current ranges of the 8,851 decreasing vertebrate species is 1,350,876 quadrats. A highly conservative estimate would indicate a similar number of local populations facing extinction. This is, of course, a very rough estimate of the total number of populations, as the number of populations of a decreasing species in each quadrat largely depends, aside from suitable habitat distribution within the quadrat, on animal body mass and trophic position (e.g., ref. 34). The assumption of one population per 10,000 km^2 might seem very conservative, as this area could accommodate many populations of small animals (e.g., 0.1-kg rodents), most of which could have been extirpated. However, 10,000 km^2 may not be sufficient for, or can barely accommodate a viable population of large carnivores (say a 330-kg Siberian tiger; ref. 34). Nonetheless, our results provide evidence of the extremely large numbers of vertebrate populations facing extinction, compared with the number of species.

Proportion of Vertebrate Species Decreasing. The proportion of decreasing vertebrates shows that there are areas across the planet with high concentrations of decreasing species in all vertebrates and regions with high proportions of decreasing species of a particular group (Figs. 2, 3, and 5). For example, in mammals, the highest percentage of decreasing species is concentrated in tropical regions, mostly in the Neotropics and Southeast Asia, whereas in reptiles, the proportional decline concentrates almost exclusively in Madagascar. Decreasing amphibians are prominent in Mexico, Central America, the northern Andes, and Brazil's Atlantic forest in the Americas; West Africa and Madagascar in Africa; and India and Southeast Asia, including Indonesia and Philippines in Asia–Southeast Asia. Finally, decreasing species of birds are found over large regions of all continents (Fig. 2).

Roughly a third (8,851/27,600) of all land vertebrate species examined are experiencing declines and local population losses of a considerable magnitude (Figs. 2–4). Such proportion of decreasing species varies, depending on the taxonomic group, from 30% or more in the case of mammals, birds, and reptiles, to 15% in the case of amphibians. Furthermore, of the decreasing species, many are now considered endangered (Fig. 4). Beyond that, roughly 30% of all decreasing species are still sufficiently common that they are considered of "low concern" by IUCN, rather than "endangered." That so many common species are decreasing is a strong sign of the seriousness of the overall contemporary biological extinction episode.

In our 10,000-km^2 quadrats, the proportion of decreasing species ranges from less than 10% to more than 50% (Fig. 2). The geographic distributions of absolute (i.e., number) and relative (i.e., percentage) of decreasing species is contrasting. Whereas tropical regions have larger numbers of decreasing species, as expected, given their higher species richness, their corresponding proportions are relatively low. In contrast, temperate regions tend to have similar or higher proportions of decreasing species, a trend dramatically prominent in the case of reptiles.

Local Population Extinctions in Mammals. Our most detailed data allow comparison of historic and present geographic range of a sample of 177 mammal species (Figs. 5 and 6). Most of the 177 mammal species we sampled have lost more than 40% of their geographic ranges in historic times, and almost half have lost more than 80% of their ranges in the period ~1900–2015. At the continental and subcontinental level, some patterns become evident (Fig. 5). The predominant category of range contraction is ≥80% in Africa (56% of the sampled mammal species), Asia (75% of the species), Australia (60% of the species), and Europe (40% of the species). In the Americas, range contractions are less marked but still considerable: 22% of the species in North America and 17% of the species in South America have experienced range contractions of at least 80%. Nevertheless, 50% of the species in North America and 28% of the species in South America have experienced a range contraction of 41% or more.

The comparison of the 1900–2015 geographic ranges showed that the 177 species of mammals have disappeared from 58,000 grid cells. On the assumption that on average each of the 10,000-km^2 occupied quadrats held a single population of the species found within it, this implies that roughly 58,000 populations of the 177 mammals we examined have gone extinct. Consider the following emblematic cases: The lion (*Panthera leo*) was historically distributed over most of Africa, southern Europe, and the Middle

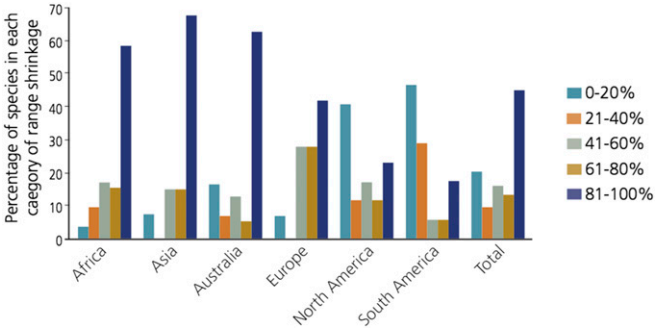

Fig. 5. The percentage of species of land mammals from five major continents/subcontinents and the entire globe undergoing different degrees (in percentage) of decline in the period ~1900–2015. Considering the sampled species globally, 56% of them have lost more than 60% of their range, a pattern that is generally consistent in Africa, Asia, Australia, and Europe, whereas in South America and North America, 35–40% of the species have experienced range contractions of only 20% or less. (See text for details.)

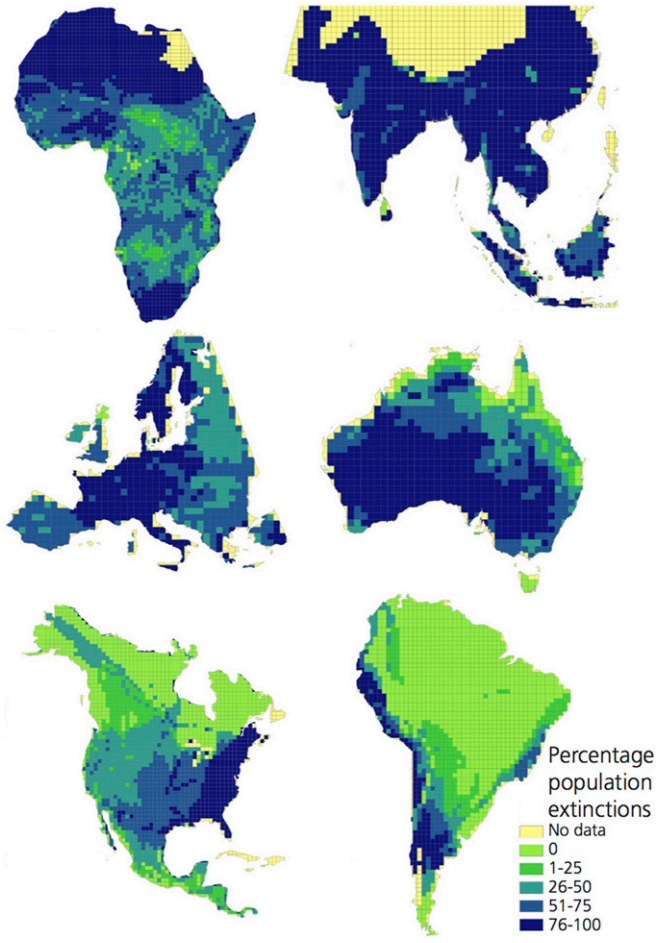

Fig. 6. Percentage of local population extinction in 177 species of mammals in 1° × 1° quadrats, as an indication of the severity of the mass extinction crises. The maps were generated by comparing historic and current geographic ranges (49) (*SI Appendix, SI Methods*). Note that large regions in all continents have lost 50% or more of the populations of the evaluated mammals. Because of the small sample size, biased to large mammal species, this figure can only be used to visualize likely trends in population losses.

East, all the way to northwestern India (*SI Appendix*, Fig. S1). It is now confined to scattered populations in sub-Saharan Africa and a remnant population in the Gir forest of India. The vast majority of lion populations are gone. In its African stronghold, it historically occupied roughly two thousand 10,000-km^2 cells, and now it is reduced to some 600 cells. Other species, such as the mountain lion (*Puma concolor*), are known to be doing better. The mountain lion has lost some of its local populations in North America, but has not suffered such disastrous losses as its Old World relative, adapting relatively well to human-dominated landscapes, and it is still found across 85% of its historic range.

Clearly, the extinction of mammal populations, although varying from species to species, has been a global phenomenon (Fig. 6). Strikingly, the predominant color code in the mammalian map is that of 70% or more of population losses, with the exception of some areas of South America and high latitudes of North America. Particularly hard hit have been the mammals of south and southeast Asia, where all of the large-bodied species of mammals analyzed have lost more than 80% of their geographic ranges. The Cape and Sahara regions in Africa, central Australia, the eastern United States, and the Atlantic forest in South America have also suffered severely from population extinctions.

Discussion

It has recently been shown, using conservative estimates of current and background species extinction rates, that Earth is now in a period of mass global species extinction for vertebrate animals (11). But the true extent of this mass extinction has been underestimated, because of the emphasis on species extinction. This underestimate largely traces to overlooking the accelerating extinction of populations. Whereas scientists have known for a long time that several relatively well-studied species have undergone major contraction of their ranges, experienced considerable population decreases, and suffered many population extinctions, the global extent of population shrinkage and extirpation has previously not been recognized and quantified.

In addition, some studies document that invertebrates and plants are suffering massive losses of populations and species (35–38). Here we extend investigation of mass extinction to terrestrial vertebrate population decreases and losses, and give estimates of the number of their species with decreasing populations. The accuracy of the estimates is strongly dependent on an unknown parameter, namely, the actual average area occupied by a vertebrate population (e.g., refs. 35, 39–41). However, even if a population would, on average, occupy an area five times larger than what we have used here (i.e., 50,000 km^2) there would still be hundreds of thousands of populations that have suffered extinction in the past few centuries. On the other hand, most vertebrates (~70%) are small species of mammals, birds, reptiles, and amphibians. If, on average, they have one population every 10 km^2 then vertebrates would have suffered more than a billion population extinctions.

Our results show that population extinction in land vertebrates is geographically omnipresent, but with notable prominence in tropical, species-rich regions. It is interesting, however, that when population extinctions are evaluated as the percentage of total species richness, temperate regions, with their typical low species diversity, show higher proportions of population loss.

There are some illustrative qualitative examples of population decreases and their consequences within terrestrial and marine vertebrates, but ours is an attempt at a quantitative evaluation of global trends in population extinctions. Recent reviews indicate that species extinctions, population decreases, and range contraction (implying population extinctions) among terrestrial invertebrates and plants are as severe as among vertebrates (e.g., refs. 35–38). For example, long-term monitoring of insect populations in the United Kingdom shows that 30–60% of species per taxonomic order have contracting ranges (36). The situation in plants has been less evaluated; thus it is difficult to compare them with animals, but there is little reason to believe that the extinction situation in plants is dramatically different (37). Furthermore, research shows that the loss of animal populations indirectly leads to changes in plant communities (20, 37, 39), frequently causing the reduction of local species richness and dominance of a few plant taxa that either experience "ecological release" in response to decreasing herbivore pressures (42, 43), and/or experience population reductions due to the decline of animals responsible for pollination or dispersal (e.g., refs. 2–3, 20). The status of biodiversity among microorganisms is too poorly known to permit us to make any comparison and generalizations about the current pulse of extinctions, although some recent research has unraveled feedbacks between local large herbivore defaunation and mycorrhizal richness (44, 45). Given what we know about genetic population differentiation, it is expected that the range contractions and declines we document here imply a considerable loss of intraspecific genetic diversity (23) but this is, clearly, an aspect that warrants further investigation.

In sum, by losing populations (and species) of vertebrates, we are losing intricate ecological networks involving animals, plants, and microorganisms (e.g., refs. 2, 8, 18, 45, 46). We are also losing pools of genetic information that may prove vital to species' evolutionary adjustment and survival in a rapidly changing global environment.

This suggests that, even if there was not ample sign that the crisis extends far beyond that group of animals, today's planetary defaunation of vertebrates will itself promote cascading catastrophic effects on ecosystems, worsening the annihilation of nature (2, 3, 46). Thus, while the biosphere is undergoing mass species extinction (11), it is also being ravaged by a much more serious and rapid wave of population declines and extinctions. In combination, these assaults are causing a vast reduction of the fauna and flora of our planet. The resulting biological annihilation obviously will also have serious ecological, economic, and social consequences (46). Humanity will eventually pay a very high price for the decimation of the only assemblage of life that we know of in the universe.

Conclusion

Population extinctions today are orders of magnitude more frequent than species extinctions. Population extinctions, however, are a prelude to species extinctions, so Earth's sixth mass extinction episode has proceeded further than most assume. The massive loss of populations is already damaging the services ecosystems provide to civilization. When considering this frightening assault on the foundations of human civilization, one must never forget that Earth's capacity to support life, including human life, has been shaped by life itself (47). When public mention is made of the extinction crisis, it usually focuses on a few animal species (hundreds out of millions) known to have gone extinct, and projecting many more extinctions in the future. But a glance at our maps presents a much more realistic picture: they suggest that as much as 50% of the number of animal individuals that once shared Earth with us are already gone, as are billions of populations. Furthermore, our analysis is conservative, given the increasing trajectories of the drivers of extinction and their synergistic effects. Future losses easily may amount to a further rapid defaunation of the globe and comparable losses in the diversity of plants (36), including the local (and eventually global) defaunation-driven coextinction of plants (3, 20). The likelihood of this rapid defaunation lies in the proximate causes of population extinctions: habitat conversion, climate disruption, overexploitation, toxification, species invasions, disease, and (potentially) large-scale nuclear war—all tied to one another in complex patterns and usually reinforcing each other's impacts. Much less frequently mentioned are, however, the ultimate drivers of those immediate causes of biotic destruction, namely, human overpopulation and continued population growth, and overconsumption, especially by the rich. These drivers, all of which trace to the fiction that perpetual growth can occur on a finite planet, are themselves increasing rapidly. Thus, we emphasize that the sixth mass extinction is already here and the window for effective action is very short, probably two or three decades at most (11, 48). All signs point to ever more powerful assaults on biodiversity in the next two decades, painting a dismal picture of the future of life, including human life.

Methods

For full methods, please see *SI Appendix*. We determined the number of decreasing vertebrate species using the IUCN (28) Red List of Threatened Species. In the IUCN, species are classified as decreasing, stable, or increasing (see also ref. 33). Either range contraction (population extinction) or reduction in numbers in extant populations determines whether a species is decreasing. We used the IUCN maps of terrestrial vertebrates (i.e., mammals, birds, reptiles, and amphibians) to create the global maps of number of species (richness) and of decreasing species, and percentage of decreasing species in relation to total species richness. The distribution of all of the species was superimposed in a 22,000 grid of 10,000-km^2 quadrats covering the continental lands. For the grid, a Lambert azimuthal equal-area projection was used (see ref. 49 for details of the projection methods). In our analyses a critical issue is how grid squares and populations correspond. This is a very difficult problem that varies with definitions of species. (In this paper, we stick with the classic biological definition of species.) The number of populations also varies from species to species; for example, a highly phylopatric species would have more populations per square than a very vagile species, and species with different mating systems would have different estimates of numbers of Mendelian populations, and these would not be the same as estimates of number of demographic units (50). For the purposes of understanding the annihilation, these differences are not critical. For example, if we have lost 90% of the lion's geographic range, whether this amounts to 10,000 demographic units or 4,000 Mendelian populations is trivial in the present context. It would be extremely useful if we had much more information on population structure for all vertebrates, but this is a major, pending agenda.

The population extinction analysis was conducted on 177 mammalian species occurring on five continents. Specifically, we analyzed 54 species in Africa, 14 in Asia, 57 in Australia, 15 in Europe, and 35 in America. The historical distribution was gathered from specialized literature (see details in ref. 26) and the current distribution from IUCN (28). Historic and current ranges were digitized as geographic information system polygons and elaborated in ArcGis 10.1 (51). For each species, we calculated the area of the historical and present distribution (in square kilometers) to estimate the percentage of lost area and the percentage of area where the species are extant. A caveat of these estimates regards how representative the sample of 177 species is. We recognize a bias in that the data include a large number of medium- and large-sized species, for which the best information is available. However, given that such medium and large species are the most seriously threatened by the predominant proximate drivers of defaunation (2, 3), the likely bias against small-sized species should not affect our overall interpretation of results.

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Anexo 2

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Biological patterning at the microscale and macroscale levels has been under intensive investigation by developmental biology, and its fundamental principles, such as the concept of the morphogens, have become textbook knowledge (1). In contrast, nanoscale biological patterning is not well studied and understood. Among the rare known examples of biological nanopatterns are the 3D nanostructures covering insect corneal surfaces (2). They were described in moths and butterflies and later some Dipterans as pseudoregularly spaced nipple-type protrusions, up to 200 nm in height and width (3–7). These nanostructures may carry antireflective, dirt-removing/self-cleaning, and hydrophobic/antiwetting functions (2, 8–12). Later, some other insects were found to possess a very different type of corneal nanocoating, such as the antireflective maze-like 30-nm-high evaginations covering corneae of the overwater eyes of Gyrinidae beetles (13). An attempt to analyze the variety of corneal nanocoatings throughout the insect class was made in the classical study by Bernhard et al. (5). However, the scanning electron microscopy technique of that time was mostly performed on platinum replicas of the insect samples and was compromised by the partial collapse of the nanoprotrusions. It permitted reliable identification of 50- to 250-nm-high nipple-type protrusions in Lepidoptera, some Dipterans, Trichopterans, and, interestingly, the primitive Thysanuras, but not identification of other types of corneal nanocoatings (5).

To use the corneal nanocoatings as the model to study nanoscale biological patterning, a comprehensive investigation across insect lineages using modern techniques must be performed. We recently applied atomic force microscopy (AFM), providing nanometer and subnanometer resolution of undamaged biological material, to investigate different types of corneal nanostructures of some Dipteran and Coleopteran insects (6, 13). Here we expand this analysis to 23 insect orders and some non-insect arthropods, describing a striking richness and beauty of the corneal nanocoatings (Fig. 1, Figs. S1–S3, Table S1, and *Detailed Description of Diverse Corneal Nanostructures Order by Order*). These nanostructures can be grouped as follows. (*i*) Nipple-like structures (Fig. 1*A* and Fig. S1) include the regularly packed protrusions of Lepidopterans (Fig. S1*A*), irregular packaging in Dipterans (Fig. S1*B*), and irregular packaging of irregularly shaped nipple-like protrusions in a range of other orders: Trichoptera (Fig. 1*A*), Mecoptera (Fig. S1*C*), Megaloptera (Fig. S1*D*), Hemiptera (Fig. S1 *E* and *F*), Psocoptera (Fig. S1*G*), Thysanura (Fig. S1*H*), Raphidioptera (Fig. S1*I*), Neuroptera (Fig. S1*J*), Orthoptera (Fig. S1*K*), and Odonata (Fig. S1*L*). (*ii*) Maze-like nanocoatings (Fig. 1*B* and Fig. S2) can be observed in Coleopterans (Fig. S2 *A* and *B*) but also in other orders such as Trichoptera (Fig. 1*B*) and Hymenoptera (Fig. S2*C*), and in some arachnids (Fig. S2 *D* and *E*). (*iii*) Parallel strands/ridges (Fig. 1*C*) formed by fusion of nipple-type protrusions can mostly be seen in Dipterans (Fig. 1 *F* and *G*) and, interestingly, in true spiders (Fig. 1*C*). (*iv*) Novel dimple-type nanocoating (Fig. 1*D* and Fig. S3) can be seen in different orders: Siphonaptera (Fig. S3*A*), Coleoptera (Fig. S3*B*), Hymenoptera (Fig. S3*C*), Hemiptera (Fig. S3 *D* and *E*), Blattodea (Fig. S3*F*), and Dermaptera (Fig. 1*D*), and, interestingly, in centipedes (Fig. S3*H*). We also see various transitions between these major forms: (*v*) nipples-to-maze transition (e.g., in

Author contributions: V.L.K. designed research; A.B., A.S., and M.K. performed research; A.S. and Y.L. contributed new reagents/analytic tools; A.B., A.S., M.K., and V.L.K. analyzed data; and A.B. and V.L.K. wrote the paper.

The authors declare no conflict of interest.

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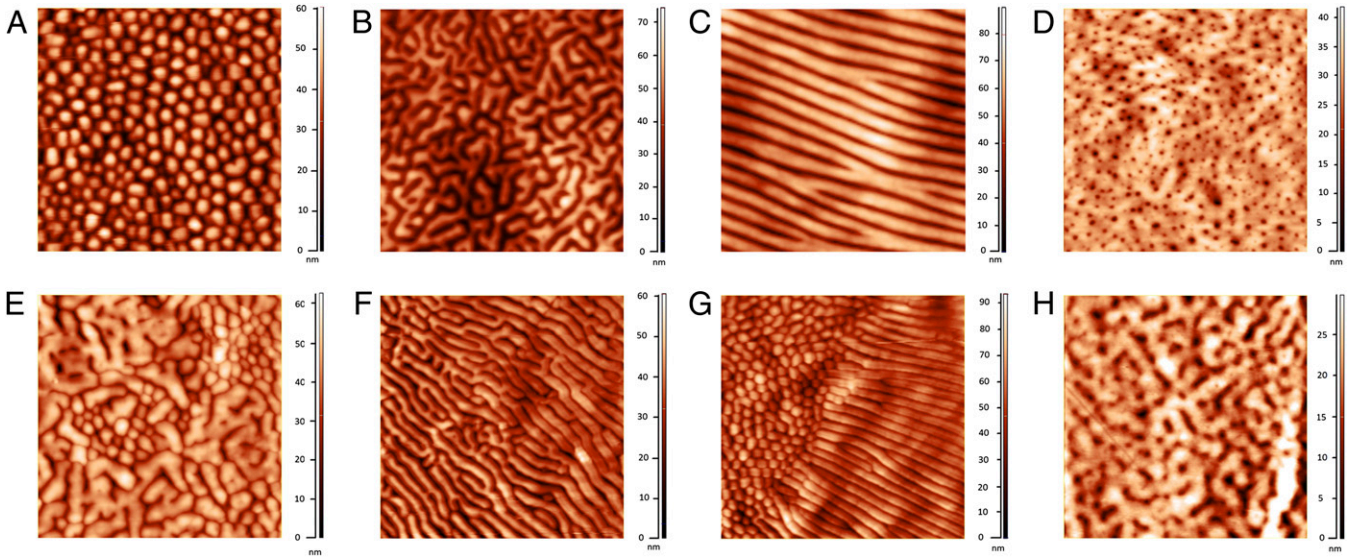

Fig. 1. The diversity of corneal nanostructural patterns among arthropod groups: (*A* and *B*) Corneal nanostructures of Trichoptera. Merged as well as undersized nipples in an irregular nipple array of the *Phryganeidae* family (*A*) and maze-like nanocoating of the Limnephilidae family (*B*). (*C*) Clearly expressed parallel strands in a true spider. (*D*) Dimpled nanopattern of an earwig (Dermaptera). (*E*) Nipples merging into maze on stonefly (Plecoptera) corneae. (*F* and *G*) Merging of individual Dipteran nipples into parallel strands and mazes: full merging of nipples into strands and mazes on the entire corneal surface in Tabanidae (*F*); partial merging of nipples in the center of Tipulidae cornea into elongated protrusions and then complete fusion into an array of parallel strands near the ommatidial edge (*G*). (*H*) Merging of individual burrows and dimples into a maze-like structure on bumblebee (Apidae, Hymenoptera) corneae. All image dimensions are 5 × 5 μm, except for *H*, which is 3 × 3 μm. Surface height in nanometers is indicated by the color scale shown next to 2D images.

Plecoptera, Fig. 1*E*); (*vi*) maze-to-strands transition (e.g., in Diptera, Fig. 1*F*); (*vii*) nipples-to-strands transition (e.g., in Diptera, Fig. 1*G*); and (*viii*) dimples-to-maze transition (e.g., in Hymenoptera, Fig. 1*H*).

The rich diversity of these nanostructures and the easiness with which the corneal nanopatterns merge one into another in closely related orders and even within the orders (Fig. 2 and *Detailed Description of Diverse Corneal Nanostructures Order by Order*) is striking and permits posing questions on the underlying molecular, developmental, and evolutionary mechanisms. Developmentally, the nipple-type protrusions were proposed to originate, during eye development, from secretion by the regularly spaced microvilli of the cone cells (5, 14). However, this idea could appear plausible when the ordered Lepidopteran nipple arrays were studied but, with the current diversity of nanostructures and transitions among them, sometimes within the same lens (Fig. 1*G*), is not satisfactory. Instead, we propose that certain mechanisms of patterning at the nanoscale are in place, and the diverse arthropod corneal nanostructures we describe here represent a model to study such nanopatterning. Further, we notice that this diversity of corneal nanostructures is remarkably similar to the complete set of the Turing patterns (Fig. 3).

In his seminal paper in 1952, Alan Turing provided a system of differential equations describing the reaction–diffusion system of two reacting morphogens—a slowly diffusing activator and a fast diffusing inhibitor—which can model various biologic, chemical, and physical patterns (15, 16). Applicability of this model to biological pattern formation has been shown in several recent examples, such as formation of colored stripes in zebrafish (17), hair follicle spacing in mice (18), and digit specification in limbs (19). The insect corneal nanopatterns we describe here differ from these examples, as they reproduce not just one of the many possible forms produced by the reaction–diffusion model but a thorough set of possible variants including the intermediate forms (Figs. 1 and 3). This remarkable completeness of coverage of the possible set of Turing structures by the arthropod corneal nanopatterns strongly argues in favor of the hypothesis that these nanopatterns are indeed a consequence of the Turing reaction–diffusion mechanisms.

We hypothesize that the Turing mechanism-based reaction–diffusion processes patterning the nanocoatings are mediated by organic components of the lens, possessing different diffusion properties and mutually influencing each other's abundance/polymerization/aggregation, the outcome of this being the stereotypical formation of the nanostructures. In previous applications of the Turing principles to biological processes, patterning at the microscale was modeled (17–19). Formal mathematical analysis shows how key parameters of the reaction–diffusion equations (primarily the diffusion coefficients of the two interacting morphogens) can result in the appearance of repeated developmental structures with the experimentally observed micrometer-scale wavelength (20). Our mathematical analysis (*Turing modeling of corneal nanopatterns*) demonstrates that nanoscale patterns are expected to form in the reaction–diffusion system acting in the colloidal or liquid crystal-type environment [which is indeed the environment of the lens of the eye (21)] where diffusion properties are reduced (compared with the liquid phase).

Although the molecular identity of the morphogens patterning corneal nanocoatings remains to be revealed, simulations of the Turing reaction–diffusion processes provide interesting hints into the potential molecular mechanisms underlying formation of different types of the nanocoatings and transitions among them (Fig. 3*I* and Fig. S4). Although different sets of the reaction–diffusion coefficients (like that of Table S2 used to obtain images on Fig. 3 and Fig. S4; see also Fig. S4*B* for schematic description of the parameters and Fig. S5 for analysis of the parameter space) can model different nanopatterns, simulations find that three of the major types of patterns we observe in insect corneae occupy defined regions within the parameter space and transit to each other as follows: dimples ↔ maze ↔ nipples (Fig. 3*I* and Fig. S4*A*). The space in these figures is populated by the incremental changes of

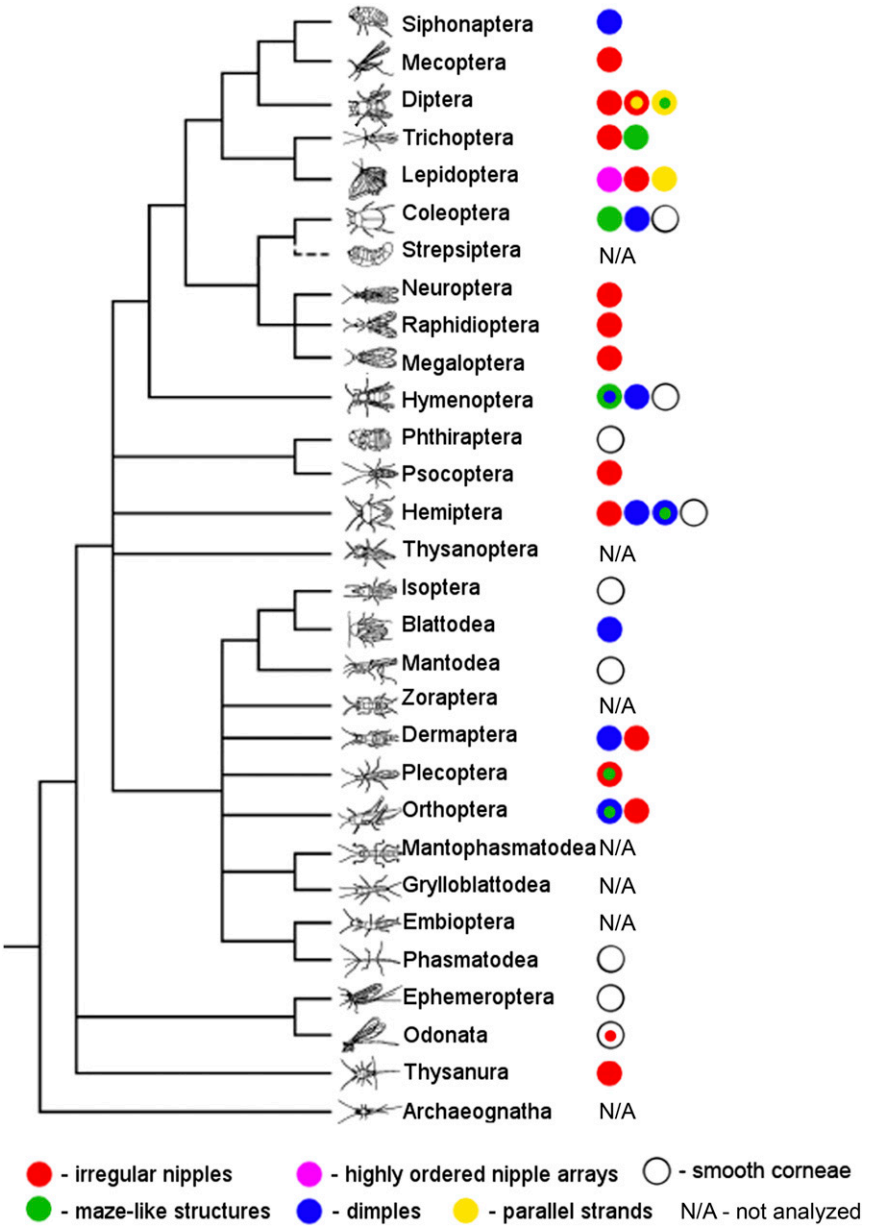

Fig. 2. Distribution of basic and intermediate nanopatterns among insect orders. Each pattern type is represented by a circle of a certain color on the diagram; double-colored circles correspond to transitional nanopatterns. Orders of which no representatives were analyzed in the present study are marked as N/A. The data on insect phylogenetic relationship are based upon ref. 24.

two of the reaction–diffusion parameters a_v and b_u describing the degree of influence of the two diffusing components (activator u and inhibitor v) on each other (Fig. S4*B*) (16, 22). Interestingly, transition from the dimple-type nanocoating to the maze-type and then further to the nipples occurs by increasing the absolute value of either of the two reaction–diffusion parameters (Fig. 3*I* and Fig. S4*A*). In this regard, it may be speculated that the initial reaction–diffusion nanopatterning system emerged when these parameters just exceeded the borderline, permitting the Turing patterns to appear (16, 22), and thus was likely of the dimple type. In this regard, it is interesting to note that the dimple pattern is not only seen in many insect groups but also in centipedes (Fig. S3*H*), which are believed to retain more characteristics of the presumed arthropod ancestor than other arthropods with sequenced genomes (23).

In the context of phylogeny, evolutionary advanced insects were initially assumed to possess fully developed nipple-type corneal nanocoatings, whereas simpler insects were reported to mostly carry less pronounced (and less functional) nanocoatings (5). Our findings suggest that this assumption is incorrect. Indeed, our study unequivocally shows that various types of the nanostructures can be seen in various insect (and wider—Arthropodan) groups without any correlation of the predominant type of the nanocoating and the evolutionary advance of the group (Fig. 2). Instead, we can also apply the Turing modeling to get insights into the evolutionary transitions among different types of corneal nanocoatings. Increase in the absolute value of either of the two a_v and b_u parameters allowed the dimple-to-maze transition, and the further increase allowed the maze-to-nipples transition (Fig. 3*I* and Fig. S4*A*). These considerations permit constructing a "morphogenetic tree" or "morphogramme"

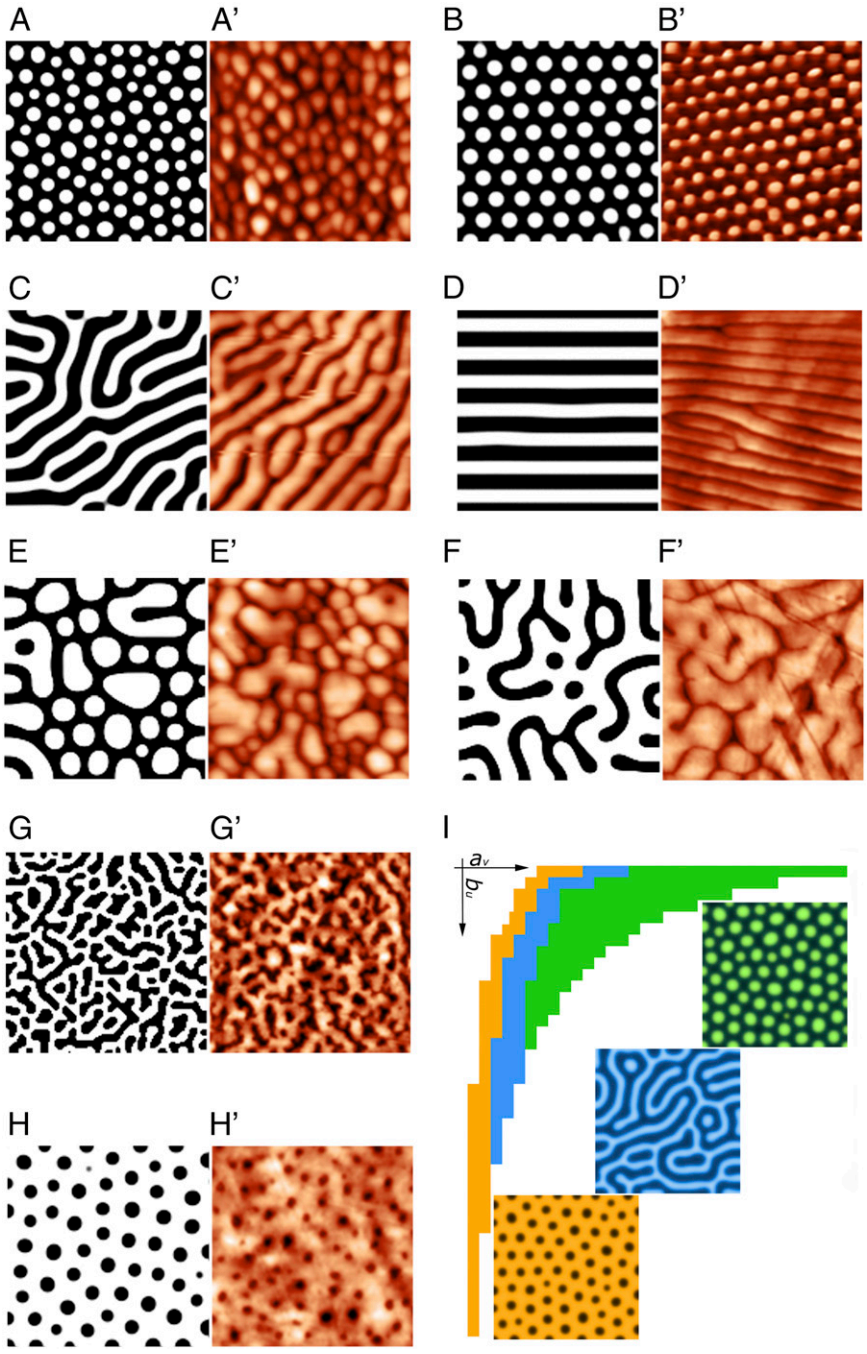

Fig. 3. The insect corneal nanostructural diversity replicates Turing patterns. Mathematically modeled Turing patterns (in black and white) and their insect counterparts. (*A* and *A′*) Irregular nipples of various sizes, characteristic e.g., for Hemipteran corneal nanocoatings. (*B* and *B′*) Highly ordered nippled nanoarrays (Lepidoptera). (*C* and *C′*) Strands merging into a maze (Diptera, Tabanidae). (*D* and *D′*) Parallel strands (Diptera, Tipulidae). (*E* and *E′*) Nipples merging into a maze (Plecoptera). (*F* and *F′*) Typical maze-like structures (Coleoptera, Gyrinidae). (*G* and *G′*) Angular maze-like structures (Coleoptera, Coccinellidae). (*H* and *H′*) A typical dimpled pattern (Dermaptera). *A′* is a fragment of Fig. S1*F*; *B′* is an image from a Pterophoridae butterfly; *C′* is a fragment of Fig. 1*F*; *D′* is a fragment of Fig. 1*G*; *E′* is a fragment of Fig. 1*E*; *F′* is an image from a *Gyrinus* beetle [overwater eye (13)]; *G′* is a fragment of Fig. S2*B*; and H′ is a fragment of Fig. 1*D*. Modeling parameters are given in Table S2. (*I*) Simulations of Turing patterns formation. Step-wise changes in the a_v and b_u parameters within the boundary conditions produce different Turing patterns: dimples (yellow zone), mazes (blue zone), and nipples (green zone). See Fig. S4*A* for more detailed representation.

of these structures (Fig. 4). In this morphogramme, different types of corneal nanocoatings are placed not based on the phylogenetic hierarchy of the insect orders (24) but instead on their morphologies and transitions among them, as justified by the Turing modeling we performed. Originating from the dimple-type nanocoatings, this morphogramme then grows into the maze type and further into the nipples type (Fig. 4). We further identify parallel ridges of some Dipterans and hexagonally packed nipples of some Lepidopterans as developments of the maze- and nipples-type structures, respectively (Fig. 4). Both represent more ordered structures and can be modeled to emerge from their less ordered predecessors through increasing of the diffusion parameter of the activator component D_u to the levels maximally allowed within the boundaries permitting the Turing patterns to form (16, 22) (Fig. S4 *C* and *D*). In contrast, increasing the diffusion parameter of the inhibitor component D_v leads to increase in the cross section of the nanostructures (nipples or ridges, respectively, in Fig. S4 *C* and *D*).

In some Dipterans, a transition from nipples to parallel ridges can be seen within the same lens, with nipples occupying the central part of the ommatidium and merging into elongated strands away from the center (Fig. 1*G*). Turing modeling predicts how such structures may be formed (Fig. S4*E*): Seen in flies with large ommatidia and lenses, these nanocoatings are likely a result of a nipples-to-maze transition within the same lens, happening during the lens formation after initial nipples in the center of the cornea have been formed. In this predefined space, parameters otherwise giving rise to mazes induce formation of parallel ridges emanating from the nippled area (Fig. S4*E*).

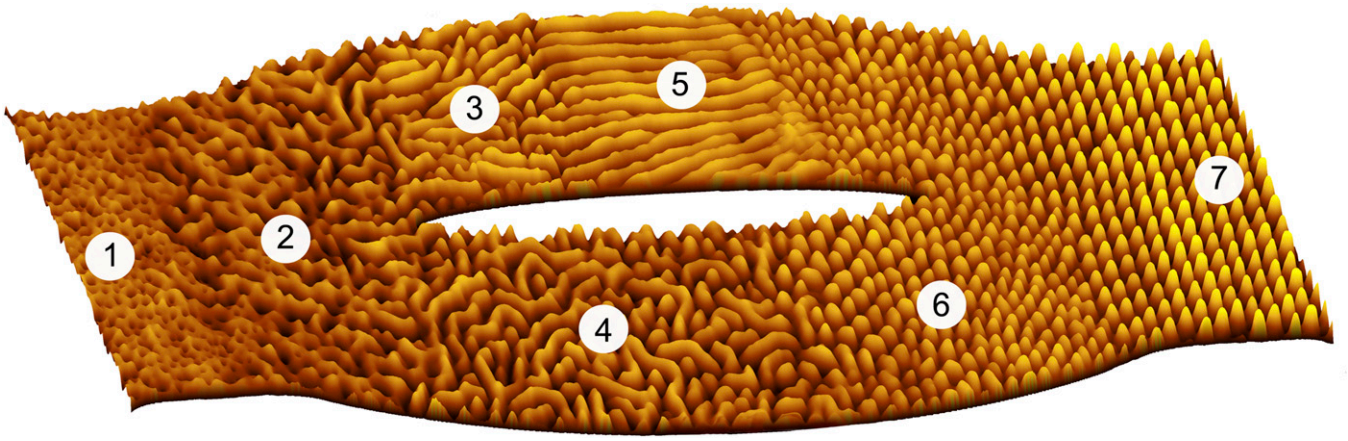

Fig. 4. Transformations of corneal nanopatterns. The morphogramme depicts the likely interconversions among the nanostructural patterns found in the insect class rather than phylogenetic relationships of the patterns. Primordial dimpled nanopatterns (1, here from a *Forficula* earwig) can transform into various maze-type nanostructures (2–4; 2 from a *Pyrrhocoris* firebug, 3 from a Tabanidae fly, and 4 from the butterfly *Protographium asius*). The latter can further transform into disordered nipples (6, here from the fruit fly *Drosophila melanogaster*), which can further become orderly packed (7, here from a Pterophoridae moth). Alternatively, parallel ridges (5, here from a Tipulidae fly) can evolve either from mazes or nipples. The figure is made of reconstructed 3D AFM images fused, for the sake of visualization not in exact scale, using MATLAB.

Detailed analysis of the physical (such as antireflective and antiwetting) properties of the diverse corneal nanostructures we present here is still to be performed, but the fact that both the nipple-type and maze-type nanostructures serve the antireflective function (2, 13) suggests the functionality of the majority, if not all, of them. The variety of these nanostructures can serve as a highly promising model, obeying the Turing mechanism of pattern formation. Insect eyes, especially those of the genetically tractable model insect *Drosophila melanogaster* (6, 25), can therefore serve as a powerful tool to further explore the precise mechanisms of the reaction–diffusion-driven processes in living organisms, to identify the molecular components governing formation of corneal nanocoatings, and to genetically engineer novel Turing nanopatterns with novel physical properties.

Methods

Insect Specimens. The dried insect samples were obtained from a collection of the Department of Entomology, Moscow State University. Fresh specimens were collected in the woods around the town of Pushchino, Moscow region. The phylogenetic tree of the insect class was taken from Su and coworkers (24).

Atomic Force Microscopy. To prepare corneal samples, the head of an insect was cut out of the body, followed by removal of the mouth apparatus with a scalpel, splitting of the head into the two hemispheres, and careful extraction of the brain tissue with forceps. Next, the cornea was cleared from the head capsule tissue as well as the underlying brain material with a scalpel. The sample was attached to a glass slide for AFM by means of two-sided scotch tape. AFM scanning of the corneal surfaces was performed with the Integra-Vita microscope (NT-MDT). For the semicontact procedure, the nitride silicon cantilever NSG 03 (NT-MDT) was used. The parameters of the cantilever were: length, 100 μm; resonant frequency, 62–123 kHz; radius, 10 nm; and force constant, 0.4–2.7 N/m. For the contact procedure, the cantilever CSG 10 (NT-MDT) was used, with the following parameters: length, 250 μm; resonant frequency, 14–28 kHz; radius, 10 nm; and force constant, 0.03–0.2 N/m. The choice between the semicontact and the contact measuring procedures was dictated by the size and curvature of the studied surface of the sample but provided essentially identical results. In each AFM experiment, several scans were made to check the reproducibility of images and the absence of possible surface damages. Measurements of height and width of the corneal nanostructures were performed by the Nova software (NT-MDT).

Turing Modeling. The 2D patterns were made using the software RDsimJ.jar (16) with the parameter values listed in Table S2.

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Anexo 3

COLLOQUIUM PAPER

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Buchnera | aphid | Muller's ratchet | selection levels | coevolution

Obligate symbiotic relationships shape the evolution of partner lineages. In symbioses that are mutually beneficial, partners evolve traits that enable and stabilize the symbiosis: this cooperative coevolution is emphasized in most studies of symbioses. Genomic work has also revealed that obligate symbiosis produces unusual genome modifications, including extreme reduction, rapid protein evolution, and codon reassignments, all of which are evident in ancient obligate symbionts of insects (1, 2). Recent studies suggest that host genomes also have acquired unusual modifications that are linked to symbiosis, including acquisition of genes from bacterial donors that seem to play a role in controlling or supporting symbionts (3–5). Below, we explore why lineages entering into obligate heritable symbiosis undergo strange patterns of genome evolution and display features that are difficult to interpret simply as adaptations for improving symbiotic function. We refer to the commitment to obligate, inherited symbiosis as the evolutionary "rabbit hole" of obligate symbiosis, implying a generally irreversible journey into a very odd world where the usual rules do not apply.

Broadly, the symbiosis rabbit hole refers to the confluence of selection and neutral evolution in generating the extreme patterns of genomic evolution observed in symbiotic partners. As we argue, these extremes are driven by three main forces: deleterious symbiont evolution due to genetic drift, within-host selection leading to symbiont selfishness, and adaptive compensation on the part of hosts (Fig. 1). The interaction of these forces results in rapid and ongoing evolutionary change in both symbiotic partners, with profound evolutionary consequences. Symbiont degeneration coupled with host compensation is a defining characteristic of heritable symbiosis. A salient feature of this relationship is that the host must maintain a viable symbiosis with a partner that has a rapidly evolving genome due to the nonadaptive fixation of mutations through drift. In sum, the host must keep pace with its symbiont as multiple forces draw it ever further down the rabbit hole.

In this perspective, we focus on insect–bacterial symbioses, especially the symbiosis of pea aphid (Hemiptera: *Acyrthosiphon pisum*) and *Buchnera aphidicola* (*Gammaproteobacteria*), for which recent experimental studies have yielded new insights into the integration of symbiotic partners. These ideas are potentially applicable to a broad range of heritable symbioses in which the symbiont is strictly clonal and restricted to living in hosts.

Evolutionary Opportunities from Symbiosis: Ecological Benefit and Lineage Expansion

On macroevolutionary time scales, symbiont acquisition has often enabled evolutionary diversification and ecological expansion. By acquiring maternally transmitted bacterial symbionts, many insect lineages have succeeded in unlocking new ecological niches, particularly ones that present nutritionally unbalanced diets. Aphids and other sap-feeding insects rely on phloem sap or xylem sap as their only food, and these diets are extremely limited in essential amino acids and some vitamins (6). Use of these unbalanced diets is possible because symbionts supply missing nutrients (7–10).

The macroevolutionary and ecological consequences of acquiring symbionts can be immense. Continuing with the same example, symbiont-dependent sap-feeding insects were among the first herbivores to exploit vascular plants (11, 12) and include highly successful clades such as aphids (5,000 described species), whiteflies (1,600 species), psyllids (3,000 species), scale insects (8,000 species), leafhoppers (>20,000 species), cicadas (2,500 species), spittlebugs (3,000 species), and planthoppers (13,000 species) (11). All possess needle-like mouthparts, or stylets, used to access plant fluid and sap diets. These groups exhibit diverse plant–parasitic lifestyles and are critical players in terrestrial ecosystems as vectors of plant disease, food to diverse predators and parasites, and mutualists to other insects including ants. In each of these sap-feeding insect groups, phylogenetic analyses

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Author contributions: G.M.B. and N.A.M. designed research, performed research, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

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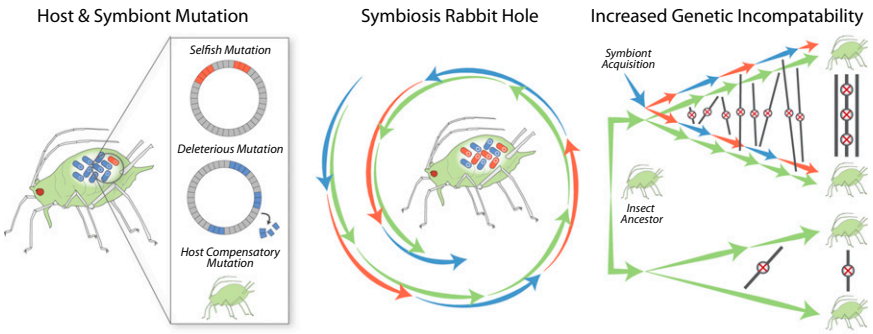

Fig. 1. Causes and consequences of symbiotic coevolution. Mutations that negatively impact the symbiosis can be fixed through genetic drift due to clonality and small population size (shown in blue) or through within-host selection for selfish symbionts that favor their own fitness over that of the host (red). In response, the host is selected to buffer these mutations (green), leading to a spiral down the symbiosis rabbit hole. This symbiont–host coevolution may drive the rapid accumulation of genetic incompatibilities between host lineages and between hosts and symbiont strains. Lineage-specific symbiont–host coevolution may lead to accelerated reproductive isolation and speciation, which could further reduce the effective size of genetically compatible host populations.

show that obligate symbionts have been vertically transmitted for millions of years, in many cases from the late Permian [>250 Mya (million years ago)] (8, 10). The diversity and abundance of insects feeding on xylem or phloem sap reflect the dominance of vascular plants in terrestrial ecosystems: symbionts provided the entry to a vast and expanding new niche that spread across the globe. As a counterexample, the Coleorrhyncha, which resembles other sap-feeding groups in originating around the same time (the Permian) and having an obligate symbiont, contains only about 24 species, reflecting ties to specific nonvascular plants (certain mosses) (13, 14).

Parallel cases of symbiont-driven ecological expansion have been documented in other insects, including cockroaches (15, 16), ants (17), lice (18), and beetles (19–21). Examples extend into other animal hosts although, in some, transmission may be partly or wholly horizontal rather than strictly vertical. Examples include vesicomyid clams (22, 23), corals (24), earthworms (25), sponges (26), tunicates (24), and flashlight fish (27). In most, phylogenetic analyses demonstrate that symbiont acquisitions occurred in a shared ancestor of a major clade. By freeing hosts from specific nutritional requirements or by providing new protection against pathogens or predators, symbiosis has enabled novel lifestyles and increased long-term fitness.

Acquiring a heritable symbiont is effectively a mutation of major effect, increasing host fitness at the population and clade level. In many, although not all, identified cases, these acquisitions have resulted in a proliferation of descendant lineages, usually comprised of species restricted to a particular dietary niche. Thus, long-term, heritable symbiosis underlies many dominant insect lifestyles and has shaped macroevolutionary and ecological patterns.

Evolutionary Hazards of Symbiosis

Becoming Irreversibly Obligate. The continuous presence of a vertically transmitted symbiont leads to the evolution of developmental dependence beyond the symbiont's original contribution; that is, hosts become addicted to their symbionts. In aphids, elimination of *Buchnera* through antibiotic treatment interferes with development, which typically stalls if *Buchnera* fails to colonize (28). Aphid females deprived of *Buchnera*, due to heat, antibiotics, or old age, produce few or no progeny, even when dietary nutrition is sufficient (29). This dependence on *Buchnera* for development reflects 150 million years of fixation of aphid mutations that are beneficial or neutral in the presence of *Buchnera* but potentially deleterious in its absence. Thus, adoption of symbionts for nutrient provisioning is a gateway to developmental dependence even when those nutrients are not needed. Indeed, so long as the symbiont is continuously present, addiction can evolve even to deleterious microbes, such as the reproductive parasite *Wolbachia* (30).

Reflecting their reliance on symbionts, hosts have evolved specialized mechanisms and tissues for housing and supporting symbionts and for transferring them from mother to progeny. In aphids, cells that are specified to become bacteriocytes show distinctive gene expression in early developmental stages, and their cellular fate is determined before *Buchnera* colonization (31, 32). Bacteriocyte expression of genes underlying amino acid metabolism complements *Buchnera* pathways for amino acid biosynthesis, reflecting extensive host–symbiont collaboration in this central nutritional function (33–35). Certain aphid genes seem to function solely in controlling or supporting *Buchnera*. For example, some highly expressed peptides are confined to bacteriocytes or surrounding sheath cells (36). An amino acid transporter expressed in bacteriocytes has altered substrate affinity that imposes negative feedback regulation of essential amino acid production by *Buchnera* (37). Finally, an aphid-encoded protein, originally of bacterial origin (but not *Buchnera*), has been shown to be localized within *Buchnera* cells although its function is not yet known (5). Taken together, these findings for the *Buchnera*–aphid symbiosis point to extensive genomic and metabolic integration of symbiotic partners and blur the distinction between symbiont and organelle.

Accommodation of symbionts may require that hosts suppress or modify immune responses (38, 39), potentially elevating risk of pathogen invasion. In aphids, many genes underlying responses to Gram-negative bacteria have been eliminated, including the immune deficiency signaling pathway (IMD), peptidoglycan receptor proteins, and antimicrobial peptides (40, 41). Potentially, these losses facilitated the evolution of symbioses with *Buchnera*, and with numerous facultative symbionts, as supported by the observation that *Buchnera* cells elicit the IMD pathway in other insects (42). This reduction in immunity seems to have consequences because aphids are susceptible to infections by bacterial pathogens during feeding and during nutritional stress (43, 44). The prospect that immune-system reduction paved the way for the elaborate symbioses in sap-feeding insects generally will likely be resolved from ongoing genome sequencing of additional insect species that vary in symbiotic associations.

As these examples illustrate, once a symbiont is required for development, hosts may become locked in, even when the original symbiotic benefit is reduced or eliminated due to changing ecological conditions or deterioration of symbiont functionality. Evidence that such deterioration indeed occurs is discussed in the next sections.

Symbiont Decay. A well-documented force affecting heritable symbionts is genetic drift leading to the fixation of neutral or deleterious mutations that cause gene inactivation, gene loss, or inefficiency of gene products (45, 46). The basis for elevated genetic drift is the drastic shift in population genetic structure that occurs when a free-living microorganism adopts an obligate symbiotic lifestyle. The genetic population size becomes largely dependent on the host population size (47), and free-living bacteria have much larger populations than do animals (48).

Furthermore, most heritable symbionts are strictly clonal, being transmitted only through host matrilines. This radical change in population structure results in less efficient selection genome-wide, leading to elevated rates of fixation of deleterious mutations (45, 46, 49, 50).

Symbiont genome decay affects genes in all functional categories (8). The first obligate symbiont genome sequenced, that of *Buchnera* of the pea aphid, was most notable for the fact that it had undergone extensive gene loss and contained no novel genes yet did retain genes for biosynthesis of essential amino acids needed by hosts (51). With more genome sequencing, it became apparent that *Buchnera* genomes in different aphid lineages continue to undergo irreversible gene loss, over long and short time scales (52–56). Similar ongoing gene loss is evident in every obligate symbiont clade for which multiple genomes have been sequenced (16, 57, 58). Many show far more extreme genome reduction than does *Buchnera.* Indeed, symbiont genomes have repeatedly evolved to be the very smallest genomes known in cellular organisms (aside from organelles), with total gene counts often <300 and sometimes <150 (1, 3, 10, 58–62). Continuing losses from established obligate symbionts include genes underlying central cellular functions and cell-envelope production, as well as genes underlying symbiotic benefits such as nutrient biosynthesis.

Essential genes that are retained are subject to elevated burdens of slightly deleterious mutations in heritable symbionts. Compared with homologs in free-living relatives, gene products have lower efficiencies and reduced thermal stability (53, 63). Symbionts also exhibit genome-wide accelerated sequence evolution and mutation-driven biases in nucleotide base composition (8, 45, 46, 64, 65). This mutation-driven bias generally favors A+T nucleotides and has extreme effects on polypeptide composition; all encoded proteins in most insect symbionts are strongly shifted toward amino acids that enable higher A+T in the DNA sequence. The negative effects of these mutations are partially masked by constitutively high expression of chaperones that help to stabilize impaired proteins (66–68), but high chaperone expression is itself metabolically costly.

These observations raise a question: How can seemingly deleterious mutations that eliminate or hinder useful symbiont functions become fixed? One explanation depends on the fluctuations in nutrient availability in environments. Host insects encounter varying ecological conditions, such as changes in host plants that affect nutrient availability. If the symbiont provisions nutrients, but the diet sometimes is enriched for those nutrients, selection to maintain the corresponding symbiont pathway will be relaxed, opening the way for inactivation of the underlying genes. The result is that the host now requires the dietary supply, leading to a long-term narrowing of its ecological range (Fig. 2). For example, in some gall-feeding aphids, *Buchnera* has lost biosynthetic pathways for some nutrients (53), probably because gall formation results in enrichment of ingested sap. This unidirectional loss of *Buchnera* capabilities potentially prevents the aphid lineage from returning to a broader feeding niche.

Loss and decay of symbiont functionality result in selection on hosts to compensate. Hosts require symbionts for nutritional benefits or for proper development so ongoing symbiont decay forces hosts to continually adapt. Host compensatory adaptations are reflected in the elaborate support systems that are beginning to be revealed from studies of symbioses of sap-feeding insects. For example, in mealybugs and psyllids, symbionts have lost most of the genetic machinery for generating cell-envelope components, and genes underlying these functions are instead found within host genomes and are highly expressed in bacteriocytes (3, 4). As hosts evolve to shore up symbiont shortcomings, the latter are able to lose even more functionality, leading to increasingly intricate host support systems. This pressure on hosts to compensate for symbiont decay explains why obligate symbionts have the smallest genomes, by far, of any cellular organisms: Their hosts evolve to compensate for symbiont gene losses, facilitating further loss of symbiont function over time. Thus, the lineage descends into the symbiosis rabbit hole, driven by genetic drift in the symbionts and compensatory adaptation by hosts (Fig. 1).

Symbiont Selfishness. Fitness of a maternally inherited symbiont is closely aligned with that of its hosts. Thus, natural selection generally favors symbiont features that benefit hosts, such as mechanisms for efficient nutrient provisioning at economical metabolic costs. Indeed, some symbiont features seem to be specific adaptations for increasing host-level fitness: e.g., in *Buchnera*, pathways for production of some essential amino acids are amplified and located on plasmids as mechanisms for overproducing or regulating the production of these nutrients (9). Nonetheless, the fitness interests of host and symbiont are not identical. The potential for the spread of "selfish" symbiont mutations persists even in the most intimate codependent associations (69). A symbiont mutation that speeds replication of the mutant cell line within a host–and thereby increases its proportional representation in the progeny–can increase in frequency even if it lowers her overall fecundity. Although mutualistic symbioses are often considered as fully cooperative, in fact, we should expect elements of a coevolutionary arms race, or Red Queen evolution, of the sort widely demonstrated for host–pathogen coevolution (70). Accordingly, the machinery underlying symbiont–host integration may represent not a stable solution to host–symbiont integration, but the current status of an ongoing struggle, driven by both conflict and concordance in evolutionary interests.

Among the clear examples of selfishness on the part of maternally inherited symbionts are mechanisms that favor female over male progeny. In most cases, males are a dead end for symbionts (but see ref. 71), and symbionts are not expected to support male reproduction (72). Numerous cases of symbionts

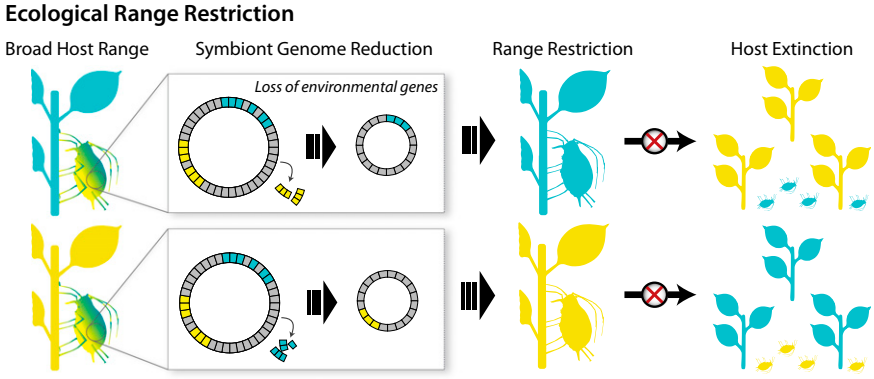

Fig. 2. Ecological range restriction by symbiont gene loss. In a specific environment, some symbiont genes may not be needed, resulting in relaxed selection for their maintenance and inactivation. In sap-feeding insects with obligate symbionts, using a food plant with abundant levels of a particular nutrient can lead to irreversible loss of symbiont genes for making that nutrient. A consequence is permanent restriction of the host's ecological range: for example, confinement to a smaller set of food plant species. As available resources change over time (e.g., due to climate change), a possible consequence of a narrower ecological niche is smaller population size or eventual extinction.

manipulating reproduction to favor production of infected matrilines, at male expense, have been documented, including many within the widely known *Wolbachia* (Alphaproteobacteria) (73), as well as *Cardinium* (Bacteroidetes) (74). In ancient obligate symbiosis, mechanisms for the propagation of symbionts have been fixed, but ongoing mutations can favor the proliferation and transmission of selfish symbionts within matrilines.

In general, a mutant symbiont cell lineage that replicates faster but does not provision nutrients to hosts might increase proportionally within progeny of a female. However, once fixed, its hosts will have lower fecundity than hosts in which all symbionts provision nutrients (47). Thus, matrilines in which selfish symbionts become fixed are negatively selected within the host population, implying strict limits on symbiont selfishness. Nonetheless, selection at the host level will not eliminate selfish tendencies of symbionts, and hosts are expected to evolve counteradaptations. Several observations do suggest elements of arms race coevolution in intimate, heritable insect symbioses. In both aphids and *Sitophilus* grain weevils, peptides resembling the classic antimicrobial peptides that are effectors of the innate immune system seem to be key in the containment of maternally inherited nutritional symbionts (36, 75). Likewise, in the obligate symbiosis of tsetse, immune components play a part in regulating symbionts (38). Thus, hosts seem to control symbionts using mechanisms related to those that limit pathogen invasion. However, host control of heritable symbiont proliferation has been investigated in only a few systems.

In addition to favoring direct controls on selfish symbionts, selection on hosts could lead to mechanisms that limit the potential for symbiont-level selection. Such adaptations could involve separation of a distinct symbiont pool used for transmission to progeny (69) or enforcing small inoculum size (47). Host controls seem most likely to evolve when symbionts replicate many times per host generation. Potentially, hosts can eliminate a bacteriocyte along with its resident bacteria if the bacteriocyte is underperforming by not provisioning sufficient nutrients or if proliferating symbiont cells become cancerous. During the life of an aphid female, bacteriocytes are lost; speculatively, this elimination could be selective, functioning as a means of disfavoring retention and transmission of selfish *Buchnera* cell lines. Although hosts might police their symbionts so as to minimize selfish tendencies and promote cooperation (76), such policing is not yet known from insect symbioses. Most likely, some selfish mutations occur and are countered by hosts. Thus, along with genomic decay through drift, symbiont selfishness is an additional pressure that ultimately tightens the specificity of host–symbiont associations.

Consequences of Symbiosis for Host Evolution

Speciation Rates. We have argued that symbionts are prone to evolve in directions detrimental to hosts, due both to genetic drift in clonal symbiont populations and to selection favoring selfish traits. An implication is that hosts are continually selected to compensate. Under this scenario, the host–symbiont interface is predicted to rely on rapidly evolving genes that quickly acquire incompatibilities between populations (Fig. 1). In effect, host and symbiont coevolution will drag each symbiotic lineage deeper into its own unique rabbit hole. Thus, incompatibilities between symbiont and host loci, or between different host loci involved in symbiotic control, are expected to emerge quickly and to accelerate the emergence of postzygotic isolating mechanisms, reinforcing reproductive isolation at early stages of lineage divergence. Incompatibilities involving loci functioning in symbioses might arise even for host loci and symbiont genotypes circulating within a population, as seems to occur for nuclear loci within populations (77).

If symbiont–host incompatibilities emerge rapidly, insect clades with obligate symbionts might have higher speciation rates than similarly aged clades without obligate symbionts. In theory, this prediction is testable using a comparative phylogenetic framework to evaluate speciation rates in host clades with and without symbionts. In practice, such tests would be difficult because we still have poor estimates of species diversity in many insect clades, and comprehensive phylogenies are nonexistent. A further prediction, and one that might be tested more readily, is that reproductive isolation in insects with symbionts will often be enforced by incompatibilities between host and symbiont loci or between different host loci that contribute to the regulation, support, and transmission of symbionts. Understanding the role of symbiosis in generating reproductive isolation can be approached through experimental investigations of symbiosis using hybridization or transfection to produce novel host–symbiont combinations (78).

If symbiosis does facilitate speciation, one of the driving forces, genetic drift affecting symbiont genomes, is exacerbated. A major determinant of the rate of fixation of deleterious mutations in symbionts is host population size (47, 50), and each speciation event generates two smaller populations. As the host strives to keep pace with its symbiont, we expect an accelerated descent into the symbiosis rabbit hole.

Ecological Range. Symbiont evolution can lead to restricted ecological range of hosts by limiting tolerance of both biotic and abiotic factors, such as nutritional availability and temperature. As discussed in *Symbiont Decay*, if a symbiont loses genes underlying pathways for provisioning its host with nutrients, due to relaxation of purifying selection during periods of temporary nutrient abundance, then the host lineage becomes permanently dependent on environmental sources (or must acquire a new symbiont) (Fig. 2). Losses of nutrient provisioning capabilities are ongoing in all groups of obligate insect symbionts for which genome comparisons within a symbiont clade are available (2, 52–55). By enforcing dietary requirements, these losses are expected to narrow the range of suitable environments for host-insect lineages (Fig. 2).

Host insects cannot completely buffer their symbionts' environment, and symbionts incur mutations that impact their environmental tolerance, particularly to heat. Obligate symbionts in insects are heat-sensitive and can be killed by temperatures that do not kill their hosts. For example, carpenter ants are limited by the heat sensitivity of their obligate symbiont, *Blochmannia* (79). Likewise, *Buchnera* numbers plummet after heat exposure (80, 81). In pea aphid populations that experience continuous cool temperatures, *Buchnera* evolves to become even more heat sensitive, due to the spread of a mutation inactivating a heat shock promoter (82, 83).

Although symbiont heat sensitivity will be constrained by prevailing temperatures, symbionts generally seem to have narrow thermal range relative to that of hosts (84). The major effect of deleterious amino acid replacements is to lower protein stability. As a general compensation for protein instability, chaperone expression in obligate symbionts is high even under nonstress conditions (68). In *Buchnera,* chaperonin (GroEL) is produced constitutively at levels equivalent to those during extreme heat shock in *Escherichia coli* (66). Other *Buchnera* chaperones are also overexpressed constitutively, and only a few genes retain any transcriptional response to heat (67). Thus, ability to compensate for environmental stressors that destabilize proteins seems to be compromised. The net effect of these tendencies in symbionts is to reduce ecological range and, thus, host population size.

Escaping the Hazards of Symbiosis: Acquiring Novel Symbioses Through Replacement and Supplementation

Once a host lineage has proceeded down the irreversible path into obligate symbiosis, it seems that there is little opportunity to exit. In a few cases, such as the leafhopper subfamily Typhlocybinae, symbionts may have been lost in connection with dietary shifts: e.g., phloem sap to parenchyma. More often, the only escape from degenerate partners seems to be to supplement or replace them

with new symbionts. Numerous clear examples of an ancient obligate symbiont being joined or replaced by a newer one are evident, including several in the sap-feeding insects of suborder Auchenorrhyncha (Fig. 3). A likely driver for adding a new symbiont is the degradation of functions in an ancient one: A new symbiont can replace or supplement functions that are lost or inefficient in the older partner.

Initially a newly acquired symbiont has a large set of biosynthetic capabilities, including some that are redundant with those of the existing symbiont. Over evolutionary time, this redundancy is eliminated, as illustrated by the perfectly complementary and nonredundant combinations of biosynthetic pathways repeatedly observed for genomes of coresident symbionts in sap-feeding insects (1, 85, 86). Depending on which genome initially loses specific biosynthetic capabilities, a likely outcome is that both old and new symbionts become obligate for the host, each maintaining distinct and complementary contributions.

New symbionts can take on functions previously carried out by more ancient symbionts. For example, the aphid *Cinara cedri* contains *Buchnera* along with a second obligate symbiont, *Serratia symbiotica*, which lives in a distinct type of bacteriocyte (87–89). The acquisition of *S. symbiotica* coincides with further gene loss in *Buchnera*: The *C. cedri Buchnera* genome is substantially smaller and lacks several amino acid biosynthetic genes present in other *Buchnera* (55). The missing pathways are retained by *S. symbiotica*, despite its genome also being reduced (87, 90). In this case and others (86), a new symbiont has replaced or supplemented capabilities of an older one. However, the new symbiont embarks on the same evolutionary path of genome decay, driven by mutation and drift.

The sequential acquisition of multiple symbionts that retain complementary biosynthetic capabilities can be reconstructed for several lineages in the sap-feeding suborder Auchenorrhyncha [e.g., cicadas, spittlebugs, leafhoppers, and sharpshooters (10,

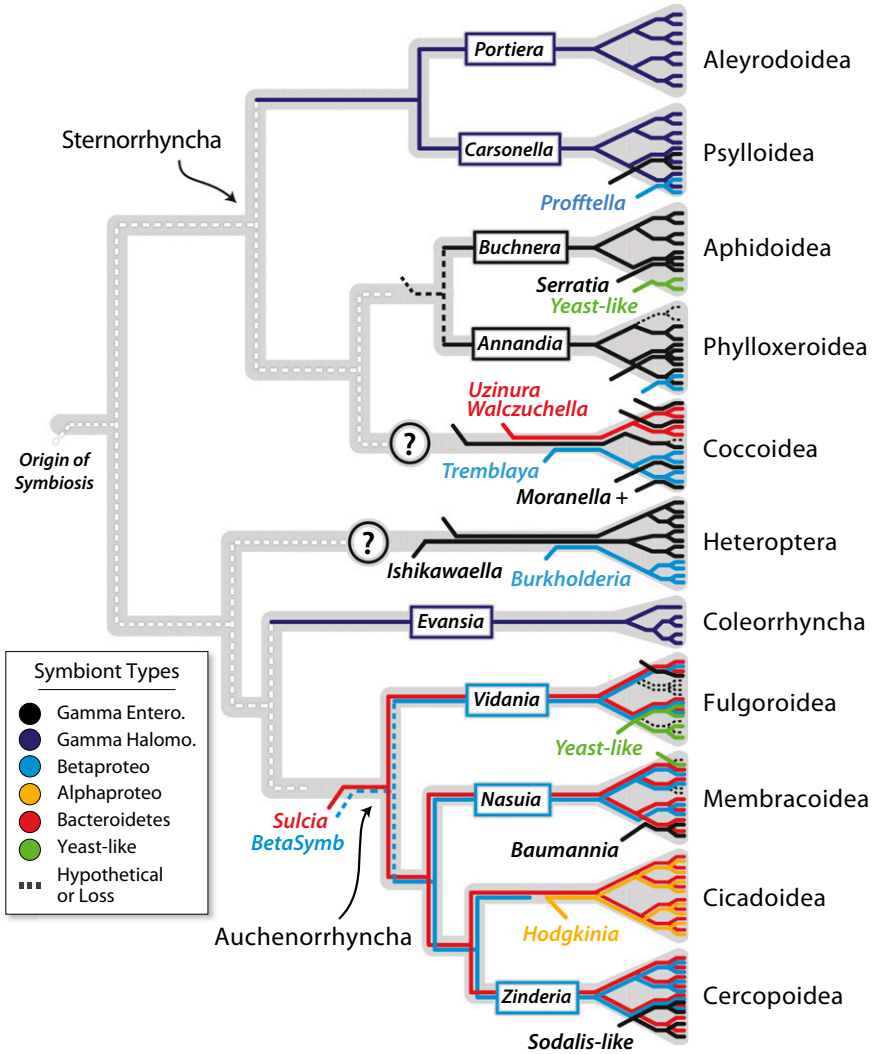

Fig. 3. Summary of the gains and losses of heritable symbionts across sap-feeding insects in the order Hemiptera. The phylogenies show evolutionary relationships of host insect groups (gray) and heritable symbionts. For color-coded lines, see *Inset* legend. Host phylogeny represents the most recent understanding, but placement of certain lineages (e.g., the Coleorrhyncha and Heteroptera) is uncertain (12, 89). Ancestral symbiont names are in boxes along their lineage; in some cases, the same symbiont lineage has different names in different insect clades. Names of acquired symbionts are shown where the symbiont is acquired on the host phylogeny. Dashed lines represent hypothetical relationships and possible origins of symbiosis deep in the evolution of the Hemiptera. The white-dashed lineage represents an ancestral symbiont that permitted the initial diversification of the Hemiptera; its identity is not yet known. Lineages that terminate at a question mark remain uncertain; host–symbiont relationships in these clades are diverse and their origins unclear. See text for citations regarding specific symbionts presented on the tree.

58, 91, 92)] and also some aphids, adelgids, and scale insects (87, 93–96) (Fig. 3). Most Auchenorrhyncha lineages contain the widespread ancestral symbiont *Sulcia muelleri*, plus a coresident partner, which varies among lineages. In each case, *Sulcia* and its partner have complementary amino acid biosynthetic pathways. The original symbiotic pair in Auchenorrhyncha was *Sulcia* plus a Betaproteobacterial symbiont; this pair originated >270 Mya and is retained by some descendant lineages (10, 92). In other lineages, including cicadas, sharpshooters, and one tribe of spittlebugs, *Sulcia* is retained, but the other symbiont is replaced by a new symbiont type (91). These replacements potentially expand the ecological niche of the host insect. For example, in the sharpshooters, a clade within the large leafhopper family Cicadellidae, *Baumannia* replaced *Nasuia* (the betaproteobacterium) and may have facilitated the dietary transition from phloem sap to xylem sap. *Baumannia* has many more biosynthetic pathways than does *Nasuia*, possibly compensating for the lack of nutrients in xylem sap (97).

Relative to the time scale of host species diversification, symbiont replacements are relatively rare. Examining the morphology of bacteriocytes in symbiont replacements gives some insight into why replacements might be so few. The *Sodalis*-like symbiont that replaced *Zinderia* in spittlebugs of tribe Philaenini (Fig. 3) has a reduced genome but retains pathways complementary to those of its *Sulcia* partner (86). This new symbiont occupies a distinct cell type from the bacteriocytes that house *Zinderia* in other spittlebugs (91). The occupation of distinct cell types by each coresident symbiont suggests that the *Sodalis*-like symbiont initially coexisted with *Zinderia* by invading separate cells of the same host. Indeed, some relatives with the *Sodalis* group are opportunistic facultative symbionts that invade multiple cell types of insects using invasion machinery closely homologous to that found in pathogenic bacteria (98). In Philaenini and some other hosts (19), *Sodalis* lineages have become obligate symbionts restricted to specialized host cells. Strikingly, the evolution of novel bacteriocytes for new symbiont acquisitions is the norm among the Auchenorrhyncha (91).

In some insect groups, multiple gains and losses of symbionts have resulted in a confusing mosaic of symbiont combinations in different host clades. For example, scale insect (Coccoidea) families display varied associations, reflecting repeated symbiont acquisitions, replacements, and losses (96, 99). Mealybugs (Pseudococcidae), one clade of scale insects, host an ancestral betaproteobacterium, *Tremblaya* spp., which coresides with a variety of partners (95). In the mealybug *Planococcus citri*, this pair is so codependent that *Tremblaya* has eliminated parts of its own translational machinery, apparently depending on gene products of its partner, which lives within the *Tremblaya* cytoplasm (3). Similarly psyllids (Psylloidea) and whiteflies (Aleyrodoidea) host ancient gammaproteobacterial symbionts (*Carsonella ruddii* and *Portiera aleyrodidarum*) that seem to descend from a single colonization of an ancestor of these related insect groups (Fig. 3). Often, this ancestral symbiont coresides with more recently acquired symbionts, such as symbionts from the *Sodalis* group or the polyketide-producing symbiont *Profftella armatura* (100). In some psyllids, *Carsonella* shows metabolic interdependence with coresident symbionts (57). In each case, the newer obligate symbiont is subject to the same genome decay process as the older symbiont (87, 90, 94, 100).

Outside the Hemiptera, one of the best-studied cases of symbiont replacement is in weevils, one of the most species-rich animal clades. Phylogenetic reconstructions for hosts and symbionts show that an ancestor of weevils was colonized 125 Mya by the symbiont clade *Nardonella* (gammaproteobacteria), which was retained in many weevil lineages but replaced in several (20, 101, 102). In *Sitophilus* grain weevils, *Nardonella* was replaced with a *Sodalis*-like symbiont that has undergone genome rearrangement and decay. Thus, an evolutionary succession of heritable symbionts may be more widespread than previously appreciated.

The Long-Term Fate of Heritable Symbiosis

Understanding Host–Symbiont Interactions. We have argued that obligate, heritable symbionts present a moving target requiring ongoing counteradaptation on the part of hosts. This view parallels the proposal that prominent features of genomes, such as size and number of introns and abundance of nongenic DNA, reflect the interplay of natural selection and genetic drift and are therefore governed by population size, in addition to natural selection (48, 103). Similarly, features of intimate symbioses must be considered in the light of the evolutionary processes that govern them, including conflicts between selection on symbionts and selection on hosts, clonality of many symbiont lineages, and genetic population sizes of hosts and symbionts. Both deleterious mutations and selfish mutations are expected to recur in symbionts, and we expect hosts to continually adapt by controlling and supporting their symbionts, and sometimes by admitting novel symbionts. These expectations are consistent with recent findings on the molecular mechanisms acting at the host–symbiont interface, from aphids and *Buchnera* symbiosis and from other insect symbioses. It is interesting to speculate on the long-term fate of heritable symbiosis in which symbiont genomes are continually declining. Potentially, these processes sometimes limit host distribution so severely that extinction results. However, most obligate insect symbioses are millions of years old so speciation rates must often outnumber extinction rates in these clades.

Differences Between Symbionts and Organelles. The most evolutionarily successful of heritable symbioses are those that gave rise to mitochondria and plastids, raising the question of why organelles have not been limiting baggage for eukaryotic hosts. Although numerous studies have documented excesses of deleterious mutations circulating within organelle genomes, these mutations are generally recent, remain at low frequencies, and do not become fixed within populations (104). An apparent reason, at least for animal mitochondria, that drift does not more often bring deleterious mutations to fixation is potent selection within the female germ line against mutations that affect mitochondrial function (105). Thus, hosts have evolved mechanisms for preventing transmission of symbionts with harmful mutations. Such mechanisms have the short-term advantage of increasing fitness of offspring and the long-term effect of limiting the accumulation of harmful mutations within lineages. The extent of mechanisms for selective symbiont transmission in heritable symbioses such as those of sap-feeding insects is unknown.

Another difference between insect symbionts and organelles is that genomes of the latter encode little of their own machinery for self-replication and depend on import of needed gene products from the host. One consequence is that they typically have fewer genes and thus present a smaller mutational target. More importantly, organelle genes that are retained are subject to strong host-level selection, limiting their deterioration or selfish tendencies. In contrast, highly reduced genomes of insect symbionts contain mostly genes involved with cell replication, transcription, and translation: Mutations in these genes will impact fitness of individual symbiont cells, where selfishness can originate. Thus, eukaryotic organelles may have escaped the symbiosis rabbit hole primarily because genes controlling symbiont replication are transferred to the host genome.

Conclusion

Symbiosis opens new ecological niches for hosts and could accelerate speciation rates. However, it can also impose long-term fitness costs. The potential negative repercussions of obligate symbiosis raise the possibility that it can limit the ecological range of hosts, reduce population sizes, or even cause extinction of some

symbiont-dependent host lineages (along with their symbionts). We have argued that acquiring a maternally inherited obligate symbiont thrusts lineages into a peculiar irreversible coevolutionary relationship that potentially increases speciation rate as well as extinction risk. Genomics-based analyses provide some supportive evidence for these disparate evolutionary consequences of obligate symbiosis.

A main driving force for this process is the genomic decay in symbionts that results from strict clonality and small genetic population size. Therefore, we emphasize that these same expectations do not apply when the symbionts undergo horizontal or environmental transmission or when they are transmitted biparentally. In such cases, the opportunity for continued DNA uptake from the environment or for homologous recombination (sex) persist, circumventing the ratchet-like loss of symbiont function and genes. This point is illustrated by the nephridial symbioses of earthworms. The ancient vertically transmitted symbiont, *Verminephrobacter*, is inherited biparentally, continues to incorporate foreign DNA, and does not undergo genome reduction (106, 107). Conversely, entrance to the symbiosis rabbit hole does not require that symbionts be intracellular: Genome decay is observed in maternally transmitted extracellular symbionts, exemplified by *Ishikawaella capsulata* in plataspid stinkbugs (108). The evolutionary rabbit hole does require that the symbiosis be beneficial to hosts, driving them to coadapt. Hosts do not adapt to maintain pathogens, which therefore must retain sufficient capabilities to function independently. Although genomic reduction occurs in host-restricted pathogens, gene loss is far more extreme in obligate symbionts (2), implying that reduction is facilitated by host adaptation. Finally, acquiring a novel symbiont can slow the descent into the symbiosis rabbit hole, but new symbionts ultimately undergo the same drastic genome decay, requiring compensatory evolution in the host.

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