Museum genomics reveals the rapid decline and extinction of Australian rodents since European settlement

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Australia has the highest historically recorded rate of mammalian extinction in the world, with 34 terrestrial species declared extinct since European colonization in 1788. Among Australian mammals, rodents have been the most severely affected by these recent extinctions; however, given a sparse historical record, the scale and timing of their decline remain unresolved. Using museum specimens up to 184 y old, we generate genomic-scale data from across the entire assemblage of Australian hydromyine rodents (i.e., eight extinct species and their 42 living relatives). We reconstruct a phylogenomic tree for these species spanning ∼5.2 million years, revealing a cumulative total of 10 million years (>10%) of unique evolutionary history lost to extinction within the past ∼150 y. We find no evidence for reduced genetic diversity in extinct species just prior to or during decline, indicating that their extinction was extremely rapid. This suggests that populations of extinct Australian rodents were large prior to European colonization, and that genetic diversity does not necessarily protect species from catastrophic extinction. In addition, comparative analyses suggest that body size and biome interact to predict extinction and decline, with larger species more likely to go extinct. Finally, we taxonomically resurrect a species from extinction, Gould's mouse (*Pseudomys gouldii* Waterhouse, 1839), which survives as an island population in Shark Bay, Western Australia (currently classified as *Pseudomys fieldi* Waite, 1896). With unprecedented sampling across a radiation of extinct and living species, we unlock a previously inaccessible historical perspective on extinction in Australia. Our results highlight the capacity of collections-based research to inform conservation and management of persisting species.

biodiversity | conservation | exon capture | phylogeny | phylogenomics

Earth is facing a biodiversity crisis, with extinction rates during the Anthropocene far outpacing background rates of extinction across geological time (1–3). While it is clear that global extinction rates are increasing, the pattern and pace of individual species extinctions are rarely well characterized by detailed occurrence records across space and time (but see ref. 4). As such, it is difficult to disentangle the relative contributions of contemporary anthropogenic impacts and longer-term population declines. For persisting species, conservation planning and management therefore lack vital context about timing and scale of declines. Species that are already genetically depauperate or inbred may be more vulnerable to novel threatening processes and may suffer faster rates of decline in response to anthropogenic change (e.g., the Tasmanian devil; refs. 5 and 6). The interaction of demographic, genetic, and environmental effects can lead to rapid population collapse via a process referred to as an "extinction vortex" (7–9). In such cases, ongoing anthropogenic impacts may act as proximate causes for decline and extinction.

The severity of anthropogenic extinction is well illustrated in Australia, where 34 species of mammals have become extinct since European colonization of the island continent in 1788 (10, 11). Native Australian rodents appear to have been disproportionately affected by this recent extinction, making up 41% of Australian mammalian extinctions since European colonization but only 19% of terrestrial mammalian-species diversity. Rodents occupy a diverse range of habitats in Australia, from mesic forest to the arid desert. They play an integral role in Australian environments as consumers of plants, fungi, and invertebrates, as a prey source for other native species, and as ecosystem engineers (12). The rapid extinction and decline of Australian rodents is likely the result of multiple proximate factors including habitat loss, predation or competition with introduced species, novel pathogens, inappropriate fire management, climate change, or interactions among these (10, 13, 14). Also, along with many other native mammals, some rodent species were intensely hunted for bounty in the latter 19th and early 20th centuries. Over 2.5 million bounties were paid for hopping mice between 1883 and 1920 in New South Wales alone (15), reflecting both the magnitude of direct anthropogenic impacts and the historical abundance of these species.

While it is clear that native rodents are particularly susceptible to the impact of human activities in Australia over the past ∼250 y, it is less obvious whether the rodent extinctions since European

Significance

Native rodents represent 41% of Australian mammal extinctions since European colonization. To determine the scale and timing of their decline, we used museum specimens to generate genome-scale data from eight extinct Australian rodents and their 42 living relatives. Relatively high genetic diversity in extinct species immediately prior to extinction in the 19th or 20th century indicates that their populations were previously large and that declines began after European colonization of Australia. This demonstrates that genetic diversity does not necessarily provide insurance against catastrophic extinction events. Our results show that extinction risk was elevated for larger-bodied rodents and varied among biomes. In addition, we taxonomically resurrect an extinct species, Gould's mouse, which survives on an island in Shark Bay, Western Australia.

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colonization were wholly the result of anthropogenic impacts since 1788, or whether a reduced adaptive capacity via preexisting genetic erosion accelerated this process in the species which are now lost. Historical accounts suggest that at least two of these extinct rodents, the white-footed rabbit rat, *Conilurus albipes*, and the lesser stick-nest rat, *Leporillus apicalis*, were common until the early 19th century (16–18). Other than these anecdotal reports and inference from sparse Holocene subfossil records (e.g., ref. 19), little is known about the abundance and historical distributions of extinct Australian rodents. As such, the historical record is insufficient to directly investigate the rate and proximate causes of declines.

If pre-European declines contributed to these 20th-century extinctions, we would expect to see patterns of longer-term genetic erosion in specimens that were collected soon after European settlement of Australia. In the extinct Australian thylacine (*Thylacinus cynocephalus*) and in the mainland extinction of the Tasmanian devil (*Sarcophilus harrisii*), genetic data from museum specimens and from subfossils, support patterns of longer-term decline (5, 20–22). In Holocene Australia, the arrival of the dingo to the mainland ∼3,500 y ago (23–25), the shift to a drier and more drought-prone climate (26, 27), and/or increases in indigenous human population sizes (28) could have contributed to these patterns.

The genetic consequences of decline are expected to be detectable in species prior to extinction (29, 30). However, where species have experienced extremely rapid population collapse, the rate of decline may outpace observable reductions in genetic diversity (30–33). For example, no evidence of genetic erosion was recovered in the rapid extinction of the Carolina parakeet (34) or the Australian paradise parrot (35). Unexpectedly high diversity at mitochondrial or microsatellite loci has also been reported in mainland remnant populations of a number of Australian marsupials that have suffered severe declines since European colonization (36–38). Using genomic data, especially from museum specimens, we can make robust inferences about the diversity of recently extinct species and gain insight into the timing of their declines. Even when species are underrepresented in collections, genome-scale estimates of heterozygosity from a single individual within a population can provide a minimum estimate for the level of genetic variation in that population or species at the time that the specimen was collected. Aside from some charismatic examples [e.g., the thylacine (20, 21) and the paradise parrot (35)] and despite widespread recent extinction in Australia, historical museum collections have remained a largely untapped genomic resource for understanding the pattern and pace of extinctions since European colonization.

In addition to a lack of information about the pace of their decline, ongoing uncertainty about the taxonomic status of extinct Australian rodent species (e.g., refs. 39–42) raises questions about the extent of biodiversity loss and limits our understanding of the severity of Australia's extinction crisis. "Old endemic" Australian rodents (Murinae: Hydromyini) are nested within a broader Australo-Papuan radiation, with an origin from Asia from ∼8 million years ago (43). The taxonomic relationships of Australian rodents have proven difficult to resolve (42), and recent phylogenetic reconstructions of extant species have challenged traditional taxonomic arrangements (44–46). All extinct Australian rodents that were collected alive are known only from small numbers of museum specimens. Many of these specimens are damaged, incomplete, or poorly preserved, making the morphological study of already ambiguous diagnostic characters in rodents even less reliable and, in some cases, not possible. As such, the evolutionary distinctiveness and phylogenetic placement of extinct Australian rodents cannot be determined in the absence of a comprehensive molecular phylogeny.

As well as potentially being more susceptible to extinction due to genetic erosion, ecological factors may predispose certain species to various proximate causes of decline and extinction. Body size has previously been associated with extinction risk in Australian mammals, especially in the "critical weight range" of between 35 and 5,500 g (14, 47–49), which includes the majority of native rodents (∼8 to 1,200 g; ref. 12) and suggests that larger rodents are at greater risk. Patterns of post-1788 extinctions in both Australian plants and animals also suggest that extinction has been highest in the arid biome (11), where lower and more variable productivity can cause species to be more susceptible to ecosystem disturbances and competition with introduced species (47). While the majority of previous studies have focused on marsupials, Smith and Quin (48) found that range declines in Australian rodents increased with body size and were most severe in arid and temperate mesic biomes. In contrast, Lawes et al. (50) found an effect of habitat type but not body size. The absence of extinct species from molecular phylogenies has meant these hypotheses have never been adequately tested using a framework that accounts for phylogenetic nonindependence. Given a molecular phylogeny including extinct taxa, comparative phylogenetic methods provide the opportunity to integrate phylogeny with ecological, environmental, and anthropogenic variables to test these associations.

Using historical and contemporary museum specimens, we generate phylogenomic data spanning 1,400 targeted exons from 87 specimens to reconstruct the evolutionary history and phylogenetic relationships of eight extinct Australian rodents and their 42 living relatives. This provides unprecedented sampling in this clade across both extinct and extant species. Using whole-exome sequencing data from a subset of 48 specimens, including five extinct species, we assess the extent of genetic erosion in extinct species at the time of specimen collection. If population collapse in recently extinct species occurred rapidly and as a direct result of human-mediated impacts since 1788, we expect to detect little or no reduction in genetic diversity in specimens collected in the 19th and early 20th centuries. Conversely, if populations had experienced prolonged periods of decline prior to European colonization, we expect reduced genetic diversity in these specimens, comparable to currently threatened or insular taxa. When accounting for phylogeny, we predict that phenotypic or ecological factors (e.g., body size and biome) will predict extinction risk and decline.

Results and Discussion

Genome-Scale Phylogeny of Australian Rodents. Using exon capture data from 1,245 independent genes, we inferred a comprehensive phylogenomic tree for 85 samples (*SI Appendix*, Fig. S1). Using a down-sampled dataset, which included one representative per species, we inferred a time-calibrated tree for 50 species (Fig. 1). Based on this extensive sampling of species and loci, we recovered a consistent, well-supported phylogeny for the Australian hydromyine rodents. Our results are congruent with previous studies of evolutionary relationships in the Pseudomys and Conilurus Divisions (45, 46) and expand our understanding of these relationships to all described species within these two divisions.

Phylogenomic Placement of Extinct Species. We resolved phylogenetic relationships of eight species of extinct Australian rodents using historical museum specimens. In total, ∼10 million years of unique branch length (i.e., evolutionary history) has been lost across these extinctions. This represents a loss of ∼11% of the total unique evolutionary history within this Australian radiation since colonization by Europeans. This loss was especially concentrated in the Conilurus Division and the genus *Notomys* (Fig. 1), with ∼31% and ∼35% of their respective evolutionary history lost to extinction within the past 150 y.

Divergence times of extinct species from their closest extant relative ranged from 0.44 to 2.24 million years ago in seven out of eight cases (Fig. 1). In the eighth case, and in contrast to all other species-level divergences, we found an exceptionally close relationship between the extinct Gould's mouse (*Pseudomys gouldii* Waterhouse, 1839) and the extant island-restricted Shark Bay mouse

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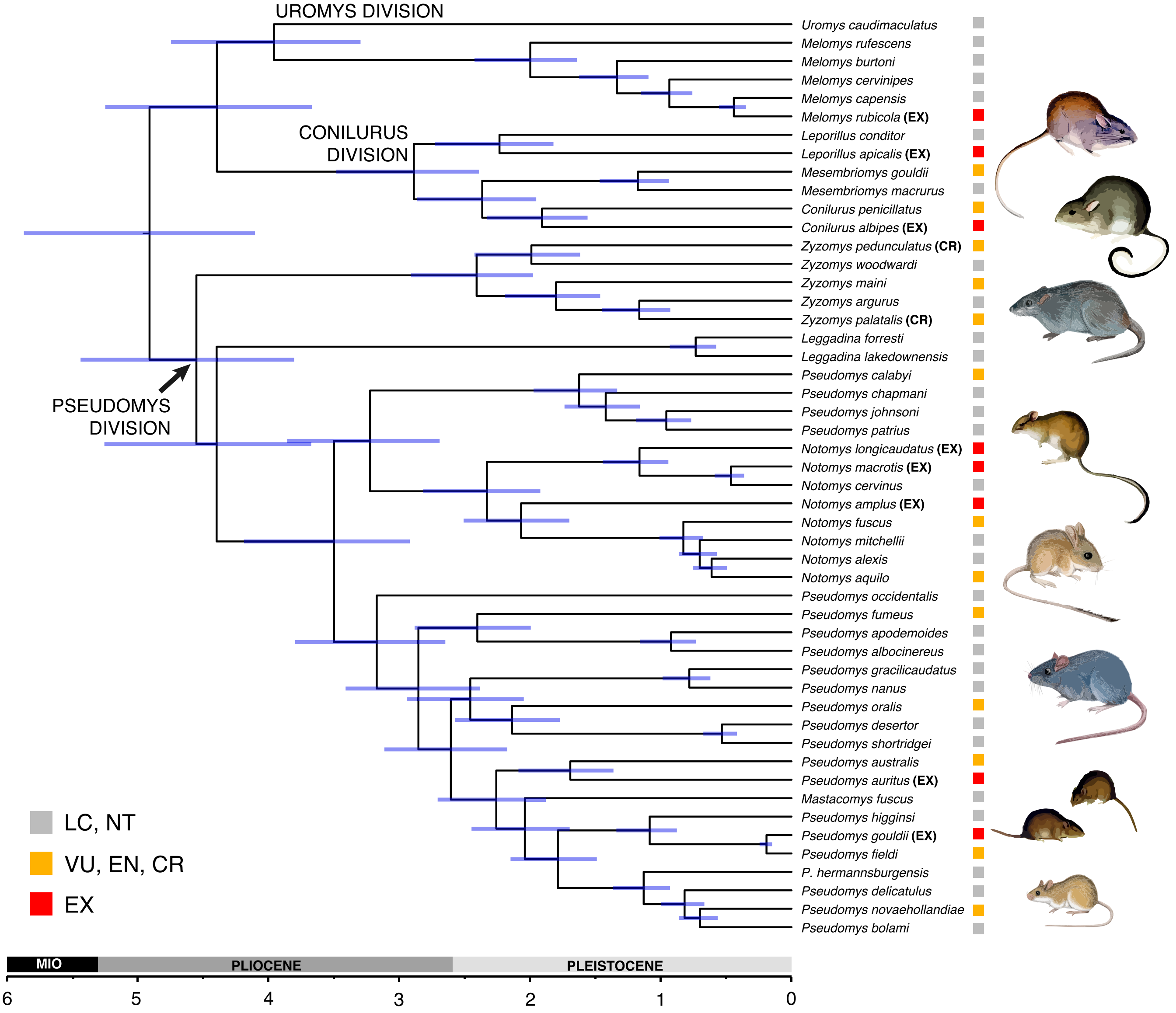

Fig. 1. Time-calibrated phylogeny of endemic Australian murine rodents based on 1,245 genes. Blue bars represent the 95% highest posterior density for each calibrated node. Colored squares indicate the current IUCN status for each species. A subset of illustrations are by Subir Shakya, and reprinted with permission from ref. 70. Top to bottom: *Ls. apicalis*, *C. albipes*, *Zyzomys woodwardi*, *N. longicaudatus*, *N. alexis*, *Pseudomys fumeus*, *P. gouldii* (×2), *Pseudomys delicatulus*.

(*Pseudomys fieldi* Waite, 1896), with an estimated divergence within the last 200,000 y (95% highest posterior density, HPD: 0.15 to 0.25 Ma). For the extinct island-endemic Bramble Cay melomys (*Melomys rubicola*), we recovered a sister relationship (95% HPD: 0.34 to 0.56 Ma) with the Cape York melomys (*Melomys capensis*). The divergence of these two *Melomys* species falls just above the intraspecies level in this clade (approximated by a path length of ∼0.0015 substitutions per site between sister species, Fig. 2*A*). Given the isolation of the Bramble Cay melomys on an ephemeral island in the Torres Strait, this is unsurprising. For the two genera in the Conilurus Division with extinct species (i.e., *Conilurus* and *Leporillus*), we recovered the expected sister relationships with their extant congeners (i.e., the extinct white-footed rabbit rat, *Conilurus albipes*, sister to the extant brush-tailed rabbit rat, *Conilurus penicillatus*; and the extinct lesser stick-nest rat, *L. apicalis*, sister to the extant greater stick-nest rat, *Leporillus conditor*). Both sister divergences occurred during the early to mid-Pleistocene (*Conilurus*, 95% HPD: 1.54 to 2.35 Ma; *Leporillus*, 95% HPD: 1.80 to 2.75 Ma) and are among the deepest phylogenetically divergent sister species of all Australian rodent lineages. In the Pseudomys Division, we resolved the phylogenetic position of five extinct species. In the genus *Notomys*, we found that the long-tailed hopping mouse (*Notomys longicaudatus*) and big-eared hopping mouse (*Notomys macrotis*) fall within the *Notomys cervinus* clade, while the short-tailed hopping mouse *Notomys amplus* represents a distinct evolutionary branch within the genus, diverging from its congeners during the early Pleistocene (95% HPD: 1.68 to 2.53 Ma). This is in contrast to morphological taxonomic assessments that allied *N. amplus* with the *N. cervinus* clade based on its larger size and lack of the derived reproductive tract characteristic of the *Notomys alexis* clade (40, 42, 51). The divergence of *N. macrotis* from *N. cervinus* was comparatively recent (95% HPD: 0.36 to 0.59 Ma), consistent with expectations of a close relationship between these taxa (52). In the genus *Pseudomys*, we recovered a sister relationship between

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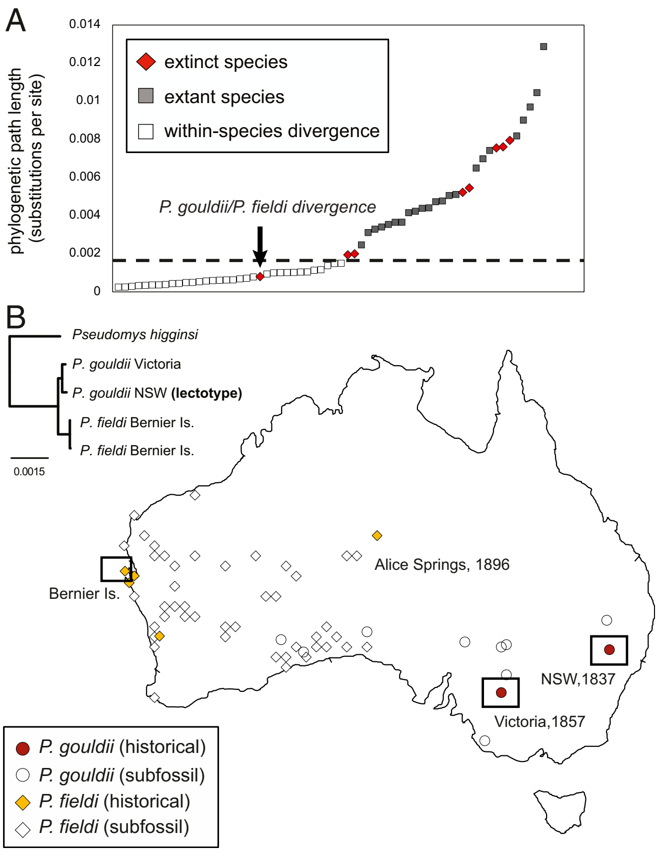

Fig. 2. (*A*) Distribution of interspecies and intraspecies divergences across all species, calculated as substitutions per site since split from sister species (above the species threshold) or split from geographically disparate subpopulation (below the species threshold). The dotted line demarcates the interspecies/intraspecies threshold based on species boundaries in Australian rodents. (*B*) Subsection taken from the full phylogeny to illustrate the close relationship between *P. gouldii* and *P. fieldi* (including from the type locality of *P. gouldii*) in relation to their otherwise closest sister species, *P. higginsi*; map of Australia showing collected specimens from live animals (colored points) and recent subfossil (open points) records of *P. gouldii* and *P. fieldi*. Sampled sites are indicated with boxes.

the extinct long-eared mouse (*Pseudomys auritus*) and the extant plains mouse (*Pseudomys australis*), with an estimated mid-Pleistocene divergence (95% HPD: 1.35 to 2.09 Ma).

Resurrection and Continental Collapse of Gould's Mouse. As a measure of relative divergence, we compared the distribution of species-level and intraspecies phylogenetic path lengths for all samples (Fig. 2*A* and *SI Appendix*, Fig. S1). The evolutionary divergence of Gould's mouse (*P. gouldii*) from the Shark Bay mouse (*P. fieldi*) falls well within the distribution of intraspecies path lengths as defined by current taxonomy (Fig. 2*A*). In addition, genetic divergence between the widely separated samples of *P. gouldii* and *P. fieldi* is consistent with a within-species isolation by distance pattern as estimated for *Pseudomys hermannsburgensis* (*SI Appendix*, Fig. S2), a species with a similarly widespread arid-zone distribution. While previously recorded across mainland Australia, the distribution of *P. fieldi* collapsed to a single 42-km^2 island in Shark Bay, Western Australia (Fig. 2*B*), from which samples in this study were obtained. *P. gouldii* is known from historically collected specimens at only two locations (Fig. 2*B*), both of which were included in this study. Samples of *P. fieldi* from Shark Bay and of *P. gouldii* from New South Wales and Victoria are all closely related phylogenetically (Fig. 2*B*). These results are consistent with previous suggestions that *P. gouldii* and *P. fieldi* are indistinguishable based on morphology (41, 53). There are also historical collection records of *P. fieldi* from two locations in mainland Western Australia and the single, badly damaged holotype specimen collected in 1896 from Alice Springs, Northern Territory, which did not yield any DNA. Our results, in combination with subfossil records of *P. gouldii*/*P. fieldi* (Fig. 2*B*), suggest that this represents a single species that had a much larger and relatively continuous range across Australia at the time of European settlement.

We propose that the lack of genetic differentiation between *P. gouldii* and *P. fieldi*, including the lectotype specimen of *P. gouldii* NHMUK ZD 1855.12.24.149 (Fig. 2 and *SI Appendix*, Figs. S1 and S2), and their indistinguishable morphology (41, 53) indicates that these names apply to a single, broadly distributed species. The name *P. gouldii* Waterhouse, 1839 takes precedence over *P. fieldi* Waite, 1896 and its junior synonym *Pseudomys praeconis* Thomas, 1910. This means that *P. fieldi* should be considered a junior synonym of *P. gouldii*. For practicality, we suggest that the common name for the remaining population of this species, the Shark Bay mouse (Djoongari), be retained.

In synonymizing the extant *P. fieldi* with *P. gouldii*, we taxonomically resurrect a species from an otherwise increasing list of mammalian extinctions in Australia since European settlement (10, 11). However, this species survived extinction via persistence on a single predator-free offshore island in Shark Bay, Western Australia, thereby only retaining remnant genetic diversity. Since European settlement, the range of collected specimens from this species spanned most of the continent (Fig. 2*B*), including the type specimens from New South Wales (*P. gouldii*) and Northern Territory (*P. fieldi*). Late Holocene subfossil records across much of Australia also capture an expansive and more contiguous distribution for this species until recent times (Fig. 2*B*) (19, 41, 54–59). This catastrophic, continental collapse is not an uncommon pattern among Australian mammals, with seven previously widespread species now restricted to islands (10, 60). Islands that harbor remnant populations of otherwise extirpated species have remained free from feral cats and foxes (10, 60). Although it is unclear exactly when mainland populations became extinct, no live individuals of *P. gouldii* have been seen or collected on the mainland since 1895. It is therefore possible that mainland populations were extirpated very rapidly and only shortly after the establishment of feral cats—one of multiple potential causative factors in their decline—across most Australian habitats by 1890 (61). The substantial expansion in the known range of *P. gouldii* revealed by this study highlights the importance of continuous, targeted conservation funding for the remaining population in Shark Bay. Current conservation efforts propose to translocate individuals to predator-free sites in western and central Australia, and these efforts could now be expanded to include such sites in eastern Australia.

Genetic Diversity in Extinct Australian Rodents. We generated a total of 48 whole exomes from 40 species in the Pseudomys and Conilurus Divisions, including five extinct species (*SI Appendix*, Table S2), from which we estimated mean individual heterozygosity per species. The other three extinct species, as included in target exon capture, did not yield sufficient DNA to obtain full whole exomes. Sequencing coverage ranged from 28× to 88× for historical specimens and from 9× to 62× for modern specimens (*SI Appendix*, Table S2), with no relationship between coverage or specimen type (i.e., bone or toepad versus fresh tissue) and heterozygosity (*SI Appendix*, Fig. S3). Based on these data, we did not find a significant difference (using a two-sample t test) between the mean heterozygosity of species that became extinct in the 20th century (0.00158) and of extant species not listed in International Union for Conservation of Nature (IUCN) threatened categories (0.00152), nor those in "large/geographically connected" populations (0.00154, Fig. 3). By contrast, mean heterozygosity was significantly (~33%) higher in these extinct species than in species currently listed as endangered

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or critically endangered (0.00107) and for those in "small/geographically restricted" populations (0.000994). In most cases, the now-extinct lineage had greater genetic diversity at the time of sample collection than its respective closest living sister species has at present (*SI Appendix*, Fig. S4). Although small mammals can persist in small but stable population sizes, estimates of heterozygosity in this study suggest that now-extinct species had comparable genetic diversity to extant species in relatively large and geographically contiguous populations and much higher values than species with small current populations. Estimates of heterozygosity calculated only from third-codon position sites were consistent with these results (*SI Appendix*, Fig. S5).

Compared to extant species that are critically endangered (e.g., *Zyzomys pedunculatus*) or from small populations (e.g., the extant island-restricted *P. gouldii*), we found no evidence for genetic erosion in any of the five extinct species at the time of their respective specimen collection: *C. albipes*, *L. apicalis*, *N. longicaudatus*, *N. amplus*, or in the mainland extinction of *P. gouldii*. Analogous to results from the recently extinct paradise parrot (35) and Carolina parakeet (34), our results suggest that the decline of these Australian rodents was extremely rapid. This is in contrast to evidence for longer-term decline preceding the extinction of the Australian thylacine (20, 21). Our results suggest that Australian rodents had not suffered significant, protracted declines in local population sizes prior to the 20th century and strongly implicate post-1788 anthropogenic impacts in Australia as the driving force in their extinction. In addition, the contrast between the heterozygosity of *P. gouldii* collected in 1857 and the present-day low diversity of the remaining island population in Shark Bay highlights the magnitude of genetic erosion that is likely in remnant populations of other species once widespread on the mainland (e.g., *L. conditor*). Severely low genetic diversity in extant species in "small/geographically restricted" populations, two of which are critically endangered, highlights the susceptibility of these populations and species to the effects of the "extinction vortex" (7–9). Without conservation intervention, the future persistence of these species is uncertain.

Particularly high heterozygosity in the extinct long-tailed hopping mouse (*N. longicaudatus*) from the central desert (Barrow Creek, NT) supports suggestions that this species was previously widespread and common owing to its frequent occurrence in subfossil remains and owl pellets from across the arid zone (19, 62, 63). As such, this species would be expected to have had a large effective population size across a relatively homogeneous landscape, facilitating gene flow and resulting in high heterozygosity. Subfossil remains also suggest that the extinct short-tailed hopping mouse (*N. amplus*) had a broad distribution across the central and western arid zone (19, 58, 63). In addition, the extinct white-footed rabbit rat (*C. albipes*) was previously abundant in Victoria and was described by John Cotton in 1846 as "the common rat of the country" (64). The extinct lesser stick-nest rat (*L. apicalis*) was reported by Krefft (16) as occurring in "great numbers" on both sides of the Murray River (i.e., in both Victoria and New South Wales); however, subsequent reports indicate that this species had become rare by the early 20th century (17). While the decline of these species appears to have been severe and rapid, such catastrophic extinction events are not unparalleled. Similarly rapid declines to extinction have occurred in some other Australian mammals [e.g., the mainland extinction of the once abundant eastern bettong (65)] and on other continents [e.g., the extinction of the passenger pigeon, previously the most abundant bird in North America (66)]. In addition to species in which longer-term declines and small population size have increased vulnerability to anthropogenic impacts (67), evidence for rapid and catastrophic collapse in previously abundant species highlights the severity of anthropogenic-induced ecological disruption. Our molecular results support conjectures that these species had large, potentially stable populations prior to European colonization of Australia. This conclusion could be further evaluated using coalescent analyses of whole-genome sequences [e.g., using pairwise sequentially Markovian coalescent methods or demographic simulations, (68)]. However, such analyses typically do not have sufficient resolution to estimate population-size changes in the very recent past or require considerably larger sample sizes than are available for most of these extinct taxa (68). The rapid collapse to extinction in Australian rodents emphasizes that species with stable populations are not immune to the impacts of rapid anthropogenic and environmental change and that genetic diversity does not always provide insurance against catastrophic extinction events.

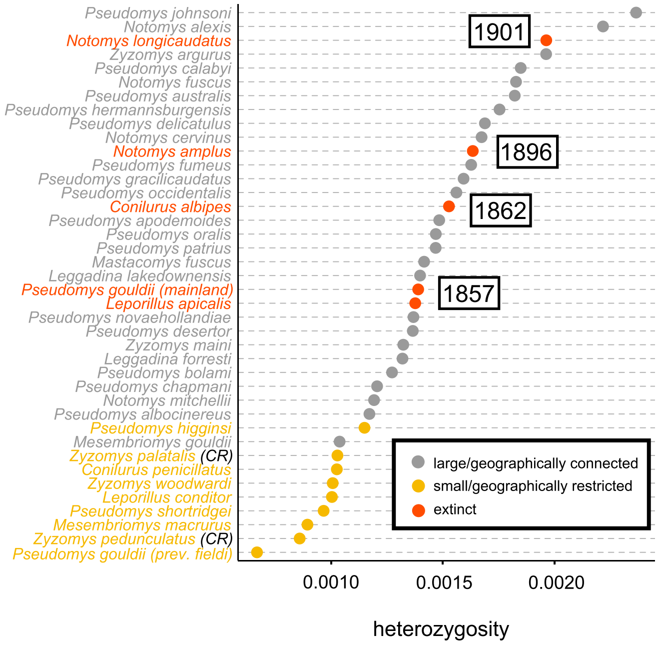

Fig. 3. Individual whole-exome heterozygosity for extinct (red, including the mainland extinction of *P. gouldii*) and extant Australian rodents. Extant species are categorized as either originating from "small/geographically restricted" populations (gold) or "large/geographically connected" (gray) populations. Year of collection is indicated for specimens of extinct species. CR indicates critically endangered status.

Predictors of Extinction Risk. Using our time-calibrated phylogeny, we used phylogenetic comparative analyses to test predictors of extinction risk in Australian rodents. The phylogenetic signal of two measures of population collapse, "proportion of extinction or severe decline" (lambda = 0.55, $P = 0.079$) and "binary extinction" ($D = 0.66$, $P = 0.20$), suggest that extinction is not significantly related to phylogeny in this group. Our comparative phylogenetic models of both "proportion of extinction or severe decline" ($P = 0.0076$) and "binary extinction" ($P = 0.044$) consistently recovered body size as a significant predictor of extinction risk. The top-ranking model of continuous decline accounted for the additive effects of both body size and biome (Akaike weight = 0.42), in which larger-bodied species were more likely to have become extinct (Fig. 4*A*) and species in the monsoon biome were less likely to have become extinct than species in the arid or temperate mesic biomes (Fig. 4*B*). The 95% confidence set of models also included models accounting for the interaction between body size and biome (Akaike weight 0.21) and for the density of non-indigenous persons (*SI Appendix*, Table S4).

Our results are consistent with previous work that has suggested that larger body size increases extinction risk (14, 47–49) and that extinction in the Australian monsoon biome has generally been less severe until recent decades (50, 69). As an example, in the

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arid-adapted genus *Notomys*, extinct species ranged from 90 to 100 g, while the largest living species, *Notomys mitchellii*, averages only 50 g. Despite being the largest of their genus, extinct *Notomys* were smaller than many monsoon and mesic species. Australian rodents in the arid biome are consistently smaller than their mesic relatives (70), and we observe a pattern in which the largest of these arid-zone specialists have been most impacted by extinction. These results support the suggestion that the body-size and extinction-risk relationship is context dependent (49, 71, 72).

Multiple interacting, proximate factors are proposed as threats to Australian native rodents, including predation by feral cats and foxes, competition with introduced rodents, anthropogenic environmental disturbance, habitat destruction by introduced herbivores, inappropriate fire management, the introduction of novel diseases, and climate change (10, 13, 14). It is possible that larger-bodied species are more susceptible to these risks generally [e.g., through preferential predation on larger species by introduced predators (48) and/or greater resilience of smaller species due to faster population growth rates (73)]. In the arid biome, the effects of predation by feral species may be exacerbated by limited and variable productivity in the landscape, grazing by introduced herbivores, and more frequent large fires (47, 74). While body size and biome arise as primary predictors of extinction risk, variation across space and time in intensive hunting, land clearing, agricultural land use, and abundance of feral species have all likely interacted to impact the pace of decline of native species and populations (10, 13–15, 50). Given a sparse historical record, the spatial and temporal variation of these factors is poorly understood, and they are therefore difficult to accurately model in phylogenetic comparative analyses. Despite this, our results reinforce the importance of considering the impacts of ecology, phenotype, and environment when managing threatening processes for native mammals in Australia.

Conclusion

Our genome-scale phylogeny reconstructs the evolutionary relationships of extinct and extant Australian rodents, confirms the distinctiveness of seven extinct species, and finds that the extinct Gould's mouse (*P. gouldii*) is conspecific with the extant but endangered Shark Bay mouse. We found no evidence of genetic erosion in extinct species at the time of their last specimen collection, highlighting the precipitous collapse of Australian rodents since European colonization. In the absence of evidence for underlying genetic erosion as a predisposing factor in extinctions, body size and biome, acting through various proximate ecological threats, contribute to explain why the decline and extinction in some native Australian rodent species has been more rapid than in others.

Materials and Methods

Additional detailed information on the samples and methods is provided in *SI Appendix*.

Sequence Data Generation and Sampling. Using the target capture approach described in ref. 45 and in *SI Appendix*, Materials and Methods, we generated exon-capture data (~1,400 loci) from 50 species (87 individuals) including 8 extinct species (*C. albipes*, *L. apicalis*, *N. longicaudatus*, *N. macrotis*, *N. amplus*, *P. auritus*, *P. gouldii*, and *M. rubicola*; *SI Appendix*, Table S1). In addition, we generated population-level sampling (15 individuals) of *P. hermannsburgensis*, a species with a present-day distribution spanning most of the Australian continent, as a reference point for testing patterns of within-species isolation by distance. For 48 individuals from 40 species, we also sequenced whole exomes using a *Mus musculus* whole-exome capture kit, which targets 54.3 Mb of protein-coding regions across the genome (75, 76). This whole-exome dataset included five of the eight extinct species for which we were able to obtain sufficient DNA yield (*SI Appendix*, Table S1). For historical DNA, extractions were performed under sterile conditions in the Museums Victoria Ancient DNA Laboratory, and libraries were prepared according to a modified Meyer and Kircher (77) protocol. We treated historical samples with PreCR Repair Mix (New England BioLabs) to repair degraded DNA. Pooled libraries of up to 92 samples (exon capture) or 20 samples (whole-exome capture) were each sequenced on 100-bp paired-end lanes of Illumina HiSeq 2500 at the Australian National University's Biomolecular Resource Facility.

Data Processing. We processed raw reads from both exon-capture and whole-exome-capture sequencing using Exon Capture Pipeline for Phylogenetics version 1.2 (https://github.com/Victaphanta/ECPP), following settings described in refs. 45 and 75, and detailed in *SI Appendix*, Materials and Methods. We filtered out loci that likely represented paralogs, based on a threshold of 3% average heterozygosity (45). To ensure high quality of the whole-exome data, we only retained exons that were captured for >80% of their total length in >80% of samples. Our final filtered exon-capture alignment consisted of 1,360 exons from 1,245 genes (1.20 Mb), and our filtered whole-exome alignment consisted of 46,293 exons from 13,168 genes (9.63 Mb, *SI Appendix*, Table S2).

Phylogenetic Reconstruction. Using target capture data from 1,245 independent loci, we estimated a maximum-likelihood phylogeny from 85 samples (50 species) of Australian rodents using IQ-TREE 1.6.12 (78). Although coding regions are under greater purifying selection compared to other genomic regions, previous studies have demonstrated the utility of exon capture in resolving phylogenetic relationships (e.g., refs. 45 and 79). To estimate a time-calibrated phylogeny using MCMCtree (80), we used a subset of 50 samples (one individual per species) with *Rattus fuscipes* as an outgroup and used a secondary calibration from ref. 43 for the 95% highest posterior-density divergence time between the Hydromyini and Rattini (11.43 to 14.00 Ma). We ran four independent runs with samples drawn every 1,000 steps from a total of 10^8 steps, with a burn-in of 10^7 steps for each run.

Evolutionary Distinctiveness of Extinct Rodents and Isolation by Distance. To determine the relative evolutionary distinctiveness of eight extinct species from their closest living relatives, we calculated the phylogenetic path length

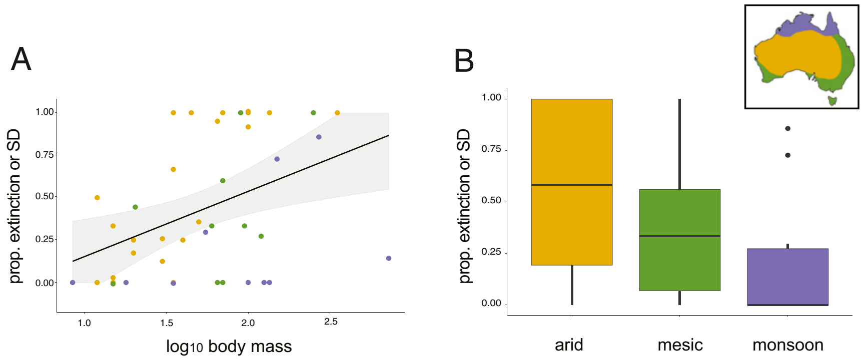

Fig. 4. Predictors of extinction and decline in Australian rodents. (*A*) Relationship between body size ($\log_{10}$ g) and the "proportion of extinction or severe decline" (SD). (*B*) Differences among biomes in the "proportion of extinction or severe decline". Inset map of Australia shows geographic regions defined as arid, mesic, and monsoon biomes, with biomes indicated by corresponding colors on both panels.

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between all sister species (or populations) in our maximum-likelihood phylogeny (i.e., the total branch length traversed along the phylogeny between tip A to tip B). Where possible, the pairs of samples used to calculate within-species divergence were from geographically separated samples. To assess whether genetic divergence between the sampled individuals of *P. gouldii* (mainland) and *P. fieldi* (island) fit an intraspecific isolation by distance pattern, we used *P. hermannsburgensis* as a comparison, a species with a modern-day distribution spanning much of the Australian arid zone. We calculated genetic distance in substitutions per site and geographic distance as $\log_{10}$ km between sampling locations.

Estimation of Population-Level Genomic Diversity. To estimate the relative genetic variation in each species in our data, we calculated average individual heterozygosity using whole-exome data from all species in the Pseudomys and Conilurus Divisions, including the five extinct species for which we obtained sufficient DNA to generate whole exomes (i.e., *C. albipes*, *L. apicalis*, *N. longicaudatus*, *N. amplus* and *P. gouldii* (total $n = 48$ samples from 40 species). As patterns of heterozygosity on fragmented landscapes primarily pertain to the N_e of the population from which the individual came, we then used contemporary distributions of species to categorize the samples we sequenced as being from either "small/geographically restricted" or "large/geographically connected" populations. We compared these to the average individual heterozygosity of five extinct species to determine whether these species were experiencing population collapse at the time of their respective collection.

Comparative Phylogenetic Analysis. We collated mean body-mass data from extinct and extant species from the literature (12, 81, 82) and classified all species into biome categories [arid, mesic, or monsoon (46, 70)]. Based on filtered occurrence records, we calculated the mean 2016 density of non-indigenous persons (via the Atlas of Living Australia, https://www.ala.org.au/) as a proxy for the density of recent anthropogenic activity across the species range (*SI Appendix*, Table S3). We coded extinction as both a binary trait, "binary extinction," and as a continuous measure, "proportion of extinction or severe decline" (based on data reported in ref. 19). To calculate the phylogenetic signal of each measure, we used the phylosig function in phytools (83) to calculate Pagel's lambda, and the D-statistic (84) using phylo.d in caper (85). We used phylogenetic generalized least squares in caper to determine whether body size, biome, and density of non-indigenous persons could predict "proportion of extinction or severe decline." We then calculated Akaike weights to determine the 95% credible set of models using the R package MuMIn (86). We also used phylogenetic logistic regression in the package phylolm (87) with 1,000 bootstrap replicates to test whether the same variables significantly predicted "binary extinction."

Data Availability. Raw sequence reads are available at the National Center for Biotechnology Information (NCBI) sequence read archive, under BioProject accession PRJNA729818 (88). Processed DNA sequence alignments are available in FigShare, at DOI:10.6084/m9.figshare.14725179 (89).

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