Multiple evolutionary origins of Australian soil-burrowing cockroaches driven by climate change in the Neogene

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Parallel evolution is the independent appearance of similar derived phenotypes from similar ancestral forms. It is of key importance in the debate over whether evolution is stochastic and unpredictable, or subject to constraints that limit available phenotypic options. Nevertheless, its occurrence has rarely been demonstrated above the species level. Climate change on the Australian landmass over the last approximately 20 Myr has provided conditions conducive to parallel evolution, as taxa at the edges of shrinking mesic habitats adapted to drier biomes. Here, we investigate the phylogeny and evolution of Australian soil-burrowing and wood-feeding blaberid cockroaches. Soil burrowers (subfamily Geoscapheinae) are found in relatively dry sclerophyllous and scrubland habitats, whereas wood feeders (subfamily Panesthiinae) are found in rainforest and wet sclerophyll. We sequenced and analysed mitochondrial and nuclear markers from 142 specimens, and estimated the evolutionary time scale of the two subfamilies. We found evidence for the parallel evolution of soil-burrowing taxa from wood-feeding ancestors on up to nine occasions. These transitions appear to have been driven by periods of aridification during the Miocene and Pliocene across eastern Australia. Our results provide an illuminating example of climate-driven parallel evolution among species.

1. Introduction

Parallel evolution occurs when phenotypic similarities arise independently among closely related species [1,2]. This phenomenon has important implications for understanding the predictability of evolution. Some have argued that evolution is fundamentally stochastic and unpredictable [3], while others have proposed that constraints commonly limit available phenotypic options, leading to parallelism and predictability [4]. Although parallel genotypic adaptation has been demonstrated across a diverse range of taxa at the intraspecific level [5–11], the widespread occurrence of parallel evolution across a clade has only rarely been shown [12,13]. The potential role of environmental change in driving parallel evolution also remains poorly understood [14].

The diverse biomes of Australia provide an opportunity to investigate potential cases of parallel evolution, and the role of environmental change in this process. The last approximately 20 Myr have seen significant climatic change in Australia, with four key periods of aridity occurring over this time frame, and relatively warm and wet conditions present in the intervening periods (reviewed by Byrne et al. [15]). The mesic forests that dominated the continent during the Palaeogene (66–23 Ma) began to be replaced by sclerophyllous forests from about 25 Ma. Notable expansion of open habitats such as sclerophyllous woodlands and savannas occurred around 15 Ma and again at around 6–8 Ma. Finally, severe periods of aridity were experienced during the glacial cycles of the Pleistocene (2.6 Myr to 11.7 kyr ago).
These periods of aridification affected large geographical areas, including much of the eastern seaboard of Australia, from northern Queensland down to southern New South Wales and Victoria, covering a distance of approximately 3000 km [15]. The aridification of mesic habitats in eastern Australia is likely to have had strong effects on the taxa distributed in these areas, providing conditions that were conducive to the parallel evolution of traits among related species. An alternative scenario is that adaptation to drier conditions occurred only once within a group of related species. An alternative scenario is that adaptation to drier conditions occurred only once within a group of related species and was followed by a radiation from this ancestor to fill the nascent arid niches.

The closely related cockroach subfamilies Panesthiinae and Geoscapheinae are a potential model system for investigating the effects of aridification on Australia’s biota. Species in Panesthiinae are known as ‘wood feeders’ and are typically found in mesic environments in Asia and eastern Australia [16]. These taxa form burrows within the rotten logs upon which they feed, and often have reduced wings (figure 1a). Species in Geoscaepheinae are known as ‘soil burrowers’ and are found primarily in dry sclerophyllous and scrubland environments of eastern Australia [17,18]. These taxa are all completely apterous (figure 1b), form unique burrows in sandy soil up to 1 m deep, and typically feed on dry leaf litter [18] (H.A.R. 1987–2012, personal observation). Members of Geoscaepheinae have a rounder body shape than those of Panesthiinae.

A molecular phylogenetic study of a limited number of taxa from Panesthiinae and Geoscaepheinae (17 and 4, respectively) showed that the former evolved in Asia, later colonizing Australia and giving rise to Geoscaepheinae [19]. The four species of Geoscaepheinae examined formed a monophyletic group that was relatively distant from the two Australian species of Panesthiinae included in the study [19]. The colonization of Australia by ancestral members of Panesthiinae was presumed to have occurred following the collision between the Australian and Asian tectonic plates around 20 Ma, although no formal analyses of divergence times were performed.

Here, we investigate the evolution of soil burrowing and the evolutionary history of these two groups of cockroaches in Australia. We included sequence data for 24 of the 26 known species of Geoscaepheinae, and 8 of 10 Australian Panesthiina species. Our study provides the first formal divergence dating analysis of the two groups, including an examination of net speciation rates. We present evidence for multiple independent origins of the soil-burrowing habit from wood-feeding ancestors during the Miocene and Pliocene, providing a strong case for climate-driven parallel evolution.

2. Material and methods

(a) Sampling and DNA sequencing

The specimens sequenced in this study, and their collection locations, are given in the electronic supplementary material, table S1; total genomic DNA extraction, PCR and sequencing were performed as described previously [20], except in the case of ITS1 (see the electronic supplementary material). Of the 142 ingroup taxa examined, new sequence data were generated for a total of 136 specimens; other sequences were obtained from GenBank. Homologous sequences from a variety of Blaberidae were used as outgroups.

(b) Phylogenetic inference, divergence dating and topology tests

To infer the phylogeny and estimate the evolutionary time scale of Panesthiinae and Geoscaepheinae, we analysed the mitochondrial-nuclear dataset using a Bayesian approach with the program BEAST v. 1.7.0 [21] and a maximum-likelihood (ML) approach with the program RAxML v. 1.0.9 [22]. In the absence of fossils assigned to either subfamily, we calibrated the clock using four fossils from representatives of other extant subfamilies in Blaberidae (the family to which Panesthiinae and Geoscaepheinae belong). We used exponential priors to reflect uncertainty in these calibrations [23], with age constraints on relevant clades (see the electronic supplementary material). The monophyly of the subfamily Geoscaepheinae was tested by comparing unconstrained trees with an artificially constrained tree topology in which the monophyly of Geoscaepheinae was enforced. Further details on the methods used for phylogenetic inference, divergence dating and topology tests are provided in the electronic supplementary material.

(c) Ancestral-state reconstruction and diversification rates

For reconstructing ancestral states and analysing diversification rates, the tree topology inferred using BEAST was pruned to contain, in most cases, a single representative of each species. Ancestral burrowing states were estimated using parsimony. Two analyses were performed: one in which reversion from soil feeding to wood feeding was permitted, and another in which this change was not permitted. The rationale for performing the second analysis is provided in the Discussion. We used the BisSE method [24] to compare the speciation rates in lineages leading to wood- and soil-burrowing taxa (see the electronic supplementary material).

3. Results

(a) Phylogenetic analyses

Bayesian and ML analyses of the concatenated nuclear and mitochondrial dataset (152 taxa in total, including outgroups) yielded similar estimates of the phylogeny (figure 2; electronic supplementary material, figures S2–S4). Early divergences in the tree lead to the following Asian lineages of Panesthiinae: (i) representatives of the genus Salganea from Santimorn, Taiwan and Kagoshima, Japan; (ii) Miapanesthi a deplanata; and (iii) Panesthi a transversa and Caeopia sticta, both from Sabah, Borneo. The fourth major lineage in the tree is divided into two larger clades, labelled A and B (figure 2).

Clade A exclusively comprises wood feeders from both Asia and Australia. The Australian taxa within this clade
Figure 2. Phylogenetic tree estimated in a Bayesian analysis of the mitochondrial-nuclear dataset (142 ingroup taxa), based on 2096 bp of concatenated fragments of COII, 12S, ITS1 and 18S. A ML analysis yielded a very similar tree (electronic supplementary material, figure S3). Posterior probabilities and ML bootstrap support values are shown on selected nodes (see key in top-left corner). Branch lengths are drawn to a time scale given in millions of years. Bars represent 95% credibility intervals for estimates of node times. *Exceptions are P. a. angustipennis from Christmas Island and the Seychelles.
form a monophyletic group, labelled as clade C. Clade B contains exclusively Australian taxa, including both wood feeders and soil burrowers (posterior probability 1.0; 100% ML bootstrap support). The wood-feeding taxa within this clade do not form a monophyletic group. Instead, some wood feeders are more closely related to soil burrowers than to other wood feeders, and vice versa.

There was a general trend for the taxa in clades containing both wood feeders and soil burrowers to be present in similar geographical regions. For example, representatives of clade D, which contains the wood-burrowing species *Panesthia sloanei* as well as *Macropanesthia rhinoceros* and other soil burrowers, are found in northern and central Queensland (electronic supplementary material, figure S1). The distribution of members of the wood-feeding-only clades C and F is closely linked to mesic areas along the eastern coast.

Our topology tests yielded strong support (log Bayes factor = 421) for the unconstrained tree topology, which contained a polyphyletic Geoscapheinae, compared with a constrained topology in which the subfamily was forced to be monophyletic. A log Bayes factor of 3 is usually considered to represent strong support [25]. The monophyly of Geoscapheinae was also rejected using the approximately unbiased likelihood-based topology test (*p* = 10^-10). The trees estimated using Bayesian and likelihood methods are not concordant with the current morphology-based generic designations. None of the four genera of Geoscapheinae (*Geoscapheus*, *Neogoscapheus*, *Macropanesthia* and *Panpanesthia*) was found to be monophyletic. No support was found for the monophyly of the wood-feeding genus *Panesthia*.

### (b) Molecular-clock analyses

The origin of the clade containing all Panesthiinae + Geoscapheinae was estimated at approximately 45.6 Ma (95% credibility interval 35.8–56.9 Ma). The time to the most recent common ancestor (TMRCA) of clade A, comprising *Panesthia* lineages from Asia and Australia, was estimated at 22.9 Ma (95% CI 17.6–28.4 Ma). Within this clade, the TMRCA of Australian *Panesthia* were estimated at 13.9 Ma (95% CI 10.4–18.1 Ma). Clade B, comprising only Australian taxa and including all species of Geoscapheinae plus *P. sloanei*, *P. tryoni*, *P. australis* and *P. obtusa*, originated at approximately 22.9 Ma (95% CI 18.1–28.9 Ma). The age of soil burrowing-only clades (containing more than one species) ranged from approximately 14.4 (within clade D) to 3.3 Ma (within clade H).

### (c) Ancestral-state reconstruction and diversification rates

Ancestral-state reconstruction of burrowing habits was performed using parsimony. The most parsimonious reconstruction, which allowed changes from wood feeding to soil burrowing and vice versa, indicated that soil burrowing evolved on three occasions. These were followed by four reversions from soil burrowing to wood feeding, in the ancestors of *P. tryoni tryoni* (on three separate occasions) and *P. tryoni tegminifera* on a single occasion (figure 3b). In the analysis that precluded reversions from soil burrowing back to wood feeding, we inferred a total of nine independent instances of the evolution of soil-burrowing from wood-feeding ancestors (figure 3b).

Using the general six-parameter model of BiSSE [24], the net speciation rate in soil-burrowing lineages was estimated at 0.617 speciation events per lineage per million years. This was significantly greater than the net speciation rate of 0.282 speciation events per lineage per million years in wood-feeding clades (*p* = 0.0045).

### 4. Discussion

#### (a) Phylogenetic relationships within Panesthiinae and Geoscapheinae

We have performed a comprehensive molecular phylogenetic analysis of the cockroach subfamilies Panesthiinae and Geoscapheinae, focusing on their Australian taxa. We found no support for the monophyly of either of these subfamilies. Instead, both were found to be polyphyletic. This result is at odds with previous assumptions that Geoscapheinae is a natural group, based on strong similarities in burrowing behaviour and gross morphology of its members [26]. Nonetheless, the relationships we found are congruent with previous studies of relationships among Geoscapheinae species based on protein allozymes [27] (note that no Panesthiinae taxa were included in that study), and studies of members of both subfamilies based on reproductive morphology. Walker & Rose [28] found that the dorsal second left phallemore (L2d) was absent in all species of Geoscapheinae, as well as in three species of Panesthiinae: *P. sloanei*, *P. t. tryoni* and *P. t. tegminifera* (i.e. those that group closely with soil-burrowing lineages of Geoscapheinae in our study). The other three species of Panesthiinae examined by Walker & Rose [28], *P. australis*, *P. cribrata* and *P. matthesii*, had fully developed L2d. Walker & Rose [28] also found that the oothecal membrane that encases eggs was present in all members of Panesthiinae, but absent in six out of 22 morphospecies of Geoscapheinae examined in our study, which correspond to members of clades D and G in figure 2. Finally, the close geographical proximity of the ranges of related wood-feeding and soil-burrowing taxa (figure 2; electronic supplementary material, figure S1) is consistent with the expected low dispersal ability of these wingless and slow-moving insects.

#### (b) Biogeography of Panesthiinae and Geoscapheinae

Our results support the hypothesis that the Australian taxa in Panesthiinae and Geoscapheinae are descendants of Asian wood feeders that invaded Australia via Wallace’s Line [19]. First, Australian lineages are nested among Asian lineages in the tree (figure 2). Second, clade B, which contains only Australian taxa, appeared at approximately 23 Ma (95% CI 18.1–28.9 Ma), which is compatible with an arrival from Asia subsequent to the collision of the Asian and Australian tectonic plates around 20 Ma. Asian taxa appear to have colonized Australia on at least two separate occasions. The most recent colonization gave rise to clade C, which contains *P. cribrata*, *P. lata*, *P. matthesii* and *P. mcnudelloides*, originating at approximately 14 Ma (95% CI 10.4–18.1 Ma) during the Miocene (figure 2). As well as being devoid of soil burrowers, this clade is more closely related to Asian taxa than to other Australian lineages. Temporary land bridges between southeast Asia and Australia may have been present from approximately 20 Ma onwards [15,29,30]. However, to chart the exact migratory route of Australian taxa requires detailed fossil
Figure 3. Ancestral-state reconstructions of burrowing habit based on the Bayesian estimate of the mitochondrial-nuclear tree, pruned so that only one sample of each putative species is shown (see text for exceptions and further details). White circles denote wood-feeding taxa; black circles denote soil-burrowing taxa; grey circles with black centres indicate evolution of soil burrowing from wood feeding; and grey circles with white centres indicate reversions from soil burrowing back to wood feeding. (a) Ancestral-state reconstruction under the assumption that reversions from the soil burrowing to wood feeding state can occur. (b) Ancestral-state reconstruction under the assumption that reversions from the soil-burrowing to wood-feeding state cannot occur.

(c) Parallel evolution of soil burrowing from wood-feeding ancestors

The ancestor of Panesthiinae and Geoscapheinae was inferred to be a wood feeder, as was the ancestor of the Australian cockroach clade B (figure 2). Soil burrowing then evolved between three and nine times independently (figure 3). Soil burrowing is likely to have been driven by aridification events in Australia, which caused the replacement of rainforest and wet sclerophyll habitats with dry sclerophyll and scrubland [15]. The transition of mesic to xeric habitats would have led to strong selection on wood-feeding taxa to adapt to these novel environments, as the availability of moist rotting wood diminished. The moisture requirements of wood feeders would have caused them to seek similarly moist conditions when their habitats dried out. This is likely to have caused selection for burrowing into soil, perhaps initially near the wood–soil interface, and later directly into the soil. A reduction in rotting wood would have also selected for evidence, which is almost certainly unattainable. Alternatively, wood-feeding species may have rafted across water in logs from their ancestral Asian range.

Our divergence date estimates are largely congruent with inferred geological events associated with the formation of the Ryukyu archipelago, and Lord Howe Island. First, the divergence event leading to Panesthia angustipennis spadica and P. a. yayeyamensis from the Ryukyu archipelago was inferred to have occurred 5.5 Ma (95% CI 3.1–8.1 Ma). This age range is broadly consistent with the hypothesized formation of the Tokara Tectonic Strait during the Pliocene, which would have led to separation of the two geographical areas in which these two lineages are found (i.e. north and south of the strait) [20].

The divergence leading to Salganea esaki and S. taiwanensis (also from either side of the strait) was inferred to have occurred much earlier, at 13.5 Ma (95% CI 9.0–19.1 Ma). Previous studies have provided evidence for the separation of Salganea spp. north and south of the Tokara Tectonic Strat during approximately 8.6–15.6 Ma, compared with a more recent date of approximately 4.4–6.9 Ma with Panesthia spp. either side of the strait [31]. These dates are somewhat similar to those found in this study. The slight differences may be due to the fact that Makawa et al. [31] used Blattabacterium endosymbiont data to estimate divergence dates.

Second, the two sampled individuals of P. lata from Roach and Blackburn Islands, near Lord Howe Island, diverged from each other around 3.2 Ma (95% CI 1.3–5.7 Ma). These were estimated to have diverged from the lineage leading to mainland Panesthia at 6.6 Ma (95% CI 4.6–8.8 Ma). Taken together, these dates are consistent with the estimated age of approximately 6 Ma for the Lord Howe Island Group [32,33].
feeding on drier substrates such as leaves and twigs. On Blackburn and Roach Islands (Lord Howe Island Group), where rotting wood is in low abundance, the species *P. lata* is known to feed on dry *Ficus* and sedge leaves, respectively (H.A.R. 2001–2003, personal observation), rather than on rotting wood as do other members of the genus *Panesthia*. Such a feeding strategy may have been adopted by the ancestors of soil-burrowing lineages as rotting wood became less available in their habitats.

Under the assumption that transitions between wood feeding and soil burrowing were equally likely during the evolution of these cockroaches, soil burrowing was inferred to have evolved on three independent occasions, followed by the reversion to wood feeding on four separate occasions in clades H, I and J (figure 3a). Such reversions may have occurred during expansions of mesic habitat in relatively warm and wet periods that prevailed in between dry and cold periods [15]. In this case, soil-burrowing cockroaches re-evolved traits necessary for wood feeding and burrowing into wood.

The scenario in figure 3a requires seven transitions, while the alternative scenario involving no reversions to wood feeding (figure 3b) requires nine transitions to soil burrowing. Although less parsimonious, we believe that the latter scenario should be considered equally. Under the scenario in figure 3a, soil burrowing evolved in the ancestors of clades H, I and J at 15 Ma (95% CI 16.4–26.4) (figure 2). These dates fall somewhat outside the key periods of aridification at 15 Ma and 6–8 Ma [15] that presumably drove the evolution of soil burrowing. Under the scenario in figure 3b, soil burrowing would have evolved on multiple occasions in clades H, I and J, between approximately 3.3 Ma (95% CI 2.1–4.7 Ma) and approximately 12.5 Ma (95% CI 8.6–17 Ma) (figure 2), which could be considered more compatible with known periods of aridification.

A further issue for consideration is the degree of morphological, behavioural and physiological specialization (and concomitant genetic changes) that accompanied the evolution of soil burrowing. Such changes facilitated the construction by soil burrowers of permanent burrows up to 1 m deep in a sandy substrate, which is vastly different from the rotting wood colonized by *Panesthiinae*. Such changes permitted soil burrowers to feed on their diet of dry and dead leaves of *Eucalyptus*, *Casuarina* and *Callitris*, and sometimes small twigs and faeces of small animals [18] (H.A.R. 1987–2012, personal observation), again very different from the rotting wood diet invariably consumed by species in *Panesthiinae*. Such specialization may have made it difficult for soil burrowers to evolve the capacity to feed and burrow into rotten wood, although such changes cannot be ruled out.

The debate surrounding the evolution of wings in stick insects (Phasmatodea) presents an analogous conundrum. Whiting et al. [34] reported the novel re-evolution of wings in stick insects on multiple occasions from a wingless ancestor. This was in contrast with the prevailing view that stick insects lost wings on multiple occasions from a winged ancestor. Trueman et al. [35] argued against the findings of Whiting et al. [34] by disputing the latter’s assumption that ancestral-state reconstructions based on maximum parsimony are the most accurate. Thus, although parsimony might support secondary gains of complex character traits, ecological, behavioural and physiological perspectives must also be considered when reconstructing ancestral states.

### 4. Diversification of wood-feeding and soil-burrowing lineages

The aridification of the Miocene and Pliocene represented a novel shift in the then lush and wet Australian continent. In turn, this had a great effect on the ancestral biota, stimulating radiations of flora and fauna adapted to the arid zone and dry sclerophyll while their mesic ancestors perished or shrunk into pockets of rainforest refugia [15,36]. Two morpho-species that appear to have been affected by Miocene reductions in mesic habitat are *P. sloanei* and *P. tryoni*. *Panesthia sloanei* is found in far north Queensland, primarily in montane environments [37]. Large genetic differences representing divergences greater than 15 Ma were found in this group. These taxa, although morphologically similar, presumably represent distinct species. Similarly, *P. tryoni*, comprising the two subspecies *tryoni* and *tegminifera*, is largely restricted to refugial habitats. The species has very deep genetic divergences going back as far as approximately 20 Ma and is polyphyletic with respect to numerous soil-burrowing taxa.

Our estimate of the evolutionary time scale (figure 2) indicates that most speciation events within lineages of either soil burrowers or wood feeders occurred in the Late Miocene and Pliocene. Climatic oscillations of the Pleistocene do not appear to have stimulated the same turnover of Australian biodiversity compared with earlier epochs, possibly because the continent already contained biota that were adapted to the alternating expansions and contractions of the arid and mesic zones. The ranges of ancestral mesic taxa expanded when the dry biomes contracted, and arid-adapted lineages would reclaim their range accordingly when the dry climate returned [38–40]. This provided biotic stability and a lack of open niches into which new species could radiate. Instead of undergoing adaptive speciation during the Pleistocene, pre-existing flora and fauna are thought to have shifted geographical ranges.

Our finding of a significantly higher speciation rate in soil-burrowing clades than in wood-feeding lineages is consistent with the hypothesis of Byrne et al. [15] that rainforest lineages should have fewer species than their sisters in sclerophyll communities. *Panesthia* contains 10 Australian representatives compared to 26 in the Geoscaphineae, although our analyses show that the former is likely to contain some additional cryptic species.

### 5. Conclusion

We have shown that wood-feeding cockroaches colonized Australia on at least two occasions. Following the first colonization event, around 20 Ma, these cockroaches presumably migrated southward within mesic environments in the eastern part of the continent. Following the onset of arid conditions in the Miocene and the Pliocene, soil burrowing evolved independently between three and nine occasions from various wood-feeding ancestors present in habitats from northern Queensland down to northern New South Wales, a distance of over 2000 km. The parallel evolution of this trait provides evidence for climate-driven niche divergence in the diversification of this group of insects, and underscores the predictability of evolution under certain circumstances. Our study provides an important example of parallel evolution above the species level, which so far remains rare in the literature. Comparisons of the genomes of wood-feeding and soil-burrowing...
sister taxa may shed light on the genetic factors that facilitated the repeated evolution of soil burrowing.

Data accessibility. All sequence data have been deposited in GenBank, and all DNA sequence alignments will be made available in Dryad. DNA sequences are available under GenBank accessions: KU577617–KU577995. An alignment showing all data in nexus format is provided as the electronic supplementary material ‘Burrowingroachdata.nex’.


Competition interests. The authors declare we have no competing interests.

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