Parallel responses of bees to Pleistocene climate change in three isolated archipelagos of the southwestern Pacific

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The impacts of glacial cycles on the geographical distribution and size of populations have been explored for numerous terrestrial and marine taxa. However, most studies have focused on high latitudes, with only a few focused on the response of biota to the last glacial maximum (LGM) in equatorial regions. Here, we examine how population sizes of key bee fauna in the southwest Pacific archipelagos of Fiji, Vanuatu and Samoa have fluctuated over the Quaternary. We show that all three island faunas suffered massive population declines, roughly corresponding in time to the LGM, followed by rapid expansion post-LGM. Our data therefore suggest that Pleistocene climate change has had major impacts across a very broad tropical region. While other studies indicate widespread Holarctic effects of the LGM, our data suggest a much wider range of latitudes, extending to the tropics, where these climate change repercussions were important. As key pollinators, the inferred changes in these bee faunas may have been critical in the development of the diverse Pacific island flora. The magnitude of these responses indicates future climate change scenarios may have alarming consequences for Pacific island systems involving pollinator-dependent plant communities and agricultural crops.

1. Introduction

Evidence of increases in global average air and ocean temperatures, and rising global sea level, is widely accepted in the scientific community [1]. Such changes are expected to have varying (and potentially very large) effects on ecosystem composition and function [2]. Potential impacts of climate change on pollinators are important because of their role in angiosperm sexual reproduction [3]. Differing phenological responses between plants and their pollinators to changing climates are particularly concerning [4], yet few empirical studies exist ([5] and references therein).

One way to explore the effects of climate change on plant–pollinator relationships is to reconstruct phylogenies of the various elements, and then to explore diversification patterns and past population sizes. This approach has been used previously to reconstruct how climate fluctuations impact the population dynamics of both terrestrial and marine organisms, including mammals [9] and fish [10]. Studies of insect populations have also revealed...
demographic responses to glacial cycles [11]. However, studies of terrestrial impacts of glacial cycles in tropical islands are scarce, despite their role in understanding island biogeography [12].

Recently, Groom et al. [13] examined population size and haplotype accumulation of halictine bees in the genus *Lasioglossum* subgenus *Homalictus* (herein referred to simply as *Homalictus*) in the Fijian archipelago. They argued that there was a decline in population size in the Late Pleistocene, approximately 20 ka, followed by a dramatic increase in population size shortly after. They argued that this pattern of decline followed by a rapid bounce-back could be a response to changing climate, with initial contraction of populations during cooling/drying conditions, followed by rapid expansion as global climate reverted to an interglacial period. The effects of human transformation of ecosystems were excluded as a confounding effect in their inferred bee demographics, because the major increase in historical population size occurred before human occupation of the Fijian archipelago (approx. 3000 years ago). However, there might be other possibilities, unrelated to climate, which could explain the observed patterns (e.g., introductions of foreign plant species that provide different food sources, adaptations unrelated to changing climate or pollinator extinctions leading to filling an available niche). One way to further examine whether climate change really did impact on bee population sizes in tropical islands is to examine changes in bee demographics in other isolated archipelagos of the southwest Pacific (SWP).

For such a scenario to be supported, changes in populations should coincide with the onset of the last glacial maximum (LGM) at 23 ka and with periods of subsequent warming commencing approximately 19 ka [14].

The SWP has a very depauperate bee fauna [15], and most (if not all) of the long-tongued bee species in Fiji represent anthropogenic dispersals [16,17]. The short-tongued *Homalictus* are the most abundant bee element of many of the SWP islands. In Fiji, there are four *Homalictus* species [17,18], six species for the archipelago of Vanuatu [15], and Samoa has the greatest recorded diversity in the SWP with 10 species [19]. Here, we use the mitochondrial cytochrome oxidase c subunit I (COI) gene to examine changes in past population sizes of *Homalictus* in three isolated (each separated by at least approx. 800 km) island archipelagos; Vanuatu, Fiji and Samoa. We explore the timing of bee colonization in each archipelago and use coalescent analyses to determine whether historical demographic changes show patterns that might indicate common responses to historical climate changes in the SWP.

2. Material and methods

(a) Study system

We collected *Homalictus* specimens visiting flowers from three isolated neighbouring archipelagos in the SWP: Vanuatu, Fiji and Samoa, spanning a total distance of approximately 4000 km (electronic supplementary material, figure S1). These island systems form highly fragmented groups of complex and relatively recent geological histories, with the largest subaerial land mass for Vanuatu, Fiji and Samoa emerging at approximately 3–8 [20], 12.5–29 [21] and 5 Ma [22], respectively. Floral hosts included numerous cosmopolitan and invasive species throughout village gardens, roadsides and native vegetation, with many flowering year-round. Identifications were based on the species descriptions of Perkins & Cheesman [19], Pauly & Villemant [23] and Michener [18].

(b) DNA processing

Extraction, amplification and sequencing of mtDNA was performed by the Canadian Centre for DNA Barcoding at the Biodiversity Institute of Ontario, using standard protocols [24]. Bidirectional sequencing using the universal primer pair LepF1/LepR2 [25] from a single leg of each specimen produced a 654 bp sequence of COI for 425 *Homalictus* specimens from Fiji, 177 from Vanuatu and 143 from Samoa. All trace files were examined using *GENEIOUS* PRO v. 6.1.6 [26], and haplotypes with one or more ambiguous nucleotides in both forward and reverse directions at the same site were removed from analyses. Identical sequences were also removed, leaving 93 unique haplotypes from Fiji, 30 from Vanuatu and 41 from Samoa. In total, 207 unique haplotypes were aligned, with the 154 Pacific representatives combined with 53 outgroup Australian species of the closely related *Lasioglossum* subgenus *Chilalictus* and further *Homalictus* sequences obtained via GenBank (see the electronic supplementary material, table S1). The resulting dataset (base frequencies—A: 33.7%, C: 14.3%, G: 11.4%, T: 40.6%) exhibits strong AT bias (74.3%) at the third codon position, as is common in Hymenoptera [27]. To avoid sequence contamination from potential *Wolbachia* infection, sequences were screened using the NCBI BLASTn database. All specimens are stored in the Schwarz Bee Collection at Flinders University, South Australia.

Phylogenetic analyses were based on unique COI haplotypes rather than on species, and correspondence between the two is discussed in the Results and Discussion sections.

(c) Phylogenetic analyses

Phylogenetic analyses were performed using Bayesian inference implemented in *BEAST* v. 1.7.5 [28] with an uncorrelated lognormal relaxed molecular clock for 50 million iterations, sampling every 1000th iteration, under a Yule process speciation model. *Chilalictus* was constrained as outgroup with a mean root height of 20 Ma (parameter *TreeModel.rootHeight* normal; mean = 20, s.d. = 3.0) as per the multi-gene phylogeny of Gibbs et al. [29]. The most suitable substitution prior was determined as GTR + I + Γ by an ABC criterion in *JMODELTEST* v. 2.1.4 [30], and individual uncorrelated lognormal rate priors set with mean (parameter *ucld.mean*) following an exponential distribution (mean = 0.33, offset = 0) and standard deviation (parameter *ucld.stdev*) followed by an exponential distribution (mean = 0.33, offset = 0). Subsequent log files were then examined in *TRACER* v. 1.5 [31] to determine whether effective sample sizes were adequate (i.e. more than 100). The resulting haplotype tree (electronic supplementary material, figure S2) was compared with an additional genetic-distance-based analysis (electronic supplementary material, figure S3). Our genetic distance analysis used a neighbour-joining technique applied to uncorrected p-distances in *PAUP* *+* 4.0b [32]. Missing gene fragments were not included when calculating pairwise distances, and trees were constructed using a heuristic search with 100 random taxon–addition sequence replicates, tree–bisection–reconnection branch swapping, and no limit on the number of shortest trees retained.

(d) Patterns of haplotype diversification

We explored temporal patterns of haplotype accumulation using log lineage through time (LTT) plots based only on unique haplotypes for each of the three major archipelago clades. One clade representing a second dispersal into Samoa and two other clades representing later dispersals into Vanuatu (electronic supplementary material, figure S2) were not included in the analyses because of the small number of haplotypes recovered from them. Separate datasets representing each archipelago were then assessed for appropriate models using *MODELTREE* v. 2.1.4 [30], and assigned a GTR + I + Γ substitution model. Datasets were unpartitioned for codon position, with default priors for
gamma distributions, and assigned a constant rate strict molecular clock of 1.0. Fixing the rate to 1.0, rather than using a relaxed clock, allows the resulting branch lengths to be interpreted in units of mean substitutions per site, which can be converted to years given a mutation rate per generation and the number of generations per year. Based on a Bayesian skyline coalescent model implemented in BEAST v. 1.7.5 [28], analyses were then run for 50 million generations with parameters logged every 5000 iterations. We then produced LTT plots separately in TRACER v. 1.5 [31] for each archipelago.

(e) Estimating changes in \( N_e \)
To determine whether the observed changes in haplotype accumulation corresponded with shifts in the effective population sizes \( (N_e) \) for the archipelagos, we produced a Bayesian skyline plot (BSP) for each clade based on the same trees produced for our LTT analyses using a constant-rate fixed strict molecular clock of 1.0 in TRACER v. 1.5 [31]. This produces a tree where branch lengths are proportional to the number of substitutions, and is required to be able to determine node ages based on an estimated number of generations per year. Because our dataset includes representatives from across a wide elevation range (0–1100 m.a.s.l.), it is possible that populations at higher elevations have fewer generations per year, and it should be considered that age estimates for these lineages may be underestimated compared with lower elevation populations.

(f) Dating estimates
There are no documented bee fossils in the Oceania region, and our analyses covered recent divergences; thus, calibrating chronogram age estimates is difficult. However, there are other approaches to molecular dating. Few data exist to assess variation in de novo mutation rates among taxa [33]. However, two studies have estimated single-nucleotide mutation rates for invertebrate mitochondrial genomes, on the nematode Caenorhabditis elegans [34] and the fruit fly Drosophila melanogaster [35]. These respective rates were within a single order of magnitude of each other (\( 9.7 \times 10^{-8} \) and \( 6.2 \times 10^{-8} \) per site per generation), and both taxa had AT biases similar to our Homalictus dataset (76% and 82%, respectively, compared with 74.3% in Homalictus). Consequently, a mutation rate in the region of \( 10^{-7} \) to \( 10^{-8} \) single-nucleotide mutations per generation might be expected for Homalictus. Other estimated rates of mutation in COI of insects were incorporated into divergence date estimates for the bee genus Bombus, where Duennes et al. [36] applied a rate range of \( 1.0 \times 10^{-7} \) to \( 1.0 \times 10^{-8} \) substitutions per site per year (one generation per year in Bombus). While these estimates differ across two orders of magnitude, they are not adjusted for varying base composition, which can have very strong effects [35]. Consequently, we deemed the Drosophila rate of \( 6.2 \times 10^{-8} \) per site per generation the most appropriate estimate for our study.

Converting the relative age of nodes based on mutation rates into chronological years requires that we know the number of generations per year for Homalictus in our study sites. Unfortunately, the only studies of Homalictus nesting biology are those of Knerer & Schwarz [37] on a temperate species from southern temperate Australia which produces two generations per year. Studies on temperate Australian Lasioglossum species in the Lasioglossum subgenus Chilalicus also indicate two generations per year [38]. Although in the tropics, Michener & Bennett [39] demonstrate almost year-round activity in populations of Halictus ligatus, which produced three broods per year. Furthermore, the neotropical halicline Megalopta genalis has been shown to have an egg-to-adult period of approximately 35 days, which would allow for up to four generations per year [40]. The number of generations per year in the tropical habitats of the SWP is therefore likely to comprise three as a minimum, but could be as many as four or more given short egg-to-adult periods and year-round activity.

Using the chronogram produced under the Bayesian skyline coalescent model analysis above, we were able to estimate (i) the likely time at which Homalictus arrived in each archipelago, and (ii) the changes in haplotype accumulation and change in effective population size based on key nodes of the tree topology. By setting the number of generations per year, we are able to convert the age of the nodes provided in mutations per site per generation to calendar years.

3. Results
Our Bayesian inference analysis indicated a single colonization of Fiji by Homalictus, two colonizations of Samoa and three for Vanuatu (electronic supplementary material, figure S2). The neighbour-joining analysis (electronic supplementary material, figure S3) shows a highly similar topology with clearly monophyletic clades for each of the three Pacific archipelagos, although their arrangement did not agree with our Bayesian approach. The Samoan clade was recovered as sister to the Fijian plus Vanuatu clades. Importantly, the distribution of branch lengths within and between major clades in each archipelago is very similar for these very different tree-building approaches, indicating that both topological and diversification patterns in our analyses are robust to very different analytical approaches.

(a) Haplotype accumulation
LTT plots are given for each of the three archipelago clades in figure 1a–c and are juxtaposed with the corresponding chronograms from our BEAST analysis (figure 1g; electronic supplementary material, figure S4). Although the number of haplotypes differs within each clade, all three curves share a highly similar shape that increases steadily before rapidly increasing at \( 5 \times 10^{-3} \) mutations per site (electronic supplementary material, figure S5). The timing of these rapid increases begins at approximately the same time point, which coincides with the emergence of a hyper-diverse haplotype clade in the Fijian representatives and the most diverse haplotype clades in the Vanuatu and Samoan clades (figure 1g). Considered separately, the changes in haplotype accumulation are striking, and together they suggest parallel temporal responses to a shared stimulus able to influence species over large spatial scales.

(b) Effective population size
Modelling the effective population size over time using BSPs (figure 1d–f; electronic supplementary material, figure S6) reveals almost identical patterns of change. The y-axis of these plots can be interpreted as \( N_e \) mutation rate per site, and if mutation rates are assumed to be constant, then this can be interpreted as a measure of relative \( N_e \) over time. Again, we see largely stable populations over the majority of time, but a dramatic drop in \( N_e \) occurs at approximately \( 5.0 \times 10^{-3} \) mutations per site before a large increase at \( 2.5 \times 10^{-3} \) mutations per site. Aligning the scales of our BSPs and chronograms indicates these changes appear to occur within a very similar time frame, and the tree topologies (figure 1g) demonstrate that this timing corresponds to both a sudden decrease and then sudden increase in \( N_e \) across each of the archipelagos.
Figure 1. (a–c) Log lineage through time (LTT) plots based on Bayesian skyline chronograms. Maximum time is the root height mean, dotted vertical line represents lower 95% highest posterior density. (d–f) Corresponding Bayesian skyline plot (BSP) for each data file of (a–c) representing colour coded archipelagos. Bold plot line indicates mean plot values; shaded area represents upper and lower confidence intervals for mean estimates. (g) Maximum credibility trees corresponding to archipelago data files of (a–f). (h) Borneo stalagmite δ¹⁸O records. Figure adapted from Meckler et al. [41] to indicate the published record for the last glacial termination from this location. Light and dark grey bars on the right show range of interglacial and glacial values, respectively, and black arrows highlight δ¹⁸O maxima interpreted as deglacial drying phases. H1, Heinrich event 1. (i) Map indicating sampling areas. (Online version in colour shows archipelagos colour-coded to preceding plots as follows: Vanuatu, blue; Fiji, red; Samoa, green; (g–h) orange vertical lines indicate (g) tree topology corresponding to key changes in LTT plot shape and (h) last glacial termination.)
(c) Age estimates
We were able to determine the approximate ages of two key components of our plots by applying the single-nucleotide mutation rate [35], and our estimated number of generations per year. First, based on node ages of 0.1322, 0.0999 and 0.051 mutations per site and four generations per year, we obtain crown age estimates of 533, 403 and 206 ka for Vanuatu, Fiji and Samoa, respectively. The crown age refers to the age of the most recent common ancestor for a particular group of taxa. If we instead assume three generations per year, then the estimates increase to 711, 537, 274 ka for Vanuatu, Fiji and Samoa, respectively. These various ages are only a fraction of the islands respective geological ages of 3–8 [20], 12.5–29 [21] and 5 Ma [22]. Second, by aligning our resulting tree topologies with both LTT and BSPs, we determined that both the increase in haplotype accumulation and change in \( N_e \) occurred at approximately 0.006 mutations per site. Based on four generations per year, this equates to 24 ka, or approximately 32 ka if three generations per year are assumed. This suggests a stimulus or stimuli common across all three isolated island groups. Likewise, if the increase in \( N_e \) occurred at 0.0025 mutations per site, then the estimated onset of increasing \( N_e \) is approximately 10 ka based on four generations per year, or 13 ka based on three generations. Based on these estimates, the changes presented in \( N_e \) appear broadly concordant with a major global change in the last glacial termination (figure 1; with Heinrich event 1 labelled H1).

4. Discussion
Numerous studies have outlined the effects of glacial cycles on Palearctic and Nearctic biota, with contractions to southern refugia during glacial maxima and then subsequent northern expansions following deglaciation [42]. Less detailed studies have inferred similar patterns in the Southern Hemisphere [43], but few have examined how biota in tropical latitudes have responded to these global cycles [44,45]. The tropical Pacific represents a major driving force behind current global climates, and is expected to have played a considerable role throughout glacial periods [46]. Despite its importance, reconstruction of climatic variation in the region over time has been limited by short or insufficiently resolved records, and confidence in extrapolating data from neighbouring regions is therefore similarly limited.

We found that the principal bee fauna in three major SWP archipelagos, spanning a distance of approximately 4000 km and representing multiple colonization events but with no subsequent interchanges, show remarkably similar patterns of haplotype accumulation since the late Pleistocene. Our LTT plots suggest marked anti-sigmoidal (S-shaped) patterns for haplotype accumulation in the Homalictus fauna from Fiji, Vanuatu and Samoa, and our phylogenetic analyses indicate that these similarities cannot be explained by dispersal events linking the bee faunas in these regions. Consequently, Homalictus bees in these regions appear to have independently responded to some factor(s) influencing each of the archipelagos, and any such factor(s) seem to have operated within a close time frame for each archipelago. For any such factor(s) to have impacted over such a large geographical scale, there are few likely explanations.

Anti-sigmoidal LTT plots can arise from both major extinction events [47], and three-phase Yule models of radiation where cladogenesis is initially high, followed by a period of low speciation and extinction rates, with a second subsequent period of high diversification rates. Discriminating between these alternatives can be very difficult [48]. However, our BSP analyses clearly indicate major declines in effective population sizes of Homalictus for each of the three archipelagos at approximately the same time point. Although single-locus demographic reconstructions are limited in their confidence with increasing time [49], very recent time scales ensure strong phylogenetic signal for conspecific variation and render the impact of saturation on our analyses negligible. Instead, confidence in estimating this time point is limited owing to potential variation in the applied mutation rate and generations per year, yet all likely scenarios commonly indicate the decrease and subsequent increase occurred either side of the LGM. The LGM involved both global cooling as well as considerable decreases in precipitation for widely separated regions neighbouring the SWP, including Australia [50], Borneo [41] and Chile [51]. Such conditions have been shown to have influenced similar contraction/expansion population dynamics in the goby fish of the SWP owing to sea-level fluctuations, determined via deep-sea sediment cores [52]. However, the impacts on terrestrial biota on this region have not been well explored.

A recent study of Borneo stalagmite \( \delta^{18}O \) records provides the closest comparison for the possible SWP climate variation and demonstrates a 100 kyr glacial–interglacial cycle throughout the Quaternary with a clear increase in atmospheric moisture since the LGM [41]. Palynology studies of the highland Lake Tagamaucia in Fiji [53] and Lynch’s Crater in northeast Australia [50] indicate a distinct change in vegetation composition beginning approximately 15 ka during a marked post-LGM warming period. With these exceptions, very few studies have attempted to reconstruct SWP climates across the LGM, so the impact of climate change in this region remains largely unexplored.

Our results suggest that some factor(s) forced near-simultaneous changes in bee populations across a wide region of the SWP. We are unable to say whether these changes are due to climate directly forcing changes in bee populations, indirect effects arising from changes in angiosperm communities, or a mixture of both. However, because bees are key pollinators in terrestrial ecosystems, it seems very likely that our inferred fluctuations in bee demography will have had very broad impacts. Island systems often harbour low species richness, which can result in a broadened range of host plant species for pollinators owing to reduced competition, resulting in keystone ‘super generalist’ species [54]. This seems very likely for Fiji, Vanuatu and Samoa, where most (if not all) non-Homalictus bees are likely to represent post-LGM and/or anthropogenic arrivals [16,17].

If the changes in bee populations that we infer here involve corresponding changes in angiosperm communities, either via shared forcing events or effects on pollinator abundance, then events surrounding the LGM are likely to have broadly impacted tropical island ecosystems as well as the better-understood Palearctic and Nearctic biota. Given predicted climate change scenarios, studies are needed to determine how climate change may impact pollinator-dependent plant communities, including agricultural crops. As tropical ecosystems are major sources of biodiversity,
incorporating a large proportion of the developing world, the consequences of disrupting plant pollinator interactions could be considerable.

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Data accessibility. DNA sequences may be accessed via corresponding GenBank Accession or BOLD ID numbers listed for all included specimens in the electronic supplementary material, table S1.

References


