Low Variation in Nuclear and Mitochondrial DNA Inhibits Resolution of Invasion Pathways across the Pacific for the Coconut Rhinoceros Beetle (Scarabeidae: *Oryctes rhinoceros*)

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Abstract. The coconut rhinoceros beetle (*Oryctes rhinoceros*) is a severe pest of coconut and other palms that has invaded the South Pacific in the last decade. The beetle can cause great economic losses, not only to agriculture but also due to indirect impacts on tropical aesthetics and tourism. In the last decade, new invasive populations of the beetle have been detected on Guam and Oahu, Hawaii. Despite the beetle's extensive invasion history and economic impacts, little is known about its invasion dynamics. We used 1,480 base pairs of cytochrome oxidase subunit I mitochondrial and 814 base pairs of carbamoyl-phosphate synthetase, aspartate transcarbamoylase, dihydroorotase nuclear DNA to conduct a population genetics analysis on eight beetle populations from Thailand, Vietnam, Taiwan, and China in the beetle's native range and Palau, American Samoa, Guam, and Hawaii in the beetle's invasive range, in an attempt to resolve invasion pathways. Genetic diversity was insufficient to generate strong evidence for *O. rhinoceros* movement patterns. Mitochondrial DNA provided a clear but poorly supported population structure. Although nuclear DNA proved to be more diverse, population structure lacked clear signal. This lack of diversity is congruent with a rapid, recent invasion. There appears to be no genetic exchange between populations once they establish, implying that they are rare, human-mediated dispersal events.

Key words: Scarabeidae, invasive species, Hawaii, *COI, CAD*

Introduction

Native to coconut growing regions between India and the Philippines, the coconut rhinoceros beetle (Scarabeidae: *Oryctes rhinoceros*) has become a major palm pest on many Pacific Islands (Gressitt 1953, Catley 1969, Hinkley 1973, Bedford 1980). Since it was first detected on Upolu, Western Samoa in 1909, the beetle has spread to several island nations including American Samoa, Palau, and Fiji (Jepson 1912, Gressitt 1953, Swaine 1966). In the last decade, an invasive population established on Guam, and in 2013 *Oryctes rhinoceros* was first detected on the Hawaiian Island of Oahu (Smith and Moore 2008, Hawaii Department of Agriculture 2014). While larvae function as decomposers feeding on decaying organic matter, the adult beetles bore into the crowns of live palm trees to feed on sap and, in doing so, damage developing fronds and potentially the meristem, killing entire trees (Young 1975, Hinkley 1966, Bedford 1976). Damage caused by tree death and loss of yield due to rhinoc-

eros beetle feeding is often extensive and detrimental to local economies (Gressitt 1953, Catley 1969, Chong 1991, Dhileepan 1992). In addition to direct agricultural losses, the beetles pose a major threat to the tropical aesthetic, which supports tourism on islands such as Guam and Oahu (Smith and Moore 2008).

Despite decades of invasions and significant damage caused by *O. rhinoceros* in the Pacific, little is known about the beetle's invasion pathways. Understanding the movement of a pest species outside of its native range is critical to devising effective management protocols (Estoup and Guillemaud 2010). Often, the successful establishment of an invasive pest and our ability to eradicate it, are dependent on the target species propagule pressure (Myers et al. 2000, Dlugosch and Parker 2008). Information on where a pest species comes from and how it moves are essential to minimizing repeat invasion and long-term control.

Molecular data is ideal for analyzing population dynamics of pest species and has been utilized to obtain crucial information on a number of invasions (Rubinoff et al. 2011, Hoos et al. 2010, Darling et al. 2008). In this study, we utilize data from both nuclear and mitochondrial genes to examine invasion pathways for the coconut rhinoceros beetle throughout the South Pacific. Using specimens from Thailand, China, Vietnam, and Taiwan in the beetle's native range, as well as from American Samoa, Palau, Guam, and Hawaii in the beetle's invasive range, we address the following questions: What is/are the source population(s) for these Pacific island invasions? What are the different pathways taken by the beetle to reach its current invasive distribution? To what degree are invasive island populations genetically distinct? This information can support management and eradication efforts on recently invaded islands such as Oahu, Hawaii, and assist in halting the spread of *O. rhinoceros* in the Pacific.

Methods

Sampling. We acquired 45 coconut rhinoceros beetle adults and larvae from the native range including nine from China, 22 from Taiwan, 13 from Thailand, and one from Vietnam, and 92 adults and larvae from the invasive range including 45 from Hawaii, 11 from Guam, 22 from Palau, and 14 from American Samoa. Samples were provided by collaborators, save the one sample collected in Vietnam by hand. Whole beetles and beetle larvae were supplied dead, either in EtOH of varying concentrations or within 24 hrs after mortality. Fresh beetles were immediately placed into 90–95% EtOH following delivery. In some cases, pre-dissected adult or larval legs (2–4 legs per sample) were provided in 70–90% EtOH. Samples were stored at –20°C prior to DNA extraction.

Laboratory work. A single leg, in the case of adult beetles, and the head or a series of legs in the case of larval beetles were dissected for DNA extraction. The remainder, if any, of each sample was placed into fresh 90–95% EtOH and deposited as a voucher stored at –80°C. DNA was extracted from all specimens using the DNeasy™ Blood & Tissue kit (Qiagen). Protocols were standard in all but the following respects: tissue digestion with proteinase K took place for 24 hours at 55°C; 80 μl of EB buffer in the case of larval legs and 120 μl in the case of adult legs was used to elute DNA. Extracts were stored at –20°C.

Polymerase chain reaction (PCR) was performed using a BioRad T100™ or C1000 Touch™ thermal cycler. Primers LCO-1490, HCO-2198, Jerry and Pat2 were used to sequence 1480 base pairs of the cytochrome oxidase subunit I (*COI*) mitochondrial gene (Folmer et al. 1994, Simon et al. 1994) under the following

PCR cycle: 3 min at 94°C, 40 cycles of 94°C for 30 s, 50°C for 30 s, and 70°C for 1 min, followed by a final 70°C extension for 10 minutes and a 4°C hold until termination. Primers CD439F and CD688R were used to sequence 814 base pairs of the carbamoyl-phosphate synthetase, aspartate transcarbamoylase, dihydroorotase (*CAD*) nuclear gene (Wild and Maddison 2008) under the following PCR cycle: 3 min at 94°C, 40 cycles of 94°C for 30 s, 53°C for 30 s, and 70°C for 1 min followed by a final 70°C extension for 10 minutes and a 4°C hold until termination. PCR products were purified using QIAquick® spin columns (Qiagen) following standard protocols. Sanger sequencing was performed at the ASGPB sequencing laboratory (http://www.hawaii.edu/microbiology/asgpb/) of the University of Hawaii at Manoa. All sequences were aligned and refined using Geneious v7.1.9 (http://www.geneious.com, Kearse et al. 2012)

Data analysis. *COI* and *CAD* data were analyzed separately. Conversion between data file formats was conducted using the PGDSpider tool (Lischer and Excoffier 2012). Several ambiguous sites identified in *CAD* sequences were resolved by running a PHASE algorithm (Stephens et al. 2001) to create haplotype pairs from multi-copy gene data in the program DnaSP v.5 (Librada and Rozas 2009). The PHASE data set was used for the overall analysis of *CAD*. Haplotype networks were constructed in the program PopART (http://popart.otago.ac.nz) using TCS Network analysis (Clement et al. 2002). DnaSP v.5 was used to estimate a series of population-level parameters including pairwise nucleotide diversity π and haplotype diversity h. Φ_{ST}—a measure of population differentiation analogous to the traditional F_{ST} statistic but optimized for nucleotide differences—assumes selective neutrality of genetic markers. We tested neutrality using Tajima's D statistic and Fu and Li's F* and D* (Tajima 1989, Fu and Li 1993). The population genetics software Arlequin v3.5 was employed for the calculation of pairwise Φ_{ST} values among the different sampling locations and for conducting an AMOVA analysis for partitioning diversity within and among populations, and among groups (Excoffier and Lischer 2010). Sampling locations were grouped according to historical records of *Oryctes rhinoceros* distribution and invasion, resulting in the following combinations: Native Population (Thailand, China, Taiwan and Vietnam), 20th Century Invasions (American Samoa and Palau), and 21st Century Invasions (Guam and Oahu, Hawaii). Records suggest a notable lag time between the two invasion periods, prompting their separation into distinct groups.

Results

COI sequence data from 127 individuals and *CAD* sequence data from 117 individuals were included in our analysis. The sample size disparity is the result of variable sequencing success rates, possibly due to degradation of some specimens prior to our receiving them. One *COI* haplotype was obtained for each individual, while two *CAD* haplotypes, one maternal and one paternal, were obtained for each individual via the PHASE process. Overall haplotype counts can be found with a number of other test statistics in Tables 1–4.

Haplotype diversity and population structure. Test statistics were generated for the 127 *COI* sequences and 234 *CAD* sequences via analysis of molecular variance (AMOVA) (Tables 5, 6), haplotype diversity (h), nucleotide diversity (π) and tests of selective neutrality (Fu and Li's F* and D* and Tajima's D) (Tables 1–4), Φ_{ST} matrices (Tables 7–8) and haplotype networks (Figures 1, 2). The native range group and two invasion groups (20th century and 21st century) were analyzed as

Table 1. Genetic variability of *COI* sequences by group.

	Native range	20th Century invasions	21st Century invasions	Total
Sample size	44	33	50	127
No. of haplotypes (Nh)	4	3	1	7
Haplotype diversity (h)	0.556	0.68	0	0.642
Nucleotide diversity (π)	0.00107	0.00147	0	0.00115
No. of segregating sites (S)	4	5	0	7
Fu and Li's F	0.54089	**1.63807**	N/A	0.50442
Fu and Li's D	–0.0638	1.13477	N/A	0.27290
Tajima's D	1.67514	**2.04528**	N/A	0.71865
Fu's F	2.152	4.493	N/A	0.905

Variability of *COI* (n = 127, 1480 bp) in *Oryctes rhinoceros* assessed in a population grouping framework. Groupings determined from historical invasion records. For Fu and Li's F and D and Tajima's D values, significant ($P<0.05$) values are in bold, while others have $P>0.10$.

discrete units in the case of the diversity and selective neutrality tests (Tables 1, 3).

The lack of variation in the *COI* gene was surprising (Tables 1, 2). A total of seven unique haplotypes were observed, with completely monotypic populations except in the case of Palau and Taiwan (Table 2). Haplotype diversity in Taiwan is heavily skewed with only two individuals possessing a secondary haplotype (h = 0.091) different from the primary at a single base pair. In Palau, the two haplotypes observed are nearly evenly distributed across 22 samples (h = 0.506) and differ by four base pairs. Levels of diversity among locations invaded in the 20th century resemble those in the native range while locations invaded in the 21st century are monotypic (Table 1). F-statistics for *COI* range from –1.0 to 1.0 with the majority of pairwise analyses yielding values of 1.0 or 0.0, corresponding to complete differentiation and identicalness (Table 7). There are very few intermediate Φ_{ST} values—all either below 0.028 or above 0.43—reinforcing the lack of genetic diversity. What diversity exists occurs primarily among populations within groups (76.35%) as opposed to between groups (5.06%) or within populations (18.59%) (Table 5). Overall Φ_{ST} is 0.81406. The prevailing signal is one of low, but segregating, genetic diversity among populations and no or almost no diversity within most populations, Palau being the exception.

The nuclear *CAD* gene possesses greater diversity than *COI* (Tables 3, 4). Twenty-three unique haplotypes were observed; nineteen of those occur between American Samoa and Palau (Table 4). Palau has seven haplotypes with eight variable sites; American Samoa has 12 haplotypes with nine variable sites. The remaining locations possessed 1–3 haplotypes with 0–3 variable sites. Haplotype diversity in American Samoa is evenly distributed across samples (h = 0.915) whereas the remaining populations exhibit a high degree of clustering with one or two clearly dominant haplotypes. F-statistics for *CAD* range from 0.0 to 1.0 with all but two significant pairwise comparisons yielding values over 0.47 (Table 8). This suggests low genetic variation between popula-

Table 2. Genetic variability of *COI* sequences by population.

	American Samoa	China	Guam	Hawaii (Oahu)	Palau	Taiwan	Thailand	Vietnam	Total
Sample size	11	8	11	39	22	22	13	1	127
No. of haplotypes (Nh)	1	1	1	1	2	2	1	1	7
Haplotype diversity (h)	0	0	0	0	0.506	0.091	0	0	0.642
Nucleotide diversity (π)	0	0	0	0	0.00137	0.00006	0	0	0.00115
No. of segregating sites (S)	0	0	0	0	4	1	0	0	7
Fu and Li's F	N/A	N/A	N/A	N/A	**1.67975**	–1.67803	N/A	N/A	0.50442
Fu and Li's D	N/A	N/A	N/A	N/A	1.09548	–1.57469	N/A	N/A	0.27290
Tajima's D	N/A	N/A	N/A	N/A	**2.37679**	–1.16240	N/A	N/A	0.71865
Fu's F	N/A	N/A	N/A	N/A	5.588	–0.957	N/A	N/A	0.905

Variability of *COI* (n = 127, 1480 bp) in *Oryctes rhinoceros* assessed in a per population framework. For Fu and Li's F and D and Tajima's D values, significant ($P<0.05$) values are in bold, while others have $P>0.10$.

tions. Existing diversity is most prominent among populations within groups (51.02%) as opposed to among groups (19.32%) or within populations 29.65%) (Table 6). Overall Φ_{ST} is 0.70346. Despite increases in diversity relative to *COI*, the *CAD* gene lacks meaningful variation for discerning population structure.

Haplotype networks. The haplotype network generated for *COI* suggests that *Oryctes rhinoceros* from Guam, Oahu, and Taiwan are nearly identical, save for two samples from Taiwan that differ by a single base pair substitution (Figure 1). China and Thailand form a monomorphic group that differs from the Vietnam haplotype by a single base pair change. American Samoa is also monomorphic. Palau is the only location containing substantial polymorphism, with two haplotypes that differ by four base pairs. This is the maximum observed difference between *COI* haplotypes. Statistical power for this analysis of population structure is reduced due to the relatively low number of polymorphic sites.

The *CAD* gene demonstrated higher overall diversity compared to the mitochondrial *COI* gene. The resulting haplotype network, however, provides a confounding depiction of the population structure with higher rates of polymorphism within and between any given population (Figure 2). American Samoa possesses the greatest overall

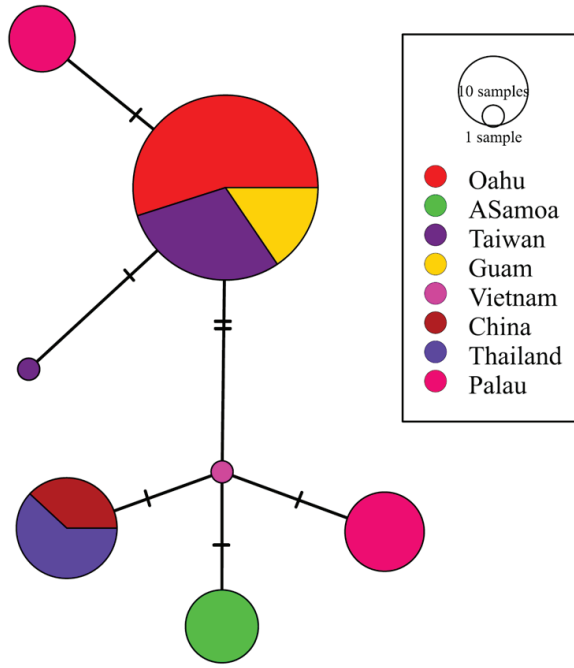

Figure 1. *COI* haplotype network. TCS Network based on 1480 base pairs of the *COI* gene region from a total of 127 individuals (8 to 39 from any given location) representing 127 total haplotypes. Hash marks represent a single base pair change. Populations are highly monotypic; Palau is the exception with two haplotypes.

Table 3. Genetic variability of *CAD* sequences by group.

	Native range	20th Century invasions	21st Century invasions	Total
Sample size	86	58	90	234
No. of haplotypes (Nh)	8	16	3	23
Haplotype diversity (h)	0.8	0.787	0.527	0.872
Nucleotide diversity (π)	0.0036	0.00342	0.00122	0.00402
No. of segregating sites (S)	9	13	2	13
Fu and Li's F	1.68994	–0.06657	1.40929	0.62505
Fu and Li's D	1.32704	0.03893	0.69383	0.21577
Tajima's D	1.61961	–0.23833	**2.36589**	1.01534
Fu's F	1.932	–5.004	2.588	–4.487

Variability of *CAD* (n = 117 individuals, 234 haplotypes; 814 bp) in *Oryctes rhinoceros* assessed in a population grouping framework. Groupings determined from historical invasion records. For Fu and Li's F and D and Tajima's D values, significant ($P<0.05$) values are in bold, while others have $P>0.10$, with the exception of Fu and Li's F value for native range, with $P>0.05$.

Table 4. Genetic variability of *CAD* sequences by population.

	American Samoa	China	Guam	Hawaii (Oahu)	Palau	Taiwan	Thailand	Vietnam	Total
Sample size	28	16	22	68	30	42	26	2	234
No. of haplotypes (Nh)	12	1	3	3	7	3	2	2	23
Haplotype diversity (h)	0.915	0	0.255	0.437	0.552	0.512	0.409	1	0.872
Nucleotide diversity (π)	0.00394	0	0.00052	0.001	0.00191	0.00091	0.00151	0.00369	0.00402
No. of segregating sites (S)	9	0	2	2	8	2	3	3	13
Fu and Li's F	0.56833	N/A	0.54409	1.14162	–0.20342	1.00138	1.25311	N/A	0.62505
Fu and Li's D	0.35088	N/A	0.85062	0.71782	0.05640	0.76579	0.96844	N/A	0.21577
Tajima's D	0.79688	N/A	–0.53159	1.58816	–0.70348	1.10305 P>0.10	1.37815	N/A	1.01534
Fu's F	–3.150	N/A	-0.472	1.76	–1.251	1.142	3.872	N/A	–4.487

Variability of *CAD* (n = 117 individuals, 234 haplotypes; 814 bp) in *Orycles rhinoceros* assessed in a per population framework. All Fu and Li's F and D and Tajima's D values are nonsignificant, with P>0.10.

haplotype diversity with 12, a sharp contrast to the monomorphic population suggested by *COI*. Guam and Oahu still associate, with three haplotypes shared between them, but neither shares a haplotype with Taiwan. China and Thailand are distinct; Guam and Oahu are relatively less diverged from China and Thailand than in the *COI* network. The maximum distance between major *CAD* haplotypes (>10 instances) is seven mutational steps with an overall maximum of nine.

Discussion

The coconut rhinoceros beetle is a serious pest with the potential to do significant agricultural and aesthetic damage to coconut palms and a variety of other species of cultural and economic importance throughout the South Pacific islands. The beetle has proven to be difficult to eradicate, with severe weather events, or other disasters, triggering population explosions due to the proliferation of breeding sites. High density booms in beetle population size decimate palm trees, and these dead palm trees act as a prime new larval substrate resulting in a positive feedback loop (USDA 2014). In some regions, suppression of *Oryctes rhinoceros* has been achieved using the *Oryctes nudivirus* (OrNV), previously classified as a baculovirus (Mohan and Pillai 1993. Jacob 1996. Prasad 2008) and to a limited extent the green muscardine fungus (*Metarrhizium anisopliae*) (Young 1974.

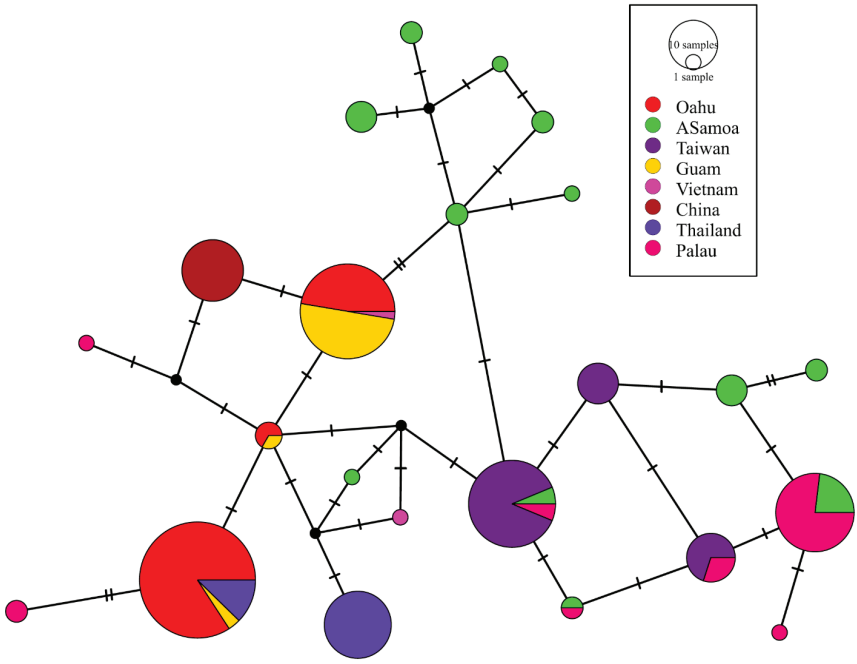

Figure 2. *CAD* haplotype network. TCS Network based off 814 base pairs of the *CAD* gene region from a total of 117 samples (8 to 39 from any given location) representing 234 total haplotypes. Hash marks represent a single base pair change. A PHASE algorithm was used to generate haplotypes from ambiguities present in the sequence data of a multi-copy nuclear gene, resulting in twice as many haplotypes as samples.

Table 5. *COI* AMOVA results.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among groups	2	38.473	0.05079 Va	5.06	FCT = 0.05056
Among populations within groups	5	46.576	0.76698 Vb	76.35	**FSC = 0.80416**
Within populations	119	22.227	0.18678 Vc	18.59	**FST = 0.81406**
Total	126	107.276	1.00455		

Molecular variance is greatest among populations within groups and is low within populations and among groups. Significant values are in bold.

Table 6. *CAD* AMOVA results.

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among groups	2	134.026	0.37353 Va	19.32	FCT = 0.19322
Among populations within groups	5	118.093	0.98640 Vb	51.02	**FSC = 0.63244**
Within populations	226	129.561	0.57328 Vc	29.65	**FST = 0.70346**
Total	233	381.679	1.93321		

Molecular variance is greatest among populations within groups; variance is lower within populations and among groups but together is equal to approximately half of the total. Significant values are in bold.

Table 7. *CAD* pairwise Φ_{ST} values.

	American Samoa	China	Guam	Hawaii (Oahu)	Palau	Taiwan	Thailand
China	**0.62907**						
Guam	**0.60083**	**0.80538**					
Hawaii	**0.67977**	**0.75109**	**0.49768**				
Palau	**0.26547**	**0.84482**	**0.80262**	**0.79731**			
Taiwan	**0.23561**	**0.88369**	**0.81971**	**0.77370**	**0.54969**		
Thailand	**0.60111**	**0.80168**	**0.67814**	**0.58419**	**0.77102**	**0.77855**	
Vietnam	0.26757	**0.90052**	0.47905	0.49603	**0.66006**	**0.67929**	0.38490

Values from *CAD* sequence data (n = 117 individuals, 234 haplotypes; 814 bp). Significant values are in bold.

Table 8. *COI* pairwise Φ_{ST} values.

	American Samoa	China	Guam	Hawaii (Oahu)	Palau	Taiwan	Thailand
China	**1.00000**						
Guam	**1.00000**	**1.00000**					
Hawaii	**1.00000**	**1.00000**	0.00000				
Palau	**0.56154**	**0.53120**	**0.44965**	**0.62253**			
Taiwan	**0.97987**	**0.97775**	–0.03587	0.02753	**0.52478**		
Thailand	**1.00000**	0.00000	**1.00000**	**1.00000**	**0.57890**	**0.98107**	
Vietnam	1.00000	1.00000	1.00000	**1.00000**	–0.11429	**0.95556**	**1.00000**

Values from *COI* sequence data (n = 117 individuals, 234 haplotypes; 814 bp). Significant values are in bold.

Gopal et al. 2006). Locations such as the Hawaiian Islands, however, enforce strict regulations on biocontrol, and the fragile nature of local ecosystems make the use of such control agents impossible. A deep understanding of the invasion pathways and population structure of the coconut rhinoceros beetle is fundamental to effective management, especially where other methods of control are not possible.

Using mitochondrial and nuclear markers to resolve invasion pathways proved to be inconclusive due to an overall lack of variation among native and invasive populations. Haplotype network analysis of *COI* provided a tentative illustration of how populations may be related to one another. For instance, Taiwan, Guam and Oahu share a unique haplotype. This lack of diversity may indicate a shared invasion history or continued genetic exchange between populations. Given the vast geographic distance between these islands, it is likely that they share ancestry from a common parental population or served as stepping stones along an invasion pathway. Our diversity and divergence statistics, however, demonstrate that our data lacks sufficient power to confidently delineate such pathways. Low genetic diversity suggests a recent, rapid invasion which likely saw multiple bottlenecks or founder's events, generating the monotypic or bitypic populations observed here. This may be exacerbated by low sample sizes from some locations such as Vietnam and China in the native range, leading to subsampling bias. Still, similarly low-diversity invasion dynamics have been observed for other Pacific invaders such as the erythrina gall wasp, and may be the product of increasing globalization and interconnectivity between Pacific islands facilitating rapid dispersal and genetic contact (Rubinoff et al. 2010).

Nuclear markers proved to be more diverse than mitochondrial markers, which we did not expect given that nuclear DNA typically evolves more slowly (Brown et al. 1979, Harrison 1989). One explanation for this increase in diversity of the *CAD* gene compared to the *COI* gene is that the maternal inheritance of the mitochondrial genome makes it more sensitive to the changes in effective population size which might occur with rare, long-distance, human-mediated dispersal events. High *CAD* haplotype diversity on American Samoa and Palau, could result from multiple invasions from a diverse native source region. Due to their diploid structure, nuclear genes are less likely to exhibit complete lineage sorting than haploid mtDNA. Thus, evidence for this would be more prominent in the nuclear genome again due to the weakened effects of drift relative to the mitochondrial genome. Gene duplication can also inflate diversity of a given gene, but we found no evidence in the literature to suggest *CAD* amplification occurs in Coleoptera. It may be the case that a new mutation in the *COI* gene resulted in a selective sweep for the haplotypes seen here (e.g., Gompert et al. 2008), though this does not seem to be a likely scenario. The observed increases in *CAD* gene diversity did not provide us with a clearer pattern of population structure or relatedness, as exemplified in the convoluted nature of the resulting haplotype network.

Although specific mitochondrial and nuclear markers were not sufficient for drawing conclusions on the invasion pathways of *O. rhinoceros*, they did confirm that it is a relatively recent invasion, consisting of a single species from a relatively limited pool of genetic diversity. Future investigations should focus on data from next-generation sequencing (NGS). This technology provides several tools for analysis based on single nucleotide polymorphisms (SNPs) from across the genome, typically non-coding regions

which evolve very rapidly. These methods have high potential for investigating invasion dynamics and population genetics, and may be sufficiently powerful to resolve pathways even in rapidly progressing modern invasions (Cristescu 2015; Rius et al. 2015). *O. rhinoceros* represents a major threat to any tropical or subtropical region that relies economically or culturally on palm trees. Once present, the beetle is a ticking time-bomb waiting for a single event to cause breeding site proliferation and trigger a destructive feedback system. Continued coconut rhinoceros beetle sampling with more extensive contributions from the beetle's vast native range coupled with these more sensitive NGS methods should be utilized to provide deeper insight into *O. rhinoceros* invasion dynamics, supporting efficient management of a pest which has already proven to be a severe economic threat.

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