Discovering Cryptic Breeding Sites by Radio Tracking Coconut Rhinoceros Beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* L., is a serious pest of coconut trees and other palms throughout Southeast Asia and on several Pacific Islands. Adults kill palms when they bore into crowns to feed on sap. Larvae feed only on dead plant material at breeding sites. Eradication is possible if all breeding sites are located and destroyed. A field trial was performed on Guam to test the feasibility of using radio-tagged adults to discover cryptic breeding sites. Of 34 radio-tagged beetles that were released, 19 were successfully tracked to landing sites, 14 were lost when they flew beyond the range of our receivers and one beetle did not fly. Two of the landing sites were confirmed breeding sites as indicated by presence of other CRB at the same site. None of the radio-tagged beetles were caught in numerous pheromone traps in the vicinity of release sites.

Using animals to find aggregations of conspecifics is not a new idea. For example, by exploiting the gregarious nature of goats, the Judas goat method is a vital tool for detecting goats at low densities and a monitoring tool to confirm eradication (Campbell and Donlan, 2005). Radiotelemetry collars are fitted to select goats, which are released and allowed to seek out other goats. Judas goats are then radio tracked, either on foot or by helicopter, and accompanying goats are shot. This approach allows the last individuals to be removed. Judas goats have been used successfully in a number of island eradication programs. Recent development of light-weight, miniaturized radio-tracking transmitters now allows application of this technique to insects and other small animals which aggregate. Here, we report on a field trial to determine the feasibility of radio-tracking coconut rhinoceros beetles (CRB), *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae) to find cryptic breeding site aggregations.

CRB is a major pest of coconut palm, *Cocos nucifera* L. (Arecales: Arecaceae). Adult beetles damage and sometimes kill palms when they bore into crowns of palms to feed on sap. Palms die when boring and feeding activity kills the apical meristem. In contrast to adults, CRB grubs cause no damage. They feed on decaying vegetation at breeding sites which include dead standing coconut palms, fallen coconut logs, rotting coconut stumps and decaying wood of many tree species. Breeding sites are also found in piles of compost, sawdust and manure where these materials are available. When CRB breeding sites are abundant following damage from typhoons, war, or large scale agricultural operations, a self sustaining positive feedback loop may be initiated where large numbers of CRB adults kill large numbers of palms creating new breeding sites which generate even more CRB. This worst case scenario was observed in the Palau Islands when CRB arrived near the end of World War II. A CRB population outbreak was fueled by availability of abundant breeding sites in the form of trees killed by military activities. Fifty per cent of coconut palms were killed by CRB throughout the Parlay Islands and some of the smaller islands lost all of their coconut palms (Gressitt, 1953).

After feeding in the crowns of palms, adults of both sexes return to breeding sites where they mate and females oviposit. Location of breeding sites is facilitated by an aggregation pheromone produced by adult males (Hallett et al., 1995). The feeding-mating-oviposition cycle occurs several times during an adults lifetime (Gressitt, 1953). Vander Meer (1987)developed a body mass index, per cent emergent weight (PEW), which is strongly correlated with the physiological and behavioral status of CRB adults. PEW can be used to estimate current state of a beetle within the feeding-mating-oviposition cycle.

CRB first was detected in Guam in the Tumon Bay tourist hotel area in September, 2007. A delimiting survey indicated that the infestation was restricted to only a small of the island ($<$500 ha) and an eradication project was launched (Smith and Moore, 2008). The project relied on mass trapping using pheromone traps to capture adults, and sanitation to remove rotting vegetation used as breeding sites. The traps were baited with the CRB aggregation pheromone, ethyl 4-methyloctanoate (Hallett et al., 1995) commercially available as oryctalure (P046; ChemTica Iternacional, Heredia, Costa Rica). Four detector dogs were trained to assist in finding breeding sites on the ground by sniffing out CRB grubs.

Despite these efforts, CRB damage in central Tumon Bay remained high and the infestation spread to all parts of Guam by 2010 making eradication unfeasible. Attempts at population suppression using *Oryctes* nudivirus (OrNV), the preferred biocontrol agent for CRB (Bedford, 1980, 1986), also failed. It has recently been determined that the Guam CRB population is genetically different from other populations in Asia and the Pacific and it is considered to be a new invasive biotype of of CRB which has escaped from biocontrol by OrNV (Marshall et al., 2015). In addition to being resistant to all currently available isolates of OrNV, it appears that the CRB-Guam biotype behaves differently. CRB breeding sites are commonly found in coconut palm crowns on Guam but only occasionally elsewhere (Moore et al., 2015) and pheromone traps baited with oryctalure are not very attractive, catching only a small proportion of beetles in the vicinity of each trap (Moore, unpublished).

Eradication of CRB from an island is difficult once this pest has become established. The only proven tactic for eradication is a vigorous sanitation program which discovers and destroys all active and potential breeding sites. The single successful CRB eradication to date occur ed on the tiny (36 km^2) Niuatoputapu Island (also known as Keppel Island), which lies between Samoa and Tonga, using sanitation alone (Gressitt, 1953; Catley, 1969). Currently available pheromone traps, although useful for detection and surveillance, are not attractive enough to provide significant population suppression in mass trapping operations. Mass trapping coupled with sanitation during 1971 through 1974 failed to eradicate CRB on two islands in Fiji **(ref needed)**. The eradication attempt was abandoned when it was determined that there was “a low but persistent population which could not be trapped. ... It appeared that possible results from the indefinite continuation of the trial were no longer commensurate with the costs” (Bedford, 1980).**Check on these quotes**

As previously mentioned, four detector dogs for CRB were trained and employed for the first time on Guam. The dogs were trained to sniff out CRB grubs and it was hoped that they would enable us to find remaining, cryptic breeding sites in the final stages of eradication. The detector dogs were a very expensive component of the Guam CRB eradication program and they were retired after two years of service when it became evident that eradication was no longer feasible.

In this article, we report results of a field trial intended to test the concept of following radio-tagged CRB to cryptic breeding sites as an alternative to using detector dogs.

Materials and Methods

Radio Tracking Equipment

All radio tracking equipment was purchased from Advanced Telemetry Systems, Isanti, Minnesota. We used A2414 glue-on transmitters which weigh 300 mg. Each of 32 transmitters used in the trial operated at a unique frequency allowing us to track multiple targets simultaneously. Two transmitters were reused on two different beetles.

We used four R410 scanning receivers equipped with three-element folding Yagi antennas (Fig. 1). Two receivers operated in the 146 to 150 MHz frequency band and two operated in the 162 to 166 MHz band.

Each of the 4 trackers used a GPS receiver (Garmin Oregon 650) to record waypoints.

Collection, Selection and Preparation of Test insects

CRB used for radio tracking were caught in pheromone traps baited with ethyl 4-methyloctanoate (Oryctalure; P046; ChemTica Iternacional, Heredia, Costa Rica). Beetles were removed from the traps within one week of capture, placed in tubs containing moist peat moss, fed fresh banana slices and allowed to rest for at least three days.

Beetles were selected for flight capability at least one day prior to use in the radio-tracking trial. Groups of about 30 beetles were placed in moist peat moss in a metal bowl which they could exit only by flight. The bowl was supported on a stand inside a flight chamber consisting of a large (121 liter) garbage container and a lid was affixed. Beetles were left in the flight chamber overnight. Those that ended up on the flight chamber floor were deemed to have flown. All others were rejected from the radio-tracking field trial.

Flight-capable beetles were marked with a unique four-digit code engraved on one elytrum using a laser engraver (Fenix Flyer, Synrad Inc., Mukilteo, WA, United States). The sex, mass and elytral dimensions of each beetle were recorded.

A transmitter was glued to the pronotum of each beetle using a hot-melt glue gun (Fig. 1). Prior to transmitter attachment, the beetle pronotum was abraded with sandpaper to improve adhesion. Each glue-on transmitter had a mass of approximately 300 mg and was secured with approximately 250 mg of adhesive.

Test insects were stored and transported in lidded plastic bins approximately (45 cm by 30 cm by 18 cm) containing about 12 cm of damp peat moss. Because not all beetles were released when first taken into the field, some beetles remained in storage for up to six days.

Release Sites

Radio-tagged CRB were released and tracked at two locations on Guam: the University of Guam Agricultural Research Station in Yigo and the War in the Pacific National Historical Park in Asan (Fig. 3).

The Yigo site is an inland agricultural experiment station farm bordered by a residential area and uncultivated forest areas that include coconut palms and other trees. At the time of the field trial, most coconut palms on the station were showing signs of CRB damage. The release site (144.872750º E, 13.531333º N) was in the middle of an uncultivated field. Beetles were released in the vicinity of three types of pheromone traps baited with oryctalure: standard vaned bucket traps (n=3 traps), barrel traps (n=31), and DeFence traps (n=4). Vaned bucket traps are the standard pheromone traps for CRB developed by Hallett et al. (1995). Barrel traps and DeFence traps are experimental designs (A. M., unpublished).

The Asan site is roughly triangular with the ocean bordering one side, coastal wetlands on another, and forested hillside on the third. The park itself is a large, open, grassy field and includes with coconut palms on the edges, many of which displayed CRB damage at the time of the study. The release site (144.708537º E, 13.473904º N) was at the middle of a large grassy field. **ASAN TRAP INFO NEEDED.**

Tracking Procedure

Beetles were transported to release sites in plastic storage bins. The lid of the bin was removed at dusk (roughly 19:30) and the container was closed at roughly 21:30. Once the containers were opened, CRB activity was carefully monitored using a thermal camera (FLIR Systems Inc., Wilsonville, Oregon; E4 infrared camera). Observation under the infrared camera revealed that a beetle's flight muscles warm to about 37º C in preparation for flight. Pre-flight beetles were briefly viewed under red light to record the identification number and determine the frequency of the radio transmitter. Though nearly all beetles flew independently, several beetles that had not yet flown by the end of experimentation were encouraged to flight by removing them from the peat moss and throwing them into the air to facilitate takeoff.

CRB were pursued on foot following release and were tracked until a landing site was determined or until the transmitter signal was lost. In either case, a waypoint was recorded at the landing site or the last point of signal reception using a GPS unit.

Landing sites were visited on the following morning, and attempts were made to more precisely determine the location of each beetle. Beetle locations were monitored over several days, and beetles and or transmitters were recovered, when possible, at the end of the experiment. CRB and transmitters were successfully recovered by digging up beetles that buried into soil or compost; however the locations of CRB tracked to coconut crowns could not be as exactly determined due to the density of the frond foliage.

Analysis

Data from the field trial were stored in two text files (Supp. detector_beetles1.csv; Supp. trap_locations.csv) and analysis was performed using an iPython notebook (Supp. detector_beetles.ipynb).

Per cent emergent weight (PEW) was calculated using the following formula derived by Moore et al. (2015) following Vander Meer and Mclean (1975) and Vander Meer (1987):

$$PEW = \frac{100 \times M}{0.021 \times L \times W - 3.58}$$

where M = current mass of beetle in grams, L, W = length and width of elytra in mm.

Results and Discussion

Weather conditions during the field trial were mainly clear with occasional periods of rain and overcast skies. On release dates, August 8 to August 14, average temperature ranged from 27°C to 29°C and relative humidity was between 80% to 88%. Beetles were generally tracked under clear skies with the exception of August 9 during which light showers occurred.

Breeding Site Discovery

Of 34 radio-tagged beetles that were released, 19 were successfully tracked to landing sites, 14 were lost when they flew beyond the range of the receivers and one beetle did not fly (Fig. 2). Most of CRB tracked to landing sites were in tree tops (n=11) and the rest were on or below the ground (n=8).

Two of the landing sites were confirmed breeding sites as indicated by the presence of other CRB at the same site.

The first breeding site we discovered by radio-tracking was in an extremely cryptic and unsuspected microhabitat: in a hole in a large rotting branch of a breadfruit tree (*Artocarpus altilis*), about six meters above the ground. In this case, the receiver had become detached and the marked beetle was not recovered. However, 3 other CRB adults were occupying the hole. It is highly likely that this breeding site was in a branch broken by high winds experienced when Typhoon Dolphin passed over Guam in May 2015, about 3 months before the radio-tracking trial.

The second breeding site we discovered was beneath a barrel trap. **... NEED DESCRIPTION**

Ten landing sites in coconut palm crowns were considered to be potential breeding sites but we did not have time or equipment to confirm these. Arboreal breeding sites may be common on Guam (Moore et al. (2015)).

Tracks and Displacement

Distance between release sites and landing sites was calculated for the 19 beetles tracked to a landing site. The remaining 14 beetles flew beyond the range of our radio receivers, about 500 m. Mean displacement could not be estimated, but median displacement was 333 m, with no significant difference between release sites (Fig. 4).

There is little information available on the natural flight range of CRB. In a laboratory experiment, Hinckley (1973) observed that tethered beetles attached to a flight mill flew between 2 and 4 km with a flight duration of 2 to 3 hours. However, field observations indicate that natural flight is limited to a few hundred meters and this distance is influenced by the availability of feeding or breeding sites.

Kamarudin and Washid (2004) performed a mark-release-recapture study in a small (4.5 ha) oil palm replanting area containing a grid of 49 pheromone traps. Beetles captured in the traps were were marked with the trap number and released. Five of these beetles were recaptured in traps and displacement was calculated. Unfortunately, the total number of marked beetles released is not reported. Displacement averaged 118 m with a range of 51 m to 186 m. The authors acknowledge shortcomings of their study: “These values may be below the actual flight potential as their flights were monitored using pheromone traps. However, the conducive environment, availability of food and abundant breeding sites in the replanting area logically play a role in the flight distance.”

Mass relationships

Masses of CRB landing on the ground were not significantly different than those landing in treetops for females (4.486 g, 4.407 g; Welch’s t = 0.117; p = 0.914) and males (3.638 g; 5.043 g; Welch’s t = -2.2749; p = 0.053).

However, mean percent emergent weight of CRB landing on the ground was higher than those landing in treetops (Fig. 5). A possible explanation for this observation is that individuals with low percent emergent weight were hungry and therefore flew to treetops in order to bore into crowns to feed and beetles with lower percent emergence weight were searching for breeding sites in which to ovipost or find mates. Thus, if the objective is to locate ground based breeding sites, beetles with low PEW should be selected.

Response to Traps

Despite the fact that beetles were released within proximity of active pheromone traps, none were trapped. The mean trap catch rate of pheromone traps at the Yigo experiment station during August 2015 was 0.03 beetles per trap-day for standard bucket traps (n=3 traps), 0.13 for barrel traps (n=31), and 0.15 for DeFence traps (n=4). In addition, no marked beetles were trapped in fish gill netting draped over a greenwaste pile at the Yigo site. This pile trapped 0.50 beetles per trap-day during August 2015. **TRAP LOCATIONS AND CATCH DATA FOR ASAN NEEDED.**

If we assume that the wild beetles respond to traps in the same way as the radio-tagged beetles, then we can estimate that the suite of traps in the vicinity of the the release points catches less than one in 33 (<3%) of wild beetles. This low trap performance is consistent with results from previous mark-release-recapture data from Guam (A. M., unpublished). It is possible that oryctalure is less attractive to individuals of the CRB Guam biotype than to individuals of other other biotypes. Results indicate that none of the currently available CRB trapping methods are useful for population reduction of CRB-Guam.

Why CRB is a Good Candidate for Radio Tracking as a Method for Finding Conspecifics

CRB has characteristics which make this species a good candidate for radio tracking. First, this is a large, powerful beetle which can fly with the additional mass of a transmitter. Second, CRB aggregates at breeding sites. And third, CRB do not fly during the day, providing time to precisely locate landing points. Although 14 tagged beetles were lost when they flew beyond the range of our receivers, it is probable that we could have tracked these to precise landing sites with the use of a helicopter.

Our feasibility study was a success in that all but one of the 34 radio-tagged beetles flew and two of these were tracked to cryptic breeding sites confirmed by the presence of other CRB. If this 'Judas beetle' method is to be used in an eradication program, there may be concerns about introduction of additional CRB adults. In this case, perhaps only sterile males should be used.

Acknowledgments

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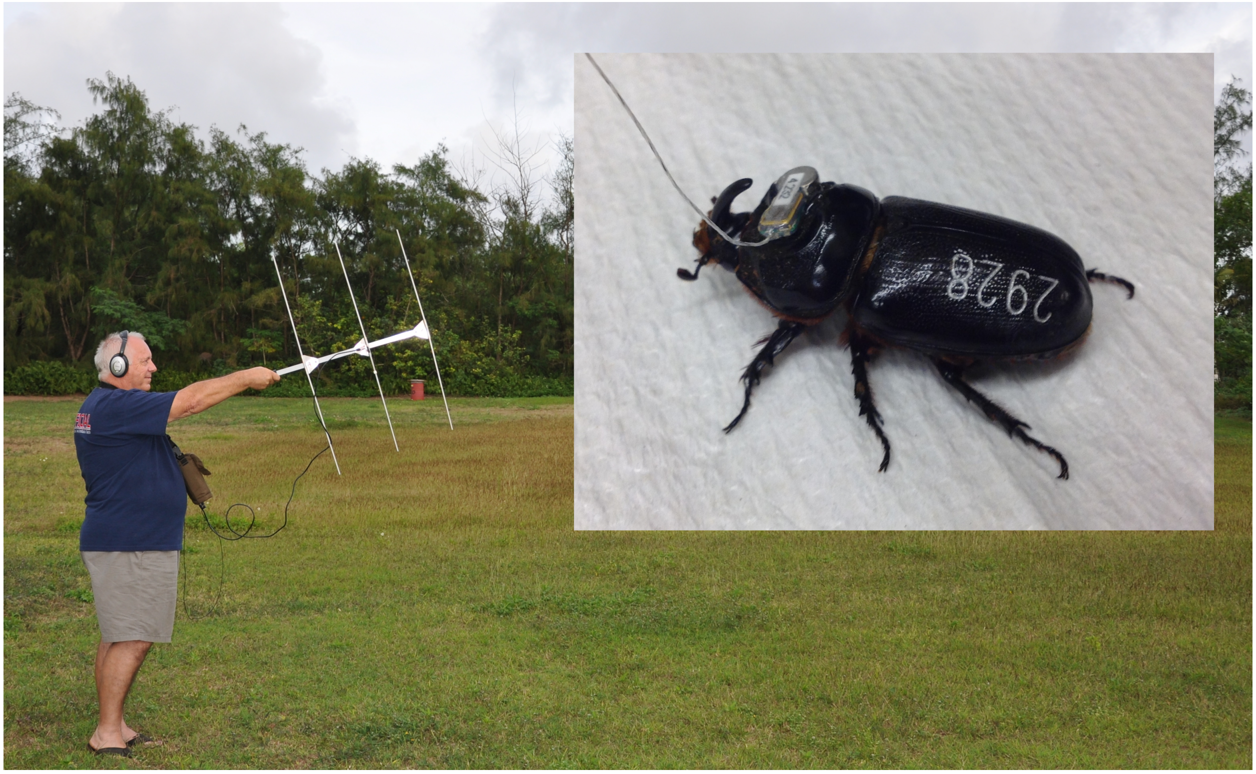


Figure 1: Radio-tracking receiver and coconut rhinoceros beetle with transmitter glued to pronotum.

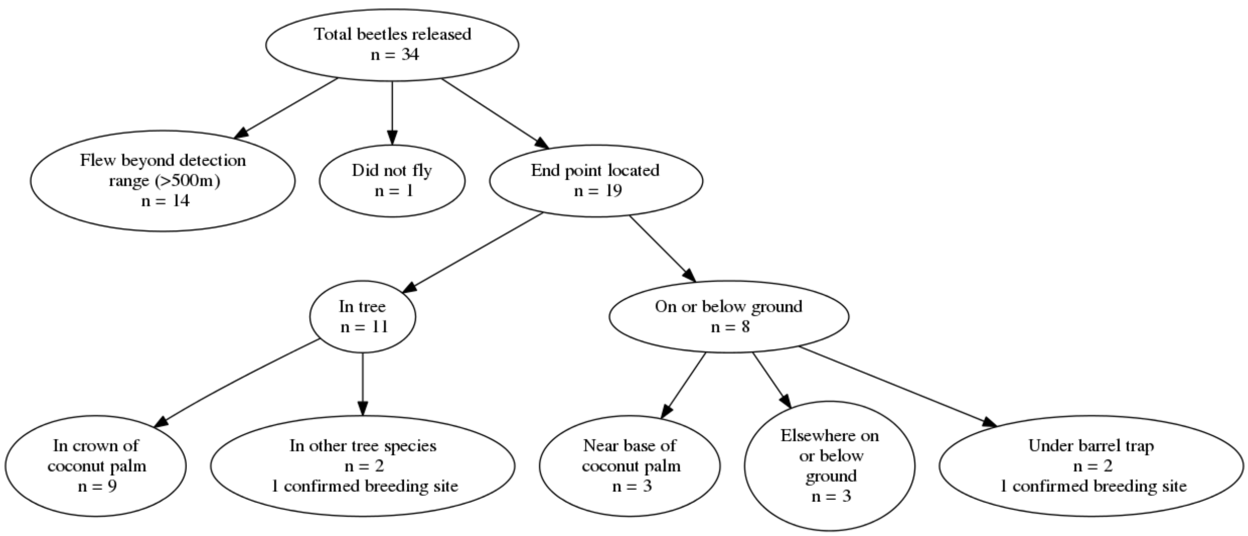
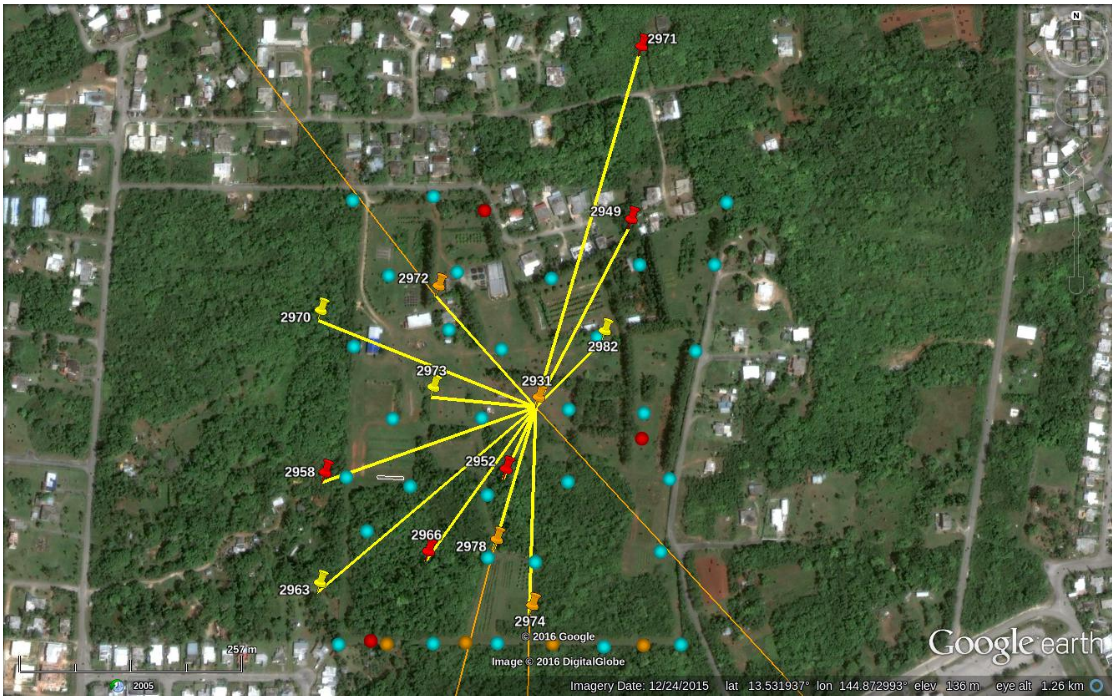
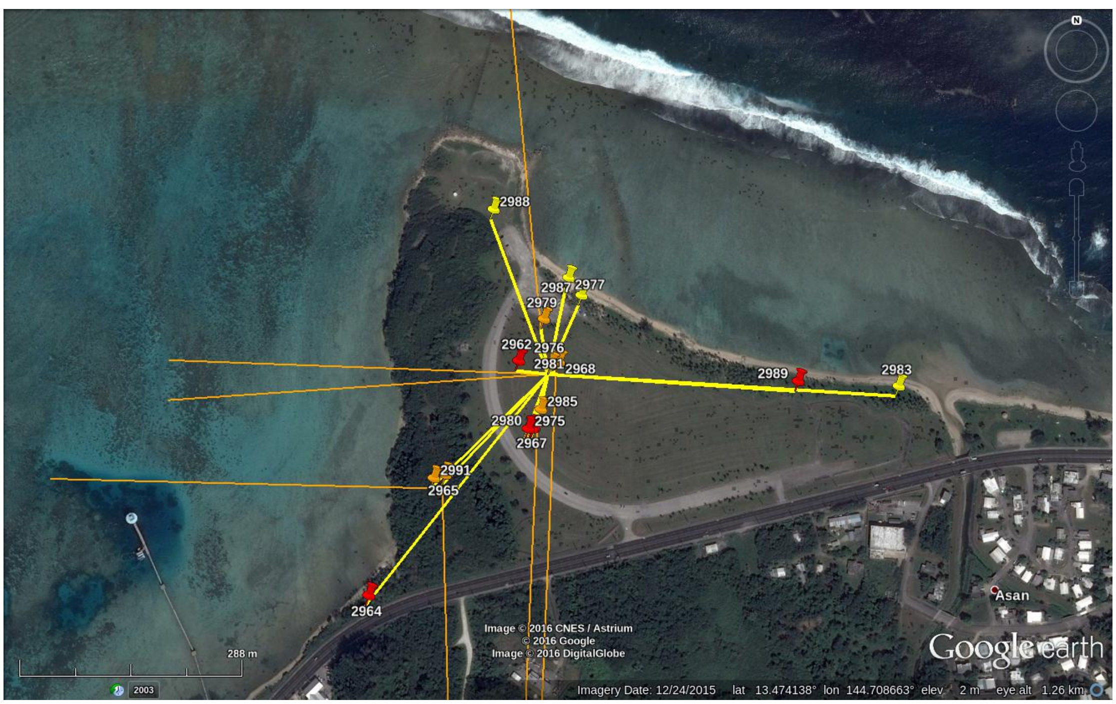


Figure 2: Outcomes for radio-tracked beetles.



(a) Yigo release site.



(b) Asan release site.

Figure 3: Results of radio-tracking coconut rhinoceros beetles at the Yigo and Asan release sites. Pushpin icons represent waypoints for tracked beetles (color code: red = beetle ended up in crown of tree; yellow: beetle ended up on or below the ground; orange: beetle was lost when it flew beyond the range of radio-tracking receivers, orange lines are extrapolated tracks based on last observed bearing of radio signal). Circular icons represent pheromone traps (color code: cyan = barrel trap; orange = DeFence trap; red = bucket trap).

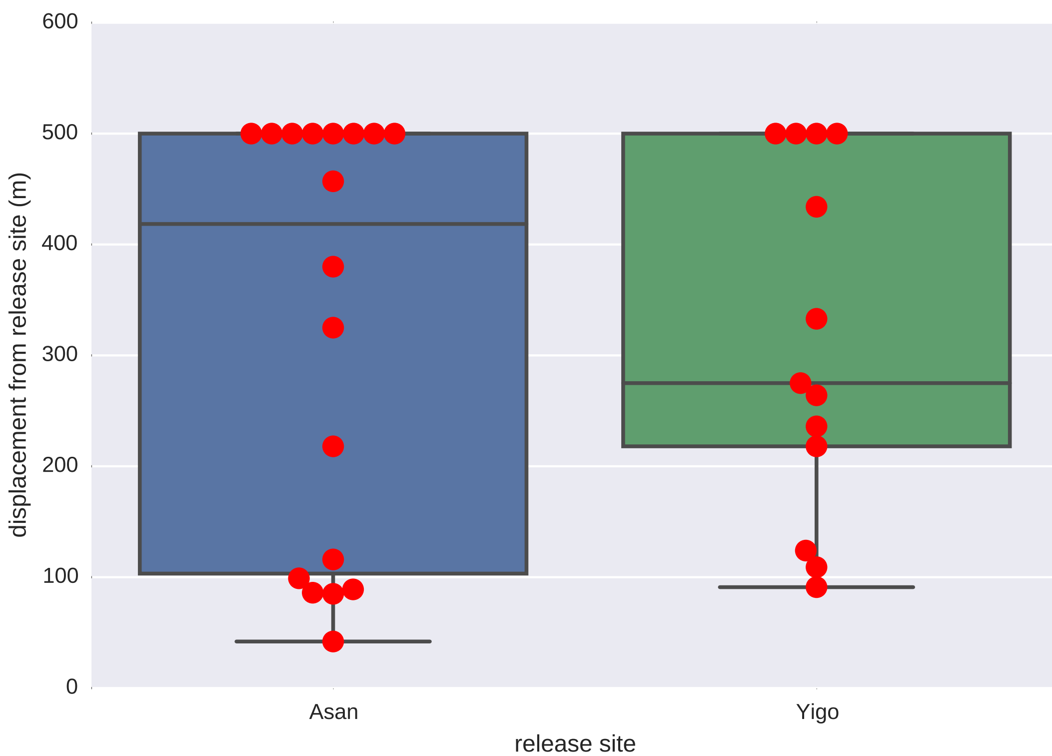
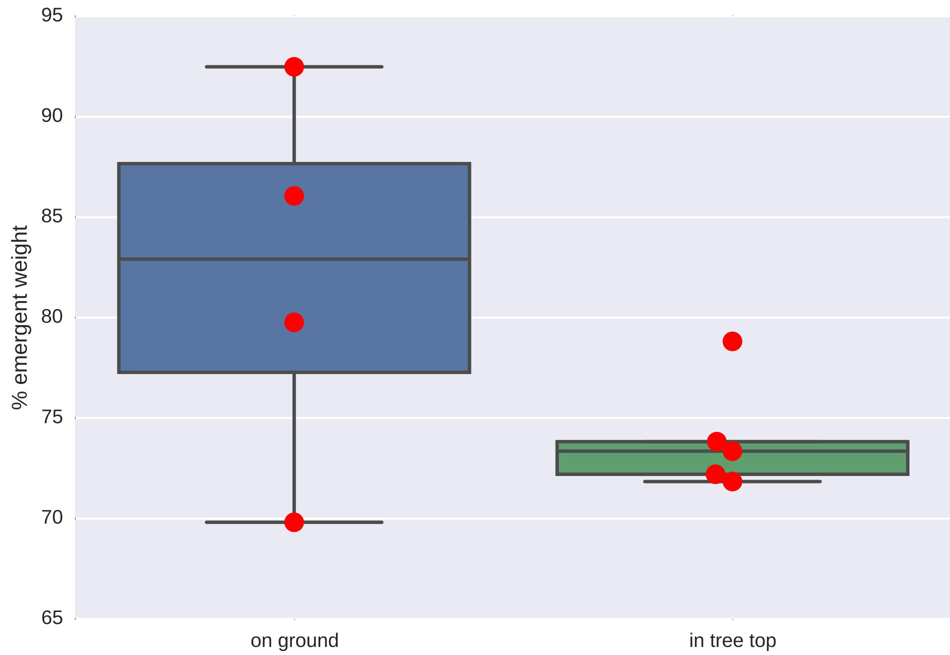
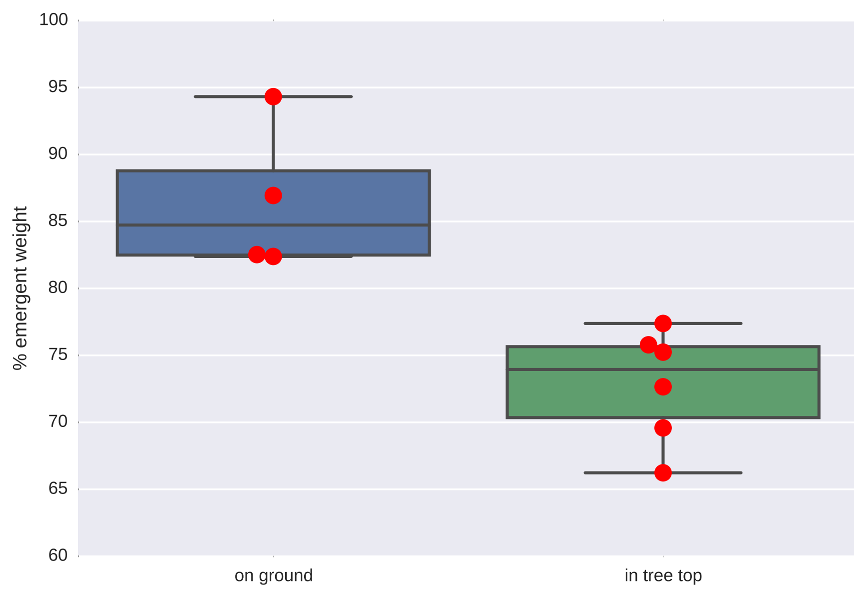


Figure 4: Displacement of beetles from release points within 48 h. The points at 500 m indicate beetles that flew beyond the range of the radio-tracking receivers. Difference between medians for beetles released at the two sites (Asan: 418 m; Yigo: 275 m) are not significantly different ($p = 0.36$; bootstrap resampling with 10,000 iterations). Pooled median displacement was 333 m.



(a) Females. Mean per cent emergent weight for female beetles tracked to ground and tree top sites were not significantly different (82%, 74% respectively; Welch's t = 1.608; p = 0.195).



(b) Males. Mean per cent emergent weight for male beetles tracked to ground and tree top sites were significantly different (87%, 72% respectively; Welch's t = 4.174; p = 0.008).

Figure 5: Percent emergent weight of beetles tracked to ground sites and tree top sites.