Diel flight activity behavior of wild caught *Anopheles farauti* s.s. and *An. hinesorum* malaria mosquitoes from northern Queensland, Australia

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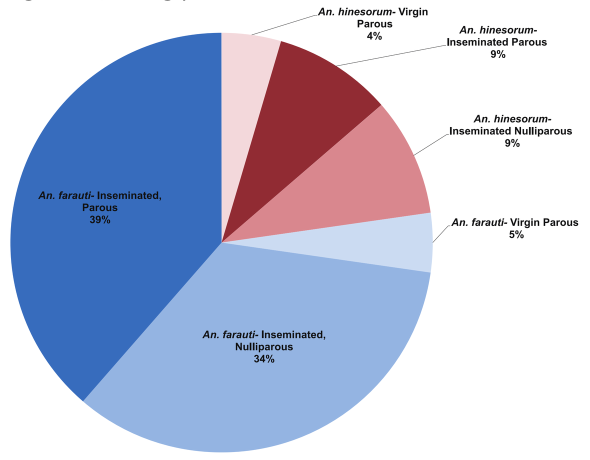
Introduction

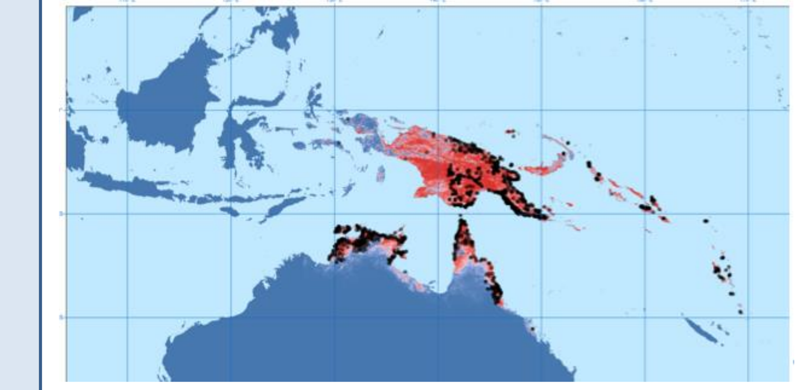
Most of the major malaria vectors in Australia, Papua New Guinea, and the Solomon Islands belong to a group of mosquitoes known as the *Anopheles punctulatus* group (Ambrose et al., 2014; Beebe et al., 2015). Originally discovered in 1901, the group was found to obtain a morphologically distinctive species named *An. farauti* in 1902. Members of the *An. farauti* complex, of which there are several distinct species, are cryptic species, *i.e.* they are morphologically indistinguishable, and can only be identified by genetic analysis (Beebe et al., 2015). *An. farauti sensu stricto* (s.s.) (herein *An. farauti*) and *An. hinesorum* mosquitoes have been identified as as two major malarial vector species in Australia, Papua New Guinea, and the Solomon Islands (Hanna et al., 2004; Cooper et al., 2009; Kabaria et al., 2011). Malaria is endemic to Papua New Guinea, and the Solomon Islands, infecting as many as 6% of the population.

Although the two species are morphologically identical, they exhibit several habitat and behavioral differences. Mosquitoes of the *An. farauti* complex vary rhythmically based on parity (Taylor, 1969, 1977; Afifi et al., 1980; Charlwood et al., 1986). Circadian and diel rhythms are an integral component of many biochemical, physiological, and behavioral processes in mosquitoes (*e.g.* Rund et al 2011, 2012), and temporal gating of mating events might play a role in the reproductive isolation that contributes to speciation. Different members of the *An. farauti* complex show differing nocturnal landing/biting profiles, which are affected by moon light and by insecticide selective pressures (Afifi et al., 1980; Burkot et al., 2013; Charlwood, 1986; Charlwood et al., 1986; Benet et al., 2004). For example, Solomon Island populations exposed to insecticides tend to bite earlier in the night. We hypothesize that the flight activity profiles of these mosquitoes will also parallel their landing catch/biting profiles.

Here we report the results of our investigation comparing the two main species of the *An. farauti* complex that are sympatric, with **the objective of discovering how the flight activity profiles of these two species differ**. Mosquitoes were trapped near to Cairns, QL, Australia in October-December 2014. We used an infrared beam break method to monitor flight activity of individual wild-caught mosquitoes to compare total flight activity, nocturnal onset and maximum peak, and accumulation of activity over the course of the 24 hour day. The long-term objective is to compare behaviors of *An. farauti* complex mosquitoes from populations derived from different locations.



Species- and reproductive state- dynamics of wild-caught sample



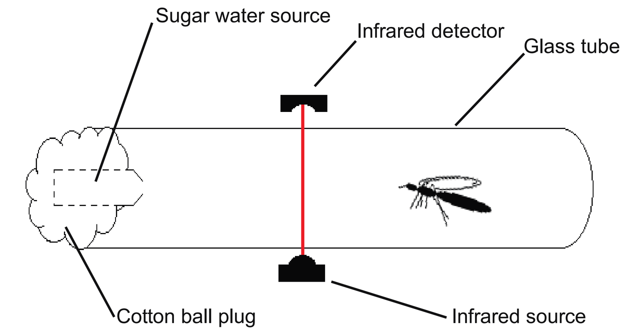
Predicted distribution of the *An. farauti* complex in northern Australia, Papua New Guinea, and the Solomon Islands. Red represents high probability areas and black represents area of recorded occurrence.

CO_2 / Octanol – baited trap

Method

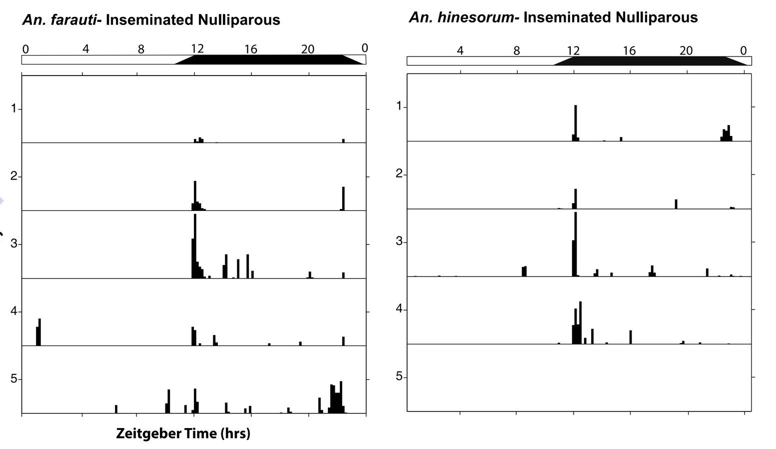
1

- Wild-caught female mosquitoes of *An. farauti* and *An. hinesorum* were placed in individual glass tubes in a Locomotor Activity Monitor (TriKinetics)



2

- Mosquito locomotor flight activity was recorded for 4-5 days under a LD cycle (dusk/dawn set to local time) under laboratory conditions



3

- Locomotor/flight activity was analyzed for a variety of measures

Build up

Nightly Onset

Max Peak in dusk-related activity

Onset to Max Peak in dusk-related activity

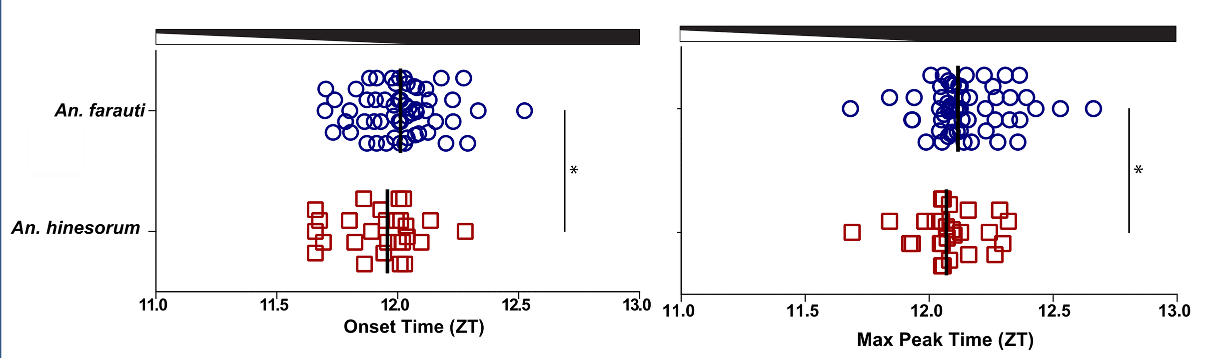
Total flight activity

Assessed *post-hoc* for reproductive state and species

See Rund et al. (2012) for more details on methods.

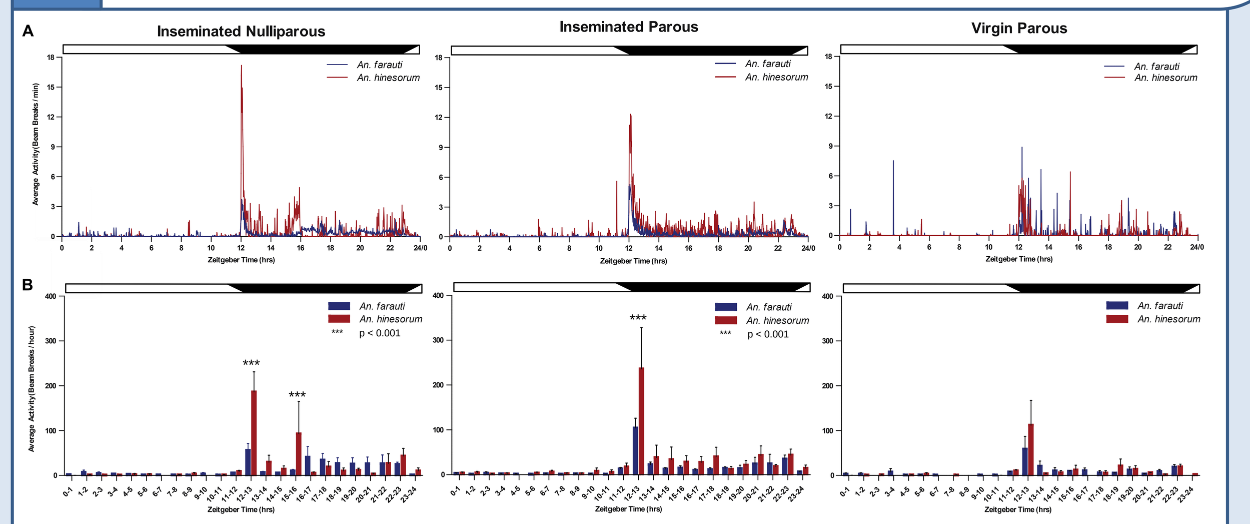
Zeitgeber time (ZT) 12 is time of lights off at the end of the 1.5 hr dusk transition/onset of night.

1 Onset and maximum peak of *An. hinesorum* mosquito flight activity precedes onset of *An. farauti* flight activity



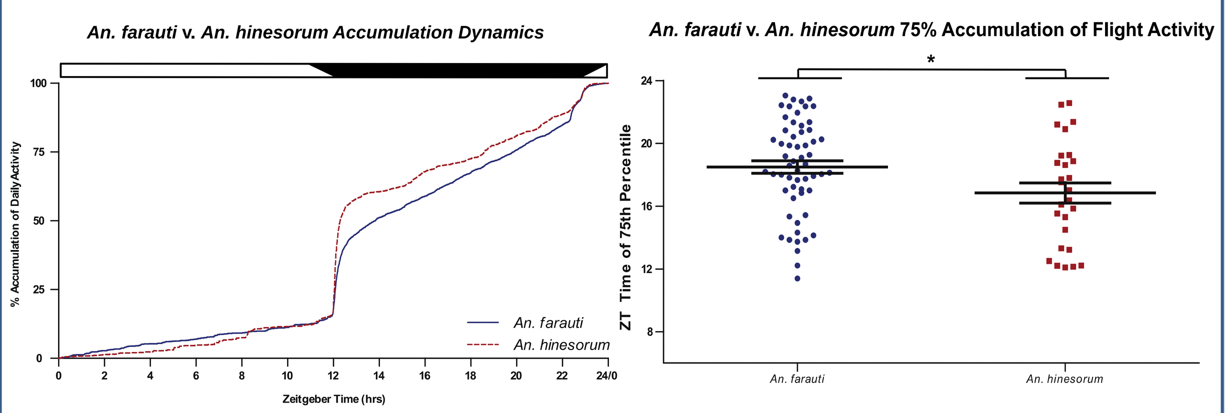
A significant difference was revealed in activity onset times through a Mann-Whitney U-test between *An. farauti* and *An. hinesorum* mosquitoes ($p < 0.05$). *An. farauti* (n = 57) activity commenced at a median time of ZT12.01, while *An. hinesorum* (n = 27) activity onset was 3 minutes earlier at a median time of ZT11.96. The mean onset of the *An. farauti* mosquitoes was ZT12.01, while the *An. hinesorum* mean activity onset time was 5 minutes earlier, commencing at ZT11.93. After the removal of a significant outlier (>2 Standard deviations from the mean), a Mann-Whitney U-test revealed a significant difference between the maximum activity peaks of *An. farauti* and *An. hinesorum* ($p<0.05$). The mean peak in *An. farauti* occurred at ZT 12.15 while the mean peak in *An. hinesorum* occurred 4 minutes earlier at ZT 12.08. The median peak time in *An. farauti* occurred at ZT 12.12, while the median peak time in *An. hinesorum* was 3 minutes earlier at ZT 12.07.

2 Activity profiles for *An. farauti* and *An. hinesorum* vary according to reproductive state



When further analyzing the swarming behaviors in regard to reproductive state, a pronounced species difference in flight activity following dusk/onset of night (ZT 12-13) and in the middle of scotophase/night (ZT 15-16) was noted amongst the reproductive state inseminated nulliparous. A two-way repeated measures ANOVA revealed a significant difference in flight activity for the interaction between time and species amongst the reproductive state inseminated nulliparous (effect of species, $F_{1,455} = 0.83$, $p = 0.38$; effect of time, $F_{23,455} = 6.99$, $p < 0.001$; interaction, $F_{23,455} = 2.99$, $p < 0.001$). A pronounced species difference in flight activity following dusk (ZT12-13) was noted amongst the reproductive state inseminated parous. Two way repeated measures ANOVA revealed a significant difference in flight activity for the interaction between time and species amongst the reproductive state inseminated parous (effect of species, $F_{1,502} = 3.08$, $p = 0.10$; effect of time, $F_{23,502} = 14.60$, $p < 0.001$; interaction, $F_{23,502} = 2.28$, $p < 0.001$). Data shown are mean +/- SEM.

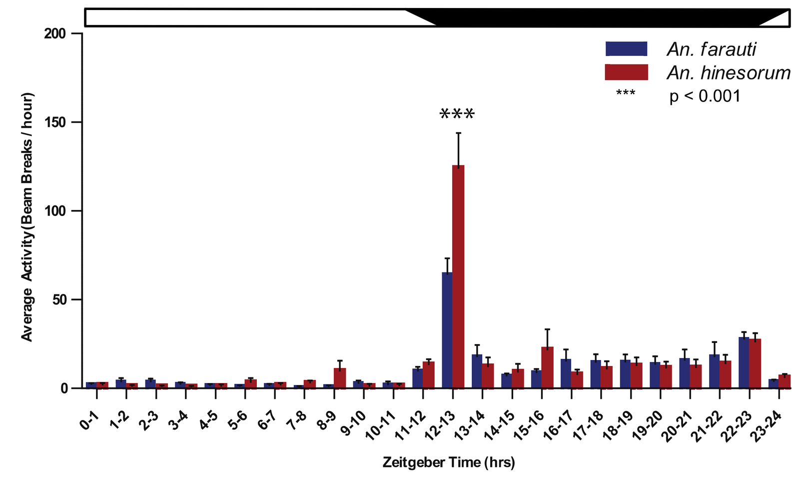
3 Flight activity increases more rapidly in *An. hinesorum* than in *An. farauti*



Left: The 24 hour accumulation dynamics show the differences between species; *An. hinesorum* shows similar accumulation over the light phase of the LD cycle [day] (ZT0 to ZT12), but differentiates during the dark phase of the LD cycle [scotophase, night] (ZT12 to ZT24/0).

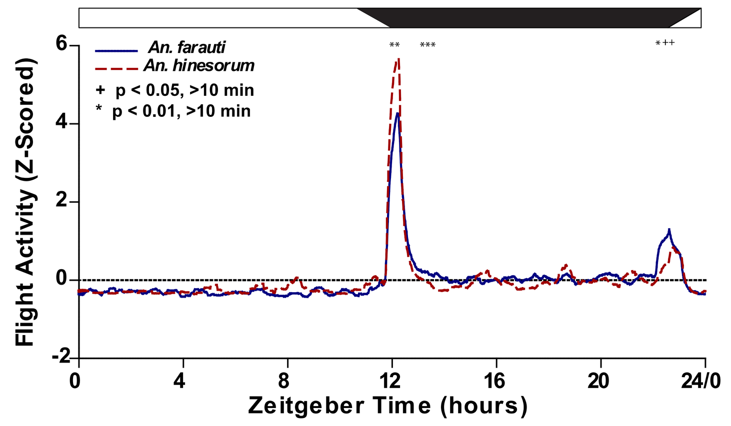
Right: When we look at the time each mosquito reached the same accumulation thresholds, significant differences were observed between species at 75% accumulation of flight activity, but did not show a significant species difference at 50 % accumulation of activity or any reproductive state difference. A Mann-Whitney *U* test revealed that there is a significant species difference in the time at which each animal reached 75% accumulation of flight activity ($U = 555.00$, $p = 0.03$; data shown are mean +/- SEM and individual data points). Although there was only a significant species difference at 75% accumulation of flight activity, *An. hinesorum* also reached 50 % accumulation of flight activity before *An. farauti.*

4 Mean average activity at dusk/onset of night of *An. hinesorum* is greater than that of *An. farauti*



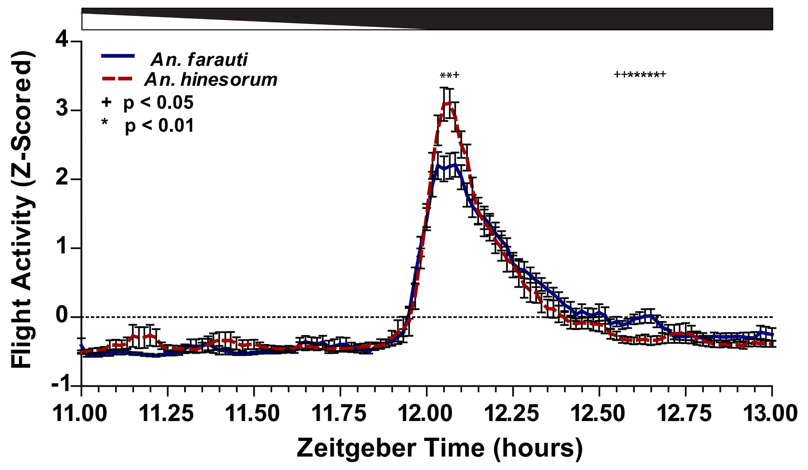
A pronounced species difference in flight activity was noted at the end of the dusk transition and onset of night (ZT12 to ZT13). In *An. gambiae* it is thought that this activity bout is associated with natural swarming behavior and/or sugar feeding associated activity (Rund et al, 2012; Gary & Foster, 2006). Total activity data were arranged into 1 hour bins for subsequent time-of-day specific analysis. A two-way repeated measures ANOVA revealed a statistically significant difference in flight activity for the interaction between time and the two species (effect of species, $F_{1,\ 2039} = 0.87$, $p = 0.35$; effect of time, $F_{23,\ 2039} = 34.16$, $p < 0.001$; interaction, $F_{23,\ 2039} = 4.09$, $p < 0.001$). When further analyzing these data in regard to reproductive state, a pronounced species difference in flight activity following sunset (ZT12 to ZT13) and in the middle of the night (ZT15 to ZT16) was noted amongst the inseminated nulliparous group (*see* Figure 2). Data shown are mean +/- SEM.

5 Z-Scored flight activity reveals species-specific differences during the early night and the onset of dawn



We next sought to determine if the dusk-related flight activity (*i.e.* possible mating swarms activity/sugar feeding associated behavior) differed between *An. farauti* and *An. hinesorum* in their temporal dynamics by analyzing build-up and decline of daily activity. The activity of each individual animal was smoothed to 30 minutes, Z-Scored and normalized for the hour analyzed. There is a an increase in activity at lights off between ZT 11:55 to ZT 12:15 in *An. hinesorum* (n=27) relative to *An. farauti* (n=58) (t-test, p < 0.01). Additionally, there was a significant decrease in activity after the peak of activity between ZT 13:39 and ZT 14:09 (t-test, p < 0.01). Furthermore, *An. farauti* shows a relative increase in activity during late night immediately preceding or at the onset of the dawn transition between ZT 22:09 and ZT 22:39 (t-test, ZT 22:09-ZT 22:18, p < 0.01; ZT 22:19-ZT 22:39, p < 0.05). Data shown are mean values.

6 Z-Scored activity shows multiple time differences at time of dusk and the onset of night, ZT 11 – ZT 13



Subsequent to the 24 hour analysis, we performed a targeted analysis of dusk associated activity as this is the expected time of swarming behavior. The activity of each individual animal between ZT 11 – ZT 13 was smoothed to 5 minutes and Z-Scored. *An. hinesorum* shows a relative increase of activity compared to *An. farauti* from ZT 12:03-ZT 12:05 (t-test, ZT 12:03-ZT 12:04, p < 0.01; ZT 12:05, p < 0.05). After the peak of activity, *An. hinesorum* shows a decrease in activity relative to *An. farauti* from ZT 12:33 to ZT 12:39 (t-test, ZT 12:35-ZT 12:38, p < 0.01; ZT 12:33-ZT12:34, ZT 12:39, p < 0.05). Analysis of mosquitoes divided by reproductive group and by both species and reproductive group yielded no significant results. Data shown are mean +/- SEM.

Conclusions & Discussion

- **These behavioral data are important for ongoing efforts to understand *An. farauti* complex mosquitoes in Australia**. In 2002 there were incidents of *Plasmodium vivax* transmission in the Cairns area of Northern Queensland, Australia. The *An. farauti* complex poses a plausible threat to a region where malaria has been eradicated (Hanna et al., 2004; Beebe et al., 2015).
- **These data also contribute to our understanding of *An. farauti* complex mosquitoes in malaria endemic areas** (Solomon Islands and Papua New Guinea; as much as 6% of population is infected).
- As the Queensland populations of *An. farauti* and *An. hinesorum* are not routinely exposed to insecticides **these data can serve as a baseline behavioral profile for comparison with mosquitoes from the Solomon Islands**, where the timing of nocturnal biting activity has been shaped by insecticide-driven selective pressure.
- ***An. hinesorum* shows an earlier onset and early maximum peak in nocturnal flight activity**. Differences observed in these components of the dusk/early night-related peak in activity might contribute to the reproductive isolation of the two species. This temporal gating of flight activity has been suggested in *An. gambaie* s.s. *and An.* coluzzii mosquitoes, which exhibit mating swarms at dusk (Rund et al., 2012; Sawadogo et al., 2013). However, it is still unclear whether such mating swarms occur in nature in *An. farauti* complex mosquitoes.
- ***An. hinesorum* has a higher sustained activity than *An. farauti* at the onset of darkness** and some species-specific differences were also observed later in the night, although these differences appear to be reproductive state/age specific.
- **A species difference in flight activity among inseminated nulliparous females at ZT 15-16** is consistent with a previous study of landing catches/host-seeking behavior in Papua New Guinea (Benet et al., 2004; Burkot et al., 2013).
- **The highest levels of activity sustained throughout the night in *An. farauti* were observed amongst inseminated nulliparous females**. Note that inseminated nulliparous mosquitoes should in theory have the highest drive to blood feed.

Acknowledgements

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