Circadian Rhythms in Mosquito Activity

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Circadian rhythms

Daily patterns of activity
- Flight
- Nectaring (males and females)
- Blood seeking (females)
- Reproduction
- Oviposition

Differ by mosquito species
- Crepuscular or nocturnal
- Diurnal
Circannual rhythms

Seasonal patterns of activity
  Peak abundance periods (spring, summer or fall)
  Diapause (as egg or adult)
  Reproduction
Differ by mosquito species
Hypotheses

1) Females should have greatest response to host odors during their species’ peak seasonal activity.

2) Males should not respond to host odors.
Hypothesis #1 Predictions

1) Species like *Culex restuans* with peak abundance in spring should be most sensitive to host odors when kept in 12:12 LD cycle.

2) Species like *Aedes albopictus* with peak abundance in summer should be most sensitive when kept in 16:8 LD cycle.
Hypothesis #2 Predictions

1) Males of *Culex restuans* or *Aedes albopictus* should not respond to host odors.

2) Light regime in incubator should have no influence on response.
Methods

- Mosquito cultures in incubators
- Assay male and female response in olfactometer to host volatiles
- Compare response of *Culex restuans* and *Aedes albopictus* from different light regimes to volatiles
Mosquito Cultures

• Mosquitoes reared in incubators kept in LD cycles of either

  – DD (continuous dark)
  – LL (continuous light)
  – 12:12 (12 hours light, 12 hours dark = spring/fall photoperiod)
  – 16:8 (16 hours light, 8 hours dark = summer photoperiod)
Mosquito incubators

- Continuous cultures maintained in laboratory in incubators in four light cycles (DD, LL, 12:12 LD, 16:8 LD) at 25°C
- Deionized water for egg deposition
- Larvae fed flake fish food
- Adults free to emerge and mate in cage
Table incubators
Mosquito cage in incubator
Mosquito species tested
*Culex restuans*

- Peak abundance spring
  (and sometimes fall)
- Permanent water breeder
- Crespuscular/nocturnal activity cycle
- Native species
Mosquito species tested
*Aedes albopictus*

- Peak abundance: summer
- Tree hole breeder
- Diurnal activity cycle
- Invasive species
Olfactometer

- Dual-port y tube design
- Custom made to Bernadette Ferraro’s specifications
- Created from recycled glass pipe from chemical labs
- Mosquitoes fly easily in tubes
Bernadette Ferraro with olfactometer
Treatment or control chamber
Environmental Measures

- Room temperature
- Humidity
- Light levels (lux)
Olfactometer assays

- “Air” trials to control for north/south port preference
- Host odors (carbon dioxide, lactic acid)
Control air trials

• Created air flow 90ml/sec from a single source across both ports as a control for port preference

• Tested *Culex restuans* from all four light regimes (DD, 16L/8D, 12L/12D, LL)

• No preference for a specific port by either males or females
<table>
<thead>
<tr>
<th>Light regime</th>
<th>North port</th>
<th>South port</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>LL</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12:12 LD</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>16:8 LD</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
### Air trials

**Culex restuans** males

<table>
<thead>
<tr>
<th>Light regime</th>
<th>North port</th>
<th>South port</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>LL</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>12:12 LD</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>16:8 LD</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Volatile chemical assay

- Air flow 90ml/sec directs air to both ports
- Chemical in treatment port (north or south)
- Air flows through treatment and control ports
- Air streams meet where arms join tube
- Insect flies up tube to y and chooses treatment arm or control arm
Assays

- Trials on separate days for each light regime (DD, LL, 12/12 LD, 16:8 LD)
- Each trial remove up to 20 male and female mosquitoes from incubator and place in releasing chamber
- Choose females that approach hand
- Record behavior for several hours and note final locations at end of day
Monitoring mosquito activity
Results for Carbon Dioxide

Contrary to Hypothesis #1, female *Culex restuans*’ response was not highest for 12:12 LD reared mosquites.

In support of Hypothesis #2, male *Culex restuans* did not respond to carbon dioxide
Female mosquitoes in treatment (carbon dioxide) or control chambers

Mean ± SE number of mosquitoes

Light regime

DD  16L8D  12L12D  LL
Male mosquitoes in Treatment (carbon dioxide) or Control Chambers

Mean ± SE number of mosquitoes

Light regime

DD  16L8D  12L12D  LL
Results for Lactic Acid Assays

• DD – Males in equal numbers in treatment and control chambers; Females only in treatment chambers
• LL - Males only in control chambers; Females only in treatment chambers.
• 12:12 LD  Males more common in control chambers; Females only in treatment chambers.
• 16:8 LD – Males more common in control chambers; Females more common in treatment chambers.
Culex restuans from DD incubators in Treatment and Control Chambers

Number of Mosquitoes

male    female

Treatment  Control
Culex restuans from LL incubators in Lactic Acid Treatment and Control Chambers
12:12 LD

Culex restuans from 12:12 LD Incubators in Lactic Acid Treatment and Control Chambers

Mean ± SE number of mosquitoes

- Treatment
- Control

male | female
16:8 LD

Culex restuans from 16:8 LD incubators in Lactic Acid Treatment and Control Chambers

Number of mosquitoes

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>female</td>
<td>3.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Conclusions - Females

• Female *Culex restuans* tended to respond more strongly to carbon dioxide and lactic acid than to control air.

• Female *Culex restuans* from 12:12 LD incubators did not have greater response than from other light regimes.
Conclusions - Males

• Male *Culex restuans* did not respond to host odors of carbon dioxide or lactic acid.

• Males did not differ according to light regime.
Aedes albopictus assays – To be continued when the lab warms up!
Future directions

• Repeat experiments with Aedes albopictus

• Assay more plant volatiles and chemical blends
Olfactometer