Prolonged dehydration resistance by the northern house mosquito, *Culex pipiens*, during its overwintering diapause

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Abstract - Throughout the winter, the northern house mosquito, *Culex pipiens*, is continually exposed to desiccating conditions, and this water deficit is further exacerbated by long periods with no access to free water. In this study, we report that mosquitoes in diapause are more tolerant of xeric conditions than their nondiapause counterparts. To counteract water loss female mosquitoes reared under conditions that induce diapause have a lower percent water content that results from a higher dry mass which in turn decreases their surface area to volume ratio. Both diapausing and nondiapausing females can tolerate a loss of approximately 30%, but diapausing reserves never do reach this limit as readily. This mosquito relies solely on drinking free water to replenish its water supply and has no ability to absorb water vapor from the atmosphere. Cuticular hydrocarbon content was nearly 3x higher in diapassing female mosquitoes than nondiapassing individuals, this enhancement impedes water loss through the cuticle. No differences were noted in sorbitol, trehalose, glycerol or the total sugar contents during diapause. Additionally, the utilization on internal lipids by diapassing *C. pipiens* was significantly lower than nondiapassing female. Thus, the increased dehydration resistance of diapassing females *C. pipiens* results from the combination of their larger size, accumulation of additional cuticular lipids, and a suppression of metabolism.

Introduction

- To survive winter the northern house mosquito, *Culex pipiens*, will enter diapause, caused by short day length, which is characterized by a molecular switch from blood feeding to sugar uptake, an increase in cold tolerance, arborization of overwintering and the accumulation of fat reserves (Rohlich and Denlinger, 2005).

- During the winter, mosquitoes are extremely susceptible to dehydration due to the dry conditions and a lack of free water. In this study, we determine the water balance characteristics for both diapausing and nondiapausing individuals and mechanisms utilized by this mosquito to suppress water loss while overwintering.

- Mosquitoes were reared under three regimens: ND25 were nondiapassing individuals reared at 18ºC, LD at 25ºC, ND18 were those reared at 18ºC, LD at 18ºC, and D18 were diapassing mosquitoes developed at 18ºC, 15ºC. Field-collected individuals were from culverts in Columbus, Ohio.

- Water balance characteristics were determined according to Benoit et al. (2003). Changes in the water content were monitored at different times after adult emergence and compared between diapausing and nondiapausing cohorts.

- Cuticular, polyalcohol and lipid contents were determined according to standard photochemical assay established by Van Hanel (1995).

Results

- Water requirements of the mosquitoes are presented in Table 1, and the highlights are an increase in dry mass for diapausing females which results in a lower percent water content. Additionally, the water loss rate was nearly 30% lower for diapausing females. This reduction continues as long as the mosquitoes are held at diapassing (D18) conditions (Fig. 1). Similar differences occurred for field-collected overwintering mosquitoes from Columbus, Ohio (Fig. 2).

- Cuticular hydrocarbons increased during diapause (Fig. 3) and lipid metabolism was reduced (Fig. 4), indicating cuticular lipids are mobilized to meet metabolic requirements. These results agree with previous observation that males do not enter and that diapause begins after adult emergence.

- Glycerol, trehalose and sorbitol are not responsible for the suppression of water loss due to a lack of correlation with the reduction in water loss rates that occurs during diapause.

- Field-collected mosquitoes have nearly identical changes in their water requirements when entering the winter as those of the lab reared individuals, indicating results correlate with those in the field.

- Overall, it appears that the increase in size (reduced surface area to volume ratio which reduce water loss), an accumulation of additional cuticular lipids, and a suppression of metabolism are responsible for the suppressed water loss rate of diapausing females *C. pipiens*.

Table 1 - Water balance requirements for male, female and pupal *Culex pipiens*. CEA, critical equilibrium humidity; CT, critical transit time; TE, terminal equivalent humidity; EL, equilibrium loss. Each value represents the mean of 30 mosquitoes.

<table>
<thead>
<tr>
<th>Value for variable</th>
<th>Value for variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mass (mg)</td>
<td>3.45</td>
</tr>
<tr>
<td>Water gain (mg)</td>
<td>2.53 ± 0.15</td>
</tr>
<tr>
<td>Water loss rate (%/h)</td>
<td>2.0 ± 0.17</td>
</tr>
<tr>
<td>Time (d) after onset of starvation</td>
<td>102 d</td>
</tr>
</tbody>
</table>

Table 2 - Amounts of polyalcohols and cuticular lipids in field-collected populations from Columbus, OH. Values represent 10 determinations.

<table>
<thead>
<tr>
<th>Value for variable</th>
<th>Value for variables</th>
</tr>
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<tbody>
<tr>
<td>Sugar (mg/mosquito)</td>
<td>124.1 ± 24.4</td>
</tr>
<tr>
<td>Trehalose (mg/mosquito)</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>Glycerol (mg/mosquito)</td>
<td>0.1 ± 0.02</td>
</tr>
</tbody>
</table>

Figure 1 - Water loss rates in females of *Culex pipiens* throughout diapause. D18 to ND25 at 40d indicates the mosquitoes were taken from ND18 and ND25 conditions after 40d to break diapause.

Figure 2 - Water balance characteristics for mosquitoes collected at monthly intervals from the field in Columbus, Ohio. WLR, water loss rate (%/d), d, dry mass (mg); m, initial mass (mg). Each point represents the mean of 30 mosquitoes.

Figure 3 - Reduction of internal lipid reserves for nondiapausing and diapausing *Culex pipiens*. Each point represents the mean of ten replicates.

Figure 4 - Amount of cuticular hydrocarbons extracted from nondiapausing and diapausing females of *Culex pipiens*. Each point represents the mean of ten replicates.

Conclusions

- Diapassing female mosquitoes are significantly more resistant to dehydration than their nondiapassing counterparts.

- Males and pupae held under diapassing condition had the same water balance profile as nondiapausing individuals, which agrees with previous observation that males do not enter and that diapause begins after adult emergence.

- Glycerol, trehalose and sorbitol are not responsible for the suppression of water loss due to a lack of correlation with the reduction in water loss rates that occurs during diapause.

- Field-collected mosquitoes have nearly identical changes in their water requirements when entering the winter as those of the lab reared individuals, indicating results correlate with those in the field.

- Overall, it appears that the increase in size (reduced surface area to volume ratio which reduce water loss), an accumulation of cuticular hydrocarbons and a reduction in metabolism are responsible for the suppressed water loss rate of diapausing female *C. pipiens*.

References


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Figure 3

Figure 4

D18
ND18
ND25
and ND25

D18
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ND25

and ND25

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- Cuticular hydrocarbons increased during diapause (Fig. 3) and lipid metabolism was reduced (Fig. 4), but sugar or polyol content did not change (data not shown). Similar differences were noted for field-collected mosquitoes (Table 2).

Conclusions
- Diapausing female mosquitoes are significantly more resistant to dehydration than their nondiapausing counterparts.
- Males and pupae held under diapausing conditions had the same water balance profile as nondiapausing individuals, which agrees with previous observation that males do not enter and that diapause begins after adult emergence.
- Glycerol, trehalose and sorbitol are not responsible for the suppression of water loss due to a lack of correlation with the reduction in water loss rates that occurs during mosquito.
- Field-collected mosquitoes have nearly identical changes in their water requirements when entering the winter as those of lab-reared individuals that are in diapause, indicating lab results correlate with those in the field.
- Overall, it appears that the increase in size (reduced surface area to volume ratio which reduce water loss), an accumulation of cuticular hydrocarbons and a reduction in metabolism are responsible for the suppressed water loss rates of diapausing female Culex pipiens.

References

| Table 1 - Water balance requirements for male, female and pupae of Culex pipiens. CEA, critical equilibrium humidity; CTT, critical transition temperature. Each value represents the mean of 60 individuals. |
| Table 2 - Amount of polyols, sugars and cuticular lipids in field-collected populations from Columbus, OH. Values represent 10 determinations. |
References

