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

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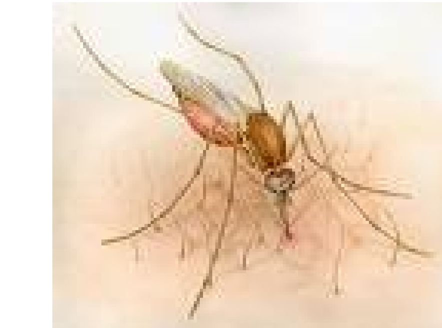

Abstract - Throughout the winter, the northern house mosquito, *Culex pipiens*, is continually exposed to desiccating conditions, and this water deficit is further exacerbated by long periods with no access to free water. In this study, we report that mosquitoes in diapause are more tolerant of xeric conditions than their nondiapausing counterparts. To counter 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 females conserve their water reserves more do not 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 diapausing female mosquitoes than nondiapausing 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 diapausing *C. pipiens* was significantly lower than nondiapausing female. Thus, the increased dehydration resistance of diapausing 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, cued by short day length, which is characterized by a molecular switch from blood feeding to sugar uptake, an increase in cold tolerance, arrestment of ovarian development and the accumulation of fat reserves (Robich 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.

Methods

- Mosquitoes were reared under three regiments: ND25 were nondiapausing individuals reared at 15H:9H, L:D at 25ºC, ND18 were those at 15H:9H, L:D at 18ºC, and D18 were diapausing mosquitoes developed at 9H:15H, L:D and 18ºC. Field-collected individuals were from culverts in Columbus, OH.

- Water balance characteristics were determined according to Benoit et al. (2005). Changes in the water content were monitored at different times after adult emergence and compared between diapausing and nondiapausing cohorts.

- Sugar, polyol and lipid contents were determined according to standard photometric assay established by Van Handel (1985).

	Pupae			Males			Females		
Characteristic	ND25	ND18	D18	ND25	ND18	D18	ND25	ND18	D18
Water content									
Initial mass (mg)	3.45 ± 0.09	3.47 ± 0.08	3.59 ± 0.11	2.01 ± 0.10	2.10 ± 0.20	2.15 ± 0.15	3.60 ± 0.10	3.66 ± 0.12	5.16 ± 0.21[a]
Dry mass (mg)	0.76 ± 0.08	0.72 ± 0.09	0.82 ± 0.09	0.58 ± 0.09	0.63 ± 0.09	0.63 ± 0.16	1.20 ± 0.11	1.26 ± 0.15	2.42 ± 0.19[a]
Water mass (mg)	2.69 ± 0.07	2.75 ± 0.07	2.77 ± 0.08	1.43 ± 0.11	1.47 ± 0.11	1.52 ± 0.19	2.40 ± 0.13	2.40 ± 0.16	2.74 ± 0.18
Water content (%)	77.9 ± 1.4	79.2 ± 1.6	77.1 ± 1.1	71.2 ± 1.8	70.8 ± 1.3	70.8 ± 1.2	66.7 ± 1.3	65.5 ± 1.4	53.1 ± 1.2[a]
Water loss									
Rate (%/h)	7.17 ± 0.16	7.26 ± 0.11	7.08 ± 0.13	5.08 ± 0.09	4.80 ± 0.10	4.71 ± 0.15	3.57 ± 0.14	3.37 ± 0.15	2.31 ± 0.16[a]
Loss tolerance (%)	29.2 ± 0.9	28.9 ± 1.1	30.1 ± 1.4	33.1 ± 1.0	33.3 ± 1.3	33.9 ± 1.1	35.4 ± 0.9	36.3 ± 1.2	35.5 ± 1.7
CTT (ºC)	36.2 ± 2.1	35.3 ± 1.7	36.3 ± 1.3	39.4 ± 2.1	38.3 ± 2.8	38.3 ± 1.9	41.2 ± 1.8	40.3 ± 1.9	41.4 ± 1.8
Water gain									
Free water uptake	NA	NA	NA	+	+	+	+	+	+
CEA (a_v)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

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.

	Date of *Culex pipiens* collection		
Concentration	Nov. 2005	Feb. 2006	July 2006
Polyols (ng/g of mosquito)			
Glycerol	52.1 ± 6.3	49.3 ± 9.2	56.5 ± 6.9
Sorbitol	31.5 ± 5.2	28.9 ± 0.6	36.6 ± 5.2
Sugar (mg/g of mosquito)			
Trehalose	1.21 ± 0.15	0.96 ± 0.10	1.22 ± 0.16
Total sugar	5.34 ± 0.29	4.72 ± 0.31	4.83 ± 0.41
Cuticular lipids (ng/mosquito)			
Hydrocarbons	440 ± 65	412 ± 62	220 ± 42[a]
Polar components	1310 ± 120	1160 ± 215	1310 ± 192

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 diapausing (D18) conditions (Fig .1). Similar differences occurred for field collected overwintering mosquitoes from Columbus, OH (Fig. 2)

- 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).

Figure 1

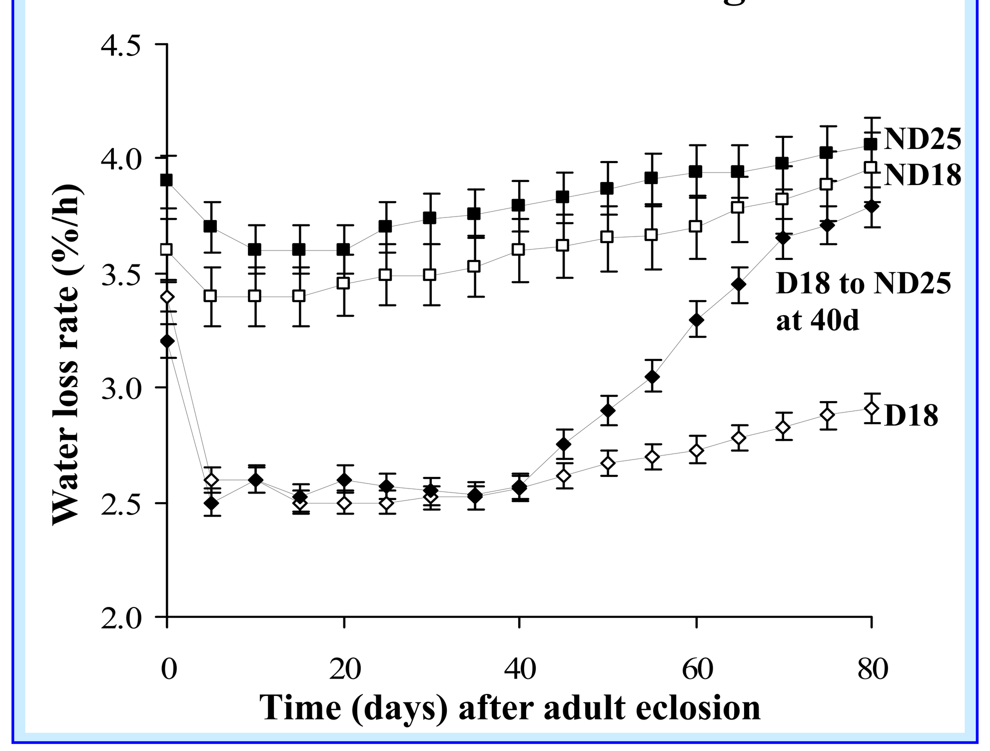

Figure 1 - Water loss rates in females of *Culex pipiens* throughout diapause. D18 to ND25 at 40d indicates the mosquitoes were moved from diapausing (D18) to nondiapausing (ND25) conditions after 40d to break diapause.

Figure 2

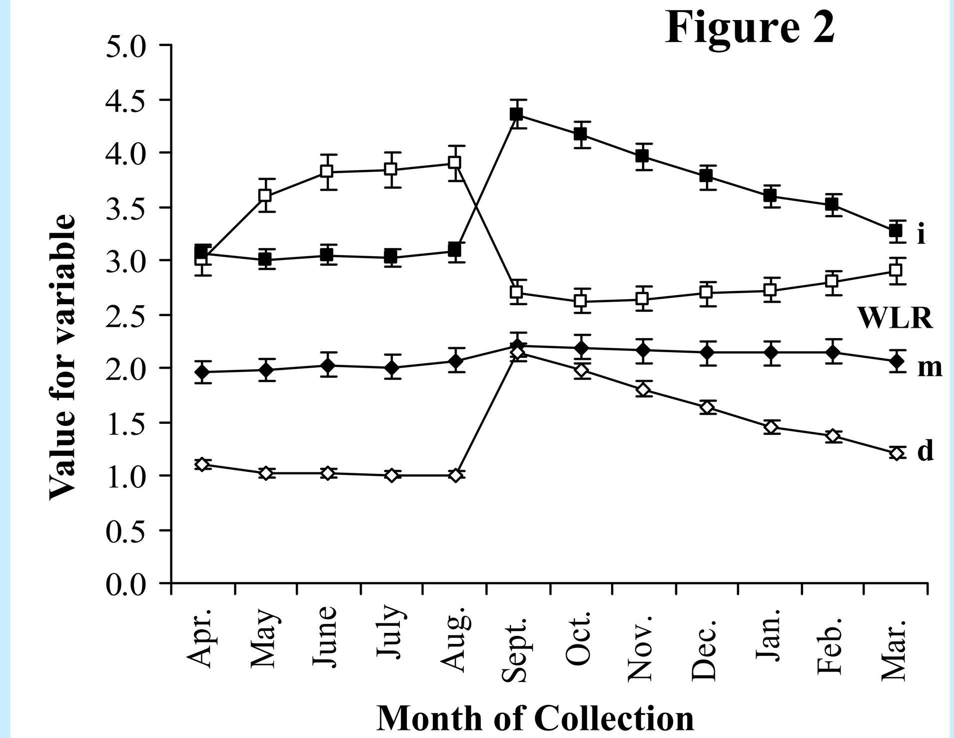

Figure 2 - Water balance characteristics for mosquitoes collected at monthly intervals from the field in Columbus, Ohio. WLR, water loss rate (%/h); d, dry mass (mg); m, water mass (mg); i, initial mass (mg). Each point represents the mean of 30 mosquitoes.

Figure 3

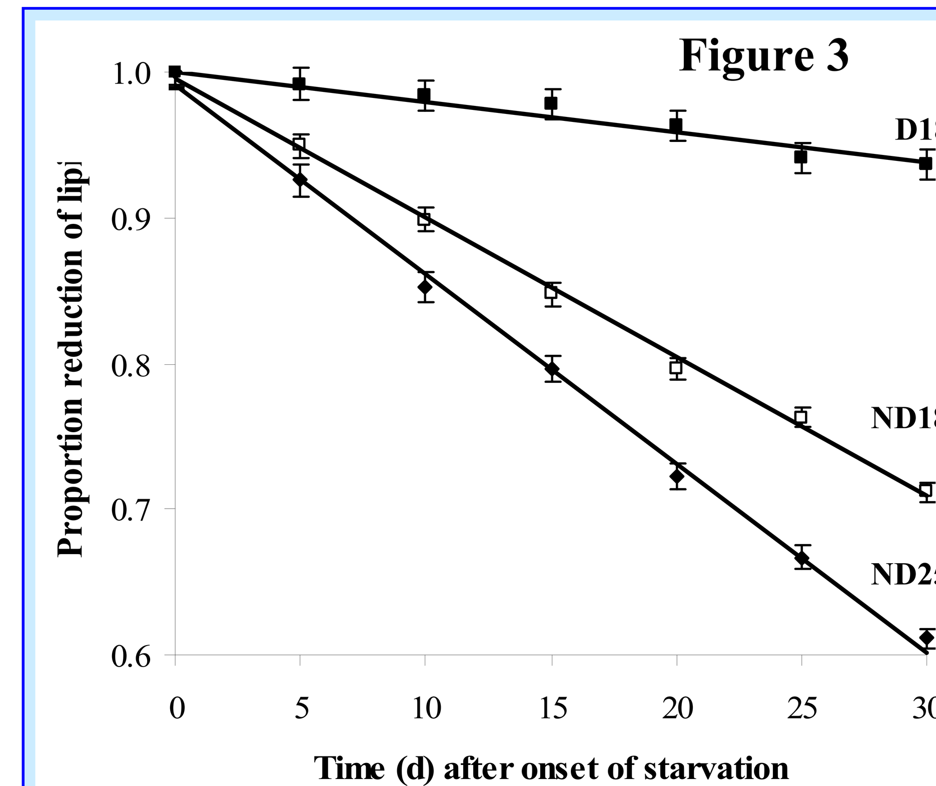

Figure 3 - Reduction of internal lipid reserves for nondiapausing and diapausing *Culex pipiens*. Each point represents the mean for ten measurements.

Figure 4

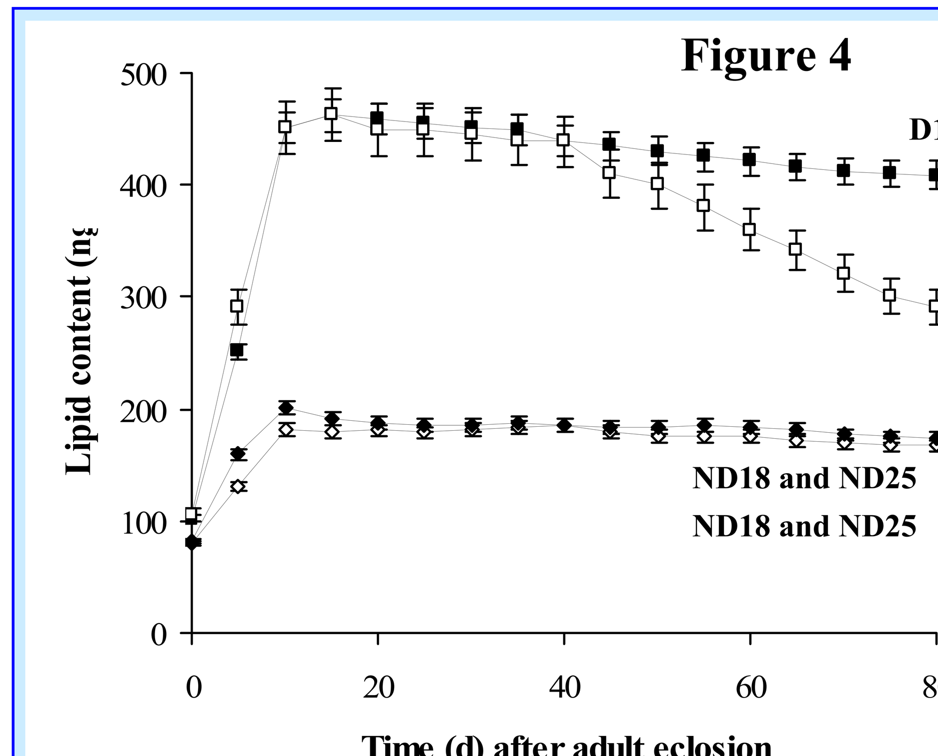

Figure 4 - Amount of cuticular hydrocarbons extracted from nondiapausing and diapausing females of *Culex pipiens*. Each point represents the mean of ten replicates.

Conclusions

- Diapausing female mosquitoes are significantly more resistant to dehydration than their nondiapausing counterparts.

- Males and pupae held under diapausing condition had the same water balance profile as nondiapausing individuals, which agrees with previous observation that males do not enter and that diapause begin 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 rate of diapausing female *Culex pipiens*.

References

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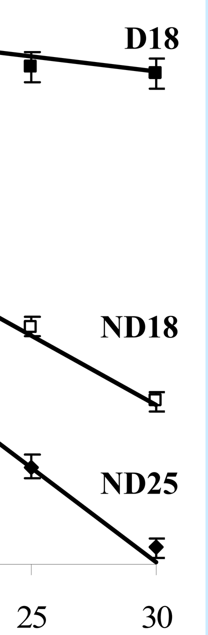

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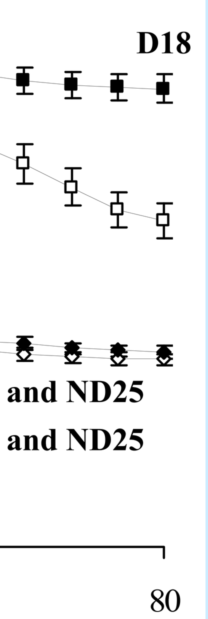

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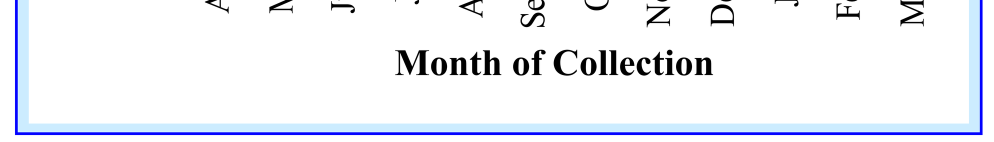

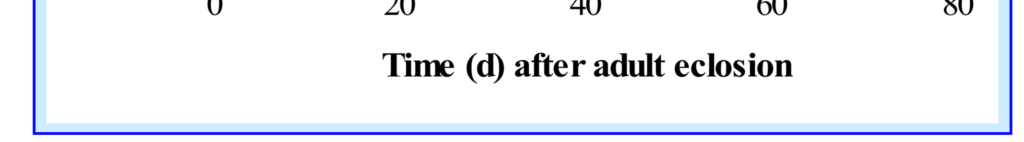

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