BODY SIZE OF *CULICOIDES VARIIPENNIS* (DIPTERA: CERATOPOGONIDAE) IN RELATION TO BLOODMEAL SIZE ESTIMATES AND THE INGESTION OF *ONCHOCERCA CERVICALIS* (NEMATODA: FILARIOIDEA) MICROFILIARIAE[1]

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Onchocerca cervicalis Railliet and Henry is a filariid parasite of horses. This parasite is found worldwide and has been reported to be highly prevalent in the United States (Klei et al. 1984). *Culicoides variipennis* Coquillett has been incriminated as a vector of *O. cervicalis* in North America (Collins and Jones 1978, Foil et al. 1984). Studies of *O. cervicalis* vector-parasite interactions may serve as a model for *O. volvulus* (Collins and Jones 1978, Foil et al. 1987).

Previous reports indicated that the ingestion of *O. cervicalis* microfilariae was unrelated to the bloodmeal weight of *C. variipennis* (Higgins et al. 1988). In that study, there was no account of size variation of the vectors; intraspecific variation of body size is known to occur seasonally in *C. variipennis* (Mullens 1987). The objectives of this experiment were to determine if engorged weight of *C. variipennis* varied with body size and if the ingestion of *O. cervicalis* microfilariae was related to the engorged weight of *C. variipennis* when controlling for body size variation of the vector.

The flies used in this study were obtained from an LSU colony of *Culicoides variipennis sonorensis* Wirth and Jones [strain 000/AA (Jones 1957)] and maintained according to procedures described in Higgins et al. (1988). Eggs used to initiate the LSU colony were obtained from the colony at the Arthropod Borne Animal Diseases Research Laboratory, USDA-ARS, Laramie, Wyoming, and a subculture of that colony at the Delta Primate Center in Covington, Louisiana. The colony was maintained at 23 ± 2°C with 14L:10D photoperiod. Upon emergence, adult flies were removed by aspiration and placed into cardboard containers covered with organza mesh cloth tops. Moistened cellucotton was placed on top of each container, and a sugar cube was attached to a no. 3 rubber stopper and inserted into each container.

Flies were fed on the ventral midline of a Shetland pony on 6 days (March 4–7, March 11 and June 15, 1987) at the St. Gabriel Louisiana State University Agricultural Experiment Station, St. Gabriel, LA. The mass feeding technique of Higgins et al. (1988) was used. Briefly, the pony was restrained in an elevated stanchion. The top of the feeding cage was placed underneath the ventral aspect of the pony, and the straps were tied along the dorsum. Prior to the introduction of flies into the feeding chamber, subsamples were made and held for 5 min at −20°C to allow evaluation of the weight and size of unfed females. The remaining flies were introduced into the feeding chamber and observed for 40 min. Flies were collected upon engorgement, placed immediately at −20°C for 5 min and then weighed on a Mettler H-20 balance to the nearest 0.01 mg. The flies were subsequently transferred to a glass slide, placed in a drop of saline solution and dissected. The head, the thorax and the abdomen were separated and teased apart. Wings were removed from the thorax and moved to the side of the preparation. The slide was then examined at 100× magnification for microfilariae that were usually alive and readily detectable.

Wings were placed between two slides and measured to the nearest 0.007 mm, from the arculus to the tip of the wing. Scales were not included. Readings were made from dried wings 3–5 days after mounting. Preliminary comparisons of the length of the left and right wings of 32 *C. variipennis* females in paired Student's *t* test revealed no significant differences ($P >$

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0.05). Subsequently, when the left wing was damaged, the right wing was measured.

An enzyme-linked immunosorbent assay (ELISA), which allows the detection in the range of 1 to 10 nl of blood (Foil et al. 1987), was used in a separate experiment. Engorged and unfed females were deposited individually into 2-ml plastic screwcap vials containing 1 ml of buffered saline solution and ground with a glass rod. The vials were immediately placed in an alcohol-dry ice bath for quick freezing and subsequently stored at −70°C until further processing. Final preparation of samples for the ELISA included thawing and vortex mixing. A 100 μl sample from the vials was used for the ELISA tests; each vial was tested 3 times. Commercially available antiserum to whole IgG for the horse was coated onto Immulon 2 plates to act as a catcher for suspect IgG. Peroxidase-conjugated antiserum was then added and followed by addition of substrate. Quantitative analysis of the IgG level was obtained by comparing the absorbance of the suspect sample to a standard curve for IgG levels from dilutions of the donor animal serum. Fifty-one flies that fed on the pony on May 12, May 26 and June 15, 1987, were processed.

The SAS (1985) *t* test procedure was used to compare the mean wing length and weight of unfed and engorged flies. Means and standard deviations are presented unless otherwise specified. Other calculations were made using the General Linear Model procedure (SAS 1985). Three variables were used in the diverse regression analyses: microfilariae, weight and wing length. The microfilariae was used as a dummy variable: absence of microfilariae was coded as zero, and presence of microfilariae was coded as one. The weight was regressed on wing length and microfilariae. Then, weight was regressed on wing length of unfed and engorged *C. variipennis* females. Residual analyses were conducted to ensure that regression assumptions were respected.

Wing length of *C. variipennis* from the two egg sources did not differ at the 0.05 level of significance. Therefore, the data from all insects were pooled for analyses. The mean wing length (WL) of flies in the experiment was 1.477 ± 0.078 mm (n = 328); there were 110 unfed (WL = 1.493 ± 0.080 mm) and 218 engorged flies (WL = 1.469 ± 0.076 mm). Average weight was 0.2501 ± 0.0587 mg for unfed females and 0.5775 ± 0.1033 mg for engorged females.

Microfilariae (MF) were detected in 37 of the 218 engorged flies (17%). The number of MF ranged from 1 to 20 per fly: 23 flies had one, 4 flies had 2, 3 and 4, respectively, 1 fly had 14 and another 20 MF. There were no differences in mean WL (1.466 ± 0.075 vs. 1.484 ± 0.079 mm) or engorged weight (0.576 ± 0.104 vs. 0.586 ± 0.098 mg) between flies that did not or did ingest MF, respectively. The linear regression analysis of the relationship between microfilariae (presence, absence) and engorged weight of *C. variipennis*, with wing length added to control for body size variation of individual flies, showed that the lines were the same (joint test for identical lines; $P = 0.62$). Therefore, engorged weight of *C. variipennis* was found to be unrelated to microfilariae presence when controlling for body size. These results support the study of Higgins et al. (1988) that showed no relation between bloodmeal weight of *C. variipennis* and the ingestion of *O. cervicalis* microfilariae.

Linear regressions of unfed and engorged weights on wing length showed that both weights increased with wing length as a population response (Fig. 1). The difference between the mean weight of engorged and unfed flies for any given wing length will be referred to as retained bloodmeal weight. The retained bloodmeal weight and the ratio of retained bloodmeal

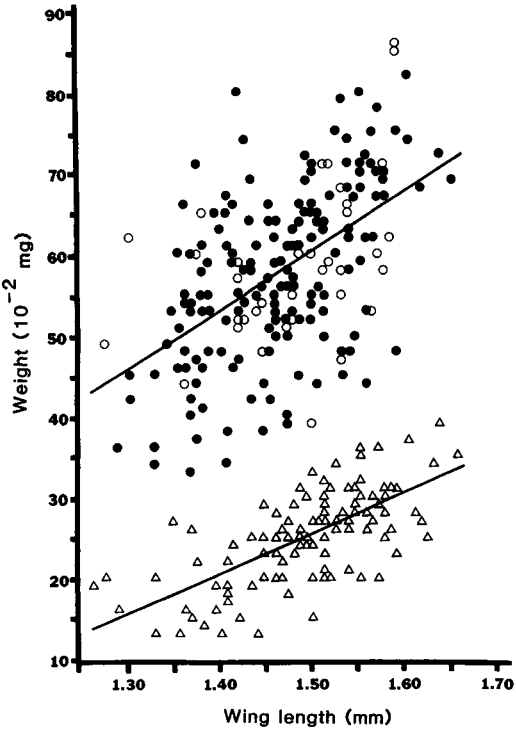

Fig. 1. Linear regressions of weight on wing length of unfed (△) and engorged (○ = ingested microfilariae; ● = without microfilariae) *C. variipennis* females. Unfed regression line: weight = −49.48 + 49.88 wing length; n = 110; $r^2 = 0.46$; residual sum of squares = 2014.88. Engorged regression line: weight = −48.94 + 72.65 wing length; n = 218: $r^2 = 0.28$; residual sum of squares = 16614.28.

Table 1. Wing length, engorged weight, unfed weight, retained bloodmeal weight (EW-UW) and the ratio of retained bloodmeal weight on unfed weight of *Culicoides variipennis* for 2 standard deviations around mean wing length (n = 328) which represents 95% of the population. Values of unfed and engorged weights (mg) derived from equations in Fig. 1.

	Standard deviation around the mean				
	−2	−1	0	+1	+2
Wing length (mm)	1.321	1.399	1.477	1.555	1.633
Engorged weight (EW)	0.4704	0.5279	0.5835	0.6401	0.6967
Unfed weight (UW)	0.1642	0.2031	0.2419	0.2808	0.3196
(EW–UW)	0.3061	0.3239	0.3416	0.3593	0.3771
Ratio (EW-UW)/UW	1.864	1.595	1.412	1.280	1.180

weight on unfed weight were calculated for one and 2 standard deviations (66 and 95% of the population, respectively) around the mean wing length. On the average, the flies retained 1.4 times their unfed weight in blood; this proportion ranged from 1.2 to 1.9 (Table 1). Engorged weight increased slower than unfed weight with body size, i.e., smaller flies retained more blood proportionally than larger flies. These results would support the hypothesis suggested for mosquitoes that there may be a critical threshold for physical or physiological repletion above which larger individuals would take less blood proportionally (Colless and Chellapah 1960).

Average engorged weight of flies processed for the ELISA was 0.495 ± 0.112 mg, and average wing length of 40 flies used during these periods was 1.40 ± 0.04 mm; both means fall within a 95% confidence interval for all engorged flies. The ELISA results indicated that the mean bloodmeal size of 51 flies was 0.443 ± 0.367 mg. The variance around the ELISA estimate bloodmeal size represented variance from the different bloodmeal weights and variance from the assay. The ELISA bloodmeal estimate was higher than 2 standard deviations above the mean retained bloodmeal weight (i.e., 0.377 mg). The greater estimate for bloodmeal size by the ELISA compared to retained bloodmeal weight is not surprising since retained bloodmeal weight does not reflect any bloodmeal concentration or excretion of fluids present in the digestive system before feeding. However, the ingestion of extravascular fluids by telmophagous flies is another variable that could influence correlation of gravimetric bloodmeal size estimates with techniques based on detection of blood components.

The ELISA data indicated that the mean bloodmeal size of *C. variipennis* is larger (23%) than the retained bloodmeal weight. This difference may reflect concentration of the bloodmeal to a lesser extent than that reported for *Culicoides arakawae* (Aarakawa) (Fujisaki et al. 1987). In that study, estimation of Hb, N and Fe content of engorged *C. arakawae* by chemical analyses showed that the mean uptake of blood in a meal was 0.36 mg, indicating a 2.5 fold concentration of ingested blood when compared to gravimetric estimates.

The average size of *O. cervicalis* MF in Louisiana was 217 ± 3.06 microns (Collins 1973). If the specific gravity of the MF is 1.0, then MF would weigh ca 1.6×10^{-6} mg; an insignificant amount compared to the bloodmeal size of *C. variipennis*. The 218 engorged flies in this study would have retained at least 74.469 mg (the mean retained bloodmeal weight from Table 1, 0.3416 mg × 218) of blood; hypothetically, the 37 MF ingested would have been ingested one per 2.013 mg of bloodmeal (MF are not randomly distributed or ingested). The difference between the retained bloodmeal weight for 2 standard deviations around the mean (95% of the population) of engorged flies was 0.071 mg (0.306–0.377, Table 1), an insignificant amount relative to the volume of bloodmeal containing one MF. Therefore, differences in engorged weight or bloodmeal size among *C. variipennis* are unlikely to be correlated with differences in MF ingestion.

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REFERENCES CITED

Colless, D. H. and W. T. Chellapah. 1960. Effects of body weight and size of blood meal upon egg production in *Aedes aegypti* (Linnaeus) (Diptera: Culicidae). Ann. Trop. Med. Parasitol. 54:475–482.

Collins, R. C. 1973. Onchocerciasis of horses in southeastern Louisiana. J. Parasitol. 59:1016–1021.

Collins, R. C. and R. H. Jones. 1978. Laboratory transmission of *Onchocerca cervicalis* with *Culicoides variipennis*. Am. J. Trop. Med. Hyg. 27:46–50.

Foil, L. D., T. R. Klei, R. I. Miller, G. E. Church, D.

D. French and J. N. Smith. 1987. Seasonal changes in density and tissue distribution of *Onchocerca cervicalis* microfilaria in ponies and related changes in *Culicoides variipennis* populations in Louisiana. J. Parasitol. 73:320–326.

Foil, L. D., D. Stage and T. R. Klei. 1984. Assessment of wild-caught *Culicoides* (Ceratopogonidae) species as natural vectors of *Onchocerca cervicalis* in Louisiana. Mosq. News 44:204–206.

Fujisaki, K., T. Kamio, S. Kitaoka and T. Morii. 1987. Quantitation of the blood meal ingested by *Culicoides arakawae* (Diptera: Ceratopogonidae). J. Med. Entomol. 24:702–703.

Higgins, J. A., T. R. Klei and L. D. Foil. 1988. Factors influencing the ingestion of *Onchocerca cervicalis* microfilariae by *Culicoides variipennis*. J. Am. Mosq. Control Assoc. 4:242–247.

Jones, R. H. 1957. The laboratory colonization of *Culicoides variipennis* (Coq.) J. Econ. Entomol. 50:107–108.

Klei, T. R., B. Torbert, M. R. Chapman and L. D. Foil. 1984. Prevalence of *Onchocerca cervicalis* in equids in the Gulf Coast region. Am. J. Vet. Res. 45:1646–1647.

Mullens, B. A. 1987. Seasonal size variability in *Culicoides variipennis* (Diptera: Ceratopogonidae) in southern California. J. Am. Mosq. Control Assoc. 3:512–513.

SAS Institute Inc. 1985. SAS User's guide: Statistics version, 5th ed. Cary, NC.