

GIEMSA STAIN AS A MARKER IN THE PITCHER-PLANT MOSQUITO, *WYEOMYIA SMITHII*

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ABSTRACT. Third and fourth instars of *Wyeomyia smithii* were reared in Giemsa stain at 4 concentrations between 4×10^{-7} and 10^{-5} g/liter. The mosquitoes retained the blue mark as adults and remained marked throughout their laboratory life. Concentration of Giemsa significantly affected eclosion success but had no significant effect on mean days to pupation or days as a pupa, male or female adult longevity, per-capita female fecundity or fertility. Larval exposure to low concentrations (4×10^{-7} or 10^{-6} g/liter) of Giemsa stain provides an effective lifetime tag for otherwise indistinguishable laboratory populations.

We undertook this study of *Wyeomyia smithii* (Coq.) to determine whether Giemsa stain would be an effective, easily detectable marker for this mosquito in laboratory populations. A reliable marker for *Wy. smithii* would allow us to determine relative longevity of adults from distinct populations when placed in mixed population cages receiving different treatments. Experiments were designed to reveal the effect of Giemsa concentration, experienced during development from larval diapause to pupation, on adult eclosion success, longevity, fecundity, fertility and persistence of the marker in the adults.

Overwintering larvae of *Wyeomyia smithii* were collected in Maine (June 1988) and maintained in the laboratory for 3 generations. Third instar diapause was induced by a short-day photoperiod (L:D = 8:16) at $21 \pm 0.5^\circ\text{C}$ and maintained for 6 months prior to experiments. Larvae which molted to the fourth instar and the third instars were compared in 2 experiments.

The Giemsa stain was prepared by adding the appropriate amount of Giemsa powder (Fisher Scientific Co., Pittsburgh, PA) to 1 liter of distilled water and heating to ensure dissolution. We tested 4 concentrations of Giemsa stain: larvae were removed from the diapause regime and placed in Giemsa (4×10^{-7} , 10^{-6} , 4×10^{-6} , and 10^{-5} g Giemsa/liter) solution. Experiments were conducted with a daily sine-wave thermoperiod ($12\text{--}28^\circ\text{C}$, mean = 21°C), 80% RH and a long-day photoperiod (L:D = 18:6). Replicates of 30 larvae in 125 ml of Giemsa solution per petri dish were compared with controls reared in distilled water. Larvae were fed (3 times/wk) an emulsion of guinea pig chow and freeze-dried brine shrimp (3:1 by volume). When cleaning was necessary fresh Giemsa solution was added to maintain a constant concentration.

Pupae were collected every Monday, Wednesday and Friday, transferred to plastic dessert dishes half-filled with unstained, distilled water and placed in cylindrical screened cages (200×150 mm) in the same room as the larvae. Adult

eclosion was recorded by removing and sexing the pupal exuviae. Adults had constant access to raisins and a cut leaf of their host plant, *Sarracenia purpurea* Linn., in a 70-ml glass jar half-filled with distilled water. The bottom of the cage was lined with filter paper kept moist with distilled water.

We conducted 2 experiments. In the first, within one day of eclosion, adults were squashed between slide and coverslip, sexed and examined against a white background under a dissecting microscope (15–30 \times magnification) for the Giemsa stain. In this experiment 2 trials of third instars were run at each of the 4 concentrations; trials of fourth instars consisted of 2 replicates each at concentrations of 10^{-6} and 4×10^{-6} g/liter and one trial each at 4×10^{-7} and 10^{-5} Giemsa concentration. In the second experiment the adults remained in the cages and were maintained until all adults had died. All dead adults were removed daily, squashed, sexed and examined for the blue marker using a dissecting microscope. Eggs were removed from leaf and jar, pupal dish and paper liner, rinsed into distilled water in a 150×25 mm petri dish and counted. Eggs were kept for 10 days and all hatched larvae were counted. Trials consisted of 2 replicates of third instars in each of the 4 Giemsa concentrations and in distilled water (controls); fourth instar trials included 2 replicates at 4×10^{-6} and at 10^{-6} g/liter, one trial at 4×10^{-7} and at 10^{-5} g/liter Giemsa concentration, and 2 control replicates in distilled water.

Frequencies were evaluated using the G-test for independence (Sokal and Rohlf 1969). Data were analyzed with the nonparametric Kruskal-Wallis test (SAS Institute 1985).

The Giemsa marker was easily observed by using the technique described above. In the first experiment where the adults were examined on the day of emergence, the blue Giemsa stain was readily visible in 100% of the individuals that had been exposed to the stain as larvae, regard-

less of concentration. In the second experiment, mean adult longevity ranged from 5.5 to 17.0 days. The blue Giemsa marker persisted throughout the adult life span in 100% of the individuals that had been exposed to the stain as larvae, regardless of concentration, but was not visible in any of the unexposed controls.

The concentration of Giemsa significantly affected eclosion success (G with 4 df = 81.46; $P < 0.001$) (Fig. 1). The Kruskal-Wallis test (χ^2 with 4 df) showed no significant effect of Giemsa concentration on mean days to pupation ($\chi^2 = 5.35$; $P > 0.05$) or days as a pupa ($\chi^2 = 2.31$; $P > 0.05$) (Table 1). Similarly, the Kruskal-Wallis test showed no significant effect of Giemsa concentration on longevity of adult males ($\chi^2 = 4.13$; $P > 0.05$) or females ($\chi^2 = 7.73$; $P > 0.05$), per-capita female fecundity ($\chi^2 = 6.13$; $P > 0.05$) or fertility ($\chi^2 = 6.55$; $P > 0.05$).

In our study, long exposure of 2-4 wk during larval development effectively marked *Wyeomyia smithii* larvae. At low concentrations (4×10^{-7} and 10^{-6} g/liter) the dye affects emergence minimally and yet produces 100% marking that persists throughout adult lifetime. The stain also has a minimal effect on life history characters of *Wy. smithii*. Previous studies (Weathersbee and Hasell 1938, Haddow 1942, Joslyn et al. 1985, Joslyn and Fish 1986, Kay and Mottram 1986) with Giemsa did not investigate the effects of the procedure on longevity, fecundity or fertility of the treated adults. Weathersbee and Hasell (1938) and Kay and Mottram (1986)

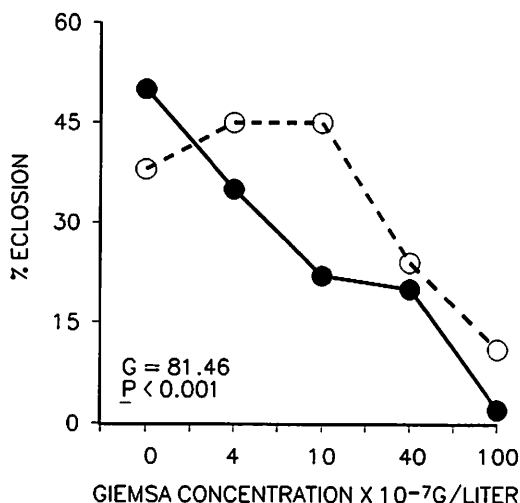


Fig. 1. Eclosion success of *Wyeomyia smithii* pupae exposed to Giemsa stain as third (O) and fourth (●) instars, including controls (0 g/liter). Concentration of Giemsa significantly affected eclosion ($P < 0.001$).

indicated that some growth retardation was evident in the treated larvae. Our studies show no significant effects of Giemsa concentration on development time or on longevity, fecundity or fertility of adult *Wy. smithii*. However, as shown in Table 1, there were parallel (albeit nonsignificant) rises in larval development times and declines in male and female longevity, female

Table 1. Life-history parameters of *Wyeomyia smithii* exposed to different concentrations of Giemsa stain, including controls reared in distilled water (0 g/liter). Values are parameter means.

	Giemsa concentration $\times 10^{-7}$ g/liter					χ^2 (K-W)*
	0	4	10	40	100	
Days to pupation						5.35
3rd instar	22	26	26	30	29	
4th instar	19	20	18	20	24	
Days as pupa						2.31
3rd instar	8	7	8	8	7	
4th instar	7	9	8	9	5	
Male longevity (days)						4.13
3rd instar	10	10	12	6	6	
4th instar	12	16	12	10	#	
Female longevity (days)						7.73
3rd instar	13	15	13	10	11	
4th instar	14	17	12	10	#	
Fecundity (eggs/female)						6.13
3rd instar	46	34	17	1	7	
4th instar	48	34	60	0	#	
Fertility (% hatch)						6.55
3rd instar	93	92	79	0	0	
4th instar	99	75	100	0	#	

No adults eclosed from these treatments.

* Kruskal-Wallis test for equality of Giemsa effects; with 4 df minimum significant ($P < 0.05$) $\chi^2 = 9.49$.

fecundity and egg hatchability among mosquitoes which had been exposed as larvae to a Giemsa concentration greater than 10^{-6} g/liter. We would therefore caution against long exposures to higher than 10^{-6} g/liter Giemsa. Used judiciously, prolonged exposure to low concentrations of Giemsa can provide a good tag for laboratory populations of *Wyeomyia smithii* which are otherwise indistinguishable as adults.

Although our experiments were not designed to test field use, the Giemsa self-marking technique has potential field applicability. An effective field marker would aid in dispersal studies. Giemsa stain could be used to determine which individual adult mosquitoes emerge from different species of pitcher plants (Bradshaw 1983) or different sub-habitats among tree hole mosquitoes (e.g., Bradshaw and Holzapfel 1983, Copeland and Craig 1990).

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REFERENCES CITED

- Bradshaw, W. E. 1983. Interaction between the mosquito, *Wyeomyia smithii*, the midge, *Metriocnemus knabi*, and their carnivorous host *Sarracenia purpurea*, pp. 161-189. In: J. H. Frank and L. P. Lounibos (eds.), *Phytotelmata: terrestrial plants as hosts for aquatic insect communities*. Plexus Publ., Medford, NJ.
- Bradshaw, W. E. and C. M. Holzapfel. 1983. Predator-mediated, non-equilibrium coexistence of tree-hole mosquitoes in southeastern North America. *Oecologia* 57:239-256.
- Copeland, R. S. and G. B. Craig, Jr. 1990. Habitat segregation among treehole mosquitoes (Diptera: Culicidae) in the Great Lakes region of the United States. *Ann. Entomol. Soc. Am.* 83:1063-1073.
- Haddow, A. J. 1942. The mosquito fauna and climate of native huts at Kisumu, Kenya. *Bull. Entomol. Res.* 33:91-142.
- Joslyn, D. J., L. B. Conrad and P. T. Slavin. 1985. Development and preliminary field testing of the Giemsa self-marker for the salt marsh mosquito *Aedes sollicitans* (Walker) (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 78:20-23.
- Joslyn, D. J. and D. Fish. 1986. Adult dispersal of *Aedes communis* using Giemsa self-marking. *J. Am. Mosq. Control Assoc.* 2:89-90.
- Kay, B. H. and P. Mottram. 1986. *In vivo* staining of *Aedes vigilax*, *Aedes aegypti* and *Culex annulirostris* larvae with Giemsa and other vital dyes. *J. Am. Mosq. Control Assoc.* 2:141-145.
- SAS Institute Inc. 1985. SAS user's guide: statistics, version 5 edition. SAS Inst. Inc., Cary, NC.
- Sokal, R. R. and F. J. Rohlf. 1969. *Biometry: the principles and practice of statistics in biological research*. W. H. Freeman and Co., New York.
- Weathersbee, A. A. and P. G. Hasell. 1938. Mosquito studies on the recovery of stain in adults developing from anopheline larvae stained *in vitro*. *Am. J. Trop. Med.* 18:531-543.