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Reproductive Potential of Stable Flies (Diptera: Muscidae) Fed Cattle, Chicken, or Horse Blood

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ABSTRACT Reproductive potential was assessed for stable fly cohorts fed cattle, chicken, or horse blood. Flies provided chicken blood oviposited 20% more eggs per day than did those fed cattle or horse blood. However, flies provided cattle or horse blood were fecund 50% longer. When both egg viability and number of eggs produced were considered, lifetime reproductive potential was almost twice as high for flies fed cattle or chicken blood than for flies fed horse blood. Maternal investment, which took egg production and volume into account, was higher in cohorts fed cattle blood (70 mm^3) when compared with the other treatments (chicken = 54 mm^3, horse = 55 mm^3). This is the first report of stable flies producing viable eggs after feeding on bird blood. Results from this study in addition to field observations indicate that stable fly interactions with birds may be limited to relatively low risk scenarios.

KEY WORDS reproductive potential, American white pelican, *Stomoxys calcitrans*, fecundity

Stable flies, *Stomoxys calcitrans* (L.), are ubiquitous blood-feeding muscids that readily feed on most warm-blooded animals (Bishopp 1913, Moon 2009). The focus of stable fly research in North America has been justified by the economic impact of this pest on dairy, feedlot, and more recently, pastured cattle (Campbell et al. 1987, 2001; Taylor and Moon 2011; Taylor et al. 2012). However, other domesticated, confined, or herding animals such as dogs, horses, and sheep are also readily attacked with the notable exception of poultry (Greenberg 1971, Hogsette and Farkas 2000). The infrequency of stable fly-chicken interactions is interesting because stable flies are often associated with poultry production (Hogsette 1979, Axtell and Arends 1990, Ruff 1999). In a California study, 98% of blood-fed stable flies aspirated from the walls of poultry houses had fed upon cattle while none had fed on chickens (Anderson and Tempelis 1970). Subsequently, Sutherland (1978) calculated maximum reproductive potential of cohorts provided blood from eight different animals. When all females that were fed chicken blood died before oviposition, a protein deficiency in chicken blood was presumed to have caused the follicles to resorb the yolk, preventing further maturation. The implication of these two studies is that stable flies are not considered pests of poultry because of limited interaction and reduced fecundity. In 2007, stable flies were observed feeding en masse on juvenile American white pelicans (*Pelecanus erythrorhynchos* Gmelin) infected with West Nile virus (WNV), challenging the validity of this assertion (Johnson et al. 2010b).

Following the introduction of WNV into a colony of pelicans in northeast Montana in 2003, mortality of prefledged pelicans increased from a 10-yr average of 4% to >30% (Madden and Restani 2003). Symptoms of WNV in moribund chicks include ataxia and immobility (Johnson et al. 2010a). Adults and asymptomatic juvenile pelicans typically fled stable fly attacks by dispersing into Medicine Lake. Stable fly attacks, which often produced open wounds, appeared limited to infected juvenile pelicans that were unable to flee. Flies congregated around the crown of the head and the eyes, where, occasionally, severe scabbing sealed the eyes shut. While stable flies have occasionally been observed biting birds, this level of attack on avian hosts has not been documented previously (Golding 1946). Because of the novelty of this behavior, the purpose of this study was to further investigate the effects of host source on stable fly daily and lifetime fecundity.

Materials and Methods

Source of Stable Flies. Stable flies were obtained from a 30-yr old colony maintained at the United States Department of Agriculture-Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, FL. The colony was established for 2 yr in the lab where the present fecundity trials were conducted.

Sources of Blood. Four liters of blood were collected from each host type during the first week of November

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2010 and treated with 3.7 g sodium citrate per liter. Cattle blood, obtained from Ranchland Packing Co., Butte, MT, was a homogenate of blood from two bulls. Chicken (Animal Technologies, Inc., Tyler, TX) and horse (Quad Five, Ryegate, MT) blood were shipped overnight on frozen cold packs to Montana State University in Bozeman, MT. Pelican blood, obtained from euthanized pelicans exhibiting advanced symptoms of WNV, was a homogenate from ten juveniles. One liter of each blood type was refrigerated at 1.6°C for immediate use while the remaining 3 liters were frozen at −17°C until needed.

Preliminary Trial. Fifty pupae were placed into each of three 30.5 cm^3 rearing cages and allowed to emerge for 2 d. At 2 d postemergence, each cohort was fed cattle, chicken, or pelican blood. A cotton ball was wetted with a 10% sucrose solution, then saturated with one blood type, wrapped in black cloth for oviposition, put into a plastic cup, and placed into the rearing cage. Daily, blood cups were replaced, eggs were counted, and dead flies were removed, enumerated, and sexed. The quantity of available pelican blood limited the authors to one cohort. Therefore, the average number of eggs laid per female per day are presented as a proof of concept and not as a means of comparison.

Formal Trial. Three cohorts of flies were randomly assigned to cattle, horse, or chicken blood. Cohorts including up to 50 nonblood-fed males and 50 nonblood-fed females 1- to 2-d old and were placed in each of nine round, 1.89 liter plastic containers on the same day. Two rectangular holes were cut on opposite sides of the containers and fitted with white cloth mesh to allow for ventilation. The lid of the container was modified so that a square was cut out and replaced with stockinette sleeve. Containers were kept in ambient room conditions where temperature and relative humidity averaged 24°C ± 0.04 and 13% ± 0.12, respectively.

One large cotton ball was saturated with a mixture 5 ml of a 10% sucrose solution and 10 ml of one blood type and wrapped in black Sheermist Batiste cloth (65 polyester/35 cotton, Walmart Stores, Inc., Bentonville, AR) to serve as a food source and oviposition substrate. Throughout the entirety of the experiment, wrapped cotton balls were placed in 118 ml plastic cups that were then put into the containers for 24 h. During each day of the trial, eggs were counted and dead flies were removed, enumerated, and sexed. Live flies were not counted. No live flies escaped during these trials. Therefore, the number of flies alive each day was calculated at the end of each trial through first totaling the number of flies that died throughout the trial ($=n$), then, starting with the first day of the trial, the number of dead flies recorded was subtracted from the total.

Up to 250 eggs were harvested from each cohort 12 d after adult eclosion (5–6 d into the oviposition period) by removing a section of the black oviposition cloth and placing it into a stable fly larval medium. Larval media comprised two parts wheat bran (223 g): one part pine shavings (36.5 g): one part Purina fly chow (200.2 g, Nestle S.A., Vevey, Switzerland), 50 mg brewer's yeast, and 1 liter water (Lysyk 1998). Diets for each container were mixed individually and placed into rectangular 1.89 liter plastic containers with white mesh cloth secured over the top. Subsequent pupae comprising the F_1 generation were removed from the larval media by water floatation, placing up to 150 pupae into clean cups for adult emergence.

Egg length and width for F_1 adults were measured with a 2 megapixel MiScope (Zarbeco, LLC, Randolph, NJ) that was calibrated with a 2 mm stage micrometer divided into 0.01 mm units. Volume for the eggs, which are prolate spheroids, was calculated as: $0.523 \times (\text{length})^2 \times \text{width}$.

Egg viability was determined by cutting sections of the black oviposition cloth containing up to 100 eggs, moistening and placing the cloth into a plastic petri dish. Dishes were placed into large plastic containers with a moist sponge and closed with lids to maintain humidity. After eggs were incubated for 3 to 4 d, viability was determined once by microscopically examining eggs for eclosion.

Statistical Analysis. The nonparametric two sample Wilcoxon signed rank test was used to detect significant differences ($P \leq 0.05$) in daily fecundity rates among cohorts within treatments, generations, and across treatments. Oviposition periods were compared between cohorts with paired *t*-tests. Lifetime fecundity was calculated as

$$F_L = F_d \times d$$

where F_L is lifetime fecundity, F_d is daily fecundity, and d is oviposition period, or the number of days the cohort produced eggs. Differences in lifetime fecundity were compared with the Kruskal–Wallis test. Variance in F_1 egg volume and viability were analyzed with one-way analyses of variance. Means were compared with Tukey's honestly significant difference (HSD) if significant differences were detected. Lifetime reproductive potential was calculated as

$$R_L = F_L \times H$$

Where R_L is reproductive potential, F_L is lifetime fecundity, and H is hatch proportion, or viability, of eggs produced.

Relative maternal investment in egg development was measured as

$$I = F_L \times V$$

where I is maternal investment, F_L is average lifetime fecundity, and V is average egg volume. Statistical analyses were performed using R software (version 2.12, R Development Core Team 2011).

Results

Preliminary results indicated that, contrary to Sutherland's (1978) findings, stable flies did oviposit when fed bird blood (Fig. 1A). During the formal study, flies provided chicken blood laid 20% more eggs per day than did flies fed cattle or horse blood (Table

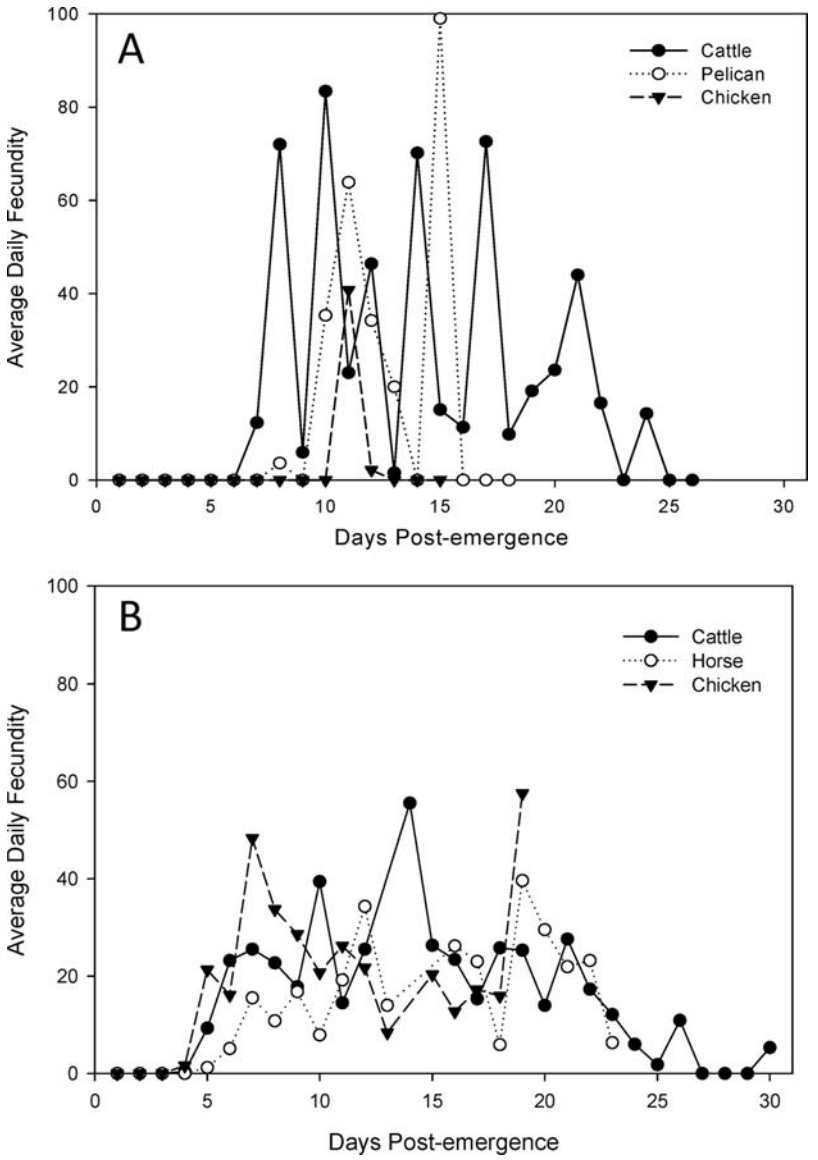

Fig. 1. (A) Average number of eggs laid per female per day in preliminary fecundity trial when cohorts were fed cattle, chicken, or pelican blood. (B) Average number of eggs laid per female per day when cohorts were fed cattle, chicken, or horse blood.

1; $P = 0.008$, $P = 0.05$, respectively). Daily fecundity did not differ between cohorts fed cattle and horse blood ($P = 0.36$). However, flies provided cattle or horse blood produced eggs for a longer period of time resulting in similar lifetime fecundity rates for all treatments, ≈340 eggs per female (Fig. 1B). Cohorts fed cattle blood oviposited and lived 1 to 2 wk longer than cohorts fed horse or chicken blood (Table 1). Eggs produced by flies provided horse blood were 30% less viable compared with the other two treatments, but this difference was not significant ($F = 3.7$; df = 2, 4; $P = 0.12$). Lifetime reproductive potentials were 323 for flies fed cattle blood, 317 for those fed chicken blood, and 172 for those fed horse blood.

Cohorts fed cattle, horse, or chicken horse blood produced eggs of similar volume ($F = 1.3$; df = 2, 3; $P = 0.39$). Females provided cattle blood invested 70.1 mm^3 in resources for their offspring. Females provided horse or chicken blood had lower investments in offspring, 55.2 and 54 mm^3, respectively.

Discussion

Lifetime fecundity was similar among stable fly cohorts fed cattle, horse, or chicken blood. While some studies have reported varying fecundity rates given different host sources (DuToit 1975, Sutherland 1978), attempts to correlate fecundity with nutritional

Table 1. Life history parameters (mean ± SE) for stable flies fed cattle, chicken, or horse blood

Host source	Oviposition period[a]		Daily fecundity[b]	Lifetime fecundity[b]	Volume[c]	Viability	Mortality$_{50}$[d]		Mortality$_{100}$[d]	
	Max	Mean					♂	♀	♂	♀
Cattle	21	16.0 ± 1.7a	19 ± 2a (3)	389 ± 47a (6)	0.18 ± 0.003a (130)	0.83 ± 0.05a (704)	14 ± 1	16 ± 2	37	37
Chicken	14	10.0 ± 1.0b	24 ± 2b (3)	337 ± 10a (5)	0.16 ± 0.002b (175)	0.94 ± 0.02a (507)	10 ± 1	9 ± 1	22	22
Horse	17	15.0 ± 3.0ab	18 ± 3a (3)	291 ± 52a (5)	0.19 ± 0.006a (31)	0.59 ± 0.13b (179)	10 ± 3	13 ± 2	23	23

Values within columns with different letters are significantly different ($P \leq 0.05$).

[a] Total no. of days during which egg deposition was observed in each cohort.

[b] Differences in daily fecundity, calculated as the avg no. of eggs laid per female per day.

[c] Egg vol (mm^3) is defined as $0.523 \times (\text{length})^2 \times \text{width}$.

[d] Average no. of days until 50 and 100% mortality for males and females in each treatment.

components have not been successful. Spates and DeLoach (1986) speculated that higher reproductive potentials of stable flies reared on a porcine blood when compared with cattle blood might have been due not to nutritional composition, but rather, the location of bloodmeal components (i.e., in the serum vs. red blood cells). Higher maternal investment and lifetime reproductive potentials of cohorts fed cattle blood in the current study suggests that even though similar numbers of eggs were developed, nutrient utilization may be altered based on blood type.

Differences in daily fecundity may have been influenced by olfactory and/or tactile variation in the blood that served as oviposition substrates. Blood used during this study was refrigerated up to 3 wk before use, and a strong, pungent odor emanated from the chicken blood that was absent in cattle or horse blood, possibly because of a difference in composition or contamination. Stable flies preferentially oviposit in substrates with active microbial communities (Romero et al. 2006). Volatiles from the oviposition substrate, then, may have stimulated or deterred oviposition.

Microbial contamination of blood may have also affected survival and/or egg development (Watson and Petersen 1991, Kraaijeveld and Wertheim 2009). Unlike horn flies (Harris 1962, Schmidt et al. 1967), lab-maintained colonies of stable flies are able to use blood that has not been treated with antimicrobials. The anterior midgut tissue of stable flies constitutively produces antibacterial peptides that are regulated by three broad classes of defense mechanisms, each with functional redundancies (Lehane et al. 1997, Munks et al. 2001). Despite the innate immunity of stable flies, the possible influence of contamination in this study cannot be dismissed. Cohorts provided chicken blood reached 50 and 100% mortality ≈4 and 15 d sooner, respectively, than those provided cattle blood.

The results from this study imply that stable fly feeding behavior is likely more of a reflection of host-parasite interaction than the nutritional quality of blood meals. After a blood source has been identified, parasite response to host defensive behavior is important in determining the extent to which feeding is successful (Schofield and Torr 2002). While some studies suggest that smaller host body size increases the effectiveness of defensive behaviors (Lehane 1991), others have been aimed more at general body condition. For example, when various hosts were restrained or unrestrained in cages, *Culex nigripalpus* Theobald mosquitoes tended to feed on restrained animals regardless of host size or species (Edman et al. 1974). Likewise, a shift in mosquito host preference from WNV-infected avian reservoirs to mammals (Kilpatrick et al. 2001) has been attributed to increased host intolerance as mosquito populations increased (Reeves 1971, Tempelis et al. 1975, Nelson et al. 1976) and chicks grew stronger and more agile (Blackmore and Dow 1972, Edman 1972). In contrast to these bird-mosquito interactions, dairy cattle in an open-field environment in southern California became ha-

bituated to increasing levels of stable fly attacks (Mullens et al. 2006).

In northeast Montana, stable fly populations were monitored near a colony of American white pelicans because of the high level of attack and feeding on local populations of WNV-infected juveniles in 2007. Adult stable fly and WNV surveillance during 2008–2010 showed that *S. calcitrans* was present near the roosting sites regardless of the health status of the colony (K.H., unpublished data). During outbreaks of WNV within the colony, juveniles with advanced symptoms were easily approached and unresponsive. In addition to stable fly attacks, other opportunistic parasites were observed including feather and pouch lice and masses of blow fly eggs covering the undersides of wings. In contrast to lethargic juveniles, healthy pelicans spent a large amount of time preening, flipping pouches inside out, and, when stable fly attacks increased, dispersed into the lake and settled along an island shore ≈0.8 km from the main roosting site. Observations of stable flies feeding on birds may therefore be limited to moribund hosts.

In summary, stable flies can oviposit viable eggs given at least two kinds of bird blood. The novel stable fly behavior reported during the WNV outbreak in the pelican colony in 2007 may be because of the availability of a relatively low risk source of blood. These results also imply that in situations where typical hosts are not present, alternate hosts may be attacked. Lack of information regarding host-stable fly interaction, especially quantitative definitions of stable fly risk-taking behavior, warrants further investigation.

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