Published in final edited form as:

J Med Entomol. 2010 March ; 47(2): 287–290.

Interspecies Predation Between *Anopheles gambiae* s.s. and *Culex quinquefasciatus* Larvae

EPHANTUS J. MUTURI^{1,2}, CHANG-HYUN KIM¹, BENJAMIN JACOB, SHANNON MURPHY³,
and ROBERT J. NOVAK¹

Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham,
Birmingham, Alabama 35294

¹ Department of Medicine, Division of Infectious Diseases, University of Alabama at Birmingham,
Birmingham, Alabama 35294.

³ Middle Tennessee State University, Murfreesboro, TN 37130.

Abstract

Interaction of aquatic stages of coexisting mosquito species may have significant influence on resulting adult mosquito populations. We used two coexisting species, *Anopheles gambiae* s.s. and *Culex quinquefasciatus* to investigate whether third instars of one species consumed first instars of the other. First instars of one species were readily consumed by a third instar of the other species irrespective food quantity. DNA of *Cx. quinquefasciatus* was detected in the eight *An. gambiae* s.s. third instars presumed to have consumed at least one *Cx. quinquefasciatus* first instar. Likewise, DNA of *An. gambiae* s.s. was detected in five of eight *Cx. quinquefasciatus* third instars presumed to have consumed at least one *An. gambiae* s.s. first instar. A small number of dead first instars was found in the controls indicating that some larvae in the treatment group may have been consumed after they had died. These findings suggest that intraguild predation between the two species may be common in nature and that it is a facultative process that is not induced by food shortage. The findings further suggest that polymerase chain reaction could be a useful technique in the study of this phenomenon in natural habitats.

In Africa, *Anopheles gambiae* s.s. (Giles) and *Culex quinquefasciatus* (Say) coexist in the same locality and aquatic stages of both species are frequently encountered in the same larval habitats such as hoof prints, pools, ditches, seepage areas, and rice fields (Fillinger et al. 2004, Muturi et al. 2007). In most of these habitats, all four larval instars of both species occur together (Mwangangi et al. 2008). Larval survival and development in these habitats is affected by biotic (such as predators, parasites, pathogens, food availability, and quality) and abiotic factors (such as temperature, rainfall, dissolved oxygen, turbidity) (Sunish and Reuben 2002, Sunish et al. 2006, Muturi et al. 2007). While the effect of these factors on mosquito population dynamics has been widely investigated, little attention has been given to predation among coexisting mosquito species. In a study to investigate the occurrence of predation and cannibalism between *An. gambiae* s.s. and *An. quadrimaculatus* (Theobald) under laboratory conditions, fourth instars of the two species were both cannibalistic and predacious (Koenraadt 2003). Further studies using *An. gambiae* s.s. and *An. arabiensis* Patton revealed that cannibalism and predation occurred probably as a result of frequent interactions within small aquatic habitats

as opposed to food shortage (Koenraadt et al. 2004). In both of these studies, the presence of a fourth instar prolonged development of surviving first instars. Other mosquito species that are reportedly predacious include genus *Toxorhynchites* spp. which prey on a wide variety of mosquito species (Russo 1986), *Lutzia tigripes* (De Grandpre and De Charmoy), which preferred *Aedes aegypti* (Linnaeus) larvae to those of *Culex* sp. or *An. gambiae* (Jackson 1953), *Lt. halifaxii* (Theobald) which fed preferentially on *An. farauti* (Laveran) (Laird 1947), and *Lt. fuscus* (Wiedemann) which preyed on several species including *An. stephensi* (Liston) (Iyengar 1920), *An. sinensis* (Wiedemann), *Ae. albopictus* (Skuse), *Cx. quinquefasciatus*, *Cx. sitiens* (Wiedemann), and *Cx. vagans* (Wiedemann) (Jin et al. 2006).

Interspecies predation may reduce larval survival or cause behavioral modifications (Holt and Lawton 1994, Peacor and Werner 2000, Peacor and Werner 2001) that may alter the fitness of emerging adults and their ability to transmit diseases. Conventional study of this phenomenon in nature is cumbersome and involves dissection of field-collected samples and examination of their gut contents under microscope. A reliable technique to identify mosquito species in the gut content is needed for evaluating the role of opportunistic predation by mosquito larvae. In the current study, we used polymerase chain reaction (PCR) technique to determine whether predation occurs between *An. gambiae* s.s. and *Cx. quinquefasciatus*. We also assessed whether food availability affected the frequency of this phenomenon under laboratory conditions.

Materials and Methods

Mosquitoes

Experiments were conducted with laboratory-adapted strains of *An. gambiae* s.s. and *Cx. quinquefasciatus* originally from Kenya, maintained at Medical Entomology Laboratory, University of Alabama at Birmingham. The mosquitoes were reared at a constant temperature of $28 \pm 1^\circ\text{C}$, 80% RH and 16 h photoperiod. Adults of *An. gambiae* s.s. and *Cx. quinquefasciatus*, respectively, were blood-fed once per week by exposing them to a restrained rabbit and quail. A 10% sucrose solution and apple slices were provided as maintenance diet. Larvae were reared in 2.5 liters trays containing de-ionized water and fed Tetramin fish food.

Experimental Design

To test the occurrence of predation between *An. gambiae* s.s. and *Cx. quinquefasciatus* and the influence of food on the frequency of predation, the following species combinations were tested in 12-well plastic plates normally used for tissue culture: (1) 10 *An. gambiae* s.s. first-instar (L1) larvae, (2) 10 *Cx. quinquefasciatus* L1 larvae, (3) 10 *An. gambiae* s.s. L1 and one *Cx. quinquefasciatus* third-instar (L3) larvae, and (4) 10 *Cx. quinquefasciatus* L1 and one *An. gambiae* s.s. L3. Each combination was tested in four replicates at 0, 0.1, and 0.2 mg of Tetramin fish food in 5 ml of de-ionized water. Counts of surviving L1 were taken at 24, 36, and 48 h. If fewer than 10 L1 were found in the well, the L3 was removed from the well, rinsed in 70% ethanol and then preserved in 100% ethanol.

Third instars from the wells in which at least one first-instar had disappeared, were subjected individually to a species diagnostic DNA-assay. *An. gambiae* DNA was detected using the rDNA-PCR method described by Scott et al. (1993) and *Cx. quinquefasciatus* DNA was detected by the method of Smith and Fonseca (2004). DNA was extracted using the Qiagen DNeasy Kit. Each mosquito was homogenized with the aid of a microtube pestle (USA Scientific, Enfield, CT) in a 1.5 ml tube containing 180 μl phosphate buffered saline (PBS) buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 , 1.47 mM KH_2PO_4) and subjected to DNA extraction according to manufacturer's recommended protocol. Isolated DNA from each mosquito was reconstituted in 100 μl AE buffer (Qiagen, 10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0) and stored at -20°C for analysis by PCR.

Diagnostic PCR assays were performed using 4.5 μ l of dH₂O, 12.5 μ l of two $\times$ ABI master mix, 0.5 μ l each of 10 μ M forward and reverse primer, and 5.0 μ l of template DNA. PCR conditions were denaturation at 94°C for 5 min followed by 37 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 5 min. Product was held at 4°C afterwards until ready to use. Gel electrophoresis was performed for 40 min and ethidium bromide was used for staining. It was expected that if an L3 had consumed an L1, both diagnostic DNA bands would be detected instead of only the known L3. DNA from *An. gambiae* s.s. and *Cx. quinquefasciatus* samples obtained from the colony served as the positive controls. Two sets of positive controls were used; each species separately (Fig. 1, lanes 20–26), and both species combined (Fig. 1, lane 3). Nuclease-free water was used as the negative control (blank).

Results and Discussion

Comparison of control (first instars of each species without third instars of the other species) and treatment groups (first instars of one species + third instars of the other species) indicated that third instars of both species are predacious on first instars of the other species although a few larvae may have been eaten after they had died (Table 1). Consumption of first instars of one species by third instars of the other species occurred irrespective of food quantity (Table 1). After 48 h, eight cases where a third instar was suspected to have consumed first instars of the other species were recorded for each species. DNA of *An. gambiae* s.s. was detected in five of eight *Cx. quinquefasciatus* third instars that were suspected to have consumed at least one first instar larva of *An. gambiae* s.s. Likewise, DNA of *Cx. quinquefasciatus* was detected in all eight *An. gambiae* s.s. third instars that were suspected to have consumed at least one *Cx. quinquefasciatus* larva (Fig. 1).

This study illustrates the occurrence of intraguild predation between *An. gambiae* s.s. and *Cx. quinquefasciatus*. However, we did not conduct behavioral studies to determine whether the first instars were eaten alive or after being killed by third instars. Some dead larvae were observed in the controls suggesting that some larvae in treatment group may have been eaten after they had died. These observations suggest that dead mosquito larvae provide a source of animal-based detritus to the surviving larvae. Previous studies have shown that mosquito larvae perform better on animal detritus than on plant detritus (Kesajaravu et al. 2007). PCR assays confirmed the presence of DNA of first instars of one species in third instars of the other species although this could not be demonstrated in some *Cx. quinquefasciatus* larvae. In addition, L3 of *An. gambiae* showed faint bands for *Cx. quinquefasciatus* first instars. The target DNA sequence for *An. gambiae* identification was 390 bp in length (Scott et al. 1993) and could have been degraded by the ‘predator’, rendering it undetectable by PCR. Similarly, DNA of *Cx. quinquefasciatus* first instars in the gut of *An. gambiae* L3 may have been degraded reducing its quality. The anterior midgut of a mosquito is highly alkaline with a pH in the range of 9–11 depending on species (Clements 1992, Corena et al. 2004). This can cause rapid degeneration of prey DNA. In some insects such as dragonfly (Libellulidae) nymphs, *An. gambiae* DNA is degraded 1 h after consumption (Morales et al. 2003). Further studies are under way to determine the duration in which DNA of the two mosquito species remains detectable after consumption.

The quantity of food available did not influence the occurrence of predation in either of the two mosquito species. Although our conclusion is limited by the small sample size, our results are in agreement with those reported for *An. gambiae* s.s. and *An. quadriannulatus* (Koenraadt 2003) indicating that predation is a facultative process. These results suggest that predation may be an important regulatory mechanism used by older larvae to reduce interspecies competition for scarce resources. In some terrestrial predators such as aphidophagous coccinellids, intra-guild predation occurs only during periods of aphid scarcity (Majerus

1994). The extent of predacious behavior of *An. gambiae* s.s. and *Cx. quinquefasciatus* in natural habitats is not well known but could be at lower frequency than observed in this study. This is because we used small experimental containers and thus first instars had little chance to escape predation. However, in most larval habitats, mosquito larvae tend to be clustered and this provides an opportunity for predation (Service 1971).

In summary, our results indicate that predation may be common between aquatic stages of *An. gambiae* s.s. and *Cx. quinquefasciatus* and that PCR is a useful tool in documenting this behavior. Field studies using a large sample size should be conducted to assess the influence of intraguild predation on adult mosquito populations and the occurrence of *Anopheles* and *Culex*-borne disease transmission.

Acknowledgments

This research was supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases Grant 01A1054889 (R.N.).

References Cited

- Clements, AN. The biology of mosquitoes. Chapman & Hall; London, United Kingdom: 1992.
- Corena M, Fiedler MM, VanEkeris L, Tu C, Silverman DN, Linserf PJ. Alkalization of larval mosquito midgut and the role of carbonic anhydrase in different species of mosquitoes. *Comp. Biochem. Physiol* 2004;137:207–225.
- Fillinger U, Sonye G, Killeen G, Knols B, Becker N. The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya. *Trop. Med. Inter. Health* 2004;9:1274–1289.
- Holt RD, Lawton JH. The ecological consequences of shared natural enemies *Annu. Rev. Ecol. Syst* 1994;25:495–520.
- Iyengar MOT. Preliminary report of a malaria survey of Calcutta and environs. *Indian Science Congress* No. 8–111. *Rev. Appl. Entomol. B* 1920;9:124.
- Jackson N. Observation on the feeding habits of predacious mosquito larvae, *Culex tigripes*. *Proc. R. Entomol. Soc. Lond. A* 1953;28:153–159.
- Jin L, Luo J, Fu Y, XU S. Prey and feeding behavior of larval *Culex (Lutzia) fuscans* (Diptera: Culicidae) in Shantou, Guangdong province, China. *J. Med. Entomol* 2006;43:785–786. [PubMed: 16892640]
- Kesavaraju B, Yee DA, Juliano SA. Interspecific and intraspecific differences in foraging preferences of container-dwelling mosquitoes. *J. Med. Entomol* 2007;44:215–221. [PubMed: 17427689]
- Koenraadt CJ, Majambere S, Hemerik L, Takken W. The effects of food and space on the occurrence of cannibalism and predation among larvae of *Anopheles gambiae* s.l. *Entomol. Experimentalis et Applicata* 2004;112:125–134.
- Koenraadt CJ, Takken W. Cannibalism and predation among larvae of the *Anopheles gambiae* complex. *Med. Vet. Entomol* 2003;17:61–66. [PubMed: 12680927]
- Laird M. Some natural enemies of mosquitoes in the vicinity of Palmamal, New Britain. *Trans. R. Soc. N.Z* 1947;76:453–476.
- Majerus, MEN. Ladybirds. Harper Collins; London, United Kingdom: 1994.
- Morales ME, Wesson DM, Sutherland IW, Impoinvil DE, Mbogo CM, Githure JI, Beier JC. Determination of *Anopheles gambiae* larval DNA in the gut of insectivorous dragonfly (Libellulidae) nymphs by polymerase chain reaction. *J. Am. Mosq. Control Assoc* 2003;19:163–165. [PubMed: 12825670]
- Muturi EJ, Mwangangi JM, Shililu J, Muriu S, Jacob B, Kabiru EW, Gu W, Mbogo C, Githure J, Novak R. Mosquito species succession and the physicochemical factors affecting their abundance in rice fields in Mwea, Kenya. *J. Med. Entomol* 2007;44:336–344. [PubMed: 17427706]
- Mwangangi J, Muturi E, Shililu J, Muriu S, Jacob B, Kabiru E, Mbogo C, Githure JI, Novak R. Contribution of different aquatic habitats to adult *Anopheles arabiensis* and *Culex*

quinquefasciatus (Diptera: Culicidae) production in a rice agroecosystem in Mwea, Kenya. J. Vector Ecol 2008;33:129–138. [PubMed: 18697315]

Peacor SD, Werner EE. Predator effects on an assemblage of consumer through induced changes in consumer foraging behavior. Ecology 2000;81:1998–2010.

Peacor SD, Werner EE. The contribution of trait-mediated indirect effects to the net effects of a predator. Proc. Nat. Acad. Sci. U.S.A 2001;98:3904–3908.

Russo R. Comparison of predatory behavior in five species of *Toxorhynchites* (Diptera: Culicidae). Ann. Entomol. Soc. Am 1986;79:715–722.

Scott J, Brogdon W, Collins F. Identification of single species specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am. J. Trop. Med. Hyg 1993;49:520–529. [PubMed: 8214283]

Service M. Studies on sampling larval populations of the *Anopheles gambiae* complex. Bull. W.H.O 1971;45:169–180. [PubMed: 5316615]

Smith JL, Fonseca DM. Rapid assays for identification of members of the *Culex* (*Culex*) *pipiens* complex, their hybrids, and other sibling species (Diptera: Culicidae). Am. J. Trop. Med. Hyg 2004;70:339–345. [PubMed: 15100444]

Sunish I, Reuben R. Factors influencing the abundance of Japanese encephalitis vectors in ricefields in India- II. Biotic. Med. Vet. Entomol 2002;16:1–9.

Sunish I, Reuben R, Rajendran R. Natural survivorship of immature stages of *Culex vishnui* (Diptera: Culicidae) complex, vectors of Japanese Encephalitis Virus, in rice fields in Southern India. J. Med. Entomol 2006;43:185–191. [PubMed: 16619597]

Fig. 1. DNA banding patterns of *Cx. quinquefasciatus* and *An. gambiae* s.s. third-instar larvae (L3) that preyed on first-instar larvae (L1). Top Row: detection of *An. gambiae* s.s. DNA; bottom row: detection of *Cx. quinquefasciatus* DNA. Lanes one and 27, molecular weight marker; lane 2, blank; lanes 3, 20–26, positive controls; lane 4–11, L3 *Cx. quinquefasciatus* that consumed L1 *An. gambiae* s.s.; lanes 12–19, L3 *An. gambiae* s.s that consumed L1 *Cx. quinquefasciatus*.

Table 1

Number of cases of interspecific predation on first instar larvae (10/test) by single third instar of *An. gambiae* s.s. (AG) and *Cx. quinquefasciatus* (CQ)

Food amt (mg)	Predator L3	Prey L1	Replicate	No. cases after 24 h	No. cases after 36 h	Total cases after 48 h
0	-	AG	4	1	0	1
1	-	AG	4	0	1	1
2	-	AG	4	0	0	0
0	-	CQ	4	1	1	1
1	-	CQ	4	0	0	0
2	-	CQ	4	0	0	0
0	CQ	AG	4	1	2	3
1	CQ	AG	4	3	3	3
2	CQ	AG	4	1	2	2
0	AG	CQ	4	3	4	4
1	AG	CQ	4	0	2	2
2	AG	CQ	4	1	2	2

Minus sign (-) indicate that no L3 was added in that treatment.