A Mallophaga, *Trinoton anserinum*, as a Cyclodevelopmental Vector for a Heartworm Parasite of Waterfowl

Abstract. *The biting louse* Trinoton anserinum *serves as the intermediate host in the life cycle of the filarial heartworm,* Sarconema eurycerca. *Microfilariae, second-, and third-stage larvae were dissected from 39 of 89 lice infesting whistling swans,* Cygnus columbianus columbianus, *in North America and mute swans,* Cygnus olor, *in the Black Sea, U.S.S.R. Infective third-stage larvae obtained from lice collected from heartworm-parasitized whistling swans were injected subcutaneously into each of two hand-reared, nonparasitized mute swan cygnets. Both of these birds developed heartworm infections, one becoming microfilaremic at 14 weeks. The results of this study provide conclusive evidence that a mallophagan serves as a natural cyclodevelopmental vector of a filarial parasite.*

The filarial heartworm, *Sarconema eurycerca*, Wehr, 1939 (*1*), was first described from the myocardium of a whistling swan, *Cygnus columbianus columbianus*, and since has been reported from six other species of swans and geese occurring in the Northern Hemisphere (*2*). *Sarconema* is one of 18 genera of nematodes of the family Dipetalonematidae which parasitize birds. The first life cycle to become known for any member of this family was that worked out by Anderson, who reported the vectors of *Splendidofilaria fallisensis* (=*Ornithofilaria fallisensis* Anderson, 1954), from the domestic duck in Canada, to be black flies of the genus *Simulium* (*3*). We now present the results of a study of the life cycle of *S. eurycerca* in whistling and mute swans, for which the biting louse *Trinoton anserinum* was shown to be the intermediate host.

The whistling swans used in this study were captured in February of 1975 and 1976 by rocket netting in their East Coast wintering grounds at Mattamuskeet and Pungo National Wildlife Refuges, North Carolina. Blood samples were taken from the tarsal vein of each bird with 3-ml heparinized syringes. These samples were kept at ambient temperature (7° to 12°C) and, within 12 hours, 0.2 ml of each was examined for the presence of motile microfilariae by the wet mount (*4*) and the capillary tube (*5*) tests. Giemsa-stained thin smears also were prepared at this time for subsequent microscopic examination. Lice, identified as *Trinoton anserinum*, were collected from swans by thorough searching of the feathers. These were either dissected alive or preserved in 70 percent ethanol for later study. Lice of the same species also were collected in August 1975, from eight molting mute swans (*C. olor*) from the Black Sea at Swan Island International Reserve, near Portova, Crimean Peninsula, U.S.S.R.

Eggs laid by mute swans in the Chesapeake Bay, Maryland (*6*), were collected and hatched in an incubator. The cygnets were hand-reared in facilities that precluded their exposure to any vector-borne parasitic helminths. These birds were experimentally infected by injecting them subcutaneously with larvae of *S. eurycerca* dissected from lice collected from parasitized captive whistling swans maintained at a rural site approximately 30 miles (1 mile = 1.6 km) away from the laboratory. Beginning on day 30 after inoculation, and at weekly intervals thereafter, blood specimens were taken from the tarsal vein of the birds and examined by the wet mount method to determine the onset of microfilaremia.

The results of the dissections of 89 *T. anserinum* collected from whistling swans, from mute swans, and from persons handling these birds are shown in Table 1. Fresh blood was found in the midgut of 59 (66 percent) of the lice and, in two of these, motile microfilariae were seen in the blood meal. These microfilariae were morphologically identical to those occurring in the blood of the whistling swans infected with *S. eurycerca*, from which these lice had been collected. Fresh blood meals also were present in four of the eight lice removed from mute swans in the Black Sea and, although no microfilariae were found in the gut contents of these, three of the lice were parasitized with filarial larvae. Comparisons of corresponding developmental stages of these larvae with those found in *T. anserinum* from *S. eurycerca*–infected whistling swans showed them to be identical. The occurrence of more than one developmental stage of *S. eurycerca* in the same louse was recorded for 24 of 39 (62 percent) of the infected mallophagans examined from whistling swans and for one of the three collected from mute swans. Late "sausage" stage as well as second-stage larvae always were found in the abdomen, whereas the larger third-stage larvae were recovered most frequently from the head, but occasionally they also were found in the thorax and the abdomen. Microscopic examination of living, parasitized lice revealed that the infective larvae were vigorously active, moving back and forth between the head and thorax of the louse and occasionally extending themselves externally well beyond its mouth parts.

The microfilaria of *S. eurycerca* is sheathed and, as originally described from the blood of a Canada goose

Table 1. Results of the dissection of 89 lice, *Trinoton anserinum*, removed from parasitized and nonparasitized whistling swans, from mute swans, and from banding assistants.

Item	Donor swans (No.)	Number of lice				
		Recovered	Infected	With blood meal	With blood meal and parasites	With parasites and no blood meal
Whistling swans*						
Positive for heartworm	13	45	27(60)†	33(73)†	24(72)‡	9(75)§
Negative for heartworm	8	23	3(13)†	15(65)†	2(13)‡	1(13)§
Lice from mute swans‖	5	8	3(37)†	4(50)†	3(75)‡	0
Lice from banders		13	6(46)†	7(54)†	2(29)‡	4(67)§
Totals	26	89	39(44)†	59(66)†	31(53)‡	14(47)§

*Origin: Mattamuskeet and Pungo National Wildlife Refuges, North Carolina. Swans were determined positive or negative for heartworm by microscopic examination of blood samples. †Percent of total lice recovered. ‡Percent of lice with a blood meal that were parasitized. §Percent of lice with no blood meal that were parasitized. ‖Origin: Portova, Crimean Peninsula, Ukraine, U.S.S.R.; blood samples were not examined for heartworm microfilariae.

Table 2. Comparison of average measurements (in micrometers) of *Sarconema eurycerca* microfilariae from different sources. A sheath was present in all.

Item	Average measurements of microfilariae from		
	Adult heartworm* (N = 25)	Circulatory system of a whistling swan (N = 12)	Circulatory system of an experimental mute swan (N = 11)
Body length	296	329	331
Body width	5.7	6.2	7.4
Cephalic space	9.1	9.5	9.3
Nerve ring	65 (21)†	73 (21)†	71 (21)†
Excretory pore	97 (32)†	110 (33)†	108 (33)†
Anal pore	254 (85)†	279 (85)†	283 (85)†
Nuclei posterior end‡	2	2	2

* Adult, female *S. eurycerca* and microfilariae from peripheral circulatory system were obtained from the same whistling swan. †Numbers in parentheses are the percent body length; the morphologic features are located as measured from the anterior end of the larvae. ‡ Posterior end space measured between the last stained nuclei and the posterior end of the larvae.

(*Branta canadensis*), measures between 310 to 345 μm in length by 5.8 to 7.6 μm in width (*7*). Morphological comparison of microfilariae from an adult *S. eurycerca* female with microfilariae from the peripheral blood of naturally infected whistling swans proved that they were identical. Further verification of the identification of this species was obtained upon postmortem examination of four captive whistling swans that had exhibited these characteristic larval forms in their blood for 11 months. All of these birds harbored adult *S. eurycerca* in the myocardium. Subsequently, the specific identity of the larval forms dissected from lice infesting the whistling swans was confirmed by injecting them subcutaneously into two mute swan cygnets that had been reared free of helminth parasites in the laboratory. The first of these birds was inoculated initially with 49 larvae and 7 days later received 13 more. On day 27, this bird died of unknown causes and at necropsy, one immature filaria was found in the myocardium. The second experimental mute swan cygnet was inoculated with 54 larvae recovered in different stages of development from lice infesting the same whistling swans. At 98 days after inoculation this bird displayed a patent infection with circulating microfilariae that were morphologically identical to those recovered previously from an adult female *S. eurycerca* and from naturally infected whistling swans (Table 2).

Historically, it has been thought that biting lice did not feed routinely on blood, but lived instead on sluffed epidermal tissue, feather barbules, and glandular sebaceous secretions of the host. Therefore, these ectoparasites have been ignored as potential cyclodevelopmental vectors for helminths parasitizing the blood vascular system of vertebrates. It now is well known that the feeding habits of species belonging to the order Mallophaga are variable. Blood has been found to be a component of the diet in many members of the suborder Amblycera, and some of these species habitually obtain blood meals (*8*). Transmission of typhus in experimental animals by the biting louse *Trimenopon hispidum* has been reported, and the occurrence of rickettsias in an ischnocerous louse of the horse, as well as in *Menacanthus stramineus*, an amblycerous ectoparasite of poultry, also has been recorded (*9*). More recently, it has been found that both *Eomenacanthus stramineus* and *Menopon gallinae* were capable of sustaining virulent *Pasteurella multocida* after removal from an infected chicken, but transmission was thought to be due to direct contamination of a wound with feces or by ingestion of the infected louse by the avian host (*10*). Therefore, these lice probably serve only as a mechanical vector for fowl cholera.

The only indication that a mallophagan might serve as an essential intermediate host of a filarial worm is to be found in the report of Dutton (*11*) who recorded the presence of larvae, thought to be those of *Filaria cypseli* Annett, Dutton et Elliot, 1901, in the fat bodies of a biting louse of the subfamily Leiothinae parasitizing a swift, *Cypselus affinis* Gray. According to Dutton these ectoparasites had been supplementing their feather diet with blood and lymph of their avian host.

In order to certify a specific arthropod as a natural intermediate host of a filarial parasite, the following basic criteria must be met: (i) the spatial and temporal relationship between the arthropod and the host must be such that an opportunity for transmission is assured; (ii) the arthropod must be capable of acquiring microfilariae from its host and developing them to the infective stage; (iii) the occurrence of various developmental stages of the larval parasite should be demonstratable in natural populations of the suspected vector; and (iv) upon inoculation into a susceptible definitive host, third-stage larvae from the infected arthropod should result in a patent infection. All of these criteria were fulfilled in our study.

The fact that fresh blood constitutes a major food item frequently found in the gut of *T. anserinum* indicates that ingestion of this substance is habitual. The finding, on two occasions, of motile microfilariae of *S. eurycerca* in the guts of lice removed from captive, microfilaremic whistling swans demonstrates that these mallophagans are capable of acquiring the first-stage larvae as a result of ingesting fresh blood. The simultaneous occurrence of second- and third-stage larvae, as well as of intermediate forms in many of these ectoparasites, signifies sequential ingestion of microfilariae through periodic feeding on fresh blood of the avian host and establishes that this louse is capable of supporting the cyclodevelopmental processes of the parasite. The transmission experiments confirm the fact that the third-stage larvae found in these lice were infective when transferred to nonparasitized swans inasmuch as they developed to patency and gave rise to circulating microfilariae of *S. eurycerca*.

Mallophaga are very host-specific and complete their entire life cycle on the vertebrates they infest. Unlike most dipteran vectors, which may only periodically overlap in both a temporal and spatial manner with their host, this biting louse is in constant association with the avian species they infest, thus enhancing the opportunity for filarial transmission.

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6. In North America the mute swan is an introduced species from Europe. At present a number of discrete, nonmigratory populations have established themselves, notably in British Columbia, Michigan, Rhode Island, New Jersey, and Maryland. In Europe the mute swan is residential in the west and migrates from colder central and northern Europe to the warmer west. Their movements are quite limited compared with the other species of swans that breed on the arctic tundra and cross continents during their migrations.
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12. We thank the following people for assistance in the field work: the managers and staff of the Mattamuskeet and Pungo National Wildlife Refuges, U.S. Fish and Wildlife Service, Department of the Interior; Dr. U. Kostin of the Swan Island International Reserve, Portova, Crimea, U.S.S.R.; as part of the Northern Waterfowl Project of the U.S.–U.S.S.R. Environmental Protection Agreement (Area V); Thomas P. LaMonica, Jeanette Evans, James Seegar, Roland Limpert, and particular thanks to Donna Seegar. We also thank Lloyd E. Rozeboom and Roy C. Anderson for criticism of this manuscript. Supported by NIH training grant 5 T01 HI00020-18, the John W. Graham Foundation, and the Swan Research Program (Chesapeake Bay Foundation).

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An Animal Behavior Model for Studying the Actions of LSD and Related Hallucinogens

Abstract. *Cats injected with LSD (*d*-lysergic acid diethylamide) exhibit a group of behaviors that appear to be specific to hallucinogenic drugs. Two of these behaviors, limb flick and abortive grooming, have an extremely low frequency of occurrence in normal cats, but often dominate the behavior of LSD-treated cats. The frequency of occurrence of this group of behaviors is related to the dose of LSD. The behavioral changes are long-lasting following a single injection of LSD, and exhibit tolerance following the repeated administration of LSD. They are not elicited by a variety of control drugs, but are elicited by other indole nucleus hallucinogens. Because the behavioral effects are specific, reliable, easy to score, and quantifiable, they represent an animal model that can be used in studies of the effects of LSD and related hallucinogens.*

An important step in understanding the effects of the major psychoactive drugs, and drugs of abuse, is frequently the development of an animal model or analog of the drug's action (*1*). Such models permit the undertaking of experiments which are precluded for moral and ethical reasons in humans. In addition, their use affords the opportunity for direct investigation of the physiological bases of drug actions. A case in point is the usefulness of "wet dog shakes" in rodents as a model for the abstinence syndrome that follows withdrawal from narcotics such as morphine (*2*).

In the course of examining the dose-response relationship for the behavioral effects of LSD (*d*-lysergic acid diethylamide) in the cat, we observed a group of behaviors that occur with a high probability in cats injected with LSD. The behaviors are also produced by drugs which are structurally or functionally related to LSD, but are not produced by potent psychoactive drugs from other classes.

In the experiments described here we used adult female cats weighing 2.0 to 3.3 kg. The cats were individually housed in standard stainless steel cat cages which also served as the observation chambers. To counterbalance the experiments, we subjected each cat to all treatments, allowing at least 8 days interval between consecutive treatments. The cats were given intraperitoneal injections of either saline or lysergic acid diethylamide tartrate (10.0, 25.0, 50.0, or 100.0 μg/kg, the dose being expressed as the salt). Behavioral observations, by raters who were "blind" to the treatment, were made during the hour immediately following drug administration. The frequency of occurrence of each behavior was tallied on a standard scoring sheet (Table 1).

Most of the descriptions of the cats' behaviors are self-explanatory, but a few require further comment. Abortive grooming is scored when the cat orients to the body surface as if to groom but does not emit the consummatory grooming response (bite, lick, or scratch), or emits the response in midair. Limb flicking is a behavior seen in normal cats almost exclusively in response to placing a foreign substance, such as water, on the hindpaw or forepaw. The paw is then lifted and rapidly flicked outward from the body. Investigatory or play behavior refers to pawing or sniffing at objects or in corners, chasing the tail, or batting at pieces of food or feces, for example. Hallucinatory-like behavior is scored when the cat looks around at the floor, ceiling, or walls of the cage and appears to be tracking objects visually, or when the cat either hisses at, bats at, or pounces at unseen objects (*3*).

The behavioral effects of LSD can be grouped into three distinct categories (Table 1). The frequency of the first group, which includes rubbing, treading, and vocalization, did not change significantly following the administration of LSD. The second group of behaviors, which includes staring, grooming, and head and body shakes, had a relatively high frequency of occurrence in saline-treated animals and then showed a three- to fivefold increase in frequency as a function of the dose of LSD. Many of these increases appear to be attributable simply to the arousal or activational effects of the drug.

Most important in the present context is the third group of behaviors that we describe as emergent in the LSD-treated animal. These behaviors were either nonexistent or occurred with a very low frequency in saline-injected animals, but emerged to the point of often dominating the behavior of LSD-treated cats. They include limb flicks, abortive grooming, investigatory or play behavior, and hallucinatory-like behavior. The first two of these have not been previously reported in studies of the effects of LSD on cats (*4*); and they are of particular interest because their highly stereotyped response topography makes them easy to observe and quantify.

The most impressive example of these emergent behaviors was the limb flick. After saline treatment, this response was seen only twice in the 1-hour observation period on 12 cats (mean, 0.2 per hour). However, when the same animals were given LSD (50 μg/kg), the response increased to a mean frequency of 45.8 per hour. This represents an increase of several orders of magnitude. It was not uncommon for individual animals to emit 20 to 30 flicks in a 15-minute period following the two higher doses of LSD. Two or three sequential flicks of different limbs were often seen within 2 to 5 seconds. It is worth noting that every animal tested showed at least some limb flicking in response to LSD.

Instances of abortive grooming occurred with a mean frequency of 5 to 8 per hour at doses of 25 to 100 μg/kg. While this does not represent a large ab-