Demography of botfly (*Cuterebra fontinella*) parasitism in white-footed mice (*Peromyscus leucopus*) in Kansas

Robert M. Timm[1,*] and Cory R. Ruedebusch[1,2]

[1]*Natural History Museum and Biodiversity Research Center & Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045, USA*

[2]*Present address: Meadowlark Consulting, 16103 Evanston Ave N, Shoreline, Washington 98133, USA*

To whom correspondence should be addressed: btimm@ku.edu

Botflies (Diptera: *Cuterebra* sp.) are widely distributed and seasonally abundant parasites of small mammals in North America. To assess their effect on rodent survival, we studied the demography of botfly parasitism on small mammals in northeastern Kansas during 1995–1999. Additional comparisons on botflies parasitizing small mammals were made to a nearby old-field grid where mark–recapture studies continued from 1975 to 2003. White-footed mice, *Peromyscus leucopus*, were infected by botfly larvae (*Cuterebra fontinella*) each year during our study. The period of infection for *P. leucopus* was June–December, with the highest proportion of mice infected in July and August. A smaller, second peak of infection in October and November indicated that *C. fontinella* reproduced bimodally at this latitude with distinct summer and autumn population peaks. During the botfly seasons of the 5 years, 140 (23.3%) of 602 *P. leucopus* captured were infected. The percentages of individuals infected during 1995, 1996, 1997, and 1999 were not significantly different (22.4%, 17.3%, 17.1%, and 22.7%, respectively). However, in 1998, 32.4% individuals were infected with a higher incidence of multiple infections and a greater number of botfly larvae per host. Nearly half (47%) of the infected individuals caught in 1998 had infections in both summer and autumn, compared with 25% during the 1995–1997 seasons. In 1999, 63% of infected mice had at least a 2nd infection during the autumn. For all 5 years, 82%, 14%, and 4% of the infected mice had one, two, or three botflies, respectively. In 1998, 24% of the infected mice carried two or more botflies at one time, compared to 14% from 1995–1997 and 11% in 1999. Overall 94% of the infected mice were adults, with no differences between sexes. The variation seen in the prevalence of botfly parasitism of white-footed mice correlates to yearly weather fluctuations. Other demographic aspects of the infected mice, such as sex ratio, age, and longevity of those infected, did not change year to year. Our data suggest that infection with *Cuterebra* has little negative or positive impact upon populations of white-footed mice.

Key words: coevolution, host–parasite relationships, Oestridae, parasite infections, parasitism, residence on grids

Los tábanos (Diptera; *Cuterebra*) son un grupo de moscas parásitas con variable abundancia estacional en Norte América. Para determinar su efecto en la supervivencia de roedores estudiamos la demografía del parasitismo por tábanos en mamíferos pequeños del noreste de Kansas desde 1995 hasta 1999. Se realizaron comparaciones adicionales del parasitismo de larvas de tábanos usando una retícula de trampas en una zona aledaña, donde estudios de captura-marcaje-recaptura fueron realizados desde 1975 hasta 2003. El ratón de patas blancas (*Peromyscus leucopus*) fue parasitado por larvas de tábanos (*Cuterebra fontinella*) en todos los años de nuestro estudio. El período de infección para *P. leucopus* fue entre junio y diciembre, con una mayor proporción de ratones infectados entre julio y agosto. Un segundo pico menor de infección sucedió entre octubre y noviembre, indicando que *C. fontinella* es bimodal en la latitud de nuestro estudio, con picos poblacionales diferenciables en el verano y el otoño. Durante lastemporadas de tábanos de los 5 años, 140 (23.3%) de 602 *P. leucopus* capturados presentaron infecciones. Los porcentajes de individuos infectados en los años 1995, 1996, 1997 y 1999 no fueron diferentes a nivel estadístico (22.4%, 17.3%, 17.1%, y 22.7%, respectivamente). Sin embargo, en 1998, un 32.4%

de los individuos fueron infectados, con una alta incidencia de infecciones múltiples, y un número mayor de larvas de tábano por hospedador. Casi la mitad (47%) de los individuos infectados capturados en 1998 tuvieron infecciones tanto en verano como en otoño, frentea un 25% durante el periodo1995–1997. En 1999, el 63% de los ratones infectados tuvo al menos una segunda infección durante la misma estación. Para todos los años, el 82%, 14% y 4% de los ratones infectados tuvieron 1, 2, o 3 larvas, respectivamente. En 1998, el 24% de los ratones infectados transportaron dos o más larvas al mismo tiempo, comparado con el 14% durante 1995–1997 y el 11% en 1999. En general, el 94% de los ratones infectados fueron adultos y no observamos diferencias en infección entre sexos. La variación observada en la prevalencia de parasitismo estuvo correlacionada con fluctuaciones climáticas anuales. Otros aspectos de la demografía de parasitismo en ratones, por ejemplo, proporción de sexos, edad, longevidad de los individuos infectados, no cambiaron entre años. Nuestros resultados sugieren que la infección por *Cuterebra* tiene poco impacto negativo o positivo en la población de ratones de patas blancas.

Palabras clave: coevolución, relaciones hospedero-parásito, parasitismo, infecciones parasitarias, residencia en cuadrículas

Parasites have been hypothesized to influence many aspects of mammalian ecology; however, the effects of parasites are difficult to quantify. Botflies of the genus *Cuterebra* (Diptera: Oestridae; Cuterebrinae) are widely distributed and seasonally abundant parasites on a number of small mammals in North America and believed to have negative effects on rodents because of their abundance, large size (especially relative to that of their host, about 22 mm long upon emergence), and the open breathing pore they maintain in their hosts. Species of *Cuterebra* generally develop on a single host species of mammal, or group of closely related species, primarily rodents and lagomorphs (Catts 1982; Sabrosky 1986; Wood 1987). Gravid female flies deposit their eggs near the areas of activity of potential hosts. When a mammal encounters botfly eggs, its body temperature triggers the eggs to hatch and the newly emerged, minute first instars enter the host through a moist natural body opening, generally the mouth. The larvae migrate through the body cavity (ca. 5 days) to the final development site, where it will mature in 19–21 days. Mature larvae emerge from the host through the open breathing pore (termed a warble) and quickly burrow underground, where they pupate and may overwinter. Adult botflies are short-lived (Catts 1982; Wood 1987).

Demographic characteristics of host populations of small mammals infected by *Cuterebra* have been the subject of several studies; however, the results and conclusions have varied considerably among authors. Studies on mice of the genus *Peromyscus* infected by *Cuterebra* at various latitudes show considerable variation in length of the botfly season. *Cuterebra fontinella* is the only species of botfly known on species of *Peromyscus* in the Eastern, Southeastern, and Midwestern United States. Southern populations experience longer botfly seasons than do northern populations. In Georgia, Durden (1995) reported botflies on cotton mice (*Peromyscus gossypinus*) throughout the winter months, with distinct summer and winter peaks of abundance. In Oklahoma, Goertz (1966) recorded botflies from June to January on white-footed (*Peromyscus leucopus*) and deer mice (*Peromyscus maniculatus*). In east-central Minnesota, however, Timm and Cook (1979) recorded botflies on white-footed mice only from mid-July to early October.

The sex ratio of infected mice in a population was reported to be male-biased (Xia and Millar 1990), female-biased in the autumn and male-biased in the winter (Galindo-Leal 1997), and not sexually biased (Dunaway et al. 1967; Miller and Getz 1969; Jaffe et al. 2005). The residence time of infected white-footed mice on trapping grids usually is reported to be longer than that of noninfected mice (Wecker 1962; Goertz 1966; Hunter et al. 1972; Jaffe et al. 2005), but the opposite pattern was reported by Miller and Getz (1969). Hunter et al. (1972) proposed that because resident mice persisted longer on the grid, they were more exposed to botfly eggs and, subsequently, more frequently infected than transient mice.

Several studies have examined the effects of botflies on host populations (Boonstra et al. 1980; Clark and Kaufman 1990; Xia and Millar 1990). The possible effects of these parasites on population dynamics of *Peromyscus* are not well understood, but are thought to interfere with reproduction (Sealander 1961; Wecker 1962; Timm and Lee 1981, 1982; Slansky 2007), direct mortality of the host (Nichols 1994), and increased risk of predation (Wecker 1962; Smith 1978). However, Timm and Cook (1979) found that white-footed mice were not reproductively hindered by botflies and have evolved a tolerance to botfly parasitism. They documented with wild-caught individuals that one or two larvae had little effect on the size of the testes of adult male mice and adult females showed no decrease in the number of embryos, corpora lutea, or placental scars (Timm and Cook 1979). It has been demonstrated in the laboratory that white-footed mice previously infected by botflies may become resistant to the physiological effects of future infections (Gingrich and Barrett 1976; Gingrich 1979). Munger and Karasov (1991, 1994) and Cramer and Cameron (2006) concluded that botflies have a relatively moderate effect on individuals, and are unlikely to have an effect on the population density of white-footed mice. Burns et al. (2005) reported that botfly infections in *Peromyscus* lead to seemingly contradictory results—enhanced survival but decreased population growth rates, which they attributed to a trade-off between the life-history traits of survival and reproduction. The rodent and rabbit botflies of the genus *Cuterebra* are restricted to the New World, and tolerance to botfly parasitism is believed to have evolved in New World hosts such as *Peromyscus*. Experimental botfly parasitism of Old World rodents (*Cricetus*, *Mus*, and *Rattus*) often resulted in the death of the host (Catts 1965). Even when the host lived, larvae seldom reached maturity.

Herein, we examined the incidence and effects of botfly parasitism in a small mammal community in northeastern Kansas. Our objectives are: (i) to document the prevalence

and seasonality of botfly parasitism in white-footed mice by focusing on the length of the botfly season; the infection rate during the botfly season; the number of botflies per animal; and the differences in infection in relation to sex, age, resident status, and persistence (= residence time) of hosts on a grid; (ii) report botfly infections in other species of small mammals in this community; and (iii) provide a new rigorous method of assessing residence times of small mammals on a grid.

Methods

This study was undertaken at the Nelson Environmental Study Area, University of Kansas Field Station in Jefferson County, ca. 12 km NNE Lawrence, in northeastern Kansas (39°03′N, 95°12′W). We undertook a mark–recapture study on small mammals and their associated botflies on a 1.6-ha forest grid from 1995 to 1999 (Welch 1998; Ruedebusch 1999). Additional comparisons were made to a nearby old-field grid where mark–recapture studies were begun in 1973 and continued to 2003 by Norman A. Slade. Herein, we use botfly data from the old-field grid collected from 1975 to 2003 for *Microtus ochrogaster*, and from 1989 to 2003 for all other species. The sites are about 750 m apart and separated by a mid-succession forest. Additional details on the old-field site were provided by Swihart and Slade (1990).

The primary study area (hereafter referred to as the forest grid) was an early- to mid-successional forest containing black walnut (*Juglans nigra*), hackberry (*Celtis occidentalis*), Osage orange (*Maclura pomifera*), and red elm (*Ulmus rubra*) as its dominant trees. Other abundant plants in this forest included rough-leaved dogwood (*Cornus drummondii*), coralberry (*Symphoricarpos orbiculatus*), wild gooseberry (*Ribes missouriense*), honey locust (*Gleditsia triacanthos*), redbud (*Cercis canadensis*), poison ivy (*Rhus radicans*), and Virginia creeper (*Parthenocissus quinquefolia*). The forest grid contained limestone rocky outcrops (<4 m high) on the northern and western edges and a small intermittent stream ran through the northeastern corner.

Trapping regimes differed somewhat between the two sites, but both were extremely effective at catching white-footed mice. Commercial scratch grain was used as bait for both grids. Upon capture, each animal was identified to species, and age, sex, reproductive condition, body mass, and parasites were recorded prior to it being released at the point of capture. Animals were aged as juveniles, subadults, or adults based on a combination of pelage color, hind foot length, and body mass following Kamler et al. (1998). All species of small mammals captured were marked on first capture following the recommendations of Wood and Slade (1990). The forest grid contained 72 trap stations, spaced about 15 m apart. Each trap station had one Sherman live trap (8 × 9 × 23 cm) covered by a wooden board. Traps were set every 2 weeks during the breeding seasons in 1995–1997 (April–June and September–November) and during the entire botfly seasons in 1998 and 1999 (June–December) to document botfly seasonality, but were set monthly at other times. The traps were opened for three consecutive days and collapsed between trapping periods.

Population estimates were calculated following Chapman's modified Lincoln–Peterson estimate for closed populations (Wilson et al. 1996). The proportion of animals harboring *Cuterebra* during each trapping period was multiplied by this population estimate, thereby giving a predicted number of animals infected. Chi-square was used to analyze differences in host sex, age, resident status, and prevalence of parasitism among years. Resident status is an indicator of whether mice permanently reside on the grid; mice can be residents or transients. Herein, residents are defined as animals caught over at least a 4-week span in 1995–1997, and caught over at least a 2-week span in 1998 and 1999. Transients are defined as animals only caught in one trapping period and not captured thereafter. Residency time (persistence) was calculated in two ways. First, residency time was calculated as the number of weeks between the first and last capture of an individual following Clark and Kaufman (1990) and papers cited therein). The second method compares the residence time of host and nonhost mice to a geometric distribution (Slade 1995). Residency time (using both methods of calculation) was subjected to an analysis of variance (general linear model—MINITAB 1996) on ranked data to assess differences in infection by botflies, sex, and year. To standardize the 4 years, persistence tests were based only on monthly trapping data. Only animals captured during the botfly season (defined as the months that botflies were found on the host) and in more than one trapping period were considered.

To assess whether hosting multiple botflies had an effect on persistence, residence time also was subjected to an analysis of variance (using both methods previously described; general linear model—MINITAB 1996) on ranked data to assess differences in botfly load and year. Three categories of botfly load were used—no botflies, one botfly, or two or more botflies simultaneously.

Botfly data on the old-field grid were collected only incidentally until 1998. For this reason, many of the comparisons of the forest grid to the old-field grid use only data from 1998 and 1999. Weather data, including the high and low temperatures for each day and the deviation from normal temperature for each month, from 1995 to 1998 were obtained from Dr. Hampton Shirer, Hillview Climate Station, Lawrence, Kansas. This project was undertaken with the approval of the Kansas Ecological Reserves and the University of Kansas Animal Care and Use Committee; animal handling protocols were in accordance with the guidelines now outlined by the American Society of Mammalogists (Sikes et al. 2016). Voucher specimens of the botflies will be deposited at the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln. Voucher specimens of the rodents were deposited at the Natural History Museum, University of Kansas, Lawrence. Primary host is defined here as the host species in which *Cuterebra* are most often found, and also that host in which the parasitic botflies appear most successful in completing its life cycle. Incidental hosts are defined here as the host species in which botflies were rarely encountered a despite numerous captures on either grid and elsewhere in Kansas.

RESULTS

Peromyscus leucopus was the dominant species of mammal on the forest grid, and accounted for 77.7% (*n* = 602) of all 775 captures. Other species regularly captured included short-tailed shrews (*Blarina brevicauda*; 10.6%, *n* = 80), eastern woodrats (*Neotoma floridana*; 5.2%, *n* = 39), prairie voles (*M. ochrogaster*; 1.9%, *n* = 15), and eastern chipmunks (*Tamias striatus*; 1.8%, *n* = 14). Incidental captures included western harvest mice (*Reithrodontomys megalotis*; *n* = 8), woodland voles (*Microtus pinetorum*; *n* = 6), hispid cotton rats (*Sigmodon hispidus*; *n* = 5), gray squirrel (*Sciurus carolinensis*; *n* = 1), meadow jumping mouse (*Zapus hudsonius*; *n* = 1), eastern mole (*Scalopus aquaticus*; *n* = 1), least shrew (*Cryptotis parvus*; *n* = 1), eastern cottontail (*Sylvilagus floridanus*; *n* = 1), and long-tailed weasel (*Mustela frenata*; *n* = 1).

Peromyscus leucopus is the mammal most commonly infected by botflies on both grids. Two botflies were raised to maturity to identify to species and a free-ranging adult female botfly was captured on the forest grid on 15 September 2001; all were identified as *C. fontinella* (Clark, 1815), a species frequently associated with white-footed mice. *Cuterebra fontinella* usually develops in the inguinal region of *P. leucopus*, but one large 3rd instar was observed on the neck. Five *N. floridana* were found infected by botflies on the forest grid in 1995. The botflies on these woodrats were on the neck and were most likely the woodrat botfly, *Cuterebra americana* (Fabricius, 1775), which is a common species in the region. On the old-field grid, other hosts for larval *Cuterebra* included *M. ochrogaster* (*n* = 10), *P. maniculatus* (*n* = 2), *R. megalotis* (*n* = 3), and *Z. hudsonius* (*n* = 1), and all are considered incidental hosts.

On the forest grid for the 5 years combined, 602 *P. leucopus* were examined and 140 (23.3%) were infected during the botfly seasons (June–December). The percentage of mice infected during 1995, 1996, 1997, and 1999 were not significantly different (22.4% [*n* = 58], 17.3% [*n* = 133], 17.1% [*n* = 82], and 22.7% [*n* = 119], respectively; $\chi^2 = 1.790$, $P = 0.617$). However, in 1998, 32.4% (*n* = 210) of mice were infected, which is significantly higher than in the other 4 years ($\chi^2 = 9.802$; $P = 0.044$). The earliest and latest dates of botfly infection were 21 June (1996 and 1998) and 5 December (1995 and 1998; Table 1), respectively. No correlation was found between population density of *P. leucopus* and proportion of individuals infected by botflies. The largest proportion of infected mice occurred in July and August. The largest proportion of mice hosting botflies over all 4 years was 86% (six out of seven individuals) in August 1995. Each year, the lowest proportion of mice infected was mid-September. The year 1998 is unusual, with 60% of the mice infected in late September. In three of the years (1995, 1997, and 1998), no infected mice were captured in mid-September; however, in mid-September 1996, 1 out of 10 mice had a botfly. A smaller second peak of infection was observed in October and November each year with 10–30% of the mice infected.

The average number of botfly larvae per mouse was 1.2 on the forest grid. For all 5 years, 82% (*n* = 115) of the infected mice carried one botfly, 14% (*n* = 20) had two botflies, and

Table 1.—Number of white-footed mice (*Peromyscus leucopus*) infested with botfly larvae (*Cuterebra fontinella*) captured during 1995–1999 seasons on the forest grid at the University of Kansas Nelson Environmental Study area, ca. 12 km NNE Lawrence, Douglas County, Kansas. The first number indicates the number of individual white-footed mice hosting at least one botfly and the following number in parentheses indicates the total number of white-footed mice caught during that trapping session.

Year	June Early[a]	June Late[b]	July Early	July Late	August Early	August Late	September Early	September Late	October Early	October Late	November Early	November Late	December Early	Total	% infested
1995	0 (7)			2 (10)		6 (7)	1 (5)	0 (5)	1 (8)		1 (8)		2 (15)	13 (65)	20.0%
1996		1 (22)		8 (14)		5 (14)	1 (10)		1 (18)	4 (20)	3 (35)		0 (23)	23 (156)	14.7%
1997	0 (13)	0 (8)		4 (9)		3 (9)		0 (14)	3 (15)	3 (15)		1 (20)	0 (6)	14 (109)	12.8%
1998	0 (11)	3 (22)	9 (21)	17 (21)	8 (21)	1 (18)	0 (18)	9 (15)	7 (16)	4 (16)	3 (22)		2 (20)	63 (221)	28.5%
1999		0 (24)	7 (20)	5 (12)	2 (11)	3 (18)		3 (15)	2 (20)	4 (16)		1 (7)	0 (14)	27 (157)	17.2%
Total	0 (31)	4 (76)	16 (41)	36 (66)	10 (32)	18 (66)	2 (33)	12 (49)	14 (77)	15 (67)	7 (65)	2 (27)	4 (78)	140 (708)	19.8%
% infestation	0.0%	5.3%	39.0%	54.5%	31.3%	27.3%	6.1%	24.5%	18.2%	22.4%	10.8%	7%	5.1%		

[a] Early = day 1–14 of the month.
[b] Late = day 15–30 (31) of the month.

4% ($n = 5$) had three botflies. A higher number of botfly larvae infecting single mice was observed in 1998, with 24% of the infected mice carrying two or more botflies at one time, compared with 14% from 1995–1997 and 11% in 1999. The average number of botflies per host was higher in 1998 than in the previous years (1.3 compared with 1.2 for the previous 3 years combined). The average number of botflies per host was lower at 1.1 in 1999. Almost half (47%) of the infected individuals caught in 1998 had infections at two different times, compared with 25% during the 1995–1997 seasons. In 1999, 10 (63%) of 16 infected mice were infected a 2nd time, when they were captured with a different botfly (or botflies), again later during the season.

During the five botfly seasons combined, 57.6% of the *P. leucopus* captured were male and 42.4% were female. Correspondingly, 62.9% (88 of 140) of the mice with botflies were male mice and 37.1% (52 of 140) were female; prevalence rates did not differ between sexes ($\chi^2 = 2.033$, $P = 0.154$). This also was true of data collected in the old-field grid. If only the months when botflies were seen from 1989 to 1999 are considered, there is no significant difference in the sex ratio of infected and noninfected mice ($\chi^2 = 0.333$, $P = 0.564$). The same was true for just the 1998 season on the old-field grid ($\chi^2 = 0.077$, $P = 0.781$), the year in which botflies were most abundant.

Infection rates were greater for adult white-footed mice than for younger age classes. Among 140 infected mice on the forest grid only eight (5.7%) were subadults, and none were juveniles. Of the noninfected mice caught during the botfly season, 97 (21%) of 462 were subadults and juveniles. These frequencies were tested with chi-square ($\chi^2 = 17.424$) and were significantly different at the 0.0001 level of confidence.

A bimodal pattern of botfly prevalence was seen, especially in 1998 (Table 1). Although the botfly season in northeastern Kansas is distinctly bimodal, annual variation occurs in the timing and prevalence of the botflies. To get a better understanding of the reasons behind the differences, we compared weather data to botfly data. An early incidence of infected mice, as seen in June of 1996 and 1998, may have resulted from particularly warm spring temperatures, allowing the pupae in the soil to mature more quickly than during a cooler spring. Although April 1998 was recorded as having normal temperatures, May 1998 was about 7.5°F warmer than normal. May 1996 was slightly warmer than normal, but June parasitism in that year was only 4.5% (1 of 22 mice). Conversely, when the spring was cool, incidence of botfly infections occurred later. April and May of 1995 and 1997 were both about 4°F cooler than average.

The occurrence of infection among transient (captured once) and resident adult mice over the 4 years is significantly different ($\chi^2 = 7.78$, $P = 0.005$), with a higher proportion of resident mice being infected compared with the transient mice. Of infected individuals, 69 were residents and 13 were transients. Of noninfected individuals, 50 were residents and 27 were transients. The incidence of infection per month in resident and transient mice was not significantly different ($\chi^2 = 0.484$, $P = 0.487$).

When using the first method to calculate persistence (number of weeks between an animal's first and last capture), resident infected mice appear to remain on the forest grid longer (median: 19 weeks, mean: 23.1 weeks, range: 4–99 weeks) than did uninfected individuals (median: 13 weeks, mean: 17.5 weeks, range: 3–89 weeks) with no differences between years or sex. The persistence of nonhost mice does not depart significantly from a geometric distribution ($\chi^2 = 13.34$, d.f. = 14, $P = 0.5$), which indicates that the probability of disappearance is independent of how long the mouse had been on the grid. Therefore, we were able to compare the residence time of nonhost animals to that of host animals starting the first time they were seen with a botfly. In the second method of calculating persistence, we compare the residence time of nonhost animals with that of host animals starting at the time they were first seen with a botfly larva. With this comparison, residence time of infected versus noninfected mice did not differ with sex, year, or infection status. No effect of simultaneous botflies was found with either method on the persistence of an individual (method 1: $P = 0.176$; method 2: $P = 0.238$). When using persistence as the first to the last capture (method 1), animals that hosted botflies seem to live longer than nonhosts and animals that host 2 or more botflies simultaneously seemed to live longer than those than those that hosted 1 ($P = 0.024$). However, when measuring persistence from when a host animal was first caught with a botfly (method 2), no effect of multiple botflies was found on the persistence of an individual on the grid ($P = 0.104$).

Discussion

Peromyscus leucopus is the most common species of small mammal infected by *Cuterebra* at the University of Kansas Field Station in northeastern Kansas. It is our assumption herein that all or most of the botflies found on *P. leucopus* were C. *fontinella*. *Peromyscus maniculatus* was captured with a botfly on only two occasions on the old-field grid in 10 years; deer mice were not captured on the forest grid. The other small mammal species captured at the Field Station with botflies include *M. ochrogaster*, *R. megalotis*, and *Z. hudsonius*. The botflies parasitizing these other rodents may have been *C. fontinella* or perhaps another species of *Cuterebra*; identifications were not made. *Microtus ochrogaster*, *R. megalotis*, and *Z. hudsonius* appear to be aberrant hosts, on which the larval *Cuterebra* probably do not develop to eclosion and/or may cause significant pathological effects on the host (Catts 1982). This is the first record of *M. ochrogaster* being infected by *Cuterebra*, with 10 infected individuals being caught in 23 years of trapping on the old-field grid with several hundred animals being handled and examined for infection by botflies. In *M. ochrogaster*, botfly larvae were observed on the side, venter, neck, and scrotum of the animals. Other species of *Microtus*, such as *M. chrotorrhinus*, *M. oregoni*, *M. pennsylvanicus*, *M. pinetorum*, and *M. townsendii*, also have been reported as incidental hosts (Timm 1985). Voles of the genus *Microtus* are

thought to have shorter history of association with botflies because the larvae tend to show a lack of site specificity on the host (Getz 1970; Boonstra et al. 1980; Galindo-Leal 1997) and the negative impact on red-backed voles (*Myodes gapperi*) observed by Lemaître et al. (2009) also may be a factor of these voles being incidentals host for botflies.

During 10 years of trapping on the old-field grid, only three *Reithrodontomys* and one *Zapus* were infected by botflies. Correspondingly, both genera rarely are reported as hosts for *Cuterebra* (Test and Test 1943; Whitaker 1963; Clark and Kaufman 1990). In addition to *C. fontinella*, two other species of *Cuterebra* are known from this area—*C. americana* (Fabricius, 1775) whose primary host is *N. floridana* and a species of *Cuterebra* we have yet to identify from *S. floridanus*.

Data from the forest grid and old-field grid and our observation of an adult *C. fontinella* from the grid in mid-September document that reproduction can occur twice in this population during the summer–autumn breeding season, with adults emerging both in late spring and again in the late summer–early autumn. Adult botflies live for only several days. Length of the botfly season and proportion of mice infected showed similar patterns on the two grids over the 5-year study, although some variation occurred. Botfly larvae were first observed in late June–early July, with infection being greatest in late July to late August. Very few mice were infected in September, but a second smaller peak of abundance occurred in October. Botfly larvae were last recorded in late November–early December. This second peak in October, along with the observation of an adult botfly in mid-September, indicates that *C. fontinella* was infecting rodents in a bimodal pattern at this latitude (39°N), with summer and autumn population peaks, suggesting that there was both early summer and early autumn reproduction.

Although parasitism by *Cuterebra* on *P. leucopus* is bimodal in northeastern Kansas, *C. fontinella* in more northern regions has a distinct single breeding season. Catts (1982) reported August–October as the most common months for *C. f. fontinella* parasitism of *P. leucopus*. Documented seasonalities for *Cuterebra* parasitism of *P. leucopus* include June–September in Maryland (Durden 1995), July–October in Minnesota (Timm and Cook 1979), and July–September in Connecticut (Miller and Getz 1969). Other documented seasonalities closer to the latitude of our study include June–September in the tallgrass prairie of Kansas (Clark and Kaufman 1990) and July–January in north-central Oklahoma (Goertz 1966). The latter study, done in a woodlot, found mice infected in August and October, but no infected mice were seen in September. This corresponds with the findings of our study that few botflies are observed in September. In southeastern Kansas, eastern woodrats (*N. floridana*) infected with the botfly *C. americana* (reported as *C. beameri*) were brought into a laboratory with adult botflies emerging in September of both years (1941 and 1942) indicating the possibility of two generations of the flies each year (Beamer et al. 1943). Smith (1977) reported botflies on *P. maniculatus* show distinct spring and autumn peaks of abundance in western Montana. *Cuterebra* were reported to have two generations during the length of the botfly season

elsewhere (Smith 1977; Wood 1987; Durden 1995; Jennison et al. 2006).

The beginning of the botfly season probably was related to spring temperature and its effect on pupal development. Warm spring temperatures probably triggered the early incidence of infected mice in June 1996 and 1998 by allowing the pupae in the soil to mature more quickly than during a cooler spring. When spring temperatures were cooler, as in 1995, 1997, and 1999, botfly infections were 1st seen in July.

We found botfly larvae in white-footed mice into December in 2 years, 1995 and 1998. The December trapping periods for 1996 and 1997 occurred a week later (15 December in both years) than those for 1995 and 1998 (5 December in both years), which suggests that by trapping later, we missed seeing infected mice at the end of those botfly seasons. Hunter et al. (1972) suggested that the early infection of botflies seen on deer mice (*P. maniculatus*) in 1 year of a 3-year study was due to a warm May. Sillman (1955) reported a delay in infection because of a particularly cool April and May.

The mean infection rate we found over the 5-year study was 1.2 botflies per host mouse. The number of botflies per host was higher in 1998 than in the previous 3 years combined. However, hosting multiple botflies at one time does not seem to have detrimental effects on the residence time of the mice on the grid. Other reported means in the literature for *P. leucopus* include 1.4 in Minnesota (Timm and Cook 1979), 1.2 in Virginia (Hensley 1976), and 1.8 in Michigan (Wecker 1962).

Prevalence of botfly parasitism of *P. leucopus* (23.3%) on our forest grid is within the range of values reported elsewhere (Goertz 1966; Dunaway et al. 1967; Miller and Getz 1969; Clark and Kaufman 1990; Durden 1995). We found no significant difference in sex ratio of infected and noninfected mice, which is consistent with findings from Georgia, Kansas, Michigan, Minnesota, and Tennessee (Wecker 1962; Dunaway et al. 1967; Timm and Cook 1979; Clark and Kaufman 1990; Durden 1995). However, other studies have reported a significantly higher rate of parasitism in males than in females (Sealander 1961; Goertz 1966; Hensley 1976; Xia and Millar 1990). Galindo-Leal (1997) reported botfly parasitism of rock mice (*Peromyscus difficilis*) to be female-biased in the autumn and male-biased in the winter.

The age structure of mice infected with botflies was primarily adults with only 6% of the infected mice being subadults, and no incidence of juveniles being infected. This corresponds with results from Ontario and Kansas (Sealander 1961; Clark and Kaufman 1990). In Minnesota, Timm and Cook (1979) also reported that older mice were more likely to be infected—of infected mice, 51% were adults, 30% were subadults, and 19% were juveniles. In Virginia however, Hensley (1976) found subadults, especially males, to have a greater prevalence of botflies than either juveniles or adults.

The reported effects of botfly parasitism on populations of *Peromyscus* have been variable depending on the species, latitude, and habitat. We defined resident status is an indicator of whether mice permanently reside on the grid; mice can be residents or transients. We found a significantly greater number of

resident mice were infected when compared to transient mice; however, a similar proportion of residents and transients were infected with botflies each month. Resident mice may be infected more often than transient mice because they are exposed to botfly eggs for a longer period of time. The similar proportion of residents and transients infected each month corresponds with the findings of Hunter et al. (1972), and further supports their hypothesis that neither residents nor transients are favored to contact the eggs and become infected.

Trapping on grids provides only a snapshot of transients (single observation) and at least two observations for residents. By simply increasing the sample size of observations of an individual (defined as residents), the probability of seeing an infection for that animal in at least one trapping session is increased. The expected infection rate would increase further if those individuals were trapped in additional months simply because they are seen more often.

No consistent relationship has been found between botfly parasitism and longevity of mice on trapping grids. Several studies have reported that infected mice (*Peromyscus*) reside on the study area longer than mice without botflies (Goertz 1966; Hunter et al. 1972; Clark and Kaufman 1990; Galindo-Leal 1997), whereas other studies report the opposite (Miller and Getz 1969; Boonstra et al. 1980). When using methods similar to those of previous studies (Goertz 1966; Hunter et al. 1972; Clark and Kaufman 1990), we found similar results: when residence time of mice is calculated simply as the number of weeks on the grid, mice with longer times on the grid have higher prevalence of parasites. This could be interpreted in two ways: mice that live longer are more likely to be infected with botflies, or perhaps the opposite is true, botflies are beneficial for the mice, because those with more botflies live longer. Because of this confounding/conflicting problem, we compared the residence time of nonhost animals to that of host animals starting at the time that the individuals were first seen with a botfly. This method was used because the probability of disappearance of the mice was statistically independent of how long they had been on the grid. By using this method of analysis, host mice and nonhost mice have no significant difference in the amount of time they remain on the grid. Therefore, greater frequency or greater incidence seems simply related to longer time under observation.

Previous studies suggesting that mice residing longer on a grid have a greater incidence of botflies explained this in a variety of ways. The suggestion was made (Wecker 1962) that infected mice do not emigrate as often as noninfected mice, possibly because botflies hinder mobility. However, Goertz (1966) concluded that infected mice had larger home ranges, and because of this may be caught more often in traps, have increased exposure to botfly eggs, or both. Hunter et al. (1972) reported no difference in the distance moved between infected and healthy mice, and suggested that mice that live on the plot longer have a longer exposure to infection, therefore have a greater incidence of parasitism by botflies. Increased persistence of infected mice on grids being equated to increased survivorship is an assumption that may not be correct if in fact infected mice only apparently have an increased persistence.

Our results, with this new more rigorous method of calculating persistence, provide evidence that host mice and nonhost mice persist on the grid a similar length of time, indicating that parasitism by botflies has no detrimental effect on the host residence time. We suggest that this method of analysis be used in future studies and to reassess previous studies.

Herein, we demonstrate the need for multiyear investigations examining the effects of parasites on a host population. Yearly variation in botfly seasonality and the proportion of mice infected could not have been documented in a 1- or even 2-year study. We found the highest rate of infection through all years was in 1998, with a higher number of larvae per host individual than we found in other years; it was clearly a "good" year for botflies. However, botflies did not have an obviously detrimental effect on the mice for the parameters measured herein that year or the subsequent year because the number of mice on the grid and their persistence was not different from that of other years. Analyses of trapping results from 1999 reveal that botfly numbers and density of *P. leucopus* were similar to those observed in the period 1995–1997. These field results provide further evidence that white-footed mice have evolved a tolerance to botfly parasitism.

The emergence times and distinct bimodal peaks in abundance we found in *C. fontinella* on white-footed mice in Kansas, along with similar findings by others, suggest that abiotic factors such as soil temperature, and perhaps soil moisture, are among the primary determinates of botfly emergence time and abundance. Abiotic factors probably play a greater role in botfly abundance than previously assumed and we suggest that other researchers explore this hypothesis with their data. One biotic factor that may play a role in botfly abundance is predation upon pupae by shrews, moles, or rodents. After *P. leucopus*, *B. brevicauda* was the most common species captured on the forest grid. There is little documentation that host population dynamics have a major impact upon botfly populations or that botflies have a major impact upon host populations.

Larval botflies are difficult to identify and that coupled with the difficulty in rearing larvae through to the adult stage has resulted in many previous studies publishing their work identifying their botflies to simply *Cuterebra* sp., or in making an assumption as to the species of botfly involved. Here we were able to raise two larvae from white-footed mice to the adult stage and captured a free-ranging adult on our forest grid—all were identified as *C. fontinella*. Future workers might explore using noninvasive molecular techniques to identify developing larvae in the host without damaging it thus obtaining species identifications for all botflies encountered, allowing them to develop and reassessing host specificity in these parasites.

Many questions remain unanswered about the details of botfly parasitism in small mammals and many specifics about the life cycle of botflies are still unknown. There is much to learn about this host–parasite relationship, but we can conclude that *Cuterebra* infections on *Peromyscus* have little negative impact upon population dynamics of the mice. Little documentation exists to show that botflies have a major impact upon

individual hosts or host populations or that host population dynamics have a major impact upon botfly populations.

LITERATURE CITED

Beamer R.H., Penner L.R., Hibbard C.W. 1943. Some notes on the biology of the pack rat cuterebrid (*Cuterebrid beameri* Hall) in Kansas. Journal of the Kansas Entomological Society 16:47–50.

Boonstra R., Krebs C.J., Beacham T.D. 1980. Impact of botfly parasitism on *Microtus townsendii* populations. Canadian Journal of Zoology 58:1683–1692.

Burns C.E., Goodwin B.J., Ostfeld R.S. 2005. A prescription for longer life? Bot fly parasitism of the white-footed mouse. Ecology 86:753–761.

Catts E.P. 1965. Host-parasite interrelationships in rodent bot fly infections. Transactions of the North American Wildlife and Natural Resources Conference 30:184–196.

Catts E.P. 1982. Biology of New World bot flies: Cuterebridae. Annual Review of Entomology 27:313–338.

Clark B. 1815. An essay on the bots of horses and other animals. Privately published by author, London, United Kingdom.

Clark B.K., Kaufman D.W. 1990. Prevalence of botfly (*Cuterebra* sp.) parasitism in populations of small mammals in eastern Kansas. American Midland Naturalist 124:22–30.

Cramer M.J., Cameron G.N. 2006. Effects of bot fly (*Cuterebra fontinella*) parasitism on a population of white-footed mice (*Peromyscus leucopus*). Journal of Mammalogy 87:1103–1111.

Dunaway P.B., Payne J.A., Lewis L.L., Story J.D. 1967. Incidence and effects of *Cuterebra* in *Peromyscus*. Journal of Mammalogy 48:38–51.

Durden L.A. 1995. Bot fly (*Cuterebra fontinella fontinella*) parasitism of cotton mice (*Peromyscus gossypinus*) on St. Catherines Island, Georgia. Journal of Parasitology 81:787–790.

Fabricius, J.C. 1775. Systema entomologiae: sistens insectorvm classes, ordines, genera, species, adiectis synonymis, locis, descriptionibvs, observationibvs. Officina Libraria Kortii, Flensburg et Leipzig, Federal Republic of Germany.

Galindo-Leal C. 1997. Infestation of rock mice (*Peromyscus difficilis*) by botflies: ecological consequences of differences between sexes. Journal of Mammalogy 78:900–907.

Getz L.L. 1970. Botfly infestations in *Microtus pennsylvanicus* in southern Wisconsin. American Midland Naturalist 84:187–197.

Gingrich R.E. 1979. Effects of some factors on the susceptibility of *Peromyscus leucopus* to infestation by larvae of *Cuterebra fontinella* (Diptera: Cuterebridae). Journal of Parasitology 65:288–292.

Gingrich R.E., Barrett C.C. 1976. Natural and acquired resistance in rodent hosts to myiasis by *Cuterebra fontinella* (Diptera: Cuterebridae). Journal of Medical Entomology 13:61–65.

Goertz J.W. 1966. Incidence of warbles in some Oklahoma rodents. American Midland Naturalist 75:242–245.

Hensley M.S. 1976. Prevalence of cuterebrid parasitism among woodmice in Virginia. Journal of Wildlife Diseases 12:172–179.

Hunter D.M., Sadleir R.M.F.S., Webster J.M. 1972. Studies on the ecology of cuterebrid parasitism in deer mice. Canadian Journal of Zoology 50:25–29.

Jaffe G., Zegers D.A., Steele M.A., Merritt J.F. 2005. Long-term patterns of botfly parasitism in *Peromyscus maniculatus*, *P. leucopus*, and *Tamias striatus*. Journal of Mammalogy 86:39–45.

Jennison C.A., Rodas L.R., Barrett G.W. 2006. *Cuterebra fontinella* parasitism on *Peromyscus leucopus* and *Ochrotomys nuttalli*. Southeastern Naturalist 5:157–167.

Kamler J.F., Pennock D.S., Welch C., Pierotti R.J. 1998. Variation in morphological characteristics of the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*P. maniculatus*) under allotopic and syntopic conditions. American Midland Naturalist 140:170–179.

Lemaître J., Fortin D., Montiglio P.-O., Darveau M. 2009. Bot fly parasitism of the red-backed vole: host survival, infection risk, and population growth. Oecologia 159:283–294.

Miller D.H., Getz L.L. 1969. Botfly infections in a population of *Peromyscus leucopus*. Journal of Mammalogy 50:277–283.

MINITAB. 1996. MINITAB reference manual, release II for Windows™. Minitab, Inc., State College, Pennsylvania, USA.

Munger J.C., Karasov W.H. 1991. Sublethal parasites in white-footed mice: impact on survival and reproduction. Canadian Journal of Zoology 69:398–404.

Munger J.C., Karasov W.H. 1994. Costs of bot fly infection in white-footed mice: energy and mass flow. Canadian Journal of Zoology 72:166–173.

Nichols L.B. 1994. The effect of bot fly (*Cuterebra*) infestation on cold-night trap mortality in cactus mice (*Peromyscus eremicus*). Southwestern Naturalist 39:383–386.

Ruedebusch C.R. 1999. Annual and demographic comparisons of botfly parasitism of small mammals in Northeastern Kansas. Master's thesis, University of Kansas, Lawrence, Kansas, USA.

Sabrosky C.W. 1986. North American species of *Cuterebra*, the rabbit and rodent bot flies (Diptera: Cuterebridae). Bulletin of the Entomological Society of America 11:1–240.

Sealander J.A. 1961. Hematological values in deer mice in relation to botfly infection. Journal of Mammalogy 42:57–60.

Sikes R.S., The Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–688.

Sillman E.I. 1955. Studies on the biology of a cuterebrid (Cuterebridae: Diptera) infesting *Peromyscus leucopus noveboracensis* Fisher, the white-footed mouse in southern Ontario. Entomological Society of Ontario Annual Report 86:89–97.

Slade N.A. 1995. Failure to detect senescence in persistence of some grassland rodents. Ecology 76:863–870.

Slansky F. 2007. Insect/mammal associations: effects of cuterebrid bot fly parasites on their hosts. Annual Review Entomology 52:17–36.

Smith D.H. 1977. The natural history and development of *Cuterebra approximata* (Diptera: Cuterebridae) in its natural host, *Peromyscus maniculatus* (Rodentia: Cricetidae), in western Montana. Journal of Medical Entomology 14:137–145.

Smith D.H. 1978. Vulnerability of bot fly (*Cuterebra*) infected *Peromyscus maniculatus* to shorttail weasel predation in the laboratory. Journal of Wildlife Diseases 14:40–51.

Swihart R.K., Slade N.A. 1990. Long-term dynamics of an early successional small mammal community. American Midland Naturalist 123:372–382.

Test F.H., Test A.R. 1943. Incidence of dipteran parasitosis in populations of small mammals. Journal of Mammalogy 24:506–508.

Timm R.M. 1985. Parasites of New World Microtus. In: Tamarin R.H., editor. Biology of New World *Microtus*. Vol. 8. Special Publication, The American Society of Mammalogists, Lawrence, Kansas, USA; p. 455–534.

Timm R.M., Cook E.F. 1979. The effect of bot fly larvae on reproduction in white-footed mice, *Peromyscus leucopus*. American Midland Naturalist 101:211–217.

Timm R.M., Lee R.E., Jr. 1981. Do bot flies, *Cuterebra* (Diptera: Cuterebridae), emasculate their hosts? Journal of Medical Entomology 18:333–336.

Timm R.M., Lee R.E., Jr. 1982. Is host castration an evolutionary strategy of bot flies? Evolution 36:416–417.

Wecker S.C. 1962. The effects of bot fly parasitism on a local population of the white-footed mouse. Ecology 43:561–565.

Welch C.K. 1998. Population and demographic response of white-footed mice, *Peromyscus leucopus*, to experimental habitat fragmentation and uneven age forest management. Master's thesis, University of Kansas, Lawrence, Kansas, USA.

Whitaker J.O., Jr. 1963. A study of the meadow jumping mouse, *Zapus hudsonius* (Zimmerman), in central New York. Ecological Monographs 33:215–254.

Wilson D.E., Cole F.R., Nichols J.D., Rudran R., Foster M.S., editors. 1996. Measuring and monitoring biological diversity: standard methods for mammals. Smithsonian Institution Press, Washington, District of Columbia, USA.

Wood D.M. 1987. Oestridae. In: McAlpine J.F., editor. Manual of Nearctic Diptera. Vol. 28. Research Branch, Agriculture Canada, Toronto, Ontario, Canada; p. 1147–1158.

Wood M.D., Slade N.A. 1990. Comparison of ear-tagging and toe-clipping in prairie voles, *Microtus ochrogaster*. Journal of Mammalogy 71:252–255.

Xia X., Millar J.S. 1990. Infestations of wild *Peromyscus leucopus* by bot fly larvae. Journal of Mammalogy 71:255–258.