A laboratory study on the effect of *Paraiotonchium autumnale* parasitism on the longevity of *Musca autumnalis*

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SUMMARY

Paraiotonchium autumnale (Nickle) (Tylenchida: Iotonchiidae) causes parasitic sterilization in female *Musca autumnalis* DeGeer (Diptera: Muscidae). In addition to sterilization, *P. autumnale* causes physiological and behavioural changes within its host. However, there have been no records of reduced host lifespan in this system. Studies were conducted in 2009 and 2010 with wild *M. autumnalis* collected as larvae from cow dung pats from Browns Valley, California, USA. Field-collected larvae were reared to adulthood and flies of the same eclosion dates were caged together, regardless of whether or not they were parasitized. Dead flies were collected daily, and parasitism status was confirmed by dissection. Due to the very different effects of *P. autumnale*-parasitism on male versus female face flies, flies were analysed separately by sex. Kaplan-Meier analysis revealed a non-significant difference in longevity between non-parasitized and parasitized flies for female and male flies in 2009. In 2010, however, significant differences ($P < 0.05$) were found between parasitized and non-parasitized female and male flies. In 2010, the median time to death for non-parasitized female flies was 20 days and 15 days for parasitized females. The 2010 median survival time for non-parasitized male flies was 33 days and 15 days for parasitized males. This is a parasite-mediated cost to the host that has not been recorded previously. Based on our results and results from another published study we suggest that the potential for sterilizing parasites to alter host longevity is condition dependent.

Key words: *Musca autumnalis*, *Paraiotonchium autumnale*, parasitic sterilization, host longevity.

INTRODUCTION

Host-sterilizing parasites are thought to have evolved in response to the tradeoff between maximum parasite reproduction and host longevity (Baudoin, 1975; Ebert and Hamilton, 1996; Lafferty and Kuris, 2009). By exploiting non-vital organs and physiological processes, parasites can maximize host tissue and energy use while minimizing parasite-induced host mortality (Obrebski, 1975). In most cases the differences in reproductive output between parasitized and non-parasitized individuals can be readily compared with egg counts and dissections. However, sterilizing parasites can not only impact reproductive output but also alter host longevity. Host longevity is a more subtle property of fitness that can largely influence the host-parasite relationship. Based on laboratory studies, the effects of sterilizing parasites on host longevity range from significantly increasing host lifespan (see Hurd *et al*. 2001) to significantly decreasing host lifespan (see Jaenike *et al*. 1995). Therefore, for a given relationship between a sterilizing parasite and its host, comparing reproductive rates between parasitized and non-parasitized hosts is not enough. It is important to determine which components of host fitness are affected by parasitism, especially when assessing host and parasite population dynamics.

At the population level, mathematical models have shown that the effects of parasitism on the host (e.g. parasitic sterilization, reduced longevity) are important determinants of host abundance in natural systems (Anderson and May, 1978; May and Anderson, 1978). Parasite-induced reductions in host fecundity and longevity reduce both the mean net reproduction rate in host populations. However, a parasite that is capable of reducing both fecundity and longevity can result in unstable population dynamics (May and Anderson, 1978). In past studies, the effect of *Paraiotonchium autumnale* (Nickle) (Tylenchida: Iotonchiidae) parasitism on natural face fly (*Musca autumnalis* DeGeer [Diptera: Muscidae]) populations has been evaluated based solely on the proportion of oviposition prevented by parasitic sterilization. For example, Krafsur *et al*. (1983) calculated modest reductions of 2·4–6·3% in net reproductive rate based on prevalence of parasitized flies in central Iowa, USA. However, this may be an underestimate if *P. autumnale* parasitism also alters face fly longevity.

An important detail to consider when comparing effects of a sterilizing parasite on its host is to assess the effects of parasitism separately by sex. Parasitism can affect male and female hosts in very different ways, which translates to different fitness costs (Poulin, 1996). For example, in the case of

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P. autumnale and face flies, parasitism results in parasitic sterilization among female flies but not among male flies (Nappi, 1973). Male face flies are considered dead-end hosts. It is possible that parasitism may be more lethal to one sex than the other especially if one sex is not a 'natural host' (Poulin, 1996; Druilhe *et al*. 2002).

Several studies have characterized the behavioural and physiological differences between parasitized and non-parasitized face flies (Stoffolano, 1967; Stoffolano and Streams, 1971; Nappi, 1973; Kaya *et al*. 1979; Chirico, 1990, 1994), but few have specifically focused on differences in longevity. A previously published longevity study reported that parasitization did not affect face fly lifespan, but no statistical analysis was done (Robinson and Combs, 1976). The sheer number of nematodes that is usually present in the abdomen of a single fly would suggest that survival would be affected. A study by Geden (1997) detected significantly decreased lifespan among female house flies (*Musca domestica* Linnaeus) (Diptera: Muscidae) parasitized with a nematode in the genus *Paraiotonchium*. Here we hypothesized that parasitized face flies would have a reduced lifespan compared to non-parasitized individuals. The main objectives of this laboratory study were to determine if *P. autumnale* alters face fly longevity and determine if there are differential effects of parasitism based on host sex.

MATERIALS AND METHODS

The impact of *P. autumnale* on the longevity of *M. autumnalis* adults was evaluated in the laboratory between May and September in 2009 and 2010. *Musca autumnalis* eggs and larvae were obtained from field-collected cattle dung pats from the University of California Sierra Foothill Research and Extension Center in Browns Valley, California. Flies that eclosed from pupae collected from these dung pats were the subjects of this study.

Natural history of the host-parasite relationship

Paraiotonchium autumnale is a host-specific nematode parasite of the face fly, that causes parasitic sterilization in female flies (Nickle, 1967; Stoffolano and Streams, 1971). This parasite's life cycle alternates between gamogenetic and parthenogenetic generations and is linked with its host's life cycle (Stoffolano, 1970). Initial invasion of hosts takes place in cow dung by mated gamogenetic female nematodes. Once inside the host's haemocoel, the gamogenetic female continues to mature as the face fly larva goes on to complete its life cycle. Following the eclosion of the adult fly, the gamogenetic female produces a generation of parthenogenetic female nematodes. These parthenogenetic females produce the second generation of sexually reproducing male and female nematodes. In female face flies, these nematodes invade the ovaries. While healthy female flies oviposit on dung pats, parasitized female face flies deposit male and female 4th-stage juvenile nematodes ('nemaposit') (Kaya *et al*. 1979; Kaya and Moon, 1980). The nematodes moult and mate in the dung. The mated female nematode is the infective stage that then searches for a face fly larva host.

Dung collection

Dung pats were collected from pastures where we observed female *M. autumnalis* activity on fresh dung pats and respiratory stalks of eggs were visible in the dung. The collection of dung pats based on these two criteria allowed for increased likelihood of obtaining an abundance of fly puparia (Teskey, 1969). A spade was used to collect an intact individual dung pat which was placed into a Sterilite® 5·7-litre plastic shoebox (34·6 cm L × 21·0 cm W × 12·4 cm H) (Sterilite Corporation, Townsend, MA, USA). Each shoebox was covered with a mesh lid and transported in a cooler to the laboratory. Ten to 15 dung pats were collected monthly.

Collection of puparia

Upon returning to the laboratory, ca. 1 kg of dry coarse grit sand (0·05–1 mm particle size) was added to each shoebox between the dung pat and the edges of the shoebox. The sand provided a medium for *M. autumnalis* pupariation. The shoeboxes were kept at ambient temperature (23 °C) and 30 ± 2% relative humidity (RH). On average, the larvae emerged from the dung pat and initiated pupariation within 7 days of collection. Two days following pupariation, a household stainless steel strainer (22·9 cm diameter) was used to separate the puparia from the sand. The puparia from dung pats collected on the same day were stored individually in 29·6 ml Solo® soufflé portion cups (Solo Cup Company, Highland Park, IL, USA). The puparia were kept in a 25 °C incubator (23 ± 2% RH) with a photoperiod of 16 h light: 8 h dark, and checked daily for eclosion.

Longevity experiment

Mating is essential for parasitized female flies to nemaposit, and because we do not know the consequences that could arise from females withholding their nematode loads, we caged male and female flies together to allow for mating and the parasite life cycle to proceed normally. Additionally, parasitized male face flies successfully mate with and inseminate parasitized and non-parasitized females, and therefore all flies in our study had a chance to mate, and we assumed there was no bias in terms of

Table 1. Parasitism class and corresponding stages of *Paraiotonchium autumnale* present in *Musca autumnalis*

Parasitism class	Stages of parasite development
C-1	First-generation gamogenetic female nematode present
C-2	Parthenogenetic females present
C-3	Second-generation gamogenetic male and female nematodes present
C-4	In female face flies, nematodes have invaded the ovaries

the costly process of mating (Nappi, 1973). Within 24 h of eclosion, 20 male and 20 female flies from the same emergence date were released into steel-mesh, sleeved cages (30·5 cm L × 30·5 cm W × 30·5 cm H). Each cage was placed 4·2 cm from a clamp-on aluminum lamp with a shade (21·6 cm diameter) with a 60 W incandescent light bulb. The photoperiod was set to 16 h light: 8 h dark which maintained the temperature of the cages between 27 °C and 29 °C and 29 ± 2% RH. Water in a 236·6 ml, 4·9 cm × 9 cm (height × diameter) hi-plas® polyethylene cup (Highland Plastics Inc. Mira Loma, CA, USA) with a moist cotton wick, 5 sugar cubes, and ca. 10·5 g of powdered skim milk in a 60 ml Petri dish were provided and replenished as needed. Slices of fresh beef liver (ca. 30 g) were hung with a paper clip inside the cages and were replaced weekly. Two hundred g of fresh cattle dung free of fly larvae was provided every 2 days in a 177 ml Solo® double poly-paper cup to allow for oviposition. There were 9 replicate cages in 2009 and 7 replicate cages in 2010.

When the flies were placed into the cages, the infection status of each fly was unknown. There is no evidence of the transfer of nematodes from parasitized to non-parasitized individuals during mating, and therefore we were not concerned about fluctuating levels of parasitism throughout the study (Nappi, 1973). The cages were checked daily and dead flies were collected and kept in a freezer (−17 °C) until they could be dissected and examined for the presence of *P. autumnale*. Flies were dissected in water, sex was determined, and nematode presence (i.e., parasitism) was determined using a dissecting microscope at 25× with transmitted light. For parasitized individuals, the status of parasite development within the fly was graded according to Kaya and Moon (1978) (Table 1).

Re-analysis of the data of Robinson and Combs (1976)

Data values from the figures published by Robinson and Combs (1976) were retrieved with graph digitizer software, DigitizeIt (Bormann, 2010). The figures were imported as JPG formats into the software. For

each figure, a list of coordinates was created based on a defined and calibrated axis. Coordinate values were in the format of number of survivors over time. The values for the longest-lived flies were reported in the paper, and these values were used to indicate the end of the study time. The difference in the numbers of survivors between each day was calculated to obtain the number of dead flies collected per day. A daily count of the number of flies at risk and the number of flies that died was established. Kaplan-Meier survival curves were plotted in the statistical program JMP® 9.0 (SAS 2010). Log-rank tests were done to compare survivorship curves between parasitized and non-parasitized female and male face flies (Fig. 2).

Data analysis

All statistical analyses were carried out in JMP® 9.0 (SAS, 2010). Kaplan-Meier curves were generated, and the log-rank test, ($P = 0.05$) was used to assess the homogeneity of the survival data from 2009 and 2010. There were no censored individuals. Data for the California flies were found to be homogeneous within both non-parasitized female ($\chi^2 = 0.5894$, log-rank = 0·4426) and male ($\chi^2 = 0.9625$, log-rank = 0·3270) flies. Homogeneity was found within parasitized male flies ($\chi^2 = 3.2913$, log-rank = 0·0696), but highly significant heterogeneity was found within parasitized female flies ($\chi^2 = 8.8465$, log-rank = 0·0029). The data were analysed separately by year for analysis since homogeneity was found only within non-parasitized groups. Subsequent analysis of the longevity data was done with additional log-rank tests based on plotted Kaplan-Meier curves. Flies were grouped by year and infection status and analysed separately by sex.

RESULTS

Kaplan-Meier analysis by year revealed a non-significant difference in longevity between healthy and parasitized flies for female ($\chi^2 = 0.5876$, log-rank = 0·4434) and male ($\chi^2 = 1.8358$, log-rank = 0·1754) flies in 2009 (Fig. 1A, C). In the 2009 dataset, the median lifespan for non-parasitized female flies was 29 days (CI = 22–33 days) and 26 days (CI = 15–32 days) for parasitized flies. For the 2009 male flies, the median lifespan was 40·5 days (CI = 28–45 days) for non-parasitized individuals, and a median time of 18·5 days (CI = 13–25 days) for parasitized individuals. Significant differences were found between parasitized and non-parasitized female ($\chi^2 = 10.4765$, log-rank = 0·0012) and male ($\chi^2 = 15.0849$, log-rank = 0·0001) flies in 2010 (Fig. 1B, D). In the 2010 dataset, the median time to death for non-parasitized female flies was 20 days (CI = 13–29 days) and 15 days (CI = 8–19 days) for parasitized females. The 2010 median survival time
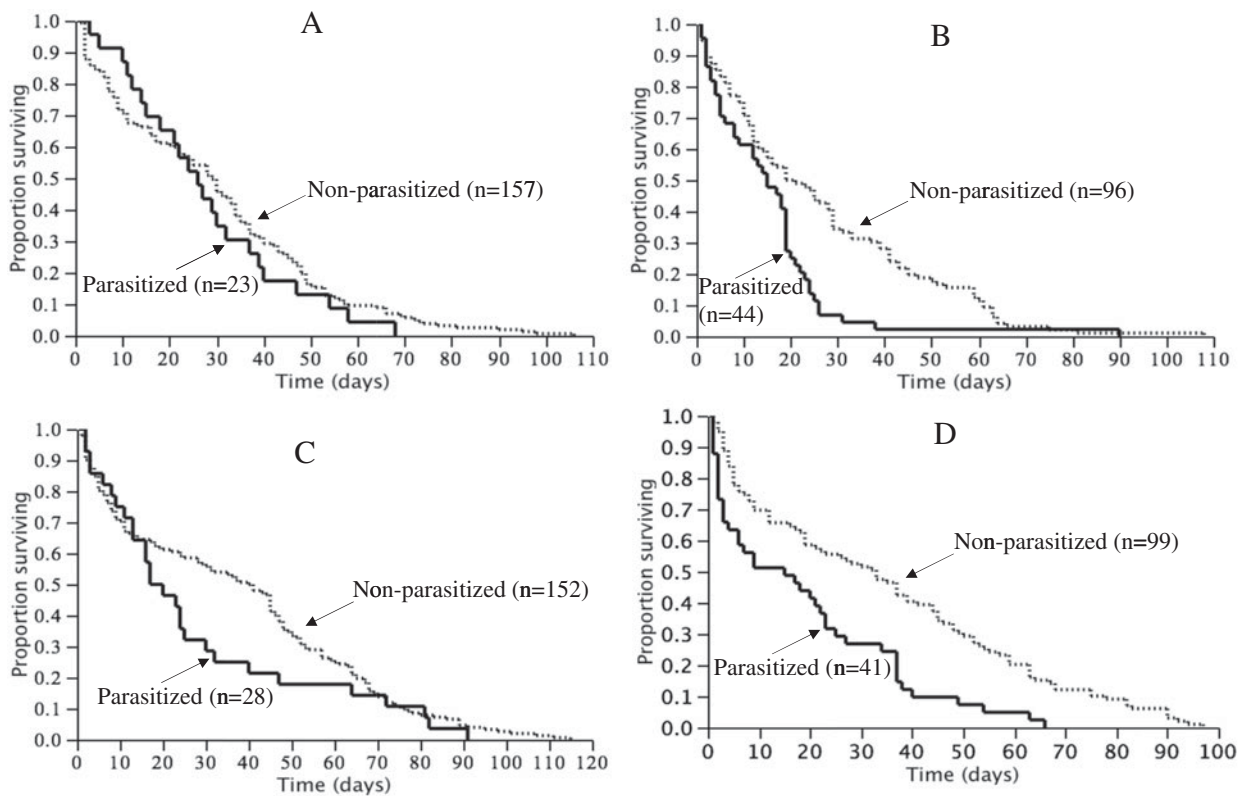
Fig. 1. Kaplan-Meier survival curves for parasitized and non-parasitized face flies. (A) Female face flies from 2009. (B) Female face flies from 2010. (C) Male face flies from 2009. (D) Male face flies from 2010.

for non-parasitized male flies was 33 days (CI = 19–39 days) and 15 days (CI = 3–23 days) for parasitized males.

The intensity of nematode parasitism was not quantified, but the first generation gamogenetic female burden usually ranged from 1 to 3 females present in female face flies and a single first generation gamogenetic female nematode was present in male face flies. Overall, 24% (CI = 15% − 35%) of parasitized female flies died prior to infection level C-4. For parasitized male face flies, 43% (CI = 32% − 55%) of male flies died prior to infection level C-3. No deaths were recorded during the C-3 stage for parasitized female flies (Table 2).

Re-analysis of the data of Robinson and Combs (1976)

The log-rank tests revealed no significant differences in longevity among parasitized female ($\chi^2 = 0.2489$, log-rank = 0.6178) (Fig. 2A) and male ($\chi^2 = 0.0944$, log-rank = 0.7586) (Fig. 2B) flies compared to their non-parasitized counterparts. The median lifespan was 63 days (CI (95%) = 58–70 days) for non-parasitized females while parasitized females had a median lifespan of 74 days (CI = 65–84 days). The median lifespan for non-parasitized males was 74 days (CI = 74–79 days) while parasitized male flies had a median lifespan of 80 days (CI = 69–84 days).

DISCUSSION

While this study is the first to report decreased longevity among *P. autumnale*-parasitized face flies, this study is not the first to report decreased longevity among hosts parasitized by nematodes in the genus *Paraiotonchium*. *Paraiotonchium muscadomesticae* Coler and Nguyen (Tylenchida: Iotonchiidae) has a similar biology to that of *P. autumnale*. This nematode, however, parasitizes houseflies. Geden (1997) reported that *P. muscadomesticae*-parasitized female houseflies live half as long as non-parasitized female flies.

Of the 67 parasitized female flies in this study, 76% harboured a C-4 class parasitism at death. *Paraiotonchium autumnale* parasitization shortens the lifespan of its host; however, this outcome may have little significance towards overall parasite reproductive and transmission success. Kaya *et al*. (1979) stated that female face flies that harbour C-4 class parasitism, change their behaviour and become dung seekers. It was reported that the parthenogenetic female nematodes continually produce second-generation gamogenetic stages so the ovaries of female flies are continuously distended. This state of the ovaries apparently provides females with the perception of a continual presence of eggs. Thus, these parasitized flies continuously seek dung to nemaposit rather than to oviposit. Host death during C-4 may be of little consequence to the parasite as
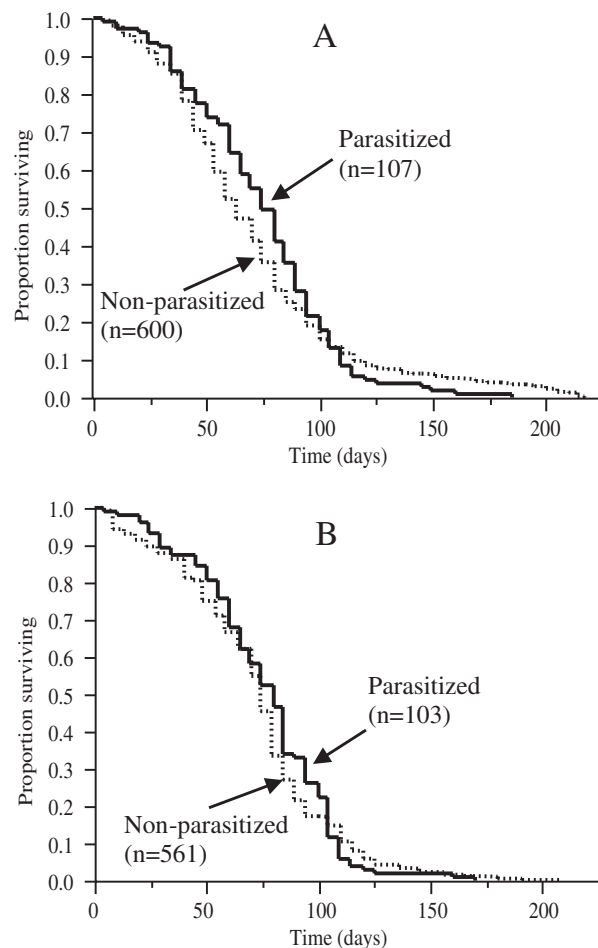
Fig. 2. Projected Kaplan-Meier survival curves for parasitized and non-parasitized face flies, *Musca autumnalis*, re-analysed with published results from Robinson and Combs (1976). (A) Female face flies. (B) Male face flies.

Table 2. Proportion of deaths associated with specific stages of parasite *Paraiotonchium autumnale* development (Table 1) ($n=67$ for female face flies and $n=69$ for male flies.)

| | Stages of *P. autumnale* development | | | |
	C-1	C-2	C-3	C-4
Female flies	15%	9%	0%	76%
Male flies	30%	13%	57%	N/A

C-4, and ovarian development and the parasite life cycle resumed after addition of protein. Previously, Turner and Hair (1967) reported longer adult longevity and zero reproductive output for non-parasitized flies deprived of dietary protein. The female flies in Robinson and Combs's (1976) study may have had extended lifespans due to the suspension of reproductive development, which is an energetically expensive process. This suspension of reproductive development may have halted parasite development and subsequent pathogenic activity, reducing the negative effects of being parasitized. The 1976 conclusion was based on starved hosts, and may have underestimated the impact of *P. autumnale* on face fly longevity. Provided adequate nutrients, decreased longevity is a more plausible outcome of *P. autumnale*-parasitized face flies. However, it is unclear if dietary protein would influence physiological processes and parasite development among male face flies in a similar fashion. It appears that host starvation can be linked to parasite-induced host death, but further investigations are needed to determine whether or not host diet plays a significant role in *P. autumnale*-induced face fly death.

By analysing female and male flies separately we have discovered that, based on the results from our 2010 study, the decrease in lifespan was much more pronounced in male face flies than female flies. Male face flies are considered dead-end hosts since there is no natural exit for *P. autumnale* to escape (Stoffolano, 1970; Nappi, 1973). A possible explanation for the pronounced effect in male face flies may be that the *P. autumnale* parasites cause substantial internal damage or interrupt vital biological processes since the nematodes are not localized within the host. Nappi (1973) reported that less than 50% of female flies oviposit when mated with a parasitized male, suggesting that sperm production also may be affected; it was also reported that the testes are not invaded by *P. autumnale*, but heavily parasitized males often lacked accessory glands. Here we have shown how the sex of the host and the role it has in the parasite's life can largely influence the magnitude of host damage.

Female and male flies are parasitized at equal frequencies (Kaya and Moon, 1980; Chirico, 1990). Are male face flies truly dead-end hosts for

long as the next generation of parasitizing nematodes is released.

A previous study by Robinson and Combs (1976) concluded that parasitism by *P. autumnale* does not affect the longevity of adult face flies based on a laboratory study in which flies were provided sugar and water as the diet. A Kaplan-Meier survival analysis with values extracted from their published figures confirms this conclusion with non-significant log-rank test values. Notably, however, the median lifespans for non-parasitized and parasitized face flies are 2–3 times longer than the values reported in our study. There are numerous factors that may have contributed to these different results. One possible explanation for this outcome is the difference in diet provided to the flies during the study. Kaya and Moon (1980) stated that dietary protein is not only necessary for ovarian development but also necessary for *P. autumnale* development. Robinson and Combs (1976) did not provide a protein supply for the flies in their study. In Kaya and Moon's (1980) study, protein-deprived parasitized female face flies harboured parasitism classes from C-1 to C-3 and rarely

P. autumnale? Nemaposition by female face flies is not a requirement for *P. autumnale* to be parasitic to face fly larvae. Second-generation gamogenetic nematodes extracted from male flies are equally capable of parasitizing hosts as nematodes from female flies (Stoffolano, 1970). Although male flies are not frequent dung visitors, it is not uncommon to see male flies on dung pats in the field or laboratory (Nappi, 1973; Kaya *et al.* 1979). Since nematodes were found in the head of male face flies, Nappi (1973) postulated that nematodes could possibly exit from the mouthparts while the flies are on the dung. However, there was no evidence that the nematodes could exit from the mouthparts of males. An alternative possibility is that nematodes are capable of escaping from dead hosts. Preliminary tests with agar revealed that *P. autumnale* could escape from dead face flies if the substrate is moist enough to cause the disintegration of the cadavers (*unpublished observations*).

Increasing evidence suggests that environmental heterogeneity and pathogen diversity can maintain high levels of host genetic variation for immune traits (Lazzaro and Little, 2009). Abiotic factors such as temperature and nutrition levels may heavily influence host susceptibility to parasites and parasite activity (Wolinska and King, 2009). Furthermore, there is evidence that exposure to higher temperatures can reduce susceptibility to low levels (Blanford *et al.* 2003). Condition-dependent host immunity and pathogen activity may explain the different results between the 2009 and 2010 flies. The temperatures for the collecting dates in 2009 were between 27 °C and 37 °C while in 2010, the temperatures were between 19 °C and 25 °C, with one day being 34 °C. The flies present in field during the 2009 collecting days may have been exposed to stressful environmental conditions that influenced susceptibility, manifested by longer lifespans of parasitized flies from this year. Additionally, the total percentage parasitism was 13% for females and 16% for males in 2009 compared to 31% for females and 29% for males in 2010. The lower percentage of parasitized flies in the 2009 cohort may also support the existence of variable immunity influenced by environmental factors. Furthermore, nutrition levels of the hosts may also have influenced the outcomes of our study. Another factor that may influence the longevity of flies is the quality of cattle dung in which the face fly larvae feed. Greenham (1972) effectively showed how the seasonal changes in the quantity and quality of forage affects the composition and formation of cattle dung, which can ultimately influence the biology of bush flies (*Musca vetustissima*) in Australia. The data indicated that the difference in the quality of dung affects survival and reproduction of coprophagous insects. Possibly, the variability of the cattle dung may have had an effect on the longevity of face flies by giving certain cohorts a nutritional advantage.

In general it would be interesting if environmental temperatures and nutritional quality can influence the relationship between sterilizing parasites and their hosts.

We have demonstrated that it is possible for *P. autumnale* parasitism to significantly reduce the *M. autumnalis* lifespan in the laboratory setting. However, given the different outcomes between the years, the impact of a sterilizing parasite on its host longevity may depend upon environmental as well as physiological factors. Whether such decreases in face fly lifespan can be measured in the field settings remains to be demonstrated. A possible way to measure *P. autumnale* impact on face fly longevity in natural systems is to characterize the age structure of parasitized and non-parasitized flies present in the field. Age structure of face flies can be characterized by measuring the pterin content and gonotrophic status of field-collected adult flies (Moon and Kaya, 1981; Krafsur *et al.* 1985, 1995; Moon and Krafsur, 1995; Butler *et al.* 2009). If prevalence of parasitism decreases with host age, then it is possible that parasitism reduces host longevity in natural systems. If significant reduction in host longevity due to *P. autumnale* is measured in the field, the overall impact of *P. autumnale* on face fly populations may have been underestimated.

For a given host-parasite relationship it is important to analyse the effect of the parasites separately by sex because the cost of parasitism may be different for conspecific males and females. Lastly, the interplay between environmental conditions and host susceptibility must be considered together to make forecasts about the impact of parasites, both on individual hosts and on host populations.

REFERENCES

Anderson, R. M. and May, R. M. (1978). Regulation and stability of host-parasite population interactions: I. Regulatory processes. *Journal of Animal Ecology* **47**, 219–247.

Baudoin, M. (1975). Host castration as a parasitic strategy. *Evolution* **29**, 335–352.

Blanford, S., Pugh, T. C. and Pell, J. K. (2003). Temperature checks the Red Queen? Resistance and virulence in a fluctuating environment. *Ecology Letters* **6**, 2–5.

Bormann, I. (2010). *DigitizeIt*. URL http://www.digitizeit.de.

Butler, S. M., Moon, R. D., Hinkle, N. C., Millar, J. G., McElfresh, J. S. and Mullens, B. A. (2009). Characterization of age and cuticular hydrocarbon variation in mating pairs of house fly, *Musca domestica*, collected in the field. *Medical and Veterinary Entomology* **23**, 426–442.

Chirico, J. (1990). Sex ratio, emergence and infection rates of *Heterotylenchus*-exposed *Musca autumnalis* (Diptera: Muscidae). *The Journal of Parasitology* **76**, 124–127.

Chirico, J. (1994). A comparison of sampling methods with respect to cattle visiting Muscidae and their nematode infections. *Medical and Veterinary Entomology* **8**, 214–218.

Druilhe, P., Hagan, P. and Rook, G. A. W. (2002). The importance of models of infection in the study of disease resistance. *Trends in Microbiology* **10**, S38–S46.

Ebert, D. and Hamilton, W. H. (1996). Sex against virulence: the coevolution of parasitic diseases. *Trends in Ecology and Evolution* **11**, 79–82.

Geden, C. J. (1997). Evaluation of *Paraiotonchium muscadomesticae* (Nematoda: Tylenchida: Iotonchiidae), a potential biological control agent of the housefly (Diptera: Muscidae). *Biological Control* **10**, 42–37.

Greenham, P. M. (1972). The effects of the variability of cattle dung on the multiplication of the Bushfly (*Musca vetustissima* Walker). *Journal of Animal Ecology* **4**, 153–165.

Hurd, H., Warr, E. and Polward, A. (2001). A parasite that increases host lifespan. *Proceedings of the Royal Society of London, B* **268**, 1749–1753.

Jaenike, J., Benway, H. and Stevens, G. (1995). Parasite-induced mortality in mycophagous *Drosophila. Ecology* **76**, 383–391.

Kaya, H. K. and Moon, R. D. (1978). The nematode *Heterotylenchus autumnalis* and face fly *Musca autumnalis*: A field study in Northern California. *Journal of Nematology* **10**, 333–341.

Kaya, H. K. and Moon, R. D. (1980). Influence of protein in the diet of face fly on the development of its nematode parasite, *Heterotylenchus autumnalis. Annals of the Entomological Society of America* **73**, 547–552.

Kaya, H. K., Moon, R. D. and Witt, P. L. (1979). Influence of the nematode, *Heterotylenchus autumnalis*, on the behavior of face fly, *Musca autumnalis. Environmental Entomology* **8**, 537–540.

Krafsur, E. S., Moon, R. D. and Kaya, H. K. (1985). Age structure of breeding face fly populations (Diptera: Muscidae). *Environmental Entomology* **14**, 590–596.

Krafsur, E. S., Moon, R. D. and Kim, Y. (1995). Age structure and reproductive composition of summer *Musca autumnalis* (Diptera: Muscidae) populations estimated by pterin concentrations. *Journal of Medical Entomology* **32**, 685–696.

Krafsur, E. S., Church, C. J., Elvin, M. K. and Ernst, C. M. (1983). Epizootiology of *Heterotylenchus autumnalis* (Nematoda) among face flies (Diptera: Muscidae) in central Iowa, USA. *Journal of Medical and Veterinary Entomology* **20**, 318–324.

Lafferty, K. D. and Kuris, A. M. (2009). Parasitic castration: the evolution and ecology of body snatchers. *Trends in Parasitology* **25**, 564–572.

Lazzaro, B. P. and Little, T. J. (2009). Immunity in a variable world. *Philosophical Transactions of the Royal Society, B* **364**, 15–26.

May, R. M. and Anderson, R. M. (1978). Regulation and stability of host-parasite population interactions: II. Destabilizing processes. *Journal of Animal Ecology* **47**, 249–267.

Moon, R. D. and Kaya, H. K. (1981). A comparison of methods for assessing age structure and abundance in populations of nondiapausing female *Musca autumnalis* (Diptera: Muscidae). *Journal of Medical Entomology* **18**, 289–297.

Moon, R. D. and Krafsur, E. S. (1995). Pterin quantity and gonotrophic stage as indicators of age in adult face flies, *Musca autumnalis* (Diptera: Muscidae). *Journal of Medical Entomology* **32**, 673–684.

Nappi, A. J. (1973). Effects of parasitization by the nematode, *Heterotylenchus autumnalis*, on mating and oviposition in the host, *Musca autumnalis. The Journal of Parasitology* **59**, 963–969.

Nickle, W. R. (1967). *Heterotylenchus autumnalis* sp. n. (Nematoda: Sphaerulariidae), a parasite of the face fly, *Musca autumnalis* De Geer. *The Journal of Parasitology* **53**, 398–401.

Obrebski, S. (1975). Parasite reproductive strategy and evolution of castration of hosts by parasites. *Science* **188**, 1314–1316.

Poulin, R. (1996). Sexual inequalities in helminth infections. A cost of being a male? *The American Naturalist* **147**, 287–295.

Robinson, J. V. and Combs, R. L. (1976). Incidence and effect of *Heterotylenchus autumnalis* on the longevity of face flies in Mississippi. *Journal of Economic Entomology* **69**, 722–724.

SAS, (2010). *JMP 9 for Mac.* SAS Institute, Cary, NC, USA.

Stoffolano, J. G. (1967). The synchronization of the life cycle of diapausing face flies, *Musca autumnalis*, and of the nematode, *Heterotylenchus autumnalis. Journal of Invertebrate Pathology* **9**, 395–397.

Stoffolano, J. G. (1970). Parasitism of *Heterotylenchus autumnalis* Nickle (Nematoda: Sphaerulariidae) to the face fly, *Musca autumnalis* De Geer (Diptera: Muscidae). *Journal of Nematology* **2**, 324–329.

Stoffolano, J. G. and Streams, F. A. (1971). Host reactions of *Musca domestica, Orthellia caesarion*, and *Ravinia i'herminieri* to the nematode *Heterotylenchus autumnalis. Parasitology* **63**, 195–211.

Teskey, H. G. (1969). On the behaviour and ecology of the face fly, *Musca autumnalis* (Diptera:Muscidae). *The Canadian Entomologist* **101**, 561–576.

Turner, E. C. and Hair, J. A. (1967). Effect of diet on longevity and fecundity of laboratory reared face flies. *Journal of Economic Entomology* **60**, 857–860.

Wolinska, J. and King, K. C. (2009). Environment can alter selection in host-parasite interactions. *Trends in Parasitology* **26**, 236–244.