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Epizootiology of *Amblyospora stimuli* (Microsporidiida: Amblyosporidae) Infections in Field Populations of a Univoltine Mosquito, *Aedes stimulans* (Diptera: Culicidae), Inhabiting a Temporary Vernal Pool

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The epizootiology of the microsporidium *Amblyospora stimuli* was studied in natural populations of a univoltine mosquito, *Aedes stimulans,* inhabiting a temporary vernal pool over an 18-year period. The yearly prevalence of benign oenocytic infections in adult females was variable, ranging from 1.0 to 9.6% (mean = 5.1%). The yearly prevalence of transovarially transmitted meiospore infections in larval populations was consistently lower but less variable, ranging from 1.3 to 5.9% (mean = 3.5%). Meiospore infections in F_1-generation larvae were significantly correlated with infections in parental-generation females, thus suggesting that larval infection rates could be substantially increased if methods were available to facilitate transmission of *A. stimuli* to a larger portion of the female population via inundative or inoculative release of infected copepods. No correlation was found when infections in filial-generation adult females were measured against meiospore infections in larvae from the preceding year. Analysis of yearly prevalence data using Fine's Fundamental Vertical Transmission Equation revealed low rates of horizontal transmission from the intermediate copepod host to female larvae in most years, ranging from 0.1 to 8.7% (mean = 3.1%). *A. stimuli* is enzootic, persists at a very low level, and has minimal impact on *Ae. stimulans* populations at this site. The low incidence rate of horizontal transmission to larvae appears to be due largely to a paucity of copepods and is a major factor that limits the abundance and subsequent proliferation of *A. stimuli* in *Ae. stimulans* populations at this locale. Results support the view that host–parasite cospeciation is an important mechanism of evolution in this group of mosquito/copepod microsporidia. © 1999 Academic Press

Key Words: Amblyospora stimuli; microsporidia; Aedes stimulans; mosquito; epizootiology.

INTRODUCTION

Microsporidia are one of the most common and widespread groups of naturally occurring parasites of mosquitoes. Isolates have been recovered from more than 100 mosquito species in 15 genera worldwide (see Hazard and Oldacre, 1975; Hazard and Chapman, 1977; Castillo, 1980; Daoust, 1983; Andreadis, 1994, for host lists) and it has been suggested that most, if not all, mosquitoes serve as hosts (Chapman, 1974; Lucarotti and Andreadis, 1995). Currently, there are 15 microsporidian genera described from mosquitoes: *Amblyospora, Crystulospora, Culicospora, Culicosporella, Edhazardia, Hyalinocysta, Goldbergia, Hazardia, Nosema, Parathelohania, Pilosporella, Polydispyrenia, Trichoctosporea, Tricornia,* and *Vavraia* (Becnel and Andreadis, 1999). Members of the genus *Amblyospora* are by far the most numerous, with over 90 species or isolates described from 79 mosquito species in 8 genera.

Significant progress has been made over the past three decades in elucidating the complex life cycles of many of these genera (see Becnel, 1994, for review). Notable findings relative to *Amblyospora* spp. have included the demonstration of: (1) transovarial transmission (Kellen and Wills, 1962) and a recognition of its importance as a mechanism of persistence (Kellen *et al.,* 1965, 1966; Andreadis and Hall, 1979), (2) host sex-dependent polymorphism with the formation of two morphologically and functionally different spore types (Hazard and Weiser, 1968), (3) meiosis (Hazard *et al.*, 1979) and a subsequent sexual cycle (Hazard *et al.*, 1985), and (4) the obligatory involvement of a copepod intermediate host in the life cycle (Andreadis, 1985a; Sweeney *et al.*, 1985).

Despite these advances in interpreting the life cycle, surprisingly few studies have examined the epizootiology of microsporidian infections in wild mosquito populations. Welch (1960) reported prevalence rates for *Amblyospora* sp. (probably *A. khaliulini*) ranging from less than 1% to a high of 32% in larval populations of *Aedes communis* inhabiting several woodland pools at one tundra and four forest locations in Manitoba, Canada, over a 2-year period. He concluded that this microsporidium was responsible for a reduction of 3 to 11% of the larval population. Sabwa *et al.* (1984) monitored the monthly prevalence of *Amblyospora* sp. (probably *A. indicola*) in field populations of *Culex*

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sitiens larvae for 1 year in two brackish water pools along the coast of Kenya. Infection rates ranged from 2 to 10% and from 2 to 15% in each respective pool but there was no significant correlation with salinity, temperature, or rainfall. Andreadis (1990) conducted a comprehensive 3-year investigation of *Amblyospora connecticus* infection in another salt-marsh mosquito, *Aedes cantator*. This microsporidium consistently produced yearly epizootics, with up to 100% infection, in late summer and early fall larval broods. These epizootics arose from the synchronized hatch of transovarially infected eggs oviposited by female mosquitoes that had acquired the microsporidium horizontally during the preceding spring generation. Similar but less extensive epizootics of *Amblyospora* spp. have also been recently documented in late summer broods of two other multivoltine *Aedes* mosquitoes, *Aedes canadensis* (90% infection) and *Aedes cinereus* (13% infection) (Andreadis, 1993). However, the underlying mechanism(s) surrounding the initiation of these events could not be determined. All other reports have typically been limited to incidental observations on the prevalence of infection, usually less than 1%, in cohorts of field-collected larvae or adult females (Kellen and Wills, 1962; Kudo, 1962; Bailey *et al.*, 1967; Chapman *et al.*, 1967, 1969; Anderson, 1968; Dickson and Barr, 1990; Goettel, 1987; Andreadis, 1994; Garcia and Becnel, 1994). The extent to which epizootics occur in other mosquito hosts, especially those that are univoltine, is unknown.

Only a few laboratory studies have attempted to quantitatively assess the contribution of both vertical (transovarial) and horizontal (oral) transmission to long-term maintenance and persistence of microsporidia in host mosquito populations. Andreadis and Hall (1979) were the first to demonstrate that mosquito-parasitic microsporidia (specifically *Amblyospora salinaria* in *Culex salinarius*) could not be maintained for more than a few generations by transovarial transmission alone and that some degree of horizontal transmission was necessary for parasite survival. This was because infections either reduced female fecundity or were not transmitted with 100% efficiency. Similar conclusions were made in laboratory studies with *A. connecticus* in *Ae. cantator* (Andreadis, 1994), *A. stimuli* in *Ae. stimulans* (Andreadis, 1985b), and *Amblyospora dyxenoides* in *Culex annulirostris* (Sweeney *et al.*, 1989). Following the discovery of the intermediate host, Sweeney *et al.* (1989) further demonstrated that the proportion of mosquito progeny acquiring each type of infection (benign or patent) from the female parent could be influenced by genetic selection. Their conclusions were derived from contained caged experiments wherein *A. dyxenoides* was maintained in laboratory colony of *Cx. annulirostris* through nine successive transovarially transmitted cycles (generations). These data were subsequently used to develop a detailed mechanistic simulation model which attempted to demonstrate effects of differing environmental conditions on host–parasite dynamics (Larkin *et al.*, 1995). Validation experiments indicated that simulated growth, peak, and decline of the mosquito population over a 4-year period conformed reasonably well with historical light trap data collected in the field for the same period. However, no precise field data on the prevalence of infection in either larval or adult populations of *Cx. annulirostris* were ever obtained to fully validate the model and assess the actual impact of this microsporidium on host mosquito populations.

Since 1981, I have continuously monitored the natural prevalence of *A. stimuli* infections in an isolated population of *Ae. stimulans*. My objectives were to: (1) sequentially document the yearly prevalence of infection in both larval and adult female populations, (2) quantitatively assess the contribution of both horizontal and transovarial transmission to long-term maintenance of the microsporidium in the field, and (3) assess the short- and long-term impact of this microsporidium on field populations of *Ae. stimulans*. The results of this investigation are reported here.

MATERIALS AND METHODS

Biology of Aedes stimulans

A. stimulans is a woodland pool mosquito with a typical northern *Aedes*-type of life cycle (Siverly, 1972; Means, 1979). It is univoltine and overwinters in the egg stage. Egg hatch typically occurs in early to mid-March in Connecticut but may take place as early as late February in some years (Andreadis, 1985b; Morrison and Andreadis, 1992). Larvae develop slowly and synchronously. Pupation occurs in early May and adult emergence is normally complete by the second week. Adult females are exceptionally long-lived and commonly survive into August. They usually remain close to breeding sites but have been reported to range up to 1.3 km from the nearest production site (Siverly, 1972).

Life Cycle of Amblyospora stimuli

The life cycle of *A. stimuli* as described by Andreadis (1985b, 1994) is summarized in Fig. 1 and detailed below. *A. stimuli* is transovarially transmitted by parental-generation female *Ae. stimulans* that were infected as larvae either horizontally or vertically the preceding spring (March–April). Oviposition occurs throughout the summer months (June–August) and the microsporidium overwinters in the diapausing egg. Parasite development in larval progeny of the F_1 generation is dimorphic and host sex dependent.

In female progeny, the microsporidium invades host oenocytes but undergoes very little multiplication. These infections are essentially benign and have no demon-

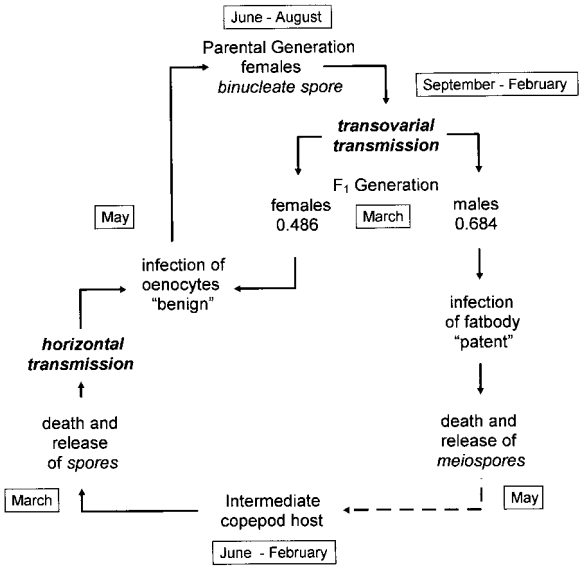

FIG. 1. Life cycle of *Amblyospora stimuli.*

strable effect on larval development. Individuals pupate normally and emerge as apparently healthy adults. Adult longevity and fecundity are similarly unaffected. Binucleate spore formation and ovarian infection occur in the female host after a blood meal is taken and another transovarial transmission cycle results. Transovarial transmission is thus continuous from one mosquito generation to the next but is incapable of sustaining the microsporidium in the host population because of low maternal transmission rates, which have been shown to be only 48.6% in female progeny produced by wild-caught females with naturally acquired infection (Andreadis, 1985b).

In male progeny, transovarially transmitted *A. stimuli* invades fatbody tissue and undergoes an extensive sporulation sequence that culminates in the production of hundreds of thousands of haploid "meiospores." These infections, which occur in 68.4% of male progeny produced by infected females, progress very slowly during the typical 7- to 8-week period of larval development. Infected larvae do not appear to be adversely affected until immediately prior to pupation in early May when almost all die as late fourth instars.

Recently completed laboratory transmission studies (Andreadis, 1994) and comparative rDNA sequence data (Vossbrinck and Andreadis, unpubl. data) have confirmed the involvement of the cyclopoid copepod *Diacyclops bicuspidatus* as the intermediate host. Although direct evidence is lacking, copepods are presumed to acquire infection by ingesting meiospores that are released into the pool upon the death of infected male larvae and to serve as an overwintering host. *D. bicupidatus* is reported to undergo diapause at the fourth copopodite stage and encyst in the bottom sediment of the dry basin of the pool (Cole, 1953).

Horizontal transmission of infection to mosquito larvae takes place sporadically during the early stages of larval development (March to mid-April) via the ingestion of a third spore type that is formed in copepods. Spores germinate within the lumen of the larval gut and initially invade epithelial cells of the gastric caeca. The microsporidium then spreads to oenocytes of adult female hosts in which it produces a benign infection that is virtually identical to that which occurs through the transovarial route. *A. stimuli* is subsequently transmitted via ovarian infection in females as described previously. This horizontal pathway of transmission thereby provides the necessary mechanism whereby *A. stimuli* can reenter the mosquito population and thus perpetuate itself. Similar horizontally transmitted infections occur in male mosquito larvae but these play no role in the transmission cycle (i.e., they are a "dead end"), as the microsporidium does not invade male gonadal tissue and there is no venereal or paternal-mediated vertical transmission.

Site Description

A wild indigenous population of *Ae. stimulans* that developed in a temporary, leaf-lined vernal pool located in an isolated 10-ha woodlot in Mt. Carmel, CT (41°24′N, 72°53′W) was studied. The area was dominated by white pine, *Pinus strobi,* and eastern hemlock, *Tsuga canadensis.* This pool is the only site where *A. stimulans* larvae have been consistently found (Andreadis, 1985; Morrison and Andreadis, 1992). Other species occasionally associated with *Ae. stimulans* during the course of the study included *Ae. canadensis, Ae. cinereus,* and *Ae. excrucians.* The pool lacks aquatic vegetation and has an annual cycle of 4 to 5 months wet phase and 7 to 8 months dry phase. It typically floods in late February to early March from melting snow and rain. Water levels decline as summer approaches and the pool is usually dry by late May or June. The maximum size of the pool was approximately 15 × 6 m with a maximum depth of 1 m in most years. Water temperatures ranged from 4°C in March and April to a high of 18°C in May. The water was moderately acidic with a pH range of 5.7 to 6.2.

Collection Methods and Assay Procedures

The natural prevalence of *A. stimuli* was recorded yearly over an 18-year period, 1981 to 1998. No collections were made from 1989 to 1991. Each of the two types of infection was monitored: patent fatbody (transovarial) infections in larvae and benign oenocytic (horizontal or transovarial) infections in adult females.

Infections in larvae were determined from gross microscopic examination of patently infected fourth instars. These were collected from the pool just prior to pupation (normally the last week in April) and brought

to the laboratory where they were immediately screened and counted. Infected larvae were readily recognizable by their milky-white opaque coloration when viewed against a black background. Cohorts of larvae not showing visible symptoms of infection were held for an additional week and examined daily. Although these patent infections are limited to males, no attempt was made to sex infected larvae and therefore prevalence rates were based on the total number of larvae (male and female) collected and examined during that year. Sample sizes ranged from 150 to 2002 larvae per year (average = 741).

Infections in adult female hosts were determined from microscopic examination (100×) of Giemsa-stained smears of individuals that emerged in the laboratory from field-collected pupae. These collections were generally made over a 4- to 5-day period, approximately 1 week after the larval collections. Field-collected pupae were individually isolated in 30-ml plastic containers and held at 20°C. Females were examined for infection 1 to 2 days after emergence. Yearly sample sizes ranged from 50 to 450 (average = 120) and prevalence rates were based on the total number of females that were examined. Diagnosis of infection was based on the presence of vegetative (diplokaryotic) stages. The source of infection (transovarial or horizontal) could not be differentiated microscopically as both routes produce identical manifestations in the adult female host. This issue was addressed in data analysis (see below).

Analysis and Interpretation of Data

Yearly prevalence data on infection rates in larvae and adults were subjected to analysis by linear regression (SigmaStat, 1995) in order to define the relationships between infection in parental-generation adult females and infection in filial-generation larvae over time.

Further analysis was done to quantitatively assess the relative contribution of both horizontal and transovarial transmission to the persistence of *A. stimuli* in natural populations of *Ae. stimulans* and to specifically determine the net incidence rate of horizontal transmission to female larvae each year. This was accomplished using observed infection rates in adult females, previously obtained data on adult longevity, fecundity, and transovarial transmission rates (Andreadis, 1985b), and the Fundamental Vertical Transmission Equation of Fine (1975, 1984) as defined below.

$$P' = \frac{\beta[P\alpha d(1 - P + P\alpha) + P\alpha v(1 - P + P\alpha - P\alpha d)]}{\beta[P\alpha d(1 - P + P\alpha) + P\alpha v(1 - P + P\alpha - P\alpha d)] + (1 - P + P\alpha - P\alpha d)(1 - P + P\alpha - P\alpha v)}$$

Since the infection has no adverse affect on the female host ($\alpha = \beta = 1$) and no vertical transmission occurs in males ($v = 0$), then the equation can be simplified to $P' = Pd$, where P' is the prevalence rate of infection among adult female progeny due to vertical (transovarial) transmission, P is the prevalence rate of infection among adult females of reproductive age (observed), d is the maternal vertical transmission rate (0.486) (Andreadis, 1985b), and $P - P'$ is the deficit in prevalence rate which must be compensated by horizontal transmission and $P - P'/1 - P'$ is the net incidence rate of horizontally acquired infections per generation.

RESULTS

The yearly prevalence of *A. stimuli* infections in larval and adult female populations of *Ae. stimulans* from 1981 through 1998 is shown in Fig. 2. The prevalence of benign oenocytic infections in adult female *Ae. stimulans* was variable, ranging from a low of 1.0% in 1993 to a high of 9.6% in 1995 with an overall average of 5.1% ± 2.8 SD (median = 4.8%). Prevalence rates in females increased steadily from 1982 to 1987 and similarly decreased from 1995 through 1998. There were two distinct periods, each lasting 2 years, in which moderately higher prevalence rates were recorded, 1985–1986 (8.5%) and 1995–1996 (8.8%).

The yearly prevalence of transovarially transmitted meiospore infections in larval populations followed a similar pattern but was consistently lower and less variable, ranging from 1.3 to 5.9% and averaging 3.5% ± 1.4 SD (median = 3.6%). Assuming a 1:1 sex ratio, it can be inferred that approximately 7% of male larvae were typically infected each year, as this type of infection is limited to males only. The highest prevalence rates were observed in 1988 and 1996 and in both instances these followed years in which comparatively high prevalence rates were recorded in parental-generation females.

Regression analysis revealed a significant positive linear correlation ($r = 0.812$, $P = 0.001$) between the prevalence of transovarially transmitted meiospore infections in filial-generation larvae and the prevalence of benign oenocytic infection in parental-generation females over the 18-year period (Fig. 3). However, no significant correlation ($r = 0.302$, $P = 0.366$) was found when infections in filial-generation adult females were plotted against meiospore infections in larvae from the preceding year (parental generation) (Fig. 4). Likewise, no significant correlation was found when infections in larvae and adult females were analyzed during the same year ($r = 0.393$, $P = 0.206$, data not shown graphically).

Analysis of yearly prevalence data in adult females using the Fundamental Vertical Transmission Equation (Fine, 1975, 1984) revealed low rates of horizontal

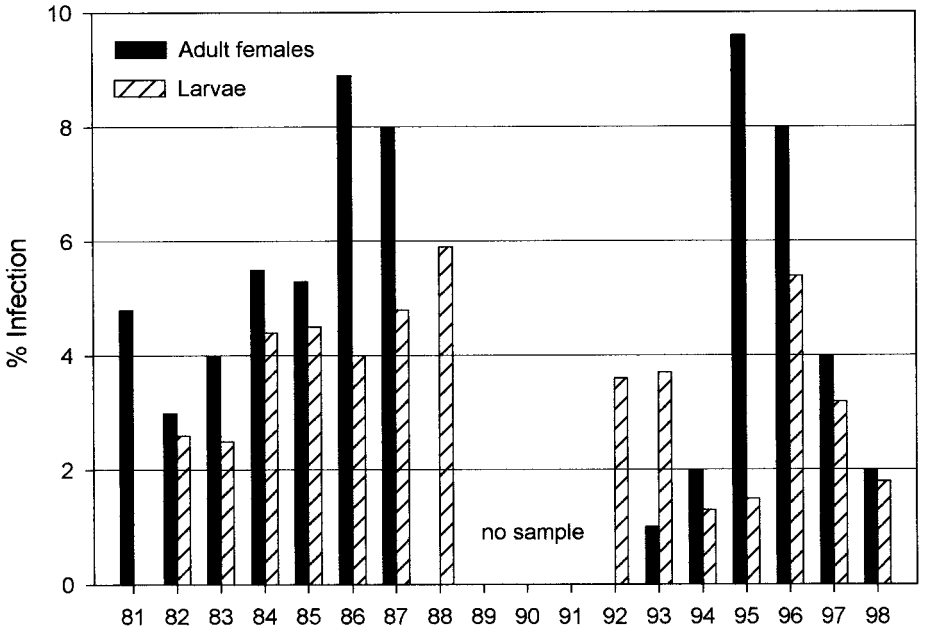

FIG. 2. Yearly prevalence of *Amblyospora stimuli* infections in adult female and larval populations of *Aedes stimulans* at Mt. Carmel, CT, 1981 to 1998.

transmission to female larvae in most years (Table 1). The estimated net incidence rate of horizontal transmission to females (i.e., number of new cases) was variable, ranging from a low of 1 per 1000 (0.1%) in 1997 and 1998 to a high of almost 9 per 100 (8.69%) in 1995. The overall average rate of transmission was 3.1% with a median of 2.7%. The estimated rate of horizontal transmission among females was greater than the estimated rate of infection due to transovarial transmission in 5 of the 11 years in which calculations were made.

DISCUSSION

Results clearly demonstrate that within this isolated population of *Ae. stimulans*, *A. stimuli* is enzootic and is maintained at a relatively low level. Although some variation was observed, on average, only 5% of adult

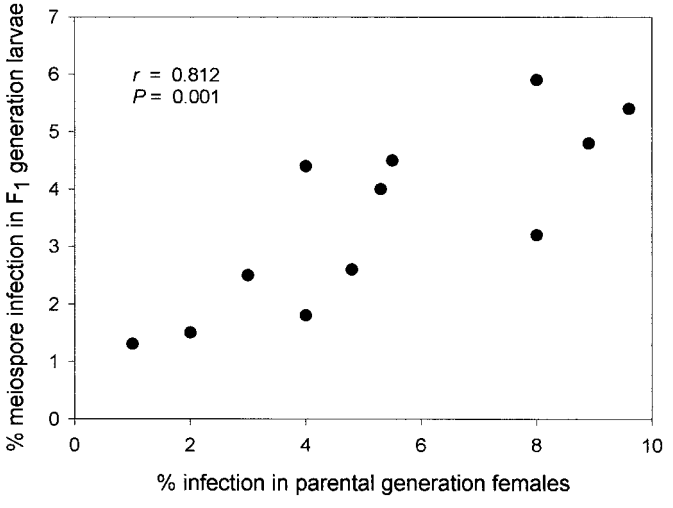

FIG. 3. Relationship between the yearly prevalence of *Amblyospora stimuli* infections in parental-generation adult female *Aedes stimulans* and the prevalence of transovarially transmitted meiospore infections in F_1-generation larvae.

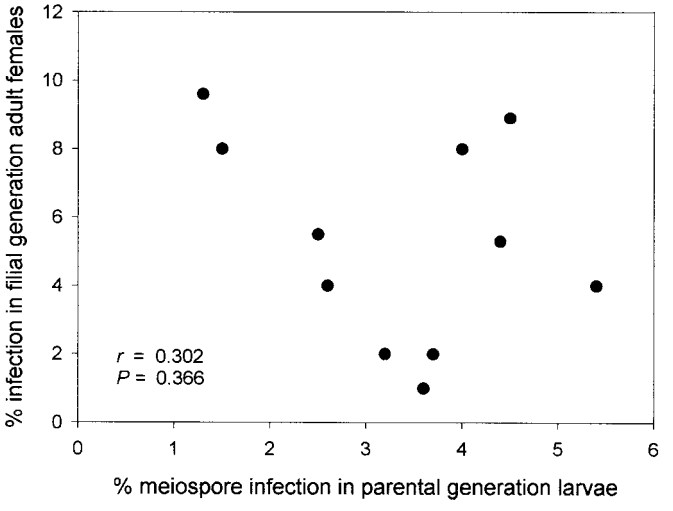

FIG. 4. Relationship between the yearly prevalence of meiospore infections of *Amblyospora stimuli* in parental-generation larval *Aedes stimulans* and the prevalence of infections in filial-generation adult females.

TABLE 1

Yearly Prevalence of *Amblyospora stimuli* Infections in Adult Female *Aedes stimulans* Showing Estimated Prevalence Due to Transovarial Transmission and Net Incidence of Horizontal Transmission as Determined Using the Fundamental Vertical Transmission Equation of Fine (1975, 1984) Where $P' = Pd$[a]

Year	Actual observed infection rate (%) P	Estimated infection rate (%) due to transovarial transmission P'	Net incidence rate (%) of horizontal transmission[b] $\frac{P - P'}{1 - P'}$
1981	4.8	—	—
1982	3.0	2.3	0.7
1983	4.0	1.5	2.5
1984	5.5	1.9	3.7
1985	5.3	2.7	2.7
1986	8.9	2.6	6.5
1987	8.0	4.3	3.9
1988–1992	—	—	—
1993	1.0	—	—
1994	2.0	0.5	1.5
1995	9.6	1.0	8.7
1996	8.0	4.7	3.5
1997	4.0	3.9	0.1
1998	2.0	1.9	0.1
Mean ± SD	5.1 ± 2.8	2.5 ± 1.3	3.1 ± 2.6

[a] $d = 0.486$.

[b] Percentage values must be converted for analysis of the equation.

females and 3.5% of larvae are infected with this microsporidium in any given year. Lethal, transovarially transmitted meiospore infections in male larvae are remarkably consistent and fluctuate little from one year to the next. The highest prevalence of infection observed within the larval population over the 18-year period was less than 6%, and there is no evidence that *A. stimuli* causes any significant epizootic activity in larval populations of *Ae. stimulans* at this location as has been observed with other *Amblyospora* spp. that infect multivoltine mosquito hosts (Andreadis, 1990, 1993). It is therefore concluded that *A. stimuli* has minimal impact on populations of *Ae. stimulans* at this specific site. The ability of *A. stimuli* to produce higher prevalence rates in *Ae. stimulans* populations in other vernal pool habitats remains to be determined.

It is difficult to draw any broad general conclusions regarding the epizootic potential of *Amblyospora* spp. in other univoltine mosquito hosts as no other species have been examined in such detail. However, results from this study are in agreement with the generally low levels of infection observed for *Amblyospora* spp. in other univoltine mosquitoes with similar life histories, *Aedes abserratus, Aedes aurifer, Ae. communis,* and *Ae. excrucians* (Welch, 1960; Anderson, 1968; Andreadis, 1994). Thus, if epizootics do occur in larval populations, they are probably quite rare.

The prevalence of meiospore infections in male larvae in the field appears to be a direct function of the prevalence of infection in parental-generation adult females from the preceding year (Fig. 3). This observation is consistent with prior laboratory studies on the life cycle of *A. stimuli* (Andreadis, 1985) and reaffirms that lethal meiospore infections in larvae can only be induced by direct transovarial transmission. The positive correlation is further significant because it implies, at least in theory, that larval infection rates could be substantially increased if methods were available to facilitate transmission of *A. stimuli* to a larger portion of the female population via horizontal means. This approach has been experimentally demonstrated with *A. connecticus* where the introduction of infected copepods into a confined area of a salt marsh pool resulted in the infection of 13.5% of the emerging adult female population of *Ae. cantator* (Andreadis, 1989). The recent discovery of *D. bicuspidatus* as the intermediate host for *A. stimuli* will allow for testing of this hypothesis in *Ae. stimulans.*

It is equally notable that no correlation was found between the prevalence of infection in adult females and the prevalence of meiospore infections in larvae from the preceding generation (Fig. 4). This suggests that increased meiospore production *per se* (i.e., infected larvae) will not necessarily translate into increased levels of horizontal transmission from copepods to mosquitoes in the following year. In the absence of any data on copepod infections, one can only speculate that horizontal transmission of *A. stimuli* to *Ae. stimulans* is directly influenced by the prevalence of infection in the intermediate host. However, it is quite likely that other factors such as host(s) densities and water levels play equally important roles. These factors need to be explored.

Analysis of the transmission data (Table 1) demonstrates that some degree of transovarial and horizontal transmission operates each year and that both modes of transmission contribute to long-term maintenance of *A. stimuli* in this population of *Ae. stimulans.* However, the net incidence rate of horizontal transmission of *A. stimuli* to female populations of *Ae. stimulans* is quite low in most years, roughly 3.0%, and this appears to be a major factor that limits the abundance and subsequent proliferation of this microsporidium at this site. Since the identity of the intermediate host was not known for the majority of this study, no attempt was made to quantify the abundance of *D. bicuspidatus* or the prevalence of infection therein. However, incidental observations revealed a paucity of copepods within the vernal pool throughout most of the period of larval development. These observations support the hypotheses of Sweeney *et al.* (1989) and Hurst (1991, 1993), who have argued that where vertical transmission is efficient and copepod populations are sparse, microsporidia will select for the production of patent meiospore

infections in male larvae and benign oenocytic infections in females, as the latter will increase the likelihood of passage to the next generation. This represents the Type I host–parasite relationship originally described by Kellen *et al.* (1965), who noted its predominance in other univoltine *Aedes* mosquitoes (*Aedes cataphylla, Aedes hexodontus, Aedes increpitus,* and *Aedes ventrovittus*) that similarly developed in temporary vernal snow pools in California. Although these researchers had no knowledge of the intermediary role of copepods in the life cycle, they surmised that this type of relationship enhanced survival of the microsporidium because of an 11-month period between the time meiospospores are produced by one generation and the time larvae of the next generation are potentially exposed. Greater reliance on continuous transovarial transmission via congenitally infected females will accordingly decrease opportunities for sexual recombination, which can only occur following passage through the copepod. However, given that relatively few copepod species have adapted to the demanding conditions associated with colonization of vernal pool habitats (Wiggins *et al.,* 1980), this reproductive strategy would appear to be more advantageous for long-term survival. This contrasts sharply with Kellen's Type III host–parasite relationship in which patent meiospore infections occur in both male and female larvae and only a few females develop benign oenocytic infections that are transovarially transmitted. With a few notable exceptions (i.e., *Amblyospora californica* in *Culex tarsalis* and *A. salinaria* in *Cx. salinarius*), this host–parasite relationship has been more frequently observed in mosquitoes with multivoltine life histories that inhabit more permanent bodies of water (Kellen *et al.,* 1965; Chapman *et al.,* 1966; Anderson, 1968; Sweeney *et al.,* 1988; Andreadis, 1990). It has similarly been argued (Sweeney *et al.,* 1989; Hurst, 1991, 1993; Lucarotti and Andreadis, 1995) that this host–parasite interaction would be expected to occur in those habitats in which copepods are intimately abundant at the appropriate time. These conditions have been observed with *A. connecticus* in *Ae. cantator* and *Acanthocyclops vernalis* (Andreadis, 1990) and with *A. dyxenoides* in *Cx. annulirostris* and *Mesocyclops albicans* (Sweeney *et al.,* 1989). Additional epizootiological studies with more species in other aquatic habitats are needed to more fully evaluate these hypotheses and the evolutionary significance of each type of host–parasite relationship. However, these and the present study support the view of Baker *et al.* (1998) that host–parasite cospeciation is an important mechanism of evolution in this group of mosquito/copepod microsporidia.

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