doi: 10.1111/j.1420-9101.2008.01665.x

Different transmission strategies of a parasite in male and female hosts

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Keywords:

Aedes aegypti;
Ascogregarina culicis;
host sex;
infectious dose;
phenotypic plasticity.

Abstract

We investigated whether a parasite with two routes of transmission responds to the different transmission opportunities offered by male and female hosts by using different transmission strategies in the two sexes. The parasite *Ascogregarina culicis*, which infects the mosquito *Aedes aegypti*, can be transmitted as its host's pupa transforms into an adult or when a female lays its eggs. As the latter transmission route is missing in males, we expected, and found, that the parasite releases a greater proportion of its infectious forms during emergence when it is within a male than when it infects a female. The transmission route, which influences the parasite's dispersal and the evolution of its virulence, was also affected by the dose of infection and the parasite's previous transmission route. Our results emphasize the complexity underlying the development of parasites and show their ability to tune their strategy to their environment.

Introduction

Do parasites adjust their phenotype to their current environmental condition? Some parasites with several transmission strategies (e.g. vertical and horizontal transmission) vary the allocation to the strategies (Agnew and Koella 1999; Kaltz and Koella 2003; Poulin 2003; Poulin and Lefebvre 2006). This variation probably reflects adaptations that increase the parasites' transmission in different epidemiological situations. For example, temperate phages that parasitize bacteria can propagate horizontally by killing their host to be released as free phages (lysis) or they can integrate in their host's genome (lysogeny) and be transmitted vertically (Levin and Lenski 1985). The phages prefer lysogeny when their host is in good condition and thus replicating rapidly, leading to efficient vertical transmission; they escape unfavourable environments by lysis when the host replicates slowly (Mittler 1996; Wang *et al.*, 1996).

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One of the variables in the epidemiological situation is the sex of a parasite's host. If males and females provide different opportunities for transmission, their parasites should use different transmission strategies in each sex. Thus, the microsporidium *Amblyospora* sp., a parasite of mosquitoes, changes its development strategy to adapt to the lower potential for transmission offered by male hosts (Andreadis and Hall 1979; Andreadis 2007). In females, the parasite develops slowly and usually does not kill the larvae. Its sporulation is synchronized with the adult's blood meal and is followed by the transovarial infection of the eggs (vertical transmission). In male hosts, where vertical transmission is not possible, the parasite develops more rapidly than in females, sporulates during the larval stage and kills the host before adulthood, leading to the liberation of infectious forms that enable horizontal infection.

Here, we consider a parasite with the possibility of two routes of transmission in the same host individual: the gregarine *Ascogregarina culicis*, a parasite of the mosquito *Aedes aegypti*. The infectious stages (oocysts) formed within each individual can be used for what we call 'local' and 'distant' transmission. Local transmission occurs when an infected mosquito releases oocysts

(which are produced in the pupa) into the breeding site where it has developed. This can happen if it dies as a late pupa or if it survives and then releases oocysts as it metamorphoses from the pupa to the adult. Locally transmitted oocysts thus remain within a breeding site. Distant transmission occurs when adult females release oocysts during oviposition (thus exposing their own offspring and other larvae to the parasite) or when infected adults of either sex die in breeding sites. Distant transmission is less likely from male than from female mosquitoes because they do not lay eggs and are unlikely to return to (and die on) a breeding site as adults. Therefore, we expected that the parasites would release a greater proportion of oocysts during the emergence of male than of female hosts.

In addition to testing this prediction, we studied the role of two other factors – the dose of infection and the parasite's previous route of transmission – on the route of transmission. We expected that a higher dose would increase pre-adult mortality and consequently favour local transmission, as it does for the microsporidium *Edhazardia aedis*, another parasite of the mosquito *Ae. aegypti*. In this system, horizontal transmission from dead larvae to other larvae within the same breeding site (thus, local transmission) increases with the dose, while vertical transmission (thus, distant transmission) decreases (Agnew and Koella 1999). While there is no *a priori* expectation for the influence of a parasite's previous transmission on its current transmission strategy, our experiment would add to the scarce literature showing that a parasite's past experience can determine its current phenotype (Tseng 2006; Little *et al.*, 2007).

Materials and methods

Study organism

The mosquito *Ae. aegypti* is widespread in subtropical and tropical regions. It has been studied in detail because of its importance in the transmission of human disease and the ease of maintaining it in the laboratory (Christophers 1960). The larvae live in small water containers, where they feed on bacteria. The larvae transform into pupae, from which the adults emerge 2 days later. Males pupate approximately 1 day before females and are smaller as adults. After a blood meal, each female generally distributes its eggs into several water containers (Colton *et al.* 2003).

Ascogregarina culicis is an Apicomplexan parasite of *Ae. aegypti* (Sulaiman 1992; Reyes-Villanueva *et al.*, 2003). Larvae are infected when they ingest the parasite's oocysts. If larvae are infected as first instars (Roychoudhury and Kobayashi 2006), the oocysts are produced during the pupal stage. In this case, some of the oocysts are released when the mosquito emerges as an adult or when pupae die, resulting in local transmission. When adults contain oocysts, these are shed by ovipositing females or are released if the adult dies in a breeding site, resulting in distant transmission. When the larvae are infected after the first instar stage, the oocysts are produced only after the emergence of the adult and thus transmitted distantly (Roychoudhury and Kobayashi 2006). Oocysts remain infective in water or in the air for up to 6 months (Roychoudhury and Kobayashi 2006). Infections by American strains of *A. culicis* have few pathogenic effects on any life stage, but some Asian strains are virulent when the infectious dose is high (Sulaiman 1992; Reyes-Villanueva *et al.*, 2003).

We obtained the mosquito colony, which has its origin in Florida, from J. Becnel (USDA, Gainesville, FL, USA). The parasites were collected in Louisiana, USA, from a natural population of mosquitoes by Dawn Wesson (Tulane University) in 2003 and maintained for almost 3 years in the mosquito colony of our laboratory using oocysts from local and distant transmission. An accidental bottleneck occurred 8 months before the experiment: only some of the oocysts shed during the oviposition of three females were saved.

Experimental design

The general design of the experiment is described in Fig. 1. From nine infected females we founded nine isolates of the parasite, each one with a fraction derived from local transmission and a fraction derived from distant transmission (giving the previous transmission route). Each of these fractions was split into three inoculates, and each of these was used to infect four mosquitoes, each of them with a different dose of oocysts.

Isolates

We refer to an isolate as the parasites obtained from a single infected female mosquito, which was reared individually in a 12-well plate in 4 mL of deionized water and infected with a standard mixture of the parasite colony. On the day they pupated, we transferred each individual into a 1.5 mL centrifugation tube with 0.5 mL of water. When they emerged, we separated the adult from the water it had emerged in and put the mosquito in a new centrifugation tube.

Fractions

To obtain locally transmitted oocysts, we collected and homogenized the water the female mosquito had emerged from and that still contained the pupal case. To obtain distantly transmitted oocysts, we crushed the female 1–3 days after emergence in its centrifugation tube with 0.5 mL of deionized water.

Inoculates and doses

We split each fraction into three inoculates and counted the oocysts in these inoculates with a haemocytometer. We exposed mosquitoes to 10, 50, 250 or 1250 oocysts of each inoculate.

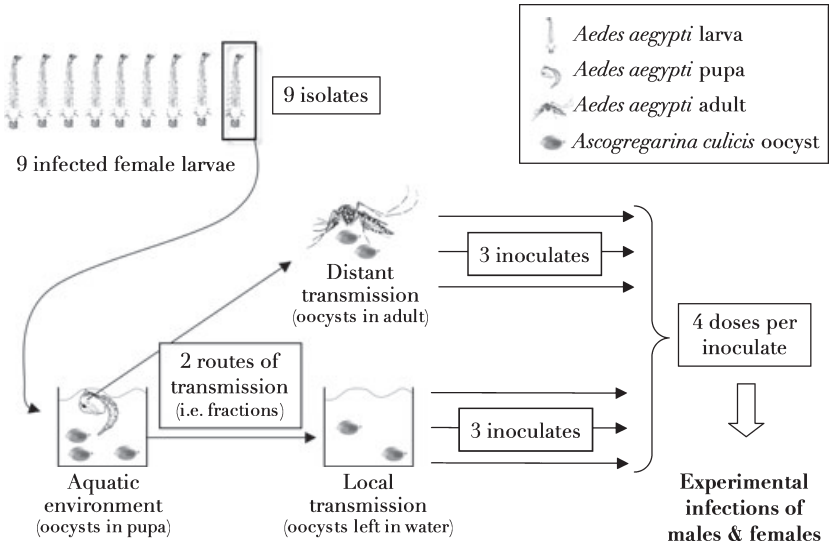

Fig. 1 Experimental design.

Infection and rearing

We synchronously hatched under low pressure a batch of eggs from our standard mosquito colony and gave them Tetramin Baby (Tetra, GmbH, Melle, Germany) *ad libitum* as food. After 24 h they were put individually into the wells of 12-well tissue culture plates with 3 mL of deionized water. They received 0.04 mg of Tetramin Baby on day 2, 0.08 mg (day 3), 0.16 mg (day 4), 0.32 mg (day 5), 0.64 mg (day 6) and 0.32 mg (following days). When they were 2 days old, they were exposed to the oocysts. When the mosquitoes pupated, they were transferred with approximately 0.15 mL of water into an open centrifugation tube within a 50-mL plastic tube covered with netting. If a pupa died, it was frozen at −20 °C. After emergence, each adult was put into a centrifugation tube and frozen at −20 °C. The water from which the adult had emerged was also frozen at −20 °C. Oocysts in the pupae, in the adults and in the water were counted with a haemocytometer.

We recorded the age at pupation of the mosquitoes to estimate a possible link of this life-history trait with the parasite's route of transmission. Only 156 of the 216 theoretical experimental replicates were inoculated and followed up until death or emergence because of insufficient oocysts for some treatment combinations and loss of a few individuals because of handling errors. Of these, 148 survived until adulthood; all of the survivors contained oocysts while four of the eight mosquitoes that died did not contain any. The experiment was performed in a climate-controlled chamber with 12 h of light per day, at 26 ± 2 °C and with 70 ± 10% relative humidity.

Statistical analysis

We analysed three traits: the total number of oocysts produced during an infection, the proportion of oocysts released during the emergence of the adult and the parasite's effect on the age of its host's pupation. We used linear mixed models with 'isolate' and 'inoculate' (nested within isolate) as the random factors and 'host's sex', 'dose' (treated as an ordinal factor to satisfy assumptions of the analyses) and 'previous route of transmission' as fixed factors. We backward-eliminated the terms with $P > 0.1$, starting with the highest-level interactions and the least significant terms. Even if they were not significant, we did not remove the isolate and inoculate terms as they controlled for the non-independence of the replicates that received oocysts from the same isolate or inoculate. In order to satisfy the assumptions of normality and homoscedasticity of the model, we log-transformed the oocyst number. We did not analyse the oocyst number of the four individuals that died before adulthood and contained oocysts because we could not determine their sex (analyses with these individuals but without the sex term gave similar results). For the analysis of the transmission route, we used the proportion of oocysts released during the host's emergence and added as covariates the total number of oocysts produced and the age at the host's pupation. The significance of each random factor was estimated with a comparison of the difference of −2 log-likelihood of the models with and without the factor to a chi-squared distribution. We used the statistical package JMP 6.0.3 (SAS Institute, Cary, NC, USA) and the REML (Restricted Maximum Likelihood) method.

Results

Oocyst number

All mosquitoes that survived to adulthood harboured at least one oocyst. The total number of oocysts was about twice as high in females (n = 80, mean = 6971,

Table 1 Final statistical models for total oocyst number and proportion of oocysts released during host's emergence (i.e. transmission route).

Trait	d.f. (Num, Denom)	Test statistic	*P*-value
Oocyst number			
Fixed factors		*F*	
Host sex	1, 130	44.92	< 0.0001
Dose of infection	3, 107	28.59	< 0.0001
Random factors		χ^2	
Isolate	1	0.014	0.9
Inoculate	1	3.67	0.054
Proportion of oocysts released locally			
Fixed factors		*F*	
Host sex	1, 120	55.32	< 0.0001
Dose of infection	3, 118	4.78	0.0035
Previous transmission route	1, 40	4.96	0.0316
Oocyst number	1, 122	62.54	< 0.0001
Random factors		χ^2	
Isolate	1	0.007	0.93
Inoculate	1	0.501	0.48

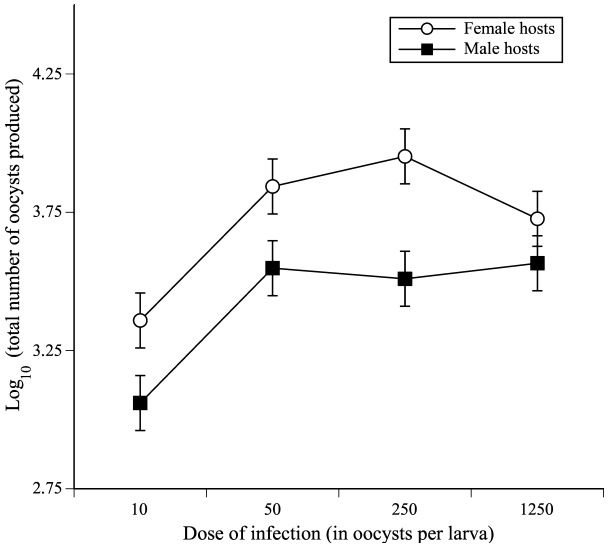

Fig. 2 Total number of oocysts produced in infected mosquitoes as a function of the host's sex and dose of infection. The dots represent means, the vertical lines are standard errors.

SE = 516) as in males (n = 68, mean = 3582, SE = 294) (Table 1, Fig. 2). There were also fewer oocysts in mosquitoes infected with the lowest dose (n = 37, mean = 2415, SE = 311) than those with the three higher doses (doses grouped together: n = 111, mean = 6413, SE = 398); the mosquitoes infected with these three doses had similar oocyst numbers. Neither the previous transmission route nor the interactions of the host's sex and the dose of infection with the other terms of the model were significant ($P > 0.1$).

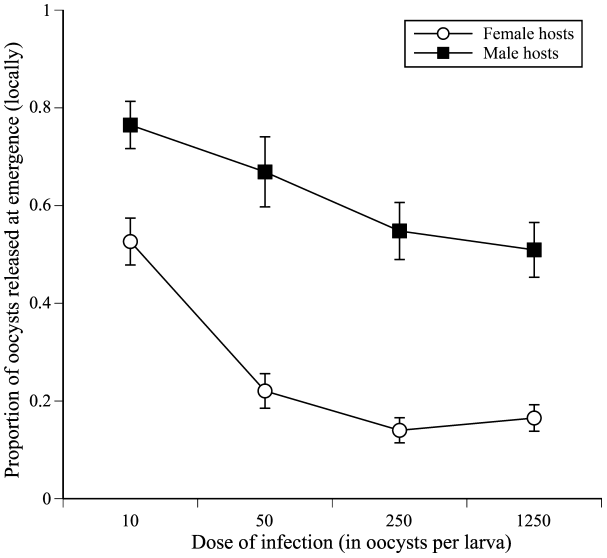

Fig. 3 Proportion of oocysts released during host's emergence (i.e. investment in local transmission) in function of host's sex and dose. The dots represent means, the vertical lines are standard errors.

Proportion of oocysts released during emergence

The proportion of oocysts released during host emergence was higher in male (n = 68, mean = 0.61, SE = 0.03) than in female hosts (n = 80, mean = 0.27, SE = 0.025) (Table 1, Fig. 3). Lower doses of infection produced relatively more local transmission than higher doses (Fig. 3), ranging from 66% at the lowest dose to 35% at the highest dose. Infections by oocysts that themselves had been locally released produced more local transmission (n = 59, mean = 0.48, SE = 0.037) than infections by oocysts retrieved from the body of an adult female (n = 89, mean = 0.39, SE = 0.032) (Fig. 4). None of the interactions of the host's sex, the dose of infection and the previous transmission route with the other terms of the model was significant ($P > 0.1$). Individuals with more oocysts released a smaller proportion of them at emergence [parameter estimate from the model: 'proportion released at emergence = $-0.12 \times$ log(number of oocysts)']. Age at pupation had no influence on the proportion of oocysts released during emergence ($F_{1,140} = 1.58$, $P = 0.21$).

Effects on the age of the host's pupation

The host's sex was the only factor significantly determining age at pupation ($F_{1,117} = 100.7$, $P < 0.0001$). On average, males pupated after 8.6 days (SE = 0.10) and females after 10.0 days (SE = 0.08).

Discussion

As we predicted, *A. culicis* released a greater proportion of oocysts during the emergence of the adult mosquito

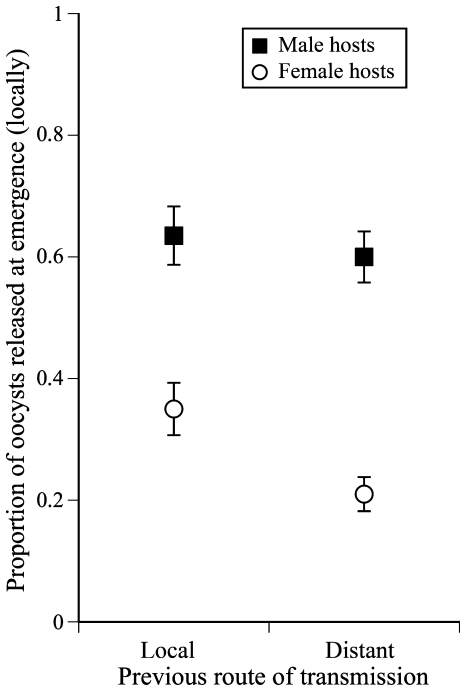

Fig. 4 Proportion of oocysts released during the host's emergence (i.e. investment in local transmission) in function of the previous route of transmission and the host's sex. Note that the interaction between these two factors is not significant ($P > 0.1$). The dots represent means, the vertical lines are standard errors.

when it was in a male host than when it was in a female host (Table 1, Fig. 3). This suggests an adaptive strategy of the parasite, as males do not produce eggs and their parasites can therefore not be transmitted during oviposition. This follows reports on several other parasites that vary their transmission strategy according to the sex of their host. Another parasite of mosquitoes, the microsporidium *Amblyospora* sp., enables its female hosts to survive to adulthood and is then transmitted vertically, but kills its male hosts as larvae and is then transmitted horizontally (Andreadis and Hall 1979; Andreadis 2007). Many parasites that are transmitted exclusively maternally, such as *Wolbachia*, *Cardinium* and *Rickettsia* bacteria, convert male hosts into functional females (Werren 1997; Bandi *et al.*, 2001; Weeks *et al.*, 2002). The fungus *Microbotryum violaceum*, which infects the dioecious *Silene latifolia* can only produce its spores in male organs. It therefore manipulates female plants to produce hermaphroditic flowers, which allows its transmission (Korpelainen 2000; Uchida *et al.*, 2003). Unlike these parasites, *A. culicis* does not preferentially kill male hosts or modify their sex, but modifies the strategy of oocyst allocation to local or distant transmission according to the sex of its host.

Such responses of parasites to host sex illustrate the broad range of adaptations that they use to cope with environmental variation. For instance, the trematode *Coitocaecum parvum* tunes its development in its intermediate crustacean host to the clues indicating the presence or absence of its (facultative) definitive fish host. In the presence of fish, the parasite develops slowly and ends up in the fish when the crustacean is eaten by it. In the absence of fish, development is accelerated, leading to the production of mature worms in the intermediate host (Poulin 2003). Parasites also adjust their reproductive phenotype to the presence or absence of conspecifics within their host. For instance, the hermaphroditic cestode *Schistocephalus solidus* delays the onset of egg production when alone in the host. An explanation for this pattern is that the worms wait for the possible arrival of mates in order to avoid the cost of selfing, expressed for example as a low hatching rate of self-fertilized eggs (Schjørring 2004). Malaria parasites decrease the proportion of male transmission stages when their relatedness with the other parasites in the host increases, thus increasing their transmission success (Reece *et al.*, 2008).

Local transmission was also influenced by the dose of infection: at the lowest dose (10 oocysts per larva) a greater proportion of the oocysts were allocated to local transmission than at higher doses. The evolutionary pressure and mechanism underlying this effect are unknown. In particular, we observed the opposite of our expectation – that higher doses would lead to higher pre-adult mortality and thus to higher local transmission – although other parasites, e.g. the microsporidian *Edhazardia aedis*, which also infects *Ae. aegypti*, do conform to this expectation. This parasite transmits horizontally by killing infected larvae and pupae, and vertically by infecting the eggs within the mother. Higher doses lead to more horizontal transmission than lower doses (Agnew and Koella 1999). As horizontal transmission is mainly local and vertical transmission is mainly distant, the effect of dose is the opposite of what we observed for *A. culicis*.

Finally, local transmission was influenced by the parasite's previous transmission route. When the oocysts used to infect an individual had been released during the emergence of the parasite's previous host, it released proportionally more oocysts at emergence than when the oocysts had been retrieved from an adult (Fig. 4). Past experience of parasites also influenced their current phenotype, as revealed in two recent studies with *Ascogregarina taiwanensis*, which infects *Aedes albopictus* (Tseng 2006), and with the bacterium *Pasteuria ramosa*, which infects the crustacean *Daphnia magna* (Little *et al.*, 2007). In both studies, the parasite was less harmful if it had previously infected food-deprived hosts than well-fed hosts. Such observations imply that transient environmental conditions can have long-lasting effects on host and parasite populations.

We suggest that the effect of sex on *A. culicis*'s route of transmission is an evolutionary adaptation of the parasite to different transmission opportunities offered by the

males and females. However, one might argue that it is a side effect of the morphological and developmental differences between male and female mosquitoes. First, male mosquitoes develop more rapidly than females, as shown by their earlier age at pupation. As the parasite's development depends on that of its host (Chen 1999; Roychoudhury and Kobayashi 2006), the phenotype of the parasites could be constrained by the host's developmental rate. However, although the age at pupation of the host was included in the statistical analysis of transmission route, the effect of host sex was significant, suggesting that it was not driven by the host's developmental speed. In addition, one would expect that the more rapid development of the males would give the parasite less time to produce the locally transmitted oocysts, which is the opposite of our observation. Second, male mosquitoes are on average smaller than females. The higher local transmission in males might therefore be a side effect of local transmission decreasing with the body size of the adult. However, in another experiment (S. Fellous and J.C. Koella, unpublished), within each sex, local transmission was lowest in the intermediate-sized individuals (see Supporting Information). This suggests that the smaller size of males than that of females cannot alone explain the large investment in local transmission of their parasites. Although other aspects of the host's sexual dimorphism, such as differential immune investment, might constrain the parasite's transmission, all available data support the fact that the difference in transmission strategy between male and female hosts is indeed adaptive.

Our discussion of the variability of *A. culicis* phenotypes with respect to its host's sex, its infectious dose and its past transmission route is based on the idea that the parasite would exhibit phenotypic plasticity (Pigliucci 2001; Pigliucci 2005). Of course, the phenotypic variations we observed might have also reflected genetic variation. Thus, for example, genotypes with more local transmission could be favoured in male hosts while genotypes with more distant transmission could be favoured in female hosts. However, even if this were the case, the prediction that the parasites in males should have more local transmission is valid, so that our results would illustrate the parasite's adaptation to the transmission opportunities offered by its different hosts.

Overall, our results show the role of the host's sex, the dose and the previous transmission route of a parasite in determining its transmission route. These effects have consequences on the epidemiology and the evolution of *A. culicis* and its host for two main reasons. First, local and distant transmissions differ in terms of dispersal distance. Increased local transmission would lower the parasite's dispersal. This would increase its average kinship within breeding sites, which could relax the competition among parasites and select for reduced virulence (Frank 1996; Chao *et al.*, 2000). Changes in a parasite's dispersal can also influence other aspects of its biology, including its local adaptation (Lively 1999; Gandon and Michalakis 2002). Secondly, when distant transmission occurs through the release of oocysts during the oviposition of an infected female, the parasite can infect its offspring. Although factors such as the number of larvae from other females in the same breeding sites and the resilience of the oocysts influence the frequency of this event, there is almost certainly more vertical transmission than for locally transmitted parasites, for which there is the possibility of vertical transmission only if the emerged female comes back to lay its eggs in the breeding site it has just left. This is, however, unlikely as female *Ae. aegypti* distribute their eggs to several breeding sites (Colton *et al.*, 2003) and deposit them on average more than 180 m away from their development site (Reiter *et al.*, 1995). As the extent of vertical transmission influences the evolutionarily stable level of virulence (Bull *et al.* 1991; Koella and Doebeli 1999), any environmental changes that increase local transmission would be likely to increase the virulence of *A. culicis*.

Conclusions

Parasites with several transmission strategies can vary their allocation to the strategies and optimize their success in the different environments they experience. As predicted, a greater proportion of *A. culicis* oocysts were used for local transmission in male than in female hosts. We argue that this reflects an adaptive strategy of the parasite in response to the low potential for distant transmission offered by males. It thus illustrates the ability of parasites to adjust their phenotype to their environment. In addition to the host's sex, the dose of infection and the parasite's past experience influenced its route of transmission, a trait that affects the dispersal and the evolution of the virulence of the parasite.

Acknowledgments

We thank Michael J. Crawley for statistical advice, and Oliver Kaltz, Yannis Michalakis, Alison Duncan, Lucie Salvaudon, Aurélie Coulon and two anonymous reviewers for helpful discussions and comments. SF thanks Brian Lazzaro for his help. SF was supported by an Allocation de Recherche of the Ministère Délégué à l'Enseignement Supérieur et à la Recherche.

References

Agnew, P. & Koella, J.C. 1999. Life history interactions with environmental conditions in a host-parasite relationship and the parasite's mode of transmission. *Evol. Ecol.* **13**: 67–89.

Andreadis, T.G. 2007. Microsporidian parasites of mosquitoes. *J. Am. Mosq. Control Assoc.* **23**: 3–29.

Andreadis, T.G. & Hall, D.W. 1979. Development, ultrastructure, and mode of transmission of *Amblyospora* sp. (Microspora) in the mosquito. *J. Eukaryot. Microbiol.* **26**: 444–452.

Bandi, C., Dunn, A.M., Hurst, G.D.D. & Rigaud, T. 2001. Inherited microorganisms, sex-specific virulence and reproductive parasitism. *Trends Parasitol.* **17**: 88–94.

Bull, J.J., Molineux, I.J. & Rice, W.R. 1991. Selection of benevolence in a host-parasite system. *Evolution* **45**: 875–882.

Chao, L., Hanley, K.A., Burch, C.L., Dahlberg, C. & Turner, P.E. 2000. Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. *Q. Rev. Biol.* **75**: 261–275.

Chen, W.J. 1999. The life cycle of *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae). *Parasitol. Today* **15**: 153–156.

Christophers, S.R. 1960. *Aëdes aegypti* (L.). The Yellow Fever Mosquito. Its Life History, Bionomics and Structure. Cambridge University Press, Cambridge.

Colton, Y.M., Chadee, D.D. & Severson, D.W. 2003. Natural skip oviposition of the mosquito *Aedes aegypti* indicated by codominant genetic markers. *Med. Vet. Ent.* **17**: 195–204.

Frank, S.A. 1996. Models of parasite virulence. *Q. Rev. Biol.* **71**: 37–78.

Gandon, S. & Michalakis, Y. 2002. Local adaptation, evolutionary potential and host-parasite coevolution: interactions between migration, mutation, population size and generation time. *J. Evol. Biol.* **15**: 451–462.

Kaltz, O. & Koella, J.C. 2003. Host growth conditions regulate the plasticity of horizontal and vertical transmission in *Holospora undulata*, a bacterial parasite of the protozoan *Paramecium caudatum*. *Evolution.* **57**: 1535–1542.

Koella, J.C. & Doebeli, M. 1999. Population dynamics and the evolution of virulence in epidemiological models with discrete host generations. *J. Theor. Biol.* **198**: 461–475.

Korpelainen, H. 2000. Labile sex expression in plants. *Biol. Rev.* **73**: 157–180.

Levin, B.R. & Lenski, R.E. 1985. Bacteria and phage: a model system for the study of the ecology and coevolution of hosts and parasites. In: *Ecology and Genetics of Host–Parasite Interactions* (D. Rollinson & R.M. Anderson, eds), pp. 227–242. Academic Press, London.

Little, T., Birch, J., Vale, P. & Tseng, M. 2007. Parasite transgenerational effects on infection. *Evol. Ecol. Res.* **9**: 459–469.

Lively, C.M. 1999. Migration, virulence, and the geographic mosaic of adaptation by parasites. *Am. Nat.* **153**: S34–S47.

Mittler, J.E. 1996. Evolution of the genetic switch in temperate bacteriophage. *I. Basic theory. J. Theor. Biol.* **179**: 161–172.

Pigliucci, M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore, MD; London.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? *Trends Ecol. Evo.*, **20**: 481–486.

Poulin, R. 2003. Information about transmission opportunities triggers a life-history switch in a parasite. *Evolution* **57**: 2899–2903.

Poulin, R. & Lefebvre, F. 2006. Alternative life-history and transmission strategies in a parasite: first come, first served? *Parasitology* **132**: 135–141.

Reece, S.E., Drew, D.R. & Gardner, A. 2008. Sex ratio adjustment and kin discrimination in malaria parasites. *Nature* **453**: 609–614.

Reiter, P., Amador, M.A., Anderson, R.A. & Clark, G.G. 1995. Dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. *Am. J. Trop. Med. Hyg.* **52**: 177–179.

Reyes-Villanueva, F., Becnel, J.J. & Butler, J.F. 2003. Susceptibility of *Aedes aegypti* and *Aedes albopictus* larvae to *Ascogregarina culicis* and *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) from Florida. *J. Invertebr. Pathol.* **84**: 47–53.

Roychoudhury, S. & Kobayashi, M. 2006. New findings on the developmental process of *Ascogregarina taiwanensis* and *Ascogregarina culicis* in *Aedes albopictus* and *Aedes aegypti*. *J. Am. Mosq. Control. Assoc.* **22**: 29–36.

Schjørring, S. 2004. Delayed selfing in relation to the availability of a mating partner in the cestode *Schistocephallus solidus*. *Evolution* **58**: 2591–2596.

Sulaiman, I. 1992. Infectivity and pathogenicity of *Ascogregarina culicis* (Eugregarinida: Lecudinidae) to *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* **29**: 1–4.

Tseng, M. 2006. Interactions between the parasite's previous and current environment mediate the outcome of parasite infection. *Am. Nat.* **168**: 565–571.

Uchida, W., Matsunaga, S., Sugiyama, R., Kazama, Y. & Kawano, S. 2003. Morphological development of anthers induced by the dimorphic smut fungus *Microbotryum violaceum* in female flowers of the dioecious plant *Silene latifolia*. *Planta* **218**: 240–248.

Wang, I.-N., Dykhuizen, D.E. & Slobodkin, L.B. 1996. The evolution of phage lysis timing. *Evol. Ecol.* **10**: 545–558.

Weeks, A.R., Tracy Reynolds, K. & Hoffmann, A.A. 2002. *Wolbachia* dynamics and host effects: what has (and has not) been demonstrated? *Trends Ecol. Evol.* **17**: 257–262.

Werren, J.H. 1997. Biology of *Wolbachia*. *Annu. Rev. Entomol.* **42**: 587–609.

Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1 Relationship between mosquito wing length, an estimator of body size, and proportion of oocysts released during emergence. A quadratic regression is fitted for each sex.

Figure S2 Relationship between mosquito wing length, an estimator of body size, and number of oocysts produced per infection. A quadratic regression is fitted for each sex.

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Received 29 April 2008; accepted 4 November 2008