Adverse effects of covert iridovirus infection on life history and demographic parameters of *Aedes aegypti*

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Abstract

Sublethal viral infections can cause changes in the body size and demography of insect vectors, with important consequences for population dynamics and the probability that individual mosquitoes will transmit disease. This study examined the effects of covert (sublethal) infection by *Invertebrate iridescent virus* 6 (IIV-6) on the demography of female *Aedes aegypti* and the relationship between key life history parameters in covertly infected female insects compared with healthy (control) insects or non-infected mosquitoes that had survived exposure to virus inoculum without becoming infected. Of the female mosquitoes that emerged following exposure to virus inoculum and were offered blood meals, 29% (43/150) proved positive for covert IIV-6 infection. The net reproductive rate (R_0) of covertly infected females was 50% lower for infected females compared to control mosquitoes, whereas non-infected exposed females had an R_0 approximately 15% lower than that of controls. Reproduction caused a significant decrease of about 13 days in mosquito longevity compared to females that did not reproduce ($P < 0.001$). Infected females lived 5–8 days less than non-infected exposed females or controls, respectively ($P = 0.028$). Infected females and non-infected exposed females both had significantly shorter wings than control insects ($P < 0.001$). There was a significant positive correlation between wing length and longevity in covertly infected female mosquitoes but not in control or non-infected exposed mosquitoes. Longer lived females produced more eggs in all treatments. There were no significant correlations between body size and fecundity or the production of offspring. There was also no correlation between fecundity and fertility, suggesting that sperm inactivation was a more likely cause of decreased fertility in older mosquitoes than sperm depletion. We conclude that covert infection by iridescent virus is likely to reduce the vectorial capacity of this mosquito.

Introduction

Studies on entomopathogens have mainly focussed on pathogen-induced insect mortality and their potential for use as biological insecticides. However, non-lethal chronic infections by insect viruses have been demonstrated to influence key demographic parameters including host fecundity, fertility, development rate, and longevity (Boots & Norman, 2000; Myers et al., 2000) which are believed to exert an important influence on host population dynamics (Anderson & May, 1981; Sait et al., 1994; Myers & Kukan, 1995; Cory et al., 1997). The majority of these studies have been performed with Lepidoptera inoculated with baculoviruses and cypoviruses (Rothman & Myers, 1996). However, caution is required when interpreting sublethal effects of insect pathogens, as many studies of this kind have assumed that the survivors of an inoculum challenge are sublethally infected but fail to demonstrate the presence of an infection (Rothman & Myers, 1996).

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Invertebrate iridescent viruses (IIVs) of the genus *Iridovirus* (*Iridoviridae*) are icosahedral particles of ~120 nm diameter with a large dsDNA genome. IIVs mainly infect soil-dwelling and aquatic insects and have been isolated from various insect orders including Diptera, Coleoptera, Ephemeroptera, and Lepidoptera, and also from terrestrial isopods (Crustacea) (Williams et al., 2000). The mechanism of transmission of these viruses is poorly understood; IIVs are highly infective by injection but of low infectivity *per os*. These viruses cause two types of infection. Patent infections are obvious to the naked eye; the insect develops an intense opalescent blue or lavender hue due to the formation of paracrystalline arrays of virus particles in the cytoplasm of host cells. Patent infections are fatal, and the insect almost invariably dies in the larval or pupal stage. Covert infections, on the other hand, are not obvious and the infected host develops to the adult stage and may reproduce. For most IIVs, covert infections can be detected by an insect bioassay involving injection of semi-purified insect homogenates into *Galleria mellonella* (L) (Lepidoptera: Pyralidae), a highly susceptible host which develops a patent infection 6–12 days post-inoculation if virus was present in the injected inoculum (Constantino et al., 2001). Molecular techniques, including polymerase chain reaction (PCR) and DNA-DNA hybridization, have also been used to detect covert IIV infections in blackflies and lepidopteran larvae (Ward & Kalmakoff, 1991; Williams, 1993, 1995). The underlying causes for such variation in virus virulence are poorly understood, although it is believed that chronic infections represent a mechanism for pathogen persistence when opportunities for horizontal transmission are limited, whereas acute virulent infections are favoured when opportunities exist for immediate horizontal transmission to susceptible hosts (Swinton et al., 2002).

Sublethal infection by *Invertebrate iridescent virus 6* caused a significant reduction in adult longevity and body size (wing length) of *Aedes aegypti* (L) compared to either controls or insects that had been exposed to virus inoculum but had not become infected (Marina et al., 1999). Numerical differences in fecundity and progeny production were also observed between covertly infected and control or non-infected exposed mosquitoes, but an increase in variability associated with covert infection prevented a formal statistical analysis (Marina et al., 1999).

Pathogen-induced reduction of adult longevity is particularly relevant in the study of vectors of disease, as lifespan can be highly influential on the vectorial capacity of the female insect (Goddard, 2000). Similarly, parameters such as body size affect the duration of the gonotrophic cycle in *A. aegypti* and hence the interval between feeding bouts during which vector-borne diseases may be transmitted (Briegel, 1990). The present study was therefore designed to examine the effect of covert IIV infection on the demography of female *A. aegypti* and the relationship between key life history parameters such as fecundity, fertility, and longevity and their interaction with body size in healthy and covertly infected female insects.

Materials and methods

Insects and virus

Eggs of *A. aegypti* were obtained from a laboratory colony maintained in the Centro de Investigación de Paludismo, Tapachula, Chiapas, Mexico. Mosquitoes used in the experiments described below were reared using filtered dechlorinated tap water. Third instar *G. mellonella* were obtained from a laboratory colony held in El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico. All procedures involving insects were performed in a laboratory at 26 ± 1 °C, 75–85% r.h., and a L12:D12 photoperiod.

Invertebrate iridescent virus 6

(IIV-6) was produced by injection into third instar *G. mellonella* as described previously (Constantino et al., 2001). Patently infected larvae were harvested at eight days post-injection and were stored at −20 °C until required. Virus was extracted and purified by trituration of infected *G. mellonella* larvae in sterile distilled water followed by centrifugation at 490 *g*, 960 *g*, and 1250 *g*, each for 10 min to remove insect debris, followed by 15 300 *g* for 10 min to pellet virus. The pellet was resuspended in 300 µL water, layered onto 30% sucrose, and subjected to 15 300 *g* for 30 min. The resulting pellet was washed once and resuspended in 1 mL sterile distilled water. The purified suspension was quantified by direct counting of a mixture of virus and polystyrene beads 460 nm diameter (Aldrich Chemical Co., MO, USA) using a scanning electron microscope (Constantino et al., 2001).

Inoculation and rearing of mosquitoes

Groups of 200 third instar *A. aegypti* were placed in a suspension of 4.4×10^7 particles/mL of IIV-6. Powdered sand (100 mg) was added to the suspension to increase the prevalence of infection. The suspension was gently agitated by air from an aquarium pump. After 6 h exposure larvae were washed by carefully passing each larva through a series of five volumes of 1 l of dechlorinated tap water to eliminate residual inoculum. Larvae were placed in rearing trays and provided with a powdered yeast and soya mixture on a daily basis. Larvae were checked twice daily for evidence of patent infection, which involved an obvious change of colour to an iridescent blue. Patently infected larvae were removed from the experimental area and

held until death to avoid any risk of contamination of other treatments. The process was repeated for control mosquitoes not exposed to virus inoculum.

Following pupation, pupae were transferred to individual 250 ml plastic cups containing 50 ml dechlorinated water. Following adult emergence 50 male and female pairs were selected from each treatment at random and each placed in 400 ml plastic cups with a fine nylon gauze lid and supplied *ad libitum* with 10% sugar solution. After a 48 h mating period the male mosquitoes were removed and transferred to plastic cups, held in groups of eight individuals/cup and supplied with 10% sugar solution and checked daily until death. Approximately 40 ml water and a wooden spatula oviposition substrate were placed in the cups containing individual female mosquitoes. Females were offered a human blood meal at four-day intervals. The number of eggs laid each day was noted for each female until death. The number of larvae that emerged from each batch of eggs was also recorded. Females that were not selected for mating were held in groups of 6–8 individuals/cup, offered 10% sugar solution and checked daily for mortality.

After death, the wing length of each mated and blood-fed female mosquito was measured from the axial incision to the outer margin of the R1 vein (Xue & Ali, 1994). The fimbrial scales were not included in this measurement and the wing was selected from the left or right side of the body at random. The abdomen of each female was then removed and homogenized in 500 µl of antibiotic solution (0.08% aureomycin). Insect debris was removed by centrifugation at 190 *g* for 5 min. Volumes of 8.4 µl of the remaining suspension were injected into 10 third-instar *G. mellonella*, which were placed in 50 ml plastic pots containing semi-synthetic diet and held at 25 °C. The presence of patent IIV infections in these larvae 12–14 days post-injection was used as an indicator of covert infection of the mosquito. The results of the insect bioassay were used to classify mosquitoes into three groups: (i) covertly infected females (ii) non-infected females that had survived exposure to virus inoculum, and (iii) control females that had not been exposed to virus. The experiment was performed three times.

Statistical analysis

The lifetime and daily fecundity, wing length, longevity and proportion of infertile eggs were subjected to analysis of variance followed by Tukey test for mean separation. Where necessary, $\log_e(x)$, $\sqrt{(x)}$ and arcsine transformations were applied to normalize the distribution of data. The regression of the proportion of infertile eggs over time was performed by repeated measures ANOVA in the SAS program (SAS Institute, 1992). Correlations between untransformed variables were investigated using Spearman's non-parametric coefficient of rank correlation (Sokal & Rohlf, 1981). Net reproductive rate (R_o) was calculated as the mean number of female offspring per female, as described by Rothman & Myers (1996). Demographic analysis was applied to calculate female mosquito life expectancy at emergence (e_o), mortality (d_x), mean age net fecundity ($\sum xL_xM_x/\sum L_xM_x$) and net maternity (L_xM_x) using life tables for all female mosquitoes that were provided with blood meals (Carey, 1993; Carey & Liedo, 1999). With the exception of net maternity, the same parameters were also calculated for the non-reproductive females fed with sugar solution alone. Only life expectancy at emergence and death rate were calculated for the male mosquitoes. Mosquito survival was also subjected to Weibull analysis using the GLIM statistical program (Numerical Algorithms Group, 1993), as described by Crawley (1993).

Results

Prevalence of patent and covert infections

Of a total of 600 mosquitoes exposed to IIV-6 inoculum, only six larvae (1%) developed a patent infection. All of these larvae died prior to pupation. Of the female mosquitoes that were offered blood meals, 43 (29%) proved positive for covert IIV-6 infection in the post-mortem bioassay. These insects were considered as a separate group in all of the following analyses.

Costs of covert infection on mosquito reproduction

A small proportion (3–7%) of blood fed mosquitoes failed to lay eggs, but this did not differ according to treatment ($\chi^2 = 1.13$, d.f. = 2, P = 0.5). Covertly infected mosquitoes laid approximately 40% fewer eggs than controls or non-infected mosquitoes that had been exposed to virus inoculum ($F_{2,206} = 8.79$, $P < 0.001$) (Table 1). The rate of egg laying was also reduced by about two-thirds in covertly infected females compared to control and non-infected exposed females ($F_{2,268} = 6.01$, P = 0.003). Overall, the mean proportion of infertile eggs did not differ between treatments ($F_{2,260} = 0.984$, P = 0.375) but increased significantly during the lifetime of female mosquitoes, possibly due to depletion or death of viable sperm ($F_{9,47} = 10.3$, $P < 0.001$, repeat measures ANOVA for the period 9–45 days post-emergence during which mosquito reproduction was observed in all treatments) (Figure 1).

Net maternity of covertly infected females, defined as the sum of average daily production of offspring by females of age x multiplied by the fraction of individuals that survived to age x, was approximately half that observed in control and non-infected exposed mosquitoes (Table 2).

Table 1 Mean (± SE) values of lifetime fecundity, longevity, wing length, rate of egg laying and percentage infertility of eggs for covertly infected female *Aedes aegypti* compared to untreated controls and non-infected insects that had been exposed to virus inoculum in the larval stage

	Treatments					
	Control		Exposed (non-infected)		Covert infection	
Variables*	n	Mean	n	Mean	n	Mean
Fecundity (total eggs/female)	121	234.4 ± 14.3[a]	102	234.3 ± 11.5[a]	40	143.3 ± 12.9[b]
Longevity (days)	125	35.1 ± 1.3[a]	107	33.4 ± 1.2[a]	43	27.9 ± 1.3[b]
Wing length (mm)	107	2.94 ± 0.001[a]	91	2.86 ± 0.001[b]	41	2.87 ± 0.002[b]
Rate of egg production (eggs/female/day)	121	6.4 ± 0.3[a]	102	6.6 ± 0.3[a]	40	4.6 ± 0.3[b]
Infertility of eggs (%)	121	45.6 ± 1.8[a]	102	49.6 ± 1.9[a]	40	51.0 ± 3.9[a]

*Fecundity, longevity, and rate of egg laying were subjected to $\sqrt{(x)}$ transformation prior to analysis. Wing length data were $\log_e(x)$ transformed and percentage infertility data were arcsine transformed prior to analysis. Figures followed by the same letter are not significantly different for comparisons of each variable between columns (ANOVA, Tukey $P < 0.05$).

The peak in daily net maternity was observed between 12 and 15 days post-emergence in all treatments, although the pattern of net maternity in covertly infected mosquitoes was lower, declined more rapidly and ceased between 8 and 20 days before reproduction ceased in non-infected exposed and control mosquitoes, respectively (Figure 2). Mean age net fecundity, representing the age at which the average female has oviposited 50% of her eggs, was 17.4 days for covertly infected mosquitoes compared to 21.8 and 20.2 days for control and non-infected exposed mosquitoes, respectively. This reflects the reduced longevity of covertly infected mosquitoes (discussed below).

Overall, the net reproductive rate (R_0) of covertly infected females was 50% lower for covertly infected females compared to control mosquitoes. Non-infected exposed females had an R_0 approximately 15% lower than that of controls (Table 2).

Adult longevity and body size

Reproduction caused a significant decrease in mosquito longevity. Control mosquitoes that were fed blood meals lived an average (± SE) of 35.1 ± 1.3 days compared to 48.1 ± 2.1 for non-reproductive control females that were fed on sugar solution ($F_{1,199} = 26.3$, $P < 0.001$). This effect

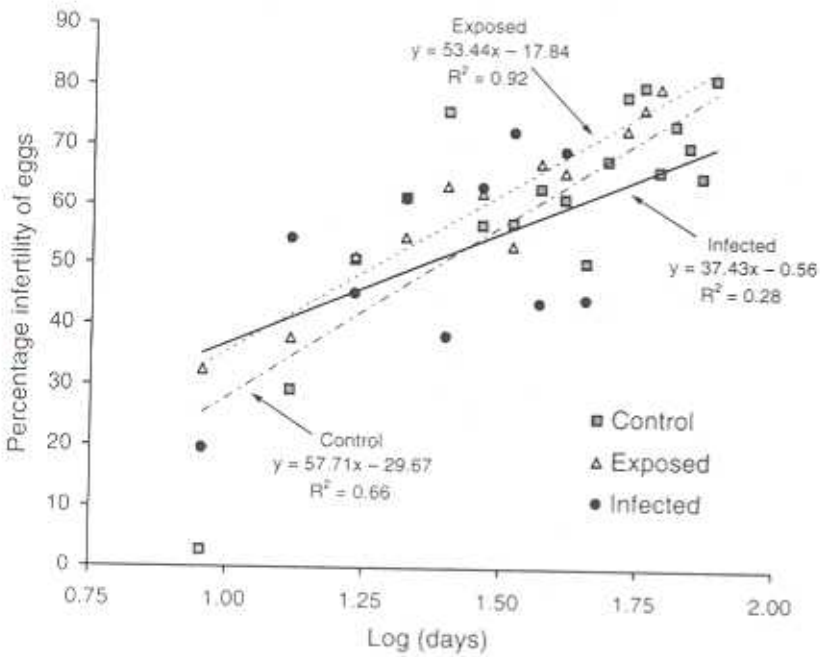

Figure 1 Linear regression of percentage egg infertility against log adult mosquito age (days) for control females (dot-dash line), non-infected exposed females (dotted line) and covertly infected females (solid line). Data pooled for four-day intervals representing the ovipositional cycles resulting from blood meals, from adult emergence until 61 days post-emergence. Statistical procedures were performed by repeat measures analysis of a subset of these data (9–45 days post-emergence).

Table 2 Net fecundity, mean age of reproduction, life expectancy, and instantaneous risk of death (Weibull hazard function) for covertly infected female *Aedes aegypti* compared to untreated controls and non-infected insects that had been exposed to virus inoculum in the larval stage

	Treatments		
Parameters	Control	Exposed (non-infected)	Covert infection
Net maternity ($\sum L_x M_x$)	228.5	223.4	131.1
Mean age net fecundity ($\sum x L_x M_x)/(\sum L_x M_x$)	21.8	20.2	17.4
Net reproductive rate (R_0)	34.8	29.3	17.2
Life expectancy at eclosion (e_0)	35.6	33.9	28.5
Hazard function (α, Weibull)	0.02474	0.02678	0.03320

was particularly clear when considering the death rate which peaked at 30 days for blood fed females and 50 days for sugar fed females (Figure 3A). Comparing the longevity of blood fed females from different treatments, covertly infected females lived on average 5–8 days less than non-infected exposed females or controls, respectively ($F_{2,172} = 3.62$, $P = 0.028$). Again, graphical representation of the death rate clearly shows how the death rate peaked higher and earlier for covertly infected females compared to those from other treatments (Figure 3B). The last covertly infected reproductive female died 15 days before the last reproductive exposed female and 35 days before the last reproductive control female (Figure 3B). The hazard function, representing the instantaneous risk of death calculated for each treatment by Weibull analysis, was accordingly greater for covertly infected females than for control and non-infected exposed females ($\chi^2 = 17.8$, d.f. = 2, $P < 0.001$) (Table 2).

Male longevity was greater than that of female mosquitoes but did not differ significantly between virus exposed males and control males ($F_{1,398} = 0.58$, $P = 0.45$). Likewise, life expectancy at eclosion was similar for virus-treated (56.3 days) and untreated control males (57.8 days). Males were not subjected to bioassay and their infection status was not determined.

Wing length was used as an indicator of body size. Covertly infected females and non-infected exposed females both had significantly shorter wings than control insects ($F_{2,236} = 9.75$, $P < 0.001$) (Table 1).

Interactions between life history parameters

There was a significant positive correlation between wing length and longevity in covertly infected female mosquitoes but not in control or non-infected exposed mosquitoes (Table 3). There was however, a significant positive correlation between longevity and fecundity for mosquitoes from all treatments. Longer lived control females and non-infected exposed mosquitoes also produced significantly more offspring, whereas the relationship was not significant for covertly infected mosquitoes (Table 3).

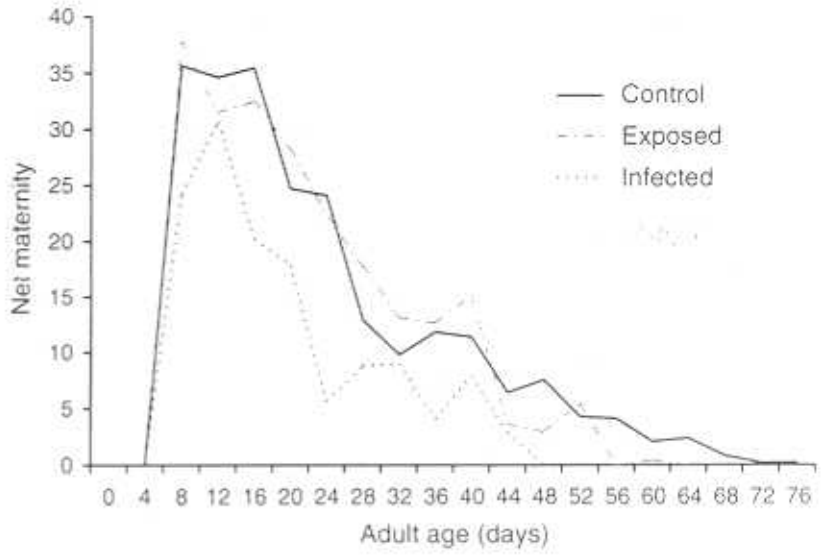

Figure 2 Net maternity index ($L_x M_x$) for control, non-infected exposed, and covertly infected blood fed mosquitoes showing reduced magnitude and shortened duration of reproduction in covertly infected mosquitoes compared to other treatments. Data were pooled at intervals of four days

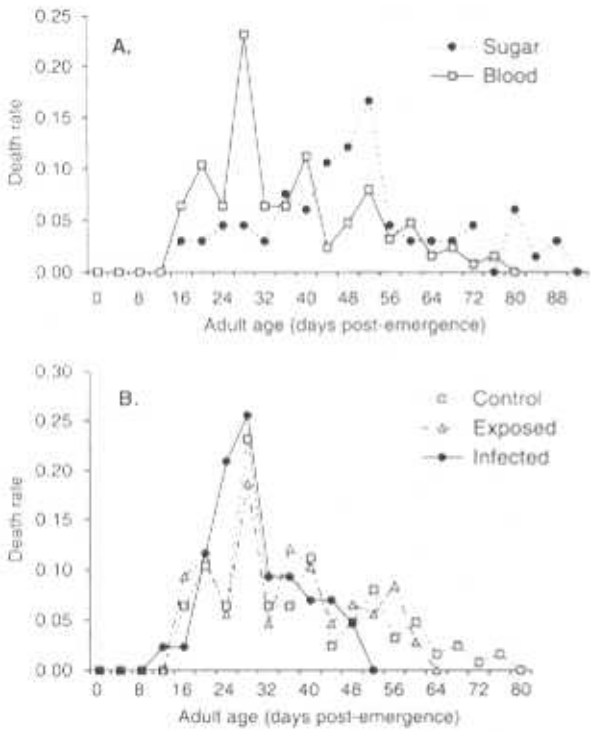

Figure 3 Comparison of mortality (d_x) of adult female *Aedes aegypti* showing (A) earlier peak in mortality in reproductively active blood fed mosquitoes from control treatment compared to non-reproductive sugar fed mosquitoes also from control treatment, and (B) earlier and greater magnitude peak mortality of covertly infected reproductive females compared to reproductive non-infected exposed and control mosquitoes. In all cases data were pooled at four-day intervals.

Increasing longevity was also associated with an increase in infertility in control and non-infected exposed mosquitoes, but not for covertly infected mosquitoes, possibly because they died before sperm depletion, or inactivation limited offspring production.

There were no significant correlations between body size and fecundity or the production of offspring. There was also no correlation between fecundity and fertility, suggesting that sperm inactivation was a more likely cause of decreased fertility in older mosquitoes than sperm depletion (Table 3).

Discussion

Sublethal infection by HV-6 caused marked reductions in the reproductive capacity, longevity, and body size of infected female mosquitoes. Although similar, our results differ from those previously reported by Marina et al. (1999), in that the sublethal effects that we observed were of greater magnitude and amenable to statistical analysis which may have been due to differences in the methodology used by Marina et al. (1999). In particular, the mating of virus inoculated females with control males and *vice versa* may have diluted the effect of each treatment on mosquito reproduction and reduced the statistical probability of detecting treatment differences in the study performed by Marina et al. (1999). Moreover, Marina et al. (1999) only considered one bout of oviposition following a single blood meal. In contrast, in the present study, males and females originating from the same treatment were mated with one another and thus were not subjected to a possible dilution of sublethal effects and the entire reproductive history of each female was monitored until death.

The combination of a lower rate of oviposition (daily net maternity) and shorter lifespan meant that covertly infected females had a net reproductive rate half that of control females, which is considerably greater than the 22% reduction reported by Marina et al. (1999). These effects may be related to body size, which has been observed to have a marked influence on the size of blood meals, the duration of the gonotrophic cycle and fecundity in *A. aegypti* (Briegel, 1990).

	Treatments		
Parameters	Control	Exposed (non-infected)	Covert infection
Longevity versus size	0.050	−0.053	0.308*
versus fecundity	0.510**	0.577**	0.414**
versus total offspring	0.304**	0.329**	0.248
versus percent infertility	0.279**	0.247*	0.046
Fecundity versus size	−0.055	−0.050	0.083
versus percent infertility	0.152	−0.022	−0.254
Body size versus total offspring	−0.088	−0.143	−0.067

Table 3 Correlation coefficients obtained following Spearman's non-parametric rank correlation of life history parameters for control, exposed and covertly infected female *Aedes aegypti* offered blood meals at 4 day intervals

*Significant correlation at $P < 0.05$ (two-tailed); **significant correlation at $P < 0.01$ (two-tailed); control n = 125, exposed n = 107, infected n = 43.

Exposure to IIV-6 inoculum also caused a small reduction in the R_0 of non-infected mosquitoes. This may be related to direct toxic effects of certain IIV proteins (Lorbacher de Ruiz, 1990) or virus-induced apoptosis and sloughing of infected gut cells resulting in reduced feeding rates and lower body size of exposed non-infected mosquitoes (C. F. Marina, unpubl.). Alternatively, individuals exposed to inoculum that did not become infected may have had an intrinsically higher resistance to infection. The metabolic costs arising from mounting an immune response may be reflected in a reduced reproductive capacity, leading to the lower R_0 value observed in this group (Ahmed et al., 2002). A genetically correlated trade-off has also been reported between immune response and immature development rate in *A. aegypti* (Koella & Boëte, 2002). We could not determine if any such effect was evident in our experimental insects because infection status was determined following death of the adult stage rather than during juvenile development.

Such effects highlight the importance of differentiating between the sublethal effects observed in the survivors of an inoculum challenge and sublethal effects attributable to viral infection. The simplest test to differentiate between infected and non-infected survivors would involve demonstrating the presence of virus in hosts as performed in the present study, and a number of the studies reviewed by Rothman & Myers (1996). Even more concrete evidence involves demonstrating an increase in the concentration of the virus in infected hosts due to viral replication as performed by Marina et al. (1999), or by detecting the presence of virus-specific RNA transcripts in host cells using reverse transcriptase RT-PCR techniques. We are currently developing such techniques for the study of covert IIV infections.

Reproduction was associated with a marked decrease in adult female longevity and a left-shifted distribution of the death rate (Figure 3a). Two different diets, blood meals and sucrose solution, were provided for reproductive and non-reproductive females, respectively. Carbohydrate sources are important to the survival of *A. aegypti* deprived of blood meals (Briegel et al., 2001), and may enhance the lifetime fecundity of blood-fed females (Briegel et al., 2002). However, the sugar supplementation of blood meals did not extend the longevity of this species compared to mosquitoes fed unmodified blood meals (Canyon et al., 1999), indicating that blood alone provides the energy reserves necessary to fulfil average life expectancy.

The trade-off between longevity and reproduction has been reported in many animal species (Reznick, 1985; Carey, 1993) and has been studied in detail in certain dipteran species, particularly *Drosophila* (Partridge & Barton, 1994) and tephritid fruit flies (Carey et al., 1998a,b). For females, survival costs to reproduction may originate from two sources. First, by directing resources away from physiological repair into egg production and second, by exposure to toxic male accessory gland compounds transferred to females during mating (Chapman et al., 1995).

The pattern of infertility was noticeably affected by viral infection. The prevalence of egg infertility increased with the period between mating and egg laying in all treatments. This correlation was compelling for control ($R^2 = 0.67$) and exposed non-infected females ($R^2 = 0.92$) but was weaker in covertly infected females ($R^2 = 0.28$) probably because covertly infected females died before sperm depletion or sperm inactivation occurred. Consequently, the correlations between longevity and infertility were positive and significant for control and non-infected exposed females and non-significant for covertly infected females. Interestingly, Sait et al. (1994) reported infertility in up to 33% of healthy *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) females that had mated with males that had been exposed to a granulovirus (Baculoviridae) in the larval stage. Subsequent studies of these males indicated that the numbers of eupyrene sperm produced by virus-exposed males was reduced by about 11% compared to control males, but the effect was not quite significant at $\alpha = 0.05$ (Sait et al., 1998).

A significant positive correlation was detected between longevity and fecundity in all treatments; longer lived females took more blood meals, laid more eggs and, in the case of control and non-infected exposed females, produced more progeny than short lived females. Unexpectedly, a significant correlation between wing length and longevity was detected in covertly infected female mosquitoes but not in control or non-infected insects. This may be a consequence of the slightly greater variability in size of covertly infected mosquitoes (Table 1), resulting in an increased probability of detecting a significant relationship between body size and longevity. Alternatively, viral infection may kill small and probably weaker individuals more quickly than large, more robust individuals, thus generating the significant correlation that was observed.

The present study confirms the observations of a previous study on the sublethal effects of IIV-6 infection in *A. aegypti* (Marina et al., 1999) and goes further in presenting statistical evidence that covert infection reduces the reproductive capacity, longevity, body size; and related demographic variables of the mosquito. Adult body size and longevity influence the duration of the gonotrophic cycle and the average number of feeding bouts per lifetime, respectively (Briegel, 1990). Covert infection by iridescent virus is therefore likely to reduce the probability that individual mosquitoes will transmit disease and may also

influence the dynamics of mosquito populations during periods in which the prevalence of covert infection is high, as has been reported for Spring-time blackfly populations in Wales (Williams, 1995). Sublethal effects of insect pathogens are renowned for being difficult to detect because individuals vary in the degree to which they are affected (Sait et al., 1994, 1998). Moreover, the mechanisms by which insect viruses cause the observed sublethal effects remain virtually unknown. Cellular studies focussing on virus-induced apoptosis and genetic disruption are required to elucidate the processes that lead to the perturbation of life history parameters observed in covertly infected insects.

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