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May the force be with you: measuring mosquito fitness in the field

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Abstract

Fitness of natural mosquito populations is discussed with relevance for the introduction of genetically modified (transonic) mosquitoes for the introgression of desired genetic traits into wild populations. Here fitness is considered with respect to selection pressures and mortality rates that affect wild mosquito populations. Selection pressures on different life stages are discussed, and examples for the estimates of population age structure and survival rate are presented. It is argued that the release of transgenic mosquitoes should be confined to males, with a critical assessment of mating success. Recent models of population replacement show that very high levels of population replacement are required for a transgenic trait to be effective, and that such replacement may take hundreds of years.

Keywords: fitness; mosquito; *Anopheles gambiae*; ecology; survival; age structure; behaviour

The future of entomological research focusing on manipulation of insect populations should belong to a strategy founded on explaining the biological mechanisms that influence insect vital rates and linking this understanding to the fundamental actuarial and demographic properties of populations.
(Carey 2001)

The concept of fitness as a technical term is a confusing one. It is confusing because it can lead philosophers to think the whole theory of natural selection is a tautology. And it is confusing even to biologists because it has been used in a least five different senses, many of which have been mistaken for at least one of the others.
(Dawkins 1982)

It must also be accepted that estimates of the probability of survival, based on the exponential model, of the sort that have been used by many of us in the past 30 years, can only be regarded as, at best, approximations.
(Gillies 1988)

Control of malaria by the release of genetically modified mosquitoes refractory to transmission is now becoming a possibility. One of the key issues involved is the effect that such a manipulation might have on the insects' ability to survive and reproduce in the environment in which they are released, in other words their fitness (Hartl and Clark 1989). A given level of success in a growing population demands a higher level of apparent success than it would in decline. Members of the *Anopheles gambiae* complex are prime targets for this technology. Releases under most of the

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conditions where *An. gambiae* occur were simulated in a spatially explicit model of transposon-based genetic mechanisms for displacing fluctuating populations of anopheline vectors. Situations where there is a long dry spell, with the likelihood of an annual bottleneck (such as occurs in the Sudan), situations where perennial breeding occurs and where there is at most a short dry spell (such as occurs in tropical islands) were simulated. They also simulated releases made at random in a meta-population of a hypothetical mosquito with characteristics like *An. gambiae* in a grid of 306 'villages'; in a linear fashion along a hypothetical road; marginally (as if on a coast) and in a clumped fashion (a situation which might occur if releases were undertaken by different authorities of differing efficiency). In all cases the model indicated that fitness of the released mosquitoes relative to those they were meant to replace was the most powerful lever from which to effect fixation. 'Fitness levels below 70% would appear likely to fail even when release ratios greatly favour transposon bearers, releases are repeated annually and all other conditions are optimal' (Kiszewski and Spielman 1998).

Fitness estimation, however, is difficult, largely because of its biological complexity. As pointed out by Williams (1992) a mere count of offspring produced will not be an accurate measure of ultimate success, unless one also knows the fitness of competing members of the population. The large sample sizes needed also hinders assessment of fitness. 'Fitness' is an operational measure applied to a genotype, usually at a single locus (Dawkins 1982). The fitness of a genotype is defined as 1- *s,* where *s* is the coefficient of selection against the genotype (Falconer 1960). The genetic code of *An. gambiae* has recently been published (Holt et al. 2002) and genome-wide sampling is becoming possible. Genome-wide sampling has the advantage over conventional single-locus population genetics in that both locus-specific and genome-wide effects can be distinguished (Black et al. 2001). The co-efficient of selection can be measured indirectly using molecular approaches and directly by assessment of lifetime fecundity. Molecular approaches, whilst estimating the effects of selection, do not measure fitness components. They are retrospective and result in statements about past natural selection without determining what the mechanism of selection is nor do they provide estimates of population prediction. Phylogenic effects do not therefore answer questions concerning what factors favour the selection of particular suite of traits nor about the trade-offs involved (Roff 1992). Nevertheless, differences between populations can help us make inferences about the selective consequences of present-day migration (Via 1994).

Population genomics is generally restricted to single-copy nuclear genes (scnDNA) that form the genetic basis of adaptations. Thus, genotype and life-history traits can be related. Life-history traits 'include biological features that confer fitness alone, or in combination, with the latter often involving trade-offs' (Statzner, Hildrew and Resh 2001). Since allelic substitution may have pleiotropic effects mortality rates need to be obtained at any of the points where selection can occur. With such information life tables can be constructed and the age-specific mortality schedule determined. The age-specific mortality schedule is the series of probabilities that an individual alive at age *x* dies before age x + 1 (Carey 2001). This serves as a foundation for all other life-table functions. Although it is clearly easiest to think about finite survival rates (and survival data should usually be reported in this form), the continuous analogue of this measure, the force of mortality (q_x), is preferred by demographers because it is not bounded by unity. The force of mortality is defined as the mortality rate that represents the limiting value of the age-specific mortality rate when the age interval to which the rate refers becomes infinitesimally small (Carey 2001). It is as likely to be

dependent on environmental as on intrinsic factors. The force of mortality typically follows a U- shaped course – from very high at the earliest stages followed by a trough created by the increase through the reproductive life span. The rate of ageing of very old individuals may, however, be much slower than that of younger individuals.

A number of approaches have been used for the estimation of selection and mortality rates. These can be split broadly into two classes: laboratory manipulation, and field sampling of different age classes. Components of selection can be determined at different stages of the life cycle. In the remainder of this article I will attempt to describe a few of the points where selection might occur. I will conclude with a brief overview of methods used to determine female longevity since this is a key component in estimating lifetime fecundities.

Egg to larva

Not all eggs that are laid will produce offspring. Mortality in eggs is probably the most difficult transition to measure and has yet to be attempted in anophelines. The biggest problem may be a suitable sample of eggs.

Larva to adult

Larvae and adult mosquitoes occupy different environments and genes which confer an advantage in the larval stage might be disadvantageous in the adult stage. Larvae are often aggregated in space and random sampling is difficult. Adequate sampling of young instars and pupae is also a difficulty when using standard sampling tools such as the mosquito dipper. This is likely to bias estimates of stage-specific survival. Total collection of larvae in quadrats (using a tin cylinder stuck into the mud) in a potential breeding site was possible in São Tomé. Instar-specific survivorship curves for sample data from Kisumu in Kenya (Service 1977) and Riboque in São Tomé are shown in Figure 1a and b and the corresponding forces of mortality in Figure 1c and d. In both cases mortality from first instar to pupae was greater than 90%. Adult mass at emergence is a measure of nutritional state whilst a larva and should be included as a co-variate in any analysis of age-specific mortality of adults. Reproductive success, in females at least, is correlated with size (Lyimo and Takken 1993; Ameneshewa and Service 1996; Takken et al. 1998). Wing length, which is well correlated with dry weight can be used as a proxy for mass (Lyimo and Takken 1993; Lounibos et al. 1995; Takken et al. 1998). At times of stress smaller larvae will not survive and hence adult sizes at emergence will be skewed. This is seen to happen in tree-hole mosquitoes, and was the case for the univoltine temperate mosquito *Aedes cantans* (Renshaw, Service and Birley 1994). It might be expected that insects occupying temporary, resource-limited habitats, as do *An. gambiae,* would suffer from such stress. Nevertheless, despite the high mortality among larvae, wing-length distributions of both male and female *An. gambiae* from Tanzania (Lyimo and Takken 1993) and São Tomé were close to normal (see Figure 2). Thus, competition (and hence differential fitness) was not observed and could not be measured.
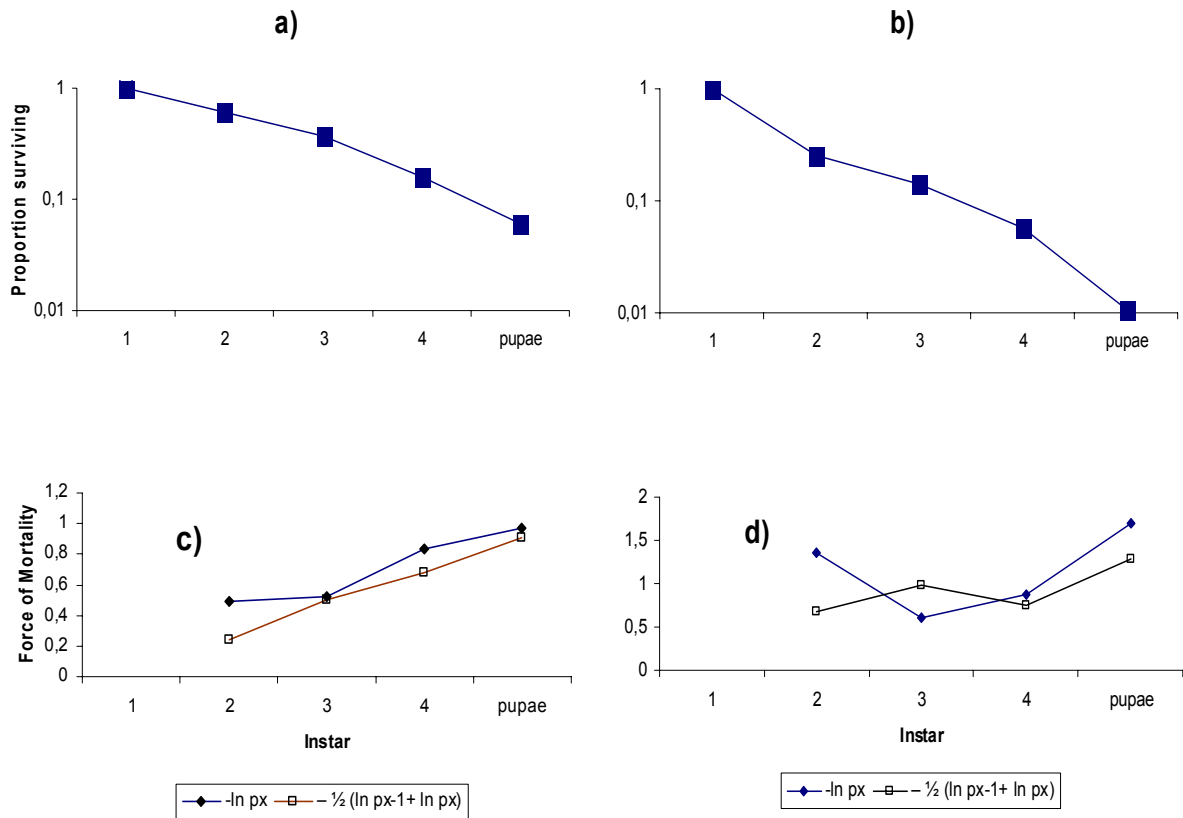
Figure 1. a) Age-distribution curves for immature *Anopheles gambiae* from borrow pits Kisumu, Kenya (Service 1977); b) from small pools in Riboque, São Tomé (n = 1583); c-d) respective force of mortality estimates (-ln p_x and − ½ (ln p_{x-1}+ ln p_x), where p_x is the age-specific survival rate, (the fraction alive at x surviving to $x+1$).
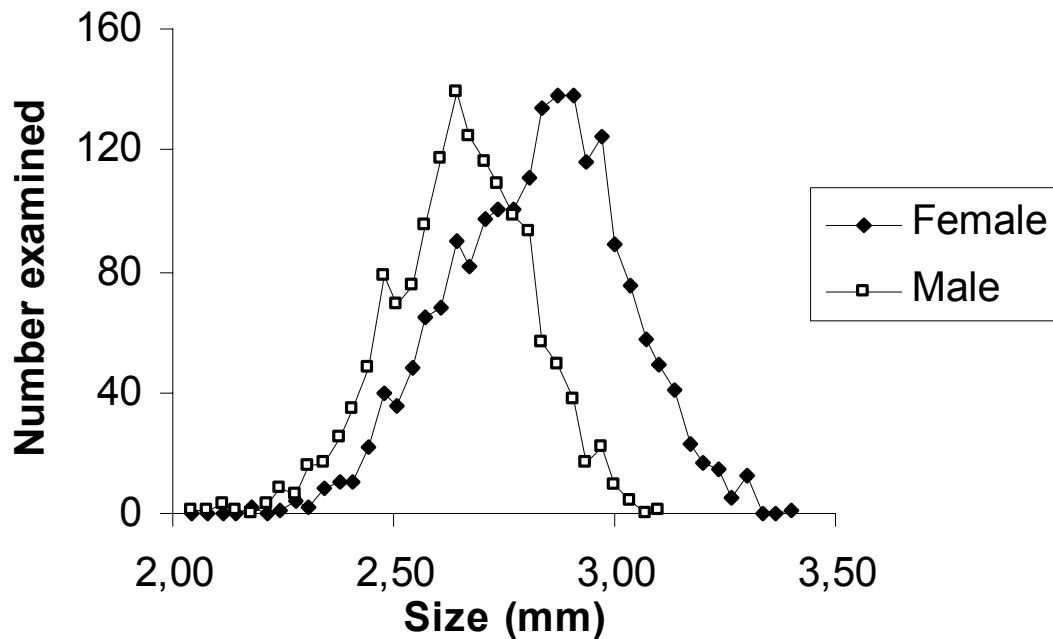

Figure 2. Male and female wing-size distributions *Anopheles gambiae* from São Tomé (n=1473 and 1932 respectively)

Teneral adults

Adult insects are perhaps at their most vulnerable during and shortly after emergence (see Plate 1), but mortality in young adults has rarely been studied. Young males can be distinguished because their terminalia on emergence are un-rotated, a process which takes from 12 to 24 hrs depending on temperature, whilst young females can be distinguished because their alimentary canal contains meconium, waste product retained from the pupal stage. Small individuals tend to survive less well than large ones (Takken et al. 1998) and the smallest ones may die before undertaking any other activity (Lyimo and Takken 1993).

NET FOR MEASURING THE OUTPUT OF MOSQUITOS FROM A MARSH—CLAIRFOND, MAURITIUS.

Plate 1. Ronald Ross in Mauritius collecting emerging adults from a breeding site of *Anopheles gambiae.*

Fitness in adult males

In many species sexual selection can have as much or more of an effect on fitness as natural selection. Sexual selection may occur among male anophelines for females. Okanda et al. (2002) found that male *An. gambiae* in cage experiments preferentially mate with larger females. In the wild, however, no effect of female size on mating success of *An. gambiae* or *An. funestus* caught in copula was observed (Charlwood et al. 2002a). Females are largely monogamous. Given the equal sex ratio at emergence an average male will only meet a single female in his lifetime; hence males are largely monogamous too. In many animals, including some anophelines (Yuval, Wekesa and Washino 1993), it is the males who must compete for females. In many species larger males secure more mates than smaller ones. Male size in *An. gambiae* from São Tomé, however, did not appear to affect the likelihood of their mating at least once (Charlwood et al. 2002a).

Young males may be at an advantage compared to older ones (Chambers and Klowden 2001). Mahmood and Reisen (1982) dissected male *An. stephensi* and examined the testes and accessory gland to determine mating success. They estimated that between 40 and 60% of males had ejaculated the previous evening, which would imply a very low survival rate. If male age influences mating success or subsequent female fecundity it is a priority that they manage to discover the swarm where they may best meet females on the day of their release. It is also important that assortative mating does not take place. Should genetically modified *An. gambiae* ever be released they will be the offspring of a population that has changed from a eurygamic one (in which mating takes place in large swarms 2m or more above the ground) to a stenogamic one (in which mating has to take place in a small cage). In the wild mating takes place at dusk over a 20-minute period. By the time it gets dark mating has largely ceased (Charlwood et al. 2002b). In colonies, because of light-on/light-off regimes the insects may be forced to mate in the dark. Selection for this ability during the colonization process may result in insects whose biological clock is slightly delayed compared to wild ones. Given the short duration of mating activity this could have a major effect on fitness.

Other factors may also influence the fitness of males. For example, insecticide-resistance genes in *Culex. pipiens* males induce a mating competition cost (Berticat et al. 2002).

Fitness in adult females

In order to pass on their genes all males have to do is to mate at least once. Females, on the other hand, have to survive a number of hazards and an individual female's contribution to the next generation can be much more variable due to the variation in the number of egg batches laid. In each oviposition cycle females need to locate a host, blood-feed, rest, locate an oviposition site and oviposit. Each of these activities is likely to involve separate risks and therefore have its own force of mortality.

Factors such as host defensiveness and infection with malaria parasites can affect the likelihood of an insect feeding to repletion on a single host and blood-feeding is likely to involve a fitness cost (Edman, Webber and Kale 1972). This cost may increase as the density of vectors attempting to feed increases. Nevertheless, the act of blood-feeding in Tanzania, at least, did not appear to be hazardous even at the exceptionally high densities sometimes observed (Charlwood et al. 1995b). Fecundity may also be affected by the quality or type of host blood used (Clements 1999). Selection should favour females that choose the most nutritious host, the trade-off here perhaps being that the most nutritious host might also be the most defensive.

Resting, especially in outdoor sites, may involve the risk of death through desiccation. Resting in man-made constructions, however, may not involve particular risk (Gillies 1988). Thus, resting-site differences may be responsible for lower survival of *An. arabiensis* in the dry season in the Kilombero Valley of Tanzania compared to the wet season. In the dry season the species exists in the absence of humans (and thus rests outside) and has a lower survival rate than in the wet season when insects live in close association with man
(compare Charlwood, Vij and Billingsley 2000; with Charlwood et al. 1995a).

The flight to and from the oviposition site may involve a risk proportional to the distance flown. Thus, in Papua New Guinea age-specific survival rates of *An. farauti* from three simultaneously sampled coastal villages differed according to oviposition-site location (Charlwood 1986).

Fitness may also be affected by infection with malaria parasites, although current evidence points to there being only a minor effect. In the wild populations infection frequencies are generally low (Hurd 2003). Manipulation that made a mosquito refractory to infection by *Plasmodium* might, nevertheless, have a positive effect on fitness. Where effects on longevity have been observed it is usually in unnatural combinations of vector and parasite and at very high infection rates rarely encountered in the wild (Ferguson and Read 2002). Infection with malaria may, however, reduce fecundity by follicular resorption (Hogg, Thomson and Hurd 1996; Hopwood et al. 2001). The only field study to examine fitness a difference between infected and uninfected insects is that of Anderson, Knols and Koella (2000). They considered that there was a lower survival among sporozoite-infected insects because there was a deficit of infected insects that fed on an unprotected host compared to those collected in a cage and unable to feed.

Estimating lifetime fecundities involves estimating female longevity. The factor which most affects fitness in females is the number of egg batches laid. Because of its importance a number of methods of estimating female longevity have been developed in the field. Available methods for the determination of longevity include:

1. Studies of the age structure of wild populations
2. Studies on the rate of disappearance of marked populations
3. Estimates of the decline of natural populations when recruitment has ceased
4. Estimations of apodeme growth
5. Estimation based on infection rates.

Each has a number of constraints and the most complete picture is obtained when a combination of methods is used. In the remainder of this article I will describe some of the methods used in more detail. One major constraint of all methods is that they assume that age groups are sampled equally in proportion to their relative density in the population. Many types of collection, however, appear to under-sample newly emerged insects. This is particularly true of the sample obtained resting inside houses, and when this collection method is used estimation of the force of mortality acting on the youngest insects is often ignored in longevity calculations (Gillies 1988). Mosquitoes for longevity determination can also come from landing or light-trap collection (Charlwood 1986; Charlwood et al. 1985; 1995a; 1995b). Light traps have been accused of under-sampling newly emerged insects (Mboera et al. 2000). In Tanzania and São Tomé under-sampling did not seem to be a problem since light-trap collections on occasion consisted of 80% newly emerged insects (Charlwood 1997; Takken et al. 1998).

Studies of the age structure of wild populations

With the exception of the first gonotrophic cycle, when two blood meals are taken, *An. gambiae* like other anophelines are generally gonotrophically concordant. Dissection of the female genital tract and ovaries enables populations to be described in terms of sharply distinguished age classes, defined in terms of the number of egg batches previously laid. Such data allow life tables to be constructed and enable age-specific mortality rates to be assessed. Should the risk of dying not increase with age, survivorship follows an exponential decline and mathematical analysis is greatly facilitated. Should mortality increase with age then a Gompertz curve will more adequately describe the data. A re-analysis of dissection data by Clements and Paterson (1981), including the benchmark data of Gillies and Wilkes (1965) from Tanzania, indicated that this was generally the case. In their study Gillies and Wilkes

(1965) dissected 3723 *An. gambiae* and 4003 *An. funestus.* The survivorship curve of the *An. gambiae* is shown in Figure 3a and the estimated force of mortality of the one-parous and older groups, determined according to equations 1-3 in Table 2 of Carey (2001), is shown in Figure 4a. The force of mortality increases steadily through adult life with a sharp rise in very old insects.
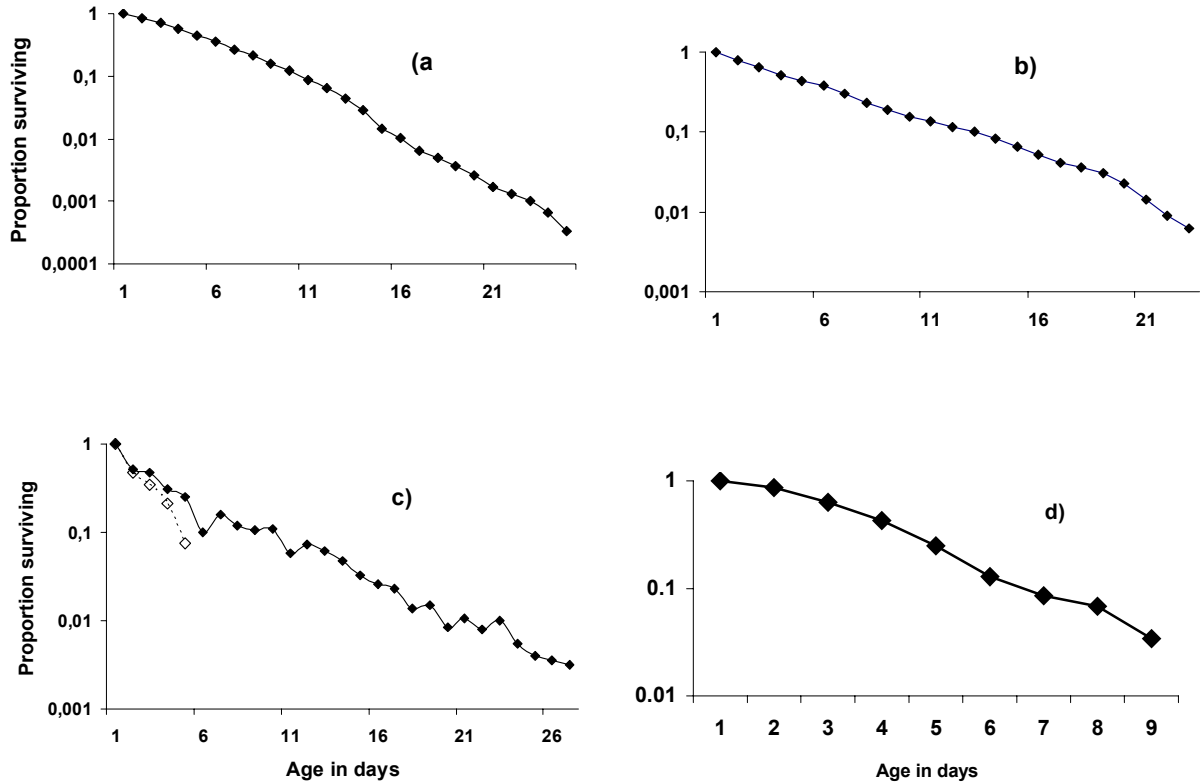

Figure 3. Survivorship curves of adult female *Anopheles gambiae* according to technique of estimation: a) physiological age according to the number of egg batches laid (from Gillies and Wilkes (1965); b) according to the rate of disappearance of marked individuals (from Gillies (1961); c) according to a decline in numbers from a light trap; solid line end of short rains 1991 (Charlwood et al. 1995a), hatched line wet season 1990 (Takken et al. 1998) and d) according to daily cuticular growth lines (Schlein 1979).

In order to obtain such age-specific mortality it is important that older insects are correctly classified. The method relies on the assumption that, following oviposition, sequential dilatations (enlargements originating from the remains of follicles), corresponding to gonotrophic age are formed in ovarioles and that there is a 1:1 relationship between gonotrophic age and the largest number of dilatations within any one ovariole. However, a more detailed examination of mosquito ovaries, using the oil-injection technique, shows that as a mosquito ages the number of ovarioles that act as reliable indicators of gonotrophic age (the gonotrophic diagnostic index) tends to decrease (Hoc and Charlwood 1990). This means that the older age groups (which are anyway rare) may be underestimated leading to overestimates of mortality in older insects. The oil-injection technique has only been applied to a limited sample of African anophelines (Hoc and Wilkes 1995).

Studies on the rate of disappearance of marked populations

Longevity and age specific life tables can also be estimated from capture-recapture experiments. Either insects reared in the laboratory are used (in which case they may well have reduced fitness compared to wild ones) or the age of released insects is unknown (which is not a problem if survival is independent of age but is a problem if it varies with age). In addition, in order to have a chance of obtaining sufficient recaptures, very large numbers of insects need to be released. This creates a local population whose dispersal and survival may be affected by its high density. The standard data set once again comes from Gillies (1961). He marked 132,000 *An. gambiae* either topically with paint or with radioisotopes and recaptured 1,019. Figure 3b shows the survivorship curve from this data and 4b the estimated force of mortality.

Gillies (1961) concluded that the mortality rate remained constant throughout the period in which marked females were recovered, and Figure 4b indicates that the force of mortality remained reasonably constant for much of the period with a sharp increase in later ages. There is a five-day periodicity in the estimated value. This may reflect availability for sampling (which was by indoor spray collection) rather than a change in the force of mortality.
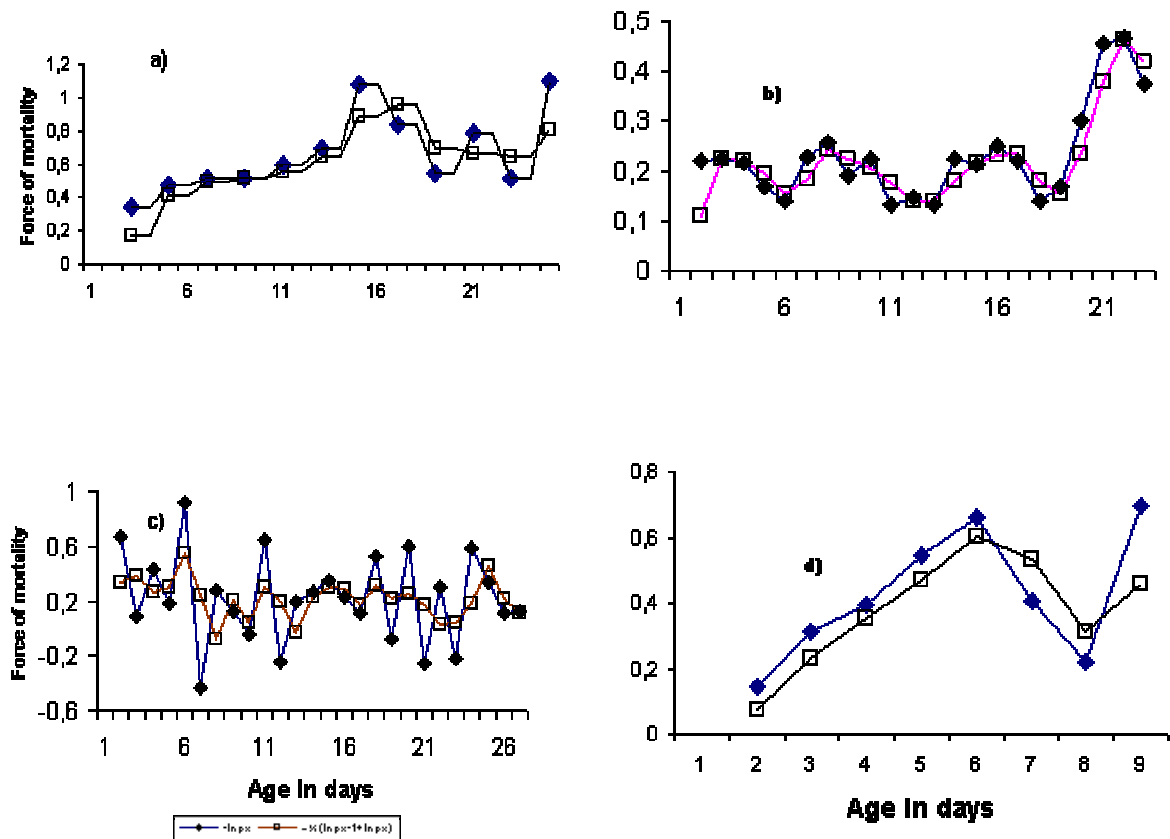

Figure 4. a-d) Estimated force of mortality ($- \frac{1}{2}$ (ln p_{x-1}+ ln p_x) derived from the data in Figure 3.

Mortality estimates from recapture data always include an unknown component of dispersal and hence migration out of the study area. It is therefore important that recapture effort extends over a sufficiently wide area so that the loss of mosquitoes by emigration is either of negligible proportion or can be estimated (Gillies 1988).

Estimates of the decline of natural populations when recruitment has ceased

Another way of estimating longevity is to monitor population declines when recruitment to the population has ceased. This was done in Tanzania in April 1991 at the end of the short rains (Charlwood et al. 1995a), when numbers of *An. arabiensis* collected declined from more than 2000 a night to less than 10 over a period of five weeks (Figure 3c). Sporozoite rates increased over this period. Longevity derived from this sample was estimated to be 0.84/day similar to estimates obtained by many other methods. As with the recapture data of Gillies (1961) mortality rate remained constant (Figure 4c), but in this case there was a considerable variation between days. This may reflect the inherent 'noise' in light-trap samples or may represent the two-day oviposition cycle experienced by a cohort such as the one studied. The mean wing length of females did not change during the population decline; hence in this case survival did not appear to be affected by female size.

Figure 3c also shows the decline observed from the same light trap with *An. arabiensis* during the wet season of May 1990 (Takken et al. 1998). The marked difference in the force of mortality (Figure 5) may reflect differences in the starting age of the two cohorts. The population at the end of the short rains in 1991 was almost exclusively parous (Charlwood et al. 1995a), whilst that from the wet season of 1990 was almost exclusively newly emerged (Takken et al. 1998). Difference in estimates of longevity may have been due to dispersal out of the catchment area of the trap by young insects. *Anopheles gambiae* shares many characteristics of other insects thatundergo the oogenesis-or-flight syndrome (Rankin, McAnelly and Bodenhamer 1986). Newly emerged females have undeveloped ovaries; they do not feed to repletion but take an incomplete meal; this meal is used for adult maintenance rather than egg development; breeding sites are temporary; there is a high mortality among larvae (and hence a premium on being the first females to find empty potential breeding sites rather than lay in already colonized sites); males form stationary swarms where mating takes place and during mating males provide a substantial mating plug which may have a nutrient function.

Evidence for dispersal in young insects is, however, only circumstantial. From his capture-recapture studies Gillies (1961) concluded that dispersal of released insects was more limited during the first two days of adult life than in older insects. This is equivalent to the time prior to mating. It takes a minimum of seven days for eggs of *An. gambiae* to develop into host-seeking adults, yet numbers of newly emerged insects following rain can increase in five days (Gillies 1954). Under such circumstances it is conceivable that the insects are migrating in from areas outside the collection site. During the 'silent years' after invading Brazil *An. gambiae s.l.* spread at a rate of 60 km a year (Lounibos 2002), although how much of this was man-assisted is not known.
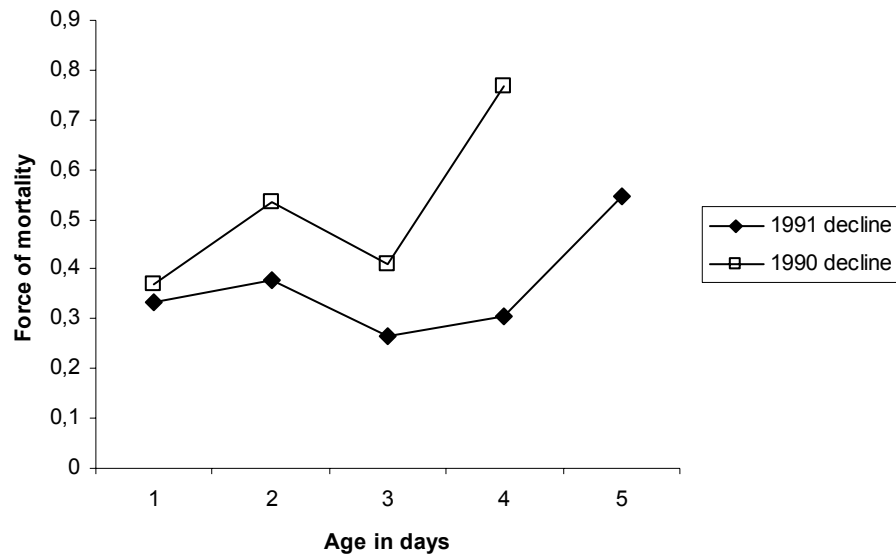
Figure 5. Estimated force of mortality ($\mu_x = - \frac{1}{2} (\ln p_{x-1} + \ln px)$) derived from declines in population density of cohorts of *An. arabiensis* from light-trap collections from a sentinel house, Namawala village, Tanzania (Carey 2001).

Estimation by assessment of apodeme growth

Contrary to popular opinion, adult insects do grow but they grow inwards. The apodemes of the thorax show daily growth layers, which can be visualized after the insects have been oxidized in potassium permanganate and stained with haematoxylin (Schlein and Gratz 1973). Just as the age of trees can be determined by counting the annual growth rings, so the age of the insect, in days, can be calculated. The technique has the advantage that the age of dry specimens can be determined but the disadvantage that it is extremely difficult to perform, and only a single paper has been published using field-collected samples (Schlein 1979). The maximum age of samples was only 9 days, compared to the more than 20 days obtained from the other methods. The survivorship curve redrawn from these data is shown in Figure 3d and the force of mortality in Figure 4d. The force of mortality increases with age, although variations beyond day 6 probably reflect the small sample size rather than real differences in mortality.

Methods which assume age-independent survival

Irreversible changes occur in the ovaries following the maturation of the first batch of eggs. Division of populations into nulliparous and parous groups has long been used to estimate longevity. Survival rate (*p)* can be determined from the formula $p^n = M$, where *n* is the number of days between emergence and first oviposition and *M* is the parous rate. The method assumes that mortality is the same for all ages and requires that samples are made over a complete population cycle (i.e. a year). If this is not done irregular recruitment to the population can lead to an error in estimates. Whilst random selection of houses in which to run collections for the estimation of population size gives better estimates than where sampling is repeated in the same houses, estimates of parity do not appear to be affected by sampling schedule (Magbity and Lines 2002).

Species like *An. gambiae,* in which the females emerge with undeveloped ovaries, may take two blood meals during their first oviposition cycle. Insects in which gonotrophic development does not occur are termed pre-gravid. Females can thus be divided into three age groups: pre-gravid, nulliparous and parous. Estimates of longevity can then be obtained using the formulae of Vercruysse (1985).

Populations of mosquitoes are rarely in equilibrium. Under these circumstances a more reliable technique for the estimation of longevity from nulliparous/parous data is to apply cross-correlation analysis to time-series data obtained on a daily basis for a minimum of 21 days (Charlwood et al. 1985; Holmes and Birley 1987). For the method to work there should be significant cross-correlation between the parous insects and the total caught one oviposition cycle earlier. More recently a method for calculating mortality rates, correcting for seasonal variations in recruitment has been described (Briet 2002).

Estimation based on infection rates

Haji et al. (1996) describe a method for determining survival in infected mosquitoes by discriminating between the most recent and other oocyst infections. They measured oocyst diameter, developed a statistical model which allows for overlap in size distributions, and estimated survival rates. The technique is a simple one and the preservation of midguts allows for batch processing of material. A suitable collection technique would be to sample gravid females as they leave houses in search of oviposition sites at dusk. This eliminates the need to identify and separate by dissection potentially infected from uninfected females. Gravid females are also the easiest category to examine for oocysts. The technique also allows for the population genetics of both the parasite and the vector to be studied (and possible interactions between them determined).

Conclusion

A transgenic release may be confined to males and it will be critical to measure mating success of these in the field. In order for a release to be successful they will need to mate with wild females on their release. In practice this means that they might have two twenty-minute periods over 48hrs, in which to find a swarming site to which females arrive, whereas, say, gravid females only need to lay eggs for the gene to be introduced into the population at the appropriate site. It may be colonization, rather than genetic manipulation, that will affect fitness of released mosquitoes. Because of inbreeding colonized mosquitoes are likely to contain deleterious recessive alleles at high frequencies. Determining the mating success of colonized versus wild mosquitoes would provide some indication of possible ratios of introduced to wild insects needed to affect introduction. Releasing marked colonized mosquitoes back into the same wild population from which they were derived would not be introducing new genes into the population. Rare- or trace-element marking has much to recommend it. It is multi-stage, multi-generational, and multi-trophic. Markers are not radioactive and are safe for the environment; there is no visible mark that might affect the insect's behaviour or interaction with other insects. They are, however, expensive and subject to local variation in trace-element concentration in the environment (Hagler and Jackson 2001). Trace-element marking (Rubidium Rb) has been used to label eggs of *Aedes* (Reiter et al. 1995). In this case females were fed on hosts injected with a trace element which was then passed from the female to the eggs. Should it be

possible to label males' accessory-gland secretion then their mating success could easily be measured by assessing mating plugs in females.

In the model developed by Kiszewski and Spielman (1998) fitness of released insects under all conditions had to be at least 80% for replacement to occur. In a recent study Catterucia, Godfray and Crisanti (2003) concluded that the genetic make-up of engineered lines is likely to have a major effect on the maintenance of the transgenic allele and that the transgene itself has a fitness cost to the mosquito. With an 80% fitness population replacement may only occur after hundreds or even thousands of generations, which in practice means tens or hundreds of years. A further model developed by Boëte and Koella (2002) indicated that, whilst fitness costs might be acceptable, the introduction of genes causing the mosquitoes to be refractory to transmission would only have an effect on transmission if they were nearly 100% effective. In control terms, hundreds of years is a long time, but on an evolutionary scale it is tiny. There is little to suppose that, without population replacement, *An. gambiae* capable of transmitting malaria will not still be a very common mosquito in hundreds of years' time. Under such circumstances pursuance of population replacement might be worth the wait.

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