Comparisons of Life-History Characteristics of a Genetic Sexing Strain With Laboratory Strains of Anopheles arabiensis (Diptera: Culicidae) From Northern Sudan

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Comparisons of Life-History Characteristics of a Genetic Sexing Strain With Laboratory Strains of *Anopheles arabiensis* (Diptera: Culicidae) From Northern Sudan

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ABSTRACT A genetic sex separation strain (GSS) has been created for *Anopheles arabiensis* (Patton) (Diptera: Culicidae), one of the major African malaria vectors, for use in controlling wild populations of this species via the sterile insect technique (SIT). This GSS strain, "ANO IPCL1," allows sex separation by a translocation linking a dieldrin resistance allele and the Y chromosome. Differences between ANO IPCL1 relative to wild strains might reflect its field performance and therefore are of concern. Of more immediate interest is how differences might affect production during mass rearing. Life-history parameters were measured for the ANO IPCL1 strain and the two wild strains from which it originated. Although developmental rate differences were found among them, none were large. However, a major observed variation was the very low intrinsic fertility of ANO IPCL1 because of the translocation itself. This resulted in a much lower rate of increase: ANO IPCL1 was able to double its population size, in 7.8 ± 0.4 d, whereas Dongola and Sennar strains could do so in 4.9 ± 0.5 and 5.6 ± 0.4 d. The presence of the Y-autosome translocation mainly affected the natural fertility of the males, and this will require amplification steps during mass rearing.

RÉSUMÉ Dans le cadre d'un projet de contrôle des populations de l'un des principaux vecteurs du paludisme en Afrique, une souche d'*Anopheles arabiensis* (Patton) (Diptera: Culicidae), "ANO IPCL1," permettant une séparation des sexes de façon génétique (GSS) a été créée pour le développement de la technique de l'insecte stérile. Cette séparation est possible grâce à une translocation liant au chromosome Y un allèle de résistance à la dieldrine. L'existence de différences entre ANO IPCL1 et les souches sauvages pourrait refléter les performances des mâles sur le terrain et il est donc important de les évaluer. Il est fondamental de comprendre comment ces différences peuvent affecter une production de masse. Les traits d'histoire de vie ont été mesurés pour ANO IPCL1 et les souches sauvages parentales. Hormis des différences mineures concernant les paramètres de développement, le processus de translocation a induit une très faible fertilité naturelle chez ANO IPCL1 entrainant un plus faible taux intrinsèque d'accroissement de la population. Une population d'ANO IPCL1 était capable de sa taille en 7,8 ± 0,4 jours, alors que Dongola et Sennar pouvaient le faire en 4,9 ± 0,5 et 5,6 ± 0,4 jours. La translocation ayant principalement affecté la fertilité des mâles, elle aura un impact important sur l'élevage de masse.

KEY WORDS genetic sexing strain, sterile insect technique, life history, fitness

The high impacts of malaria on human health and on countries' economies continue to motivate control campaigns targeting either the parasite or the vector. The female mosquito *Anopheles arabiensis* (Patton) (Diptera: Culicidae) is one of the major African vectors of malaria (Coetzee et al. 2000). A feasibility study of the use of the sterile insect technique (SIT) for *An. arabiensis* as part of an areawide integrated pest management project (Klassen and Curtis 2005) is currently being conducted in northern Sudan and La Reunion (Robinson et al. 2009, Boyer et al. 2011). SIT effectiveness in reducing diseases is based on the prediction of a lower probability of contact between the vectors and humans resulting from the progressive reduction of the vector population. The means to accomplish this purpose is to release large numbers of mass-reared and sterilized males in situ where they would mate with wild virgin females. For insects, such as mosquitoes, in which only females bite and thus are

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able to transmit diseases, it is mandatory that only males be released. Beside the public health concerns, the release of sterile females together with males has been shown to reduce the dispersal and the mating efficiency of sterile male fruit flies (Hendrichs et al. 1995, Rendon et al. 2000). Indeed, when both females and males are released, males can mate with the released females and therefore do not necessarily need to fly farther to mate with wild females. It is therefore useful to be able to efficiently remove females before release.

In contrast to aedines and culicines, anopheline pupal size differences between sexes are not large enough to allow accurate mechanical separation (Dame et al. 1974). So far, besides separating adults (e.g., providing toxicant in the bloodmeal; Lowe et al. 1981), the only alternative is the use of a genetic sexing strain (GSS) that confers a certain resistance only to males. For that purpose, the GSS named "ANO IPCL1," based on dieldrin resistance, has been selected for *An. arabiensis* at the Food and Agriculture Organization/International Atomic Energy Agency Insect Pest Control Laboratory (IPCL, Seibersdorf, Austria). Natural resistance to dieldrin exists in the strain of *An. arabiensis* from Sennar, Sudan (El Gaddal et al. 1985, Du et al. 2005). A translocation linking the resistance allele with the Y chromosome was induced via gamma irradiation and identified by backcrossing candidate males with virgin females from the Dongola strain, which has no resistance to any insecticide.

The success of SIT projects is based on the survival and mating capacity of the released males, and it is important to understand how these are altered by the translocation in the GSS compared with the wild parents. Ultimately, this can only be determined by large cage trials or open field releases. In the context of mass rearing, it is fundamental to understand how differences can be accommodated to achieve sufficient levels of production. The assumption is that the wild strains from which the GSS is produced is a reasonable comparator for a GSS. Here, we compare the three closely related Sudanese strains of *An. arabiensis:* Dongola, originating from Sudan; Sennar originated in the Gezira irrigation area, Sudan; and ANO IPCL1, derived from both. The comparison of life-history traits between these strains is discussed in the context of mass production.

Materials and Methods

Mosquito Stocks and Rearing Methods. The experiments were conducted using three strains of *An. arabiensis.* The Dongola strain (available from the Malaria Research and Reference Reagent Resource Center (MR4) as MRA-856) was colonized in 2004 from specimens collected near the village of Dongola, Sudan, and has been maintained for 125 generations at the IPCL. It is pure breeding for a dieldrin susceptibility trait. Sennar (MRA-334) was first colonized in 1969 by the Malaria Training Center in Sudan. Sennar, which carries the resistance to dieldrin, was used to establish ANO IPCL1. The ANO IPCL1 is maintained by backcrossing resistant males to Dongola females. All three strains were reared in a climate-controlled room maintained at 27 ± 1°C, 60 ± 10% RH, and a photoperiod of 12:12 (L:D) h, including dusk (1 h) and dawn (1 h). Larvae were reared in plastic trays (40 by 29 by 8 cm) at a density of ≈500 first-instar larvae (L1) per tray that contained ≈1.5 liter of deionized water and were fed larval diet consisting of finely ground (224-μm sieved) Koi Floating Blend (Aquaricare, Union Hill, NY). Pupae were collected and placed in small plastic cups inside a fresh adult cage for emergence. Adults were kept in standard 30 by 30 by 30-cm insect cages (Megaview Science Education Services Co., Ltd., Taichung, Taiwan) and continuously supplied with sugar water (10% wt:vol sucrose solution with 0.2% methylparaben, Benedict et al. 2009). Females were blood fed weekly on defibrinated bovine blood and provided a standard oviposition cup consisting of plastic cups with black lining containing a wet sponge over which a filter paper was placed.

Immature Development of Three Strains. To determine the egg hatch rate, 400–500 eggs (24 h old) of each strain were hatched in deionized water at 27 ± 1°C. This was replicated three times for each strain, with eggs originating from three different cages. The hatch rate was determined by microscopic examination of eggs after 48 h.

Larval development and survival were determined by transferring 100 larvae (<4 h old) of each strain to 20 by 20 by 8-cm plastic trays filled with 500 ml of deionized water. This was replicated three times for each strain, with larvae originating from egg batches collected from three different cages. Larval diet was provided on a daily rate per tray (days 1 and 2, 25 mg; days 3 and 4, 50 mg; day 5, 100 mg; and days 6 and 7, 150 mg).

Trays were rearranged daily within the space being used for the experiment to randomize the effects of local conditions within the room. Pupae were removed on the day they formed and transferred into individual tubes for emergence; eclosion time, sex, and survival were recorded. Digital photos of the left wings (or right where left wings were damaged) were taken and measured using analysis B software (Olympus Soft Imaging Solutions, Münster, Germany). Wings were measured from the distal edge of the alula to the end of the radius vein (excluding fringe scales).

Adult Fecundity and Longevity. One hundred newly emerged males and females (ratio 1:1) were placed in 30 by 30 by 30-cm plastic cages (Megaview Science Education Services Co., Ltd.) with constant access to sugar water. Starting at day 8, a mechanically defibrinated bovine bloodmeal was provided weekly through a Parafilm membrane (American Can, Neenah WI). A standard oviposition cup was added in the cage 48 h after blood feeding for en masse oviposition. The egg paper was removed the following day, and eggs were counted under a microscope. Dead adults were removed daily and their sex determined. Three replicates were performed for each strain.

Statistical Analyses. The analyses were conducted using MINITAB statistical software (Minitab, State

Table 1. Egg hatch rate, L1 survival to pupa and adult, and sex ratio (all means with CI in parentheses) for the three *An. arabiensis* strains studied

Strain	Egg hatch rate	Survival rate from L1 to pupa	Survival rate from L1 to adult	Sex ratio[a]
Dongola	0.95a (0.91–0.98)	0.81a (0.76–0.85)	0.78a (0.72–0.82)	0.50a (0.41–0.58)
Sennar	0.82b (0.78–0.85)	0.92b (0.88–0.94)	0.85c (0.80–0.88)	0.53a (0.51–0.55)
ANO IPCL1	0.27c (0.26–0.27)	0.92b (0.88–0.95)	0.91b (0.88–0.94)	0.50a (0.37–0.63)

Within columns, values followed by different lowercase letters are statistically different; ANOVA was performed for egg hatch rate and sex ratio analysis, and logistic regression was performed for survivorship analysis ($P < 0.05$).

[a] Sex ratio was calculated as the proportion of males out of the total number of adults.

College, PA), Excel (Microsoft, Redmond, WA), and R (R Development Core Team 2011). Egg hatch rates and sex ratio data were arcsine transformed and compared between strains by using two-tailed one-way analysis of variance (ANOVA) and Tukey's post hoc tests ($P < 0.05$). Survivorship of *An. arabiensis* larvae (from first-instar larva [L1] to pupa) was compared between strains using a logistic regression ($P < 0.05$). Mean time to pupation was defined as the average duration (in days) from the L1 until pupation. Fecundity was calculated as the number of eggs laid per female per day. One-way ANOVA and Tukey's post hoc tests were used to compare the developmental duration between strains for each sex, the wing measurements between strains and sexes, and female fecundity between strains. Kaplan–Meier survival analyses ($P < 0.05$) were conducted to determine adult survivorship differences between the strains.

The net reproductive rate, R_0, was calculated for each strain based on the daily survivorship and fecundity. *R0* was defined as the average number of offspring a female produced in her lifetime and was calculated as $R_0 = \Sigma(l_x m_x)$, where l_x is the age-specific survivorship, and m_x is the age-specific fecundity. Per-capita intrinsic growth rate, r, defined as the number of progeny born to each female mosquito per unit of time, was calculated using the Euler–Lotka equation $1 = \Sigma_{x=0}\, e^{-rx}\, l_x m_x$ (Hedrick 1984, David et al. 1995), where x was the mosquito age. The generation time in days, T_c, is defined as the average length of time between the hatching of an individual and the hatching of its offspring and was calculated as $T_c = \Sigma\, x(l_x m_x)/\Sigma(l_x m_x)$. The doubling time for the population size was calculated as $T_d = \ln 2/r$. One-way ANOVA and Tukey's post hoc tests ($P < 0.05$) were used to compare differences in the net reproductive rate, generation time, intrinsic growth rate, and time for population size doubling between the strains. Results are expressed as mean ± SEM.

Results

Development of *An. arabiensis* Immatures. Under similar conditions, the mean fertility of the three genotypes differed significantly ($F = 234.33$, df = 2, $P < 0.001$; Table 1). The mean percentage of egg hatch of ANO IPCL1 was low (26.8 ± 0.2%) compared with those of Dongola (94.5 ± 1.8%) and Sennar (81.8 ± 1.7%).

The mean survivorship from L1 to pupa was 92% for Sennar and ANO IPCL1 (Table 1). In contrast, Dongola survival rate to the pupal stage was significantly lower than that of Sennar ($Z = -3.95$, df = 299, $P < 0.001$) and ANO IPCL1 ($Z = -4.10$, df = 299, $P < 0.001$). The survival rate to adult eclosion followed the same pattern. For the three strains the sex ratio was similar ($F = 0.120$, df = 2, $P = 0.89$) and averaged 51.0 ± 2.4%.

Mean time to pupation was ≈6 d for the three strains. However ANO IPCL1 showed a significantly faster development compared with Dongola and Sennar for both females and males ($F = 29.27$, df = 2, $P < 0.001$ and $F = 63.19$, df = 2, $P < 0.001$, respectively; Table 2). The same pattern was found for the duration time until emergence, because eclosion from pupae collected in the morning occurred in the evening following the day of pupation.

Wing lengths were significantly shorter for both males ($F = 15.95$, df = 2, $P < 0.001$) and females ($F = 4.57$, df = 2, $P < 0.05$) of the Sennar strain compared with both Dongola and ANO IPCL1 (Fig. 1); however, there was no difference between Dongola and ANO IPCL1. The difference between sexes was significant for Dongola ($t = 4.82$, df = 19.9, $P < 0.001$), ANO IPCL1 ($t = 8.17$, df = 50.9, $P < 0.001$), and Sennar ($t = 5.32$, df = 26.7, $P < 0.001$).

Adult Fecundity and Longevity. Four blood-feedings and ovipositions occurred, each separated by 1 wk; they are referred to as the four gonotrophic cycles (GCs) of the females. Because mortality was checked daily, the mean fecundity per female could be calculated as the number of eggs laid en masse divided by the number of females alive in the cage. For all strains, the mean fecundity per female was low on the first oviposition opportunity and was the highest on the second (Fig. 2). A strong decrease of fertility was

Table 2. Developmental duration (mean ± SEM) from L1 to pupa formation and to adult emergence

Strain	Mean time to pupation (d)		Mean time to eclosion (d)	
	Female	Male	Female	Male
Dongola	6.4 ± 0.05a	6.2 ± 0.04a	7.8 ± 0.04a	7.5 ± 0.05a
Sennar	6.6 ± 0.05c	6.3 ± 0.04a	7.8 ± 0.05a	7.4 ± 0.05a
ANO IPCL1	6.1 ± 0.05b	5.8 ± 0.04b	7.4 ± 0.05b	6.9 ± 0.04b

Within columns, values followed by different lowercase letters are statistically different ($P < 0.05$; ANOVA).

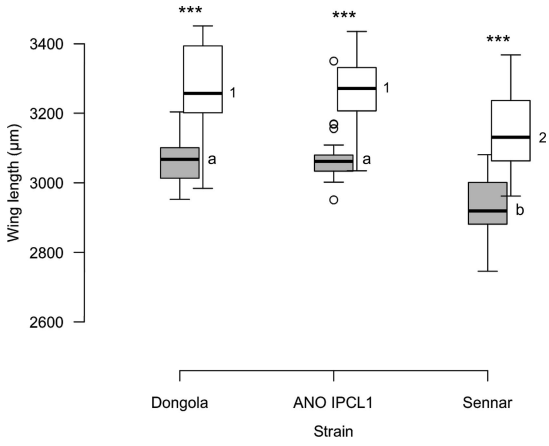

Fig. 1. Box plots of wing length measurements for males (gray box) and females (white box). The line represents the median of the sample, the box itself shows the upper and lower quartiles, the whiskers show the range (i.e., the largest and smallest values), and circles indicate outliers. Boxes with the same letter or number were not statistically different from each other, $P < 0.05$ (ANOVA between strains). Asterisks (***) indicate a significant difference between sexes within the same strain ($P < 0.001$).

observed on the third and fourth GCs. There was no significant difference between strains except during the fourth GC where Sennar showed a higher fecundity than the two other strains ($F = 11.9$, df = 2, $P < 0.01$).

Small but significant differences were observed when comparing the Kaplan–Meier estimates of the adult survival curves between the three strains (Fig. 3). Survival of Dongola males was slightly higher compared with Sennar males ($\chi^2 = 4.2$, df = 1, $P < 0.05$) and ANO IPCL1 males ($\chi^2 = 7.8$, df = 1, $P < 0.01$). When the Peto & Peto modification of the Gehan–Wilcoxon test was used to give more weight to the early deaths, it seemed that in both cases the difference was significant only for the early days of mortality. Concerning the longevity of females, no difference was observed between the three strains ($\chi^2 = 2.6$, df = 2, $P = 0.272$).

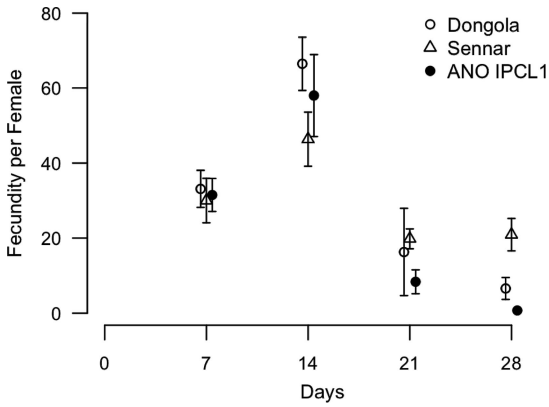

Fig. 2. Fecundity (mean ± SEM) per female for the four gonotrophic cycles, for the three strains of *An. arabiensis*.

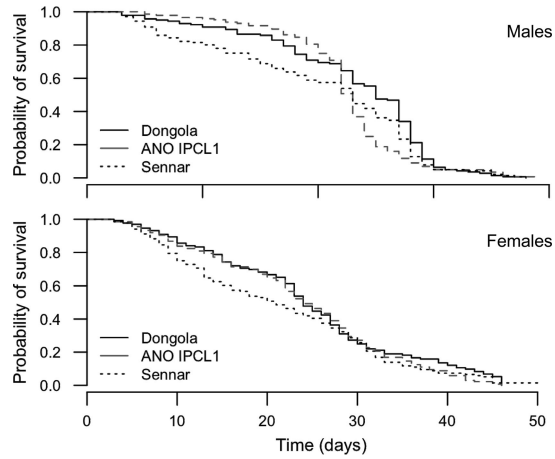

Fig. 3. Adults survival curves for the three strains of *An. arabiensis*. Kaplan–Meier curves (estimate of the survivor function) for males and females.

Life–History Parameters. The variations of the main life-history parameters of different strains are given in Table 3. The generation time (T_c) values were close for the three strains, although a significant difference was found ($F = 10.45$, df = 2, $P < 0.05$) between the strains ANO IPCL1 and Sennar at a level of significance of 0.01 (Tukey's post hoc honestly significant difference [HSD] test). The net reproductive rate (R_0) varied greatly between the strains ($F = 8.95$, df = 2, $P < 0.05$), ranging from 8.70 for the ANO IPCL1–31.62 for the Dongola strain. Tukey's post hoc HSD tests indicated a significant difference between these two groups at the 0.05 level of significance. The intrinsic rate of natural increase (r) was a more comprehensive measurement of fitness (Birch 1948) and was significantly lower for ANO IPCL1 compared with Dongola and Sennar ($F = 14.87$, df = 2, $P < 0.01$). Therefore, the ANO IPCL1 strain was theoretically able to double its population size in 7.8 ± 0.4 d, whereas Dongola and Sennar strains could do so in 4.9 ± 0.5 and 5.6 ± 0.4 d, respectively. The difference between ANO IPCL1 and the two other strains was significant ($F = 18.35$, df = 2, $P < 0.01$).

Discussion

The ANO IPCL1 strain was created from a cross between males from the Sennar strain and females

Table 3. Calculated generation time (in days; mean ± SEM), net reproductive rate, intrinsic rate of increase, and doubling time of the population for the three strains

Strain	Avg T_c	Avg R_0	Avg r	Avg T_d
Dongola	25.5 ± 0.4ab	31.6 ± 5.7a	0.14 ± 0.01a	4.92 ± 0.28a
Sennar	26.6 ± 0.2a	21.9 ± 3.4ab	0.12 ± 0.01a	5.60 ± 0.36a
ANO IPCL1	24.9 ± 0.2b	8.7 ± 1.0b	0.09 ± 0b	7.82 ± 0.41b

Within columns, values followed by different lowercase letters are statistically different ($P < 0.05$; ANOVA).

from the Dongola strain of *An. arabiensis*, with the purpose of mass rearing, sterilization, and release for SIT programs against this malaria vector. The chromosome rearrangement has obvious effects on the egg hatching rate, but it is possible that other undetected effects, including behavioral, developmental, and neurological effects, might exist.

With the exception of fertility rate, the observed life history of ANO IPCL1 is similar to that of Dongola and Sennar. ANO IPCL1 however showed a slightly higher larval-to-adult survivorship and shorter developmental time, which will impact positively on a mass production system. The sex ratio for the three strains was 50%, demonstrating similar survivorship for male and female larvae. Similar probabilities of survival over time were observed for adults in laboratory cages and at a low density. Sennar adult size, reflected by wing length, was significantly smaller than for the two other strains. A high larval survivorship can lead to a lower food availability and thus a lower food intake, which results in smaller size (Agnew et al. 2002, Gilles et al. 2011). In the case of Sennar, the larval survival rate was similar to the one of ANO IPCL1; however, its development took slightly longer. It has recently been documented that longer larval development was strongly correlated with shorter wing length in this species (Gilles et al. 2011) and in *Aedes aegypti* (L.) (Agnew et al. 2002), which is concordant with the developmental data for the Sennar strain. When comparing strains heterozygous for dieldrin resistance with homozygous resistant or susceptible strains of *Anopheles gambiae* Giles and *Anopheles stephensi* Liston, Rowland (1991) reported a faster development for heterozygous larvae, but no difference concerning adult survival and size were observed. In both his study and ours, no strong developmental effects seem to be attributable to the dieldrin resistance itself.

In ANO IPCL1, the females do not carry the translocation; hence, the genetic background of these females is similar to that of Dongola females to which ANOP IPCL1 males are often backcrossed. It is then not surprising to observe no difference in terms of egg number or adult female survivorship between the two strains. However, all GSS based on a Y-autosome translocation show a reduction of male fertility, attributable to the genetic behavior of the translocation during meiosis. This inherited sterility of translocation-carrying males is proportional to the complexity of the translocation, i.e., the more autosomes involved the higher the sterility level (Franz 2000, Robinson 2002). The egg-hatching rate of ANO IPCL1 was low; however, 90% of the first instar larvae survived until adulthood.

In a mass-rearing facility, a high number of broodstock adults will be required for the egg production to counteract the low fecundity; thus, it will be essential to optimize the survivorship of the immature stages. This emphasizes the importance of adjusting the number of females present in a mass-rearing cage according to the operational sex ratio (i.e., the ratio of sexually active males to receptive females at any time) so that an adequate number of females produce progeny. Although the generation time remains similar, the translocation of the ANO IPCL1 induced an important fitness cost regarding the demographic parameters with a lower intrinsic rate of increase and a longer period needed to double the population size. The demographic parameters values for the Dongola and Sennar strains were similar to those reported for different strains of this species in Kenya, which varied from 0.08 to 0.169 according to the landcover types (Afrane et al. 2007). A similar study on *An. gambiae* indicated a higher intrinsic rate of increase, ranging from 0.205 and 0.230 according to the various landcover types (Afrane et al. 2006). However, the comparison of demographic parameters between different studies remains complex because of variations between protocols, highlighting a need for standardization. We estimate that starting from a small GSS colony in which undesired recombinants are eliminated by selection and manual sex separation for crossing, two generations of amplification will be required to produce a sufficient number of mosquitoes for production of 10 million males per week (unpublished data). If one of the wild-type strains could be used for this purpose, or a GSS with higher fertility were available, one amplification stage could probably be eliminated, thus reducing the cost of production.

The use of male-linked translocation systems for population control has often been investigated. The semisterile males from GSS strains released in a wild population can affect directly the rate of increase of the latter by the decreased average fertility levels, as shown in several models (Serebrovsky 1940, Curtis and Hill 1968, Laven 1969). McDonald and Rai (1971) developed a model showing the possibility of eradicating a wild population of *Ae. aegypti* after six generations following successive releases of 50% sterile male-linked translocated males. High sterility levels of translocated males might be required for a successful control program as the initial impact on the reduction of the wild population fertility would then be high and would avoid the compensation because of a decreased larval competition in the natural larval sites (Curtis 1975, Krishnamurthy et al. 1975, Service 1985, Yakob et al. 2008). Laven et al. (1971) reported a progressive and rapid reduction of wild population of *Culex pipiens* L. from southern France after 1 yr of releases of a translocated strain in which males were 50% sterile. However, during the years following the end of the releases, the number of larvae carrying the translocation rapidly decreased, suggesting immigration, dilution, or both by remaining wild individuals by failure to completely replace normal male karyotypes (Cousserans and Guille 1974), underlining the difficulty of controlling population densities when the population is not isolated and if releases are not continuous. The fitness costs associated with the semisterility of ANO IPCL1 might put this strain at a disadvantage with the wild strains; therefore, its use for population replacement does not seem viable. However, under continuous releases, this natural semisterility might be sufficient to progressively reduce a wild population. In addition, the larvae resulting from matings between

wild females and semisterile males would maintain the larval competition for food in the larval sites and thus avoid creating conditions of overcompensatory dynamics that might improve the survival of wild larvae (Yakob et al. 2008). If the released males were fully sterilized by irradiation, these fitness costs would only affect the mass-rearing production, because no progeny would survive in the field.

In conclusion, this study of these life-history parameters detected few differences between the ANO IPCL1 and its parental strains Dongola and Sennar in the laboratory. Although important characteristics of ANO IPCL1 such as competitiveness remain to be determined and there is natural sterility of 73%, the strain shows potential for reduction of wild population size over generations. The life-history characteristics of ANO IPCL1 as determined in the laboratory show qualities consistent with the requirements for a successful SIT program such as fast development, good immature and adult survivorships, and high fecundity.

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