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Selection of a strain of *Culex pipiens* highly susceptible to *Wuchereria bancrofti*

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ABSTRACT

The selection of a strain of *Culex pipiens* highly susceptible to *Wuchereria bancrofti* may better facilitate studies of vector–parasite relationships. To increase the susceptibility of *Cx. pipiens* to *W. bancrofti* infection over successive generations, a standard system of pairwise mating was used for two generations, followed by mass mating and selection. Inbreeding depression was avoided by pooling families whenever necessary. Offspring of the crosses were exposed to microfilaria carriers, blood-fed females were dissected 12-13 days later and prevalence was assessed. After four generations of genetic selection, susceptibility to the parasite exceeded 95%. Susceptibility dropped when selection pressure was removed (generations F_{10} to F_{13}). Resuming selection pressure produced a highly susceptible mosquito strain with 94% prevalence at F_{16}. Fluctuations in mosquito susceptibility from F_{16} to F_{25} (85.5-97.1%) were not significant, indicating that a high level of susceptibility was maintained over these generations.

KEYWORDS: *Culex pipiens*, *Wuchereria bancrofti*, filariasis, genetic selection, susceptibility.

INTRODUCTION

Human lymphatic filariasis is a mosquito-borne disease resulting from infections with *Wuchereria bancrofti, Brugia malayi* and *B. timori*. This malady affects more than 120 million people (Ottesen *et al.* 1997). Because of the health-related and economic impact this disease has on human populations, sustained research efforts attempt to clarify complex relationships existing between filarial parasites and vector mosquitoes. Susceptibility to parasitic infection varies in different mosquitoes and even among different geographical strains of the same mosquito. Early studies by Huff (see Thathy *et al.* 1994) showed that susceptibility of *Culex pipiens* to Plasmodium cathemerium could be increased by selection and seemed to behave as a simple recessive trait. Kartman (1953) obtained by mass selection strains of *Aedes aegypti* more susceptible and more refractory to Dirofilaria immitis than the original stock of mosquitoes. A standard system of pairwise and mass selection was used to elevate genetically controlled (Macdonald 1962a) susceptibility of *Ae. aegypti* to *B. malayi* (Macdonald 1962b).

In Egypt, filariasis caused by *W. bancrofti* is transmitted by the mosquito *Cx. pipiens*. The disease has made a remarkable resurgence in Egypt over the last decades (Harb *et al.* 1993). Our multidisciplinary group has extensively examined various epidemiological and entomological aspects related to this infection (Gad *et al.* 1994; Gad *et al.* 1996a,b; Farid *et al.* 2000). Various studies, in particular those needing mass production of infective worms for immunological investigations (Helmy *et al.* 2000) required the use of a mosquito colony that is highly susceptible to the filarial parasite.

The present study gives an account of the isolation, by artificial selection, of a strain of *Cx. pipiens* that is highly susceptible to infection with *W. bancrofti*.

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MATERIALS AND METHODS

Mosquito collection and exposure to *W. bancrofti* carriers: Parental *Cx. pipiens* originated from larvae collected on the outskirts of Cairo in a highly polluted drainage canal in El Moassassah (Qaliubiya Governorate), and were reared at 27 ± 2°C, 60-70% R.H. and 16L:8D. Emerging mosquitoes were supplied with a 10% sugar solution, but sucrose was removed shortly being exposure to *W. bancrofti*-infected volunteers. Three- to 5-d-old females were allowed to feed on the forearms of informed and consenting microfilaremic subjects for 20 min between 22:00 and 02:00. To determine microfilaremia at the time of mosquito feeding (Table 1), thick blood films were prepared from 50µl finger prick blood samples taken (whenever consent was obtained) immediately before feeding (WHO 1987).

Selection of *Cx. pipiens* lines susceptible to *W. bancrofti*: Twelve hours after the infective feed (38mf/50µl blood), parental blood-engorged mosquitoes were transferred individually into labeled plastic vials (3 cm diameter, 7 cm height) containing water for oviposition and supplied with sucrose. Individual egg rafts were placed in rearing cups and labeled. Twelve days post blood meal, which is the extrinsic incubation period of the parasite (Gad *et al.* 1988), surviving mosquitoes were cold-killed and dissected for the presence and number of infective (L3) worms. Prevalence rate was the percentage of surviving females that contained one or more L3(s).

F_1 progenies of those infective mothers having a high intensity of parasitic infection (21-53 L3/female) were used to initiate selection for parasite susceptibility. Female pupae from selected mothers were separated from males and individual virgin females (F_1) from one sub-line were pairwise mated with individual males from another sub-line. Crosses were conducted in both directions. Groups of 3- to 5-d-old mated F_1 females, having common parents, were released in separate 15x15x15 cm cages and transported to a *W. bancrofti*-endemic village for exposure to a microfilaremic volunteer (59 mf/50µl blood). Blood-engorged F_1 females were allowed to oviposit individually and were assessed for infection 12-13 days after blood feeding.

F_2 progenies from sub-lines showing high prevalence rates were selected. F_2 virgin females were separated from males, and sister-brother pairwise mating was allowed to take place in individual vials. Groups of mated F_2 females from each sub-line were exposed to an infective blood meal, blood-fed mosquitoes were allowed to oviposit, and then were evaluated for filarial worm susceptibility as described. Because inbreeding problems arose rapidly, mass mating was used starting from the 3rd generation and continued throughout the remaining selection procedure. A strain of mosquitoes was considered highly susceptible when susceptibility exceeded 85% for at least 3 successive generations.

Synchronization of mosquito development: Because batches of mosquitoes emerged asynchronously over a period of approximately 10 days, an attempt was made to synchronize adult emergence. To obtain enough young males and females for mating at any one time for simultaneous exposure to a microfilaremic carrier, larvae that hatched first were kept at 25° C, whereas those hatching later were held at 29°C. Mosquitoes emerging over a 3-day period then were collected in an adult holding cage as one batch for a single feeding experiment.

Statistical analysis: Prevalence rates of groups of mosquitoes were compared by Chi-square analysis performed on a Personal Computer using Epi-info software (Epi Info 5.01-CDC, Atlanta, GA, USA).

RESULTS

Prevalence of the parental field strain of *Cx. pipiens* exposed to blood containing 38 mf/50 µl was 48.9% (N = 137). This value served as the baseline for evaluating further increase in

mosquito susceptibility. Progenies of ten parental females (F_0) designated A, B, C, D, E, G, H, K, S, and M (Fig.1) were used to initiate selection for *W. bancrofti* susceptibility.

The F_1 progeny of one female line from the F_0 generation was pairwise mated with that of another female line. Mating took place in both directions (Fig.1). Resulting sub-lines in the F_1 generation were designated BA, AB, DC, CD, GE, EG, KH, HK, MS, and SM (Fig.1). Individuals in the sub-lines were exposed to a microfilaremic volunteer (59 mf/50 µl) and the resulting susceptibility of the F_1 sub-lines ranged from 33.3 to 63.6%. Combined susceptibility for F_1 females (54.1%, N = 122), however, did not vary significantly (Chi^2 = 0.50, P = 0.48) from that of the parental generation. Because susceptibility of mosquitoes in sub-line DC and GE was low, their progenies were not retained for further selection.

Siblings in the F_2 generation were pairwise mated, and following exposure to infected blood, 22 families yielded prevalence rates from 50 to 100%. The combined prevalence of F_2 females (89.6%) was significantly greater than that of the F_1 generation (Chi^2 = 63, P = <0.001). BA3, CD1 and SM1 had no progeny and the susceptibility of BA2 females was low (50.0%); consequently, these sub-lines were eliminated. Pairwise sib-mating was allowed to take place in sub-lines BA1 and AB5, CD2 and EG1, and KH1 and CD2. Pairwise-mated mosquitoes were exposed to infected blood and prevalence resulting from this exposure ranged from 88.9-100%.

Because sib-mating of individuals in the F_2 generation resulted in few or no progeny, the F_3 progenies of the remaining F_2 families were pooled and mass-mated according to prevalence rates of their mothers (Fig. 1). Group S, Z, T, and M included F_3 progenies of F_2 mothers with 100, 92.3-94.7, 81.3-90.0, and 81.3-94.7% prevalence, respectively. Prevalence of infection in these groups, following exposure to infected blood (75 mf/50 µl), ranged from 72.0-95.0%, and their combined prevalence (86.8%, N = 190) did not vary significantly from that of the F_2 (Chi^2 = 0.60, P = 0.44). Because susceptibility of group S was less than 80% (N = 50), individuals in this group were discarded.

Mass mating and selection for susceptibility of the Z, T, and M groups from the F_4 to the F_6 generations (Fig.1) resulted in combined prevalence rates of 94.9 to 96.4%, with no significant difference (Chi^2 = 0.49, P = 0.78) among generations. However, at the F_7 generation, the susceptibility of M females dropped to 80.0% (N= 65), resulting in significantly reduced combined prevalence of this generation (Chi^2 = 4.3, P = 0.039) relative to that of F_6. Therefore, group M was removed from the study. Combined prevalence rates of females in groups Z and T (89.2%, N = 111 and 92.7%, N = 192, respectively) did not vary significantly (Chi^2 = 0.71, P = 0.40) between F_8 and F_9.

To amplify the populations of selected groups, F_{10} individuals in groups Z and T were combined (designated ZT) and selection pressure was removed from F_{10} to F_{13}. Because prevalence rates of ZT mosquitoes dropped to 33.3% (N = 33) and 63.9% (N =36) at the F_{12} and F_{13} generations, respectively, selection for susceptibility was resumed beginning with generation F_{14} (Table 1). High mosquito susceptibility to filarial worm infection was observed at the F_{16} and F_{17} generations (>90%). The reduced prevalence observed in the F_{18} females (80.9%) probably resulted from the low microfilaremia of ingested blood (9 mf/50 µl).

The mass-mated ZT line maintained an average prevalence rate of 92.5% from F_{19} to F_{25}. Fluctuations of susceptibility rates (Table 1) for these generations (85.5-97.1%) were not significant (Chi^2 = 9.41, P = 0.152). Moreover, when F_{18} was excluded from the computations, fluctuations in susceptibility (85.5-97.1%) from F_{16} to F_{25} were not significant (Chi^2 = 9.83, P = 0.277). Consequently, we believe susceptibility of ZT females is now stabilized at a level approximately twice as great as the original parental population.

DISCUSSION

Single-pair mating and selection for susceptibility to *W. bancrofti* from individual infective mosquitoes was the basic procedure used herein for two generations. Isofemale line selection schemes are known to produce rapid responses to selection for parasite susceptibility or refractoriness in insect vectors (Collins *et al.* 1986; Feldmann & Ponnudurai 1989). However, the use of pairwise sib-mating strategies for our *Cx. pipiens* strain resulted in a rapid degeneration of mosquito fitness in these lines. We overcame this problem by using between- and within-family selection through two generations and obtained 90% susceptibility at the F_2 generation and enough mosquitoes to proceed further.

Table 1: Selection for susceptibility of *Cx. pipiens* to *W. bancrofti* infection.

Generation	Mf/50ul blood	N fed	Infectivity %	Selection*
Parent	38	137	48.9	Y
F_1	59	122	54.1	Y
F_2	-	288	89.6	Y
F_3	75	190	86.8	Y
F_4	-	139	96.4	Y
F_5	-	181	95.0	Y
F_6	-	215	94.9	Y
F_7	-	198	88.9	Y
F_8	-	111	89.2	Y
F_9	60	192	92.7	Y
F_{10}†	-	-	-	-
F_{11}†	-	-	-	-
F_{12}	-	33	33.3	N
F_{13}	-	36	63.9	N
F_{14}	-	74	71.6	Y
F_{15}	54	65	66.2	Y
F_{16}	-	82	93.9	Y
F_{17}	-	135	92.6	Y
F_{18}	9	84	80.9	Y
F_{19}	-	71	94.4	Y
F_{20}	-	70	97.1	Y
F_{21}	-	88	94.3	Y
F_{22}	33	55	85.5	Y
F_{23}	18	114	92.9	Y
F_{24}	93	84	88.1	Y
F_{25}	47	50	94.0	Y

† Not exposed to microfilaremic blood, *Y = yes, N = no.

Although selection progresses rapidly by keeping the descendants of individual females separate and selecting within families, rather than pooling the progeny of all selected mosquitoes (Feldmann & Ponnudurai 1989), pooling and mass selecting was necessary to revert inbreeding defects at the F_3 generation. Impaired female insemination, marked pupal mortality or a distortion of the sex ratio in favour of females among inbred mosquitoes were observed at intervals. Inbreeding depression has been reported for individuals derived directly from several generations of sib-mating (Huff 1929; Macdonald 1962b; Feldmann & Ponnudurai 1989) and is known to result from the accumulation of deleterious genes (Macdonald 1962b).

Pooling of selected families ensured that sufficient mosquitoes were available for selection and attenuated the severity of problems associated with inbreeding. Mass selection of individual pools carried out until F_9 maintained susceptibility levels at a minimum of 90%. Fluctuations in susceptibility of laboratory stocks of mosquitoes to malarial parasites, filarial worms, and viral infections are not uncommon (Rutledge *et al.* 1970; Al-Mashhadani *et al.* 1980). However, withdrawal of selection pressure for four generations drastically reduced the susceptibility of selected mosquitoes to the filarial parasite. Resuming selection maintained an average 92.5% susceptibility for 12 more generations. This, however, was not accompanied by a parallel increase in intensity of infection (data not displayed). In a classic series of studies, Macdonald (1962a & b, 1963) obtained by familial selection of *Ae. aegypti* to semiperiodic *B. malayi* through 15 generations a strain with 84% susceptibility compared to an initial 17-31%. Failure to obtain 100% susceptibility may have several causes. Both non-genetic and genetic factors influence the susceptibility of mosquitoes to infection. Variation in parasite stocks is an important parameter. Some filarial worm susceptible

mosquitoes may not ingest microfilariae during blood feeding on an infected host, especially if the microfilaremia is low. This is particularly true for *W. bancrofti* infection in Egypt, where the microfilaremia of human hosts rarely exceeds 60 mf/50 µl blood. Environmental circumstances relating to temperature, humidity, age and nutrition of the mosquito, as well as relative size of the infective meal also may interfere with levels of susceptibility (Al-Mashhadani *et al.* 1980; Thathy *et al.* 1994). A highly susceptible strain may not be homozygous for the gene(s) responsible for filarial worm susceptibility. The females of a strain may be homozygous initially, but dilution with heterozygotes can take place, because there is no means for determining the genetic structure of males (Macdonald 1962a).

A sex-linked recessive gene, designated f^m, was reported to control susceptibility of *Ae. aegypti* to *B. malayi*, *B. pahangi*, and *W. bancrofti* (Macdonald & Ramachandran 1965). When dealing with a sex-linked recessive character, the gene involved may not be expressed for a few generations, particularly if selection is made from a few individuals Macdonald (1962a). This could explain the nonsignificant increase in the susceptibility of *Cx. pipiens* to the Bancroftian parasite after one generation of paired mating. However, we have been unable to select, by pairwise mating, a strain of *Cx. pipiens* refractory to *W. bancrofti* (data not shown). Susceptibility of selected mosquitoes did not drop below 20% after five generations of selection. Furthermore, strong fluctuations in refractoriness characterized successive generations, indicating that the genetics of *Cx. pipiens* susceptibility *to W. bancrofti* is likely more complex than that described by Macdonald and co-workers for *Ae. aegypti.* More studies are needed to assess the genetic basis of *Cx. pipiens* susceptibility to *W. bancrofti.*

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الملخص العربي

انتخاب سلالة من بعوض *كيولكس بيبنز* ذات درجة حساسية مرتفعة لطفيل الفيلاريا *فوشيريريا بانكروفتى*

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٣. قسم صحة الحيوان والعلوم الطبية الحيوية – جامعة ويسكونسين – ماديسون – الولايات المتحدة الأمريكية

يعتبر انتخاب سلالة من بعوض *كيولكس بيبنز* ذات درجة حساسية مرتفعة لطفيل الفيلاريا *فوشيريريا بانكروفتى* يمكن أن يسهل دراسة العلاقات المختلفة بين البعوض الناقل والطفيل . ولرفع درجة حساسية بعوض *كيولكس بيبنز* للإصابة بطفيل الفيلاريا *فوشيريريا بانكروفتى* على مدى أجيال متتابعة، فقد استخدمت طريقة قياسية من التزاوج الفردي على مدى جيلين ثم تبعه تزاوج وانتخاب جماعي. وقد أمكن تجنب الضعف الناتج عن التوالد الداخلي عن طريق ضم بعض العائلات عند الضرورة. تم تغذية النسل الناتج من التزاوج على أفراد مصابة بطفيل الفيلاريا ثم تشريح إناث البعوض في اليوم الثاني عشر أو الثالث عشر بعد العدوى ثم تحديد معدل العدوى. وبعد ٤ أجيال من الانتخاب الوراثي ارتفعت درجة حساسية البعوض للطفيل إلى اكثر من ٩٥%. بينما انخفضت درجة حساسية البعوض للطفيل عند التحرر من ضغط الانتخاب من الجيل العاشر إلى الجيل الثالث عشر. وقد أدى استئناف الضغط الانتخابي إلى ارتفاع حساسية البعوض للطفيل إلى ٩٤% عند الجيل السادس عشر. وقد وجد أن تفاوت درجة حساسية البعوض للإصابة بالطفيل من الجيل السادس عشر إلى الجيل الخامس والعشرون (٨٥,٥-٩٧,١%) كان بدون فروق معنوية، مما يوضح أن درجة الحساسية المرتفعة قد تم الحفاظ عليها على مدى هذه الأجيال.

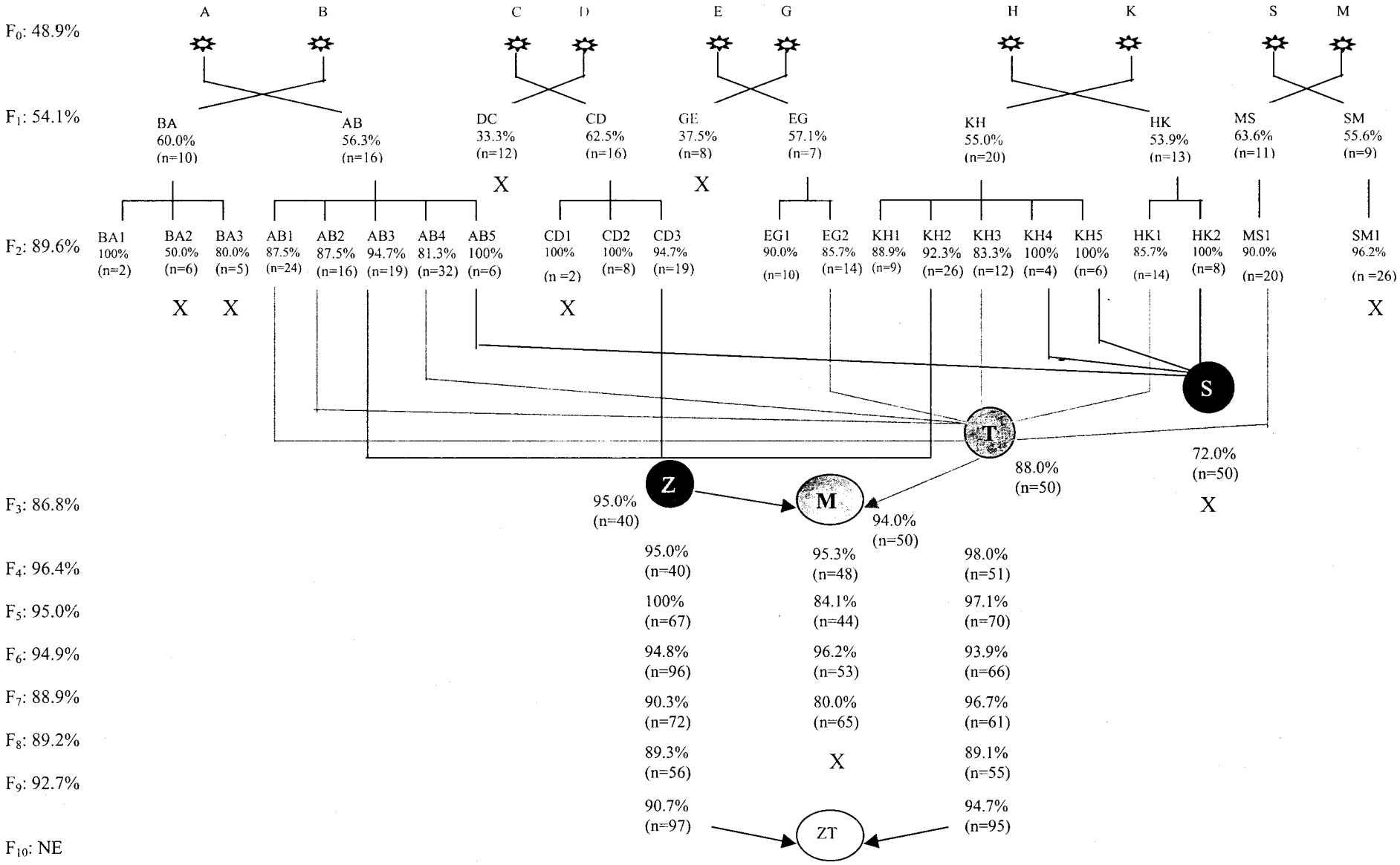

Fig 1. Selection of a *Cx. pipiens* strain highly susceptible to *W. bancrofti*. Left hand column shows mosquito generation (F): % prevalence. NE = no exposure. ✡ indicates a positive single female line. ⚤ indicates pairwise mating. ⫲ indicates pairwise sib-mating. X indicates that the family ended. Colored lines indicate pooling of families.