Glossina fuscipes fuscipes Newstead is one of the major vectors of human trypanosomosis that seriously hampers economic growth in infested areas. Large numbers of tsetse flies are required for the sterile male technique and research. Yet attempts to colonize this species in the laboratory in many instances have not been effective. The mating behaviour of Glossina fuscipes fuscipes Newstead (Diptera: Glossinidae) from Rusinga Island, Kenya, was studied in the laboratory with the aim of determining factors that affect their reproductive performance. Their performance was compared with that of a population from the International Atomic Energy Agency (IAEA) in Seibersdorf, Austria, that originated from Central African Republic (C.A.R.). Single mating showed that females from the C.A.R. population were more receptive compared to the Rusinga population. In the Rusinga population, young females aged one to two days post-emergence were most receptive and had higher spermathecal values compared to older females while in the C.A.R. population, these two parameters were high irrespective of age. The mean spermathecal values and fecundity of females given chance to mate with males more than once was statistically similar to those that mated once. Fecundity was lower in the Rusinga population compared to the C.A.R. one, and was independent of age and mating regime. Blood meal indices and pupal weights were also lower for the Rusinga population compared to the C.A.R. population. It is concluded that the mating behaviour of the Rusinga population of Glossina fuscipes fuscipes differs from that of C.A.R. and the former may require a longer time to establish in the laboratory than was provided for in the current study.