Effect of Host Egg Densities on Sex Ratio and Laboratory Performance of *Trichogrammatoidea* sp. nr. *lutea* Girault (Trichogrammatidae:Hymenoptera) Collected from *Plutella xylostella* Linnaus in Kenya.

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ABSTRACT

Four native lines of Trichogrammatoidea sp. nr. lutea Girault collected from Plutella xylostella Linnaus in coastal Kenya were reared on Corcyra cephalonica Sainton (Lepidoptera: Pyralidae) in the laboratory. This experiment was done to explore the possibility for enhancing the potential impact of these native egg parasitoids in terms of favourable sex ratio in the progeny using different host egg densities and competing females during mass production in the laboratory. In addition, other important reproductive parameters such as parasitization success and progeny production were also evaluated. When adult females were placed in-groups of four to oviposit just enough eggs they require to parasitize, the offspring sex ratio was female biased unlike when placed as single females. In group females, host egg patch size (the number of host eggs exposed per adult parasitoid) did not seem to affect the progeny sex ratio, but in test with single females, the offspring sex ratio significantly increased with increasing patch size. Results from this study suggested that placing ovipositing females in groups could be used to maximise percentage of female offspring during mass rearing in the laboratory for field release of T. sp. nr. lutea.

Key words: Trichogrammatids, Mass production, *Helicoverpa armigera,* tomato, parasitoids
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INTRODUCTION

Egg parasitoids (trichogrammatids) have successfully been utilized for biological control of several lepidopteran pests worldwide. In Kenya, *Plutella xylostella* Linnaus is known as a major pest of crucifers as well as African bollworm *Helicoverpa armigera* Hubner, a serious pest on tomatoes [1, 2, 3, 4]. These pests can successively be suppressed by parasitoids in many countries [5]. Naturally occurring egg parasitoids are most promising biological control agents for inundative releases against important lepidopteran pests, including augmentative biological control of *H. armigera*, [2,4]. They are more preferred than larval or pupal parasitoids because they attack the host at the egg stage before it emerges to larval stage (caterpillars), which is the destructive stage for the pest. In mass production of trichogrammatid egg parasitoids the sex ratio in the progeny is considered an important trait affecting the impact of the released adults. Indeed, the failure of numerous biological projects involving parasitoid wasps have been tentatively attributed to male-biased sex ratios [6].

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Several factors are known to affect offspring sex ratios through female manipulating fertilization of their eggs. One important factor which can be used to manipulate sex ratio in parasitic hymenopterans is the host density that the female encounters for it to make decisions which offspring sex it needs to allocate in the patch. *Trichogramma* responds to the number of hosts it encounters in the field, single or eggs laid in patches. As host density increases, it becomes more probable that a wasp will find sufficient number of hosts to oviposit its entire egg compliment. Under these conditions it is advantageous for the wasp to reduce the number of eggs laid in each host in order to produce larger progeny of greater fecundity and reproductive fitness [7]. For most *Trichogramma* species, as patch size increases, sex ratios are increasingly female biased [7,8]. During progeny allocation, cumulative sex ratios decrease with patch size when closely spaced hosts are parasitized [7]. Male progeny are allocated to the first and second hosts parasitized and at intervals of several female eggs thereafter.

The density of conspecific females on a host patch has also a particular effect on sex ratio [9, 10, 11]. So *Trichogramma* species are able to make adaptive responses to changes in both the size of the resource patches and the number of wasps competing with them. This also has an implication for mass production of trichogrammatids. The overall objective of this study was to explore the possibility for enhancing the potential impact of the native trichogrammatid egg parasitoids in terms of favorable sex ratio in the progeny using different host egg densities together with using competing females during mass production in the laboratory of *T.* sp. nr. *lutea*, a naturally occurring egg parasitoid on lepidopteran pests in Kenya.

MATERIALS AND METHODS

Parasitoid culture and maintainance
Four native lines of *T.* sp. nr. *lutea* used in this experiment were collected from the coastal part of Kenya, as they emerged from *P. xylostella* eggs in brassica crops. They were reared at the International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya insectary on Rice Moth, *Corcyra cephalonica* Sainton host eggs at a temperature of 27±2°C, relative humidity of 65±10% and a photoperiod of 12L: 12D. The individuals used were from isofemale lines established from a single egg mass parasitized by a single female. The four were selected following results of preliminary tests 16] which indicated high lifetime fecundity, progeny production and longevity. The factitious host C. *cephalonica* was used instead of the target pest *P. xylostella* because of ease rearing.Egg cards were made by sticking the eggs on Xerox paper using diluted adhesive gum (Gum arabica). The wasps were fed on honey solution (200ml: 3g gelatin: 100ml distilled water) made as minute streaks on the Xerox paper. All the host eggs provided were fresh and irradiated using UV-lamp to prevent them from hatching into host larvae, which predates on unemerged and parasitized eggs.

Effects of host egg patch size on sex ratio, parasitism, and progeny production of *T.* sp. nr. *lutea*
To test the influence of the egg patch size on the sex ratio of the females, host egg densities of containing 8, 16 and 32 eggs were per adult female were used. These patch sizes were selected on the basis that the parasitoid is likely to encounter small to very big host patches in the field. They were counted under light microscope before they were offered to the test insects. One individual female per vial represented a replication and a total of ten replications were made for each of the patch treatment. In another set of experiment, we kept four females together as a group per vial as to represent a replicate with similar egg densities per adult female.

The egg patches were replaced and changed daily as long as the females lived for both sets. They were placed in separate vials to await emergence. They were kept at 27±2°C, 65±10% RH and 12L: 12D. The lifetime number of parasitized (blackened) eggs, and adult progeny which emerged were counted and recorded, together with the number of females out of the total progeny (number of adults). The number of parasitized eggs per replicate was determined by counting black host eggs as described by Flanders (1965). Parasitism was confirmed by blackening of the vitelline membrane of host egg. The number of females out of the emerged adults was also recorded. The sexes were distinguished primarily on the basis of males having longer, more setose antennae than females [12].

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Data analysis

The data on numbers were log transformed to normalize the distribution, while the percentage data were transformed using the arcsine function. Analysis of Variance (ANOVA) was performed and Student Newman Keul's (SNK) test was adopted as a post- ANOVA test, to rank the means of those that were significantly different at P=0.05 The analyses were performed using Generalized Linear Models procedure (GLM) [13]. Sex ratio was expressed as % of females [(total progeny)*100]; % emergence as [(no. of emerged adults/blackened eggs)*100] and % parasitism as [(no. of blackened eggs/blackened eggs+non-blackened eggs) *100].

RESULTS

Parasitism efficiency

The mean(±SE) number of eggs parasitized per isolated female on different patch sizes differed significantly (F=9.36; df=2,33; p=0.0003), with least number of eggs parasitized on host patch size containing 8 eggs (1.86±0.12 eggs) (Table 1). The number of eggs parasitized was low at low and high host egg densities; the same trend was realized with percent parasitism per adult female at respective egg patches. The overall mean number of eggs parasitized per female when the adult females were placed in groups of four was generally higher (6.49 eggs) (Table 2) than when they were singly placed (3.75 eggs) (Table 1) on different egg patch sizes.

Table 1. Means (±SE) of reproductive parameters of *Trichogrammatoidea*. sp.nr. *lutea* in test with single females at different host egg patch sizes.

Adult: Egg ratio	No. of parasitized eggs per adult	No. of adult emerged	No. of females emerged out of adults	Percentage of parasitized eggs	Percent emergence of parasitized eggs	Percent of females out of adult progeny
1:8	1.86±0.12 c	1.83±0.23 c	0.50±0.2 c	23.30±0.15 **b**	98.75±1.01 a	27.65±1.20 **b**
1:16	5.32±1.85 a	4.80±1.64 a	2.00±0.99 a	33.22±0.62 a	90.28±1.32 b	41.86±1.64 a
1:32	4.08±0.96 b	3.58±0.86 b	1.29±0.23 b	12.76±0.31 c	87.67±1.25 b	36.21±1.72 a
Grand mean	**3.75**	**3.40**	**1.26**	**23.09**	**92.36**	**34.96**
F	**9.36**	**14.56**	**42.96**	**7.69**	**6.39**	**3.69**
df	**2,33**	**2,33**	**2,33**	**2,33**	**2,33**	**2,33**
P value	**0.0003**	**0.0004**	**0.0012**	**0.0003**	**0.0001**	**0.0012**

Means in the same column followed by the same alphabetical letter are not significantly different at (P>0.05) by SNK.

Adult emergence from parasitized eggs

The overall mean percentage of parasitized eggs with adult emergence was generally high both in test with single females and those placed together in groups. Emergence of parasitized eggs by females placed singly had a mean of 92.36% (Table 1) whereas emergence of parasitized eggs by group females of four was 85.41% (Table 2). There were significant differences in the number of emerged eggs per adult in test with single females on different host egg patch sizes (F=6.39; df=2,33; p=0.0001) and when they were in groups (F=3.96; df=2,33; p=0.0029).

Proportion of females (sex ratio) in the progeny

The overall percentage of females per adult in test with single females on different host egg patch sizes was generally low (34.96%) (Table 1). This was in contrast with individual females when placed in groups of four

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(72.11%) (Table 2), where dominance of females was realized in all egg densities. There was significant (F=3.69; df=2,33; p=0.0012) differences in proportion of females produced per adult at different egg patch sizes in tests with single females (Table 1) and no significant (F=4.12; df=2,33; p=0.8320) differences were found at different egg patch sizes in tests with group of four females (Table 2).

Table 2. Means (±SE) of reproductive parameters of *Trichogrammatoidea*. sp.nr. *lutea* in test with group of four females at different host egg patch sizes

Adult: Egg ratio	No. of parasitized eggs per adult	No. of adult emerged	No. of females in emerged adults	Percentage of parasitized eggs	Percent emergence of parasitized eggs	Percent of females out of adult progeny
1:8	1.74±0.16 **b**	6.89±1.25 **b**	5.12±1.58 **b**	21.73±1.53 **a**	88.97±1.23 **a**	74.36±1.52 **a**
1:16	4.98±0.13 **c**	4.45±0.98 **c**	3.21±0.69 **c**	29.36±1.32 **b**	89.28±1.56 **a**	72.13±1.36 **a**
1:32	12.77±1.23 **a**	10.14±2.12 **a**	7.15±1.09 **a**	39.99±2.38 **a**	79.54±1.47 **b**	70.50±1.56 **a**
Grand mean	6.49	7.16	5.06	30.36	85.41	72.11
F	16.35	9.37	10.06	5.36	3.96	4.12
df	2,33	2,33	2,33	2,33	2,33	2,33
P value	0.0003	0.0004	0.0041	0.0003	0.0029	0.8320

Means in the same column followed by the same alphabetical letter are not significantly different at (P>0.05) by SNK.

DISCUSSION

It was strongly clear from the results obtained that the sex ratio was female biased when the females attacked the egg masses when in groups. When the same egg mass giving female biased sex ratio is attacked by single isolated females, the sex ratio tends to male bias on small size host egg masses and female biased as the number of eggs per egg mass increases. The same trend was realized with parasitization capacity of these wasps but only when they attack the host eggs as isolated individuals. This is in consistent with observations made on *Trichogramma* sp. nr. *sibericum* (Hymenoptera: Trichogrammatidae) by Li, *et al*., (1993) on parasitization capacity but not on sex ratio. The authors also found out that the sex ratio is unaffected by increase in number of host eggs per patch in group females as it was the case with this study. Similar results on parasitism capacity were obtained for other parasitoids with female biased progeny, *T. evanescens* Westwood [14, 15]. Several authors have recorded that for most *Trichogramma s*pecies, as patch size increases, sex ratios are increasingly female biased [8,14,15]. These parasitoids show a mechanism in which when the wasps oviposit on egg masses, they allocate their offspring in a non-random distribution that ensures the presence of sons to mate all daughters depending on the egg mass size; the sex ratio approaches to male bias from small egg mass as well as from egg masses too large to be exploited by one single female; while in the middle size egg mass situations the sex ratio becomes more female biased as the number of eggs per egg mass increase. On the other hand, according to Waage and Lane (1984) and Green *et al.* (1982), the most efficient and simple mechanism females could use lies in their laying son and daughter eggs in a particular order, and such sequence will enable female to produce accurate sex ratios without counting the number of host eggs in a patch and to calculate what fraction of their progeny should be male and female. The parasitoids in the present study seem to have followed the former pattern, leading to a female biased sex ratio as number of eggs per egg mass increased when they attacked the eggs as isolated females. From this study, by placing parasitoids in groups to attack just enough eggs they require for parasitization, offspring female percentage is maximized for this promising trichogrammatid during mass production in the laboratory before field release. One can also predict the field

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performance any promising parasitoid where it encounters different host egg masses to make a decision on sex allocation of its offspring in order to maximise its reproductive fitness.

CONCLUSION

The overall results of this study suggested that host egg density could be used to manipulate sex ratio during mass rearing in the laboratory in order to optimise percentage of females for field release of *T.* sp. nr.*lutea.* Furthermore, placing ovipositing females in groups could be used to maximise percentage of female offspring during mass rearing in the laboratory before field releases.

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