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POPULATION VARIABILITY AND DEVELOPMENTAL TIME STUDIES OF *COTESIA FLAVIPES* CAMERON (HYMENOPTERA: BRACONIDAE) REARED ON DIFFERENT POPULATIONS OF *CHILO PARTELLUS* SWINHOE (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT: Variability of two populations of *Cotesia flavipes* and *Chilo partellus* was studied for reproductive success and developmental time under laboratory conditions. Fourth instar larvae of two populations of *C. partellus* (Ziway and Melkasa) were exposed to a day old mated adult female of two populations of *C. flavipes* (Ziway and Melkasa) to study the variability in reproductive success. The developmental time of the two geographical populations of *C. flavipes* reared on *C. partellus* obtained from the two different locations was also studied under four temperatures (20°C, 25°C, 28°C and 30°C). The experiments were designed in a complete randomized design. When both the parasitoid and the host were from the same location, the number of dead larvae inside the host and dead cocoons were significantly lower ($p<0.0.5$) and the total progeny was significantly higher than when the parasitoid and the hosts were from different locations. However, percent of female progeny was not significantly affected irrespective of the origin of the parasitoid and the host population. The developmental time decreased as the temperature increased from 20°C to 30°C. The significance of population variation in relation to biological control is discussed.

Keywords/phrases: *Chilo partellus*; *Cotesia flavipes*; Melkasa population; population interaction; Ziway population.

INTRODUCTION

Cereal crops, particularly maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench) are the main food crops in eastern and southern African countries (Minja, 1990). In Ethiopia, these crops rank first and second in terms of yield per hectare and total production, respectively. They account for about 41% of the total crop production of the country (CSA, 2000).

Among the various insect pests attacking maize and sorghum, lepidopterous stem borers are considered to be the most damaging (Seshu Reddy and Sum, 1992). In Ethiopia, four lepidopteran and two coleopteran stem borers attack maize and sorghum (Emana Getu *et al*., 2001). Of the various insect pests

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attacking maize and sorghum in eastern and southern Africa, the maize stalk borer, *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and the spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) are by far the most important (Kfir, 1998; Emana Getu *et al*., 2002). *Chilo partellus* was introduced accidentally from Asia to Africa in the early 1930's (Tams, 1932) while *B. fusca* is indigenous to Africa (Harris and Nwanze, 1992). *Chilo partellus* is a polyphagous insect pest, has a wider host range and attacks sorghum, maize, pearl millet, finger millet, rice, wheat, sugar cane, foxtail, and various wild grass species (Sithole, 1990).

Cotesia flavipes Cameron (Hymenoptera: Braconidae) is an important parasitoid of graminaceous stem borers in the Oriental region. It has been utilized for biological control worldwide. *Cotesia flavipes* is now established and significantly suppresses *C. partellus* in eastern and southern African countries including Ethiopia (Cugala and Omwega, 2001; Emana Getu *et al*., 2001; Matama *et al*., 2001; Nsami *et al*., 2001; Songa *et al*., 2001).

Local parasitoid populations may be adapted to their sympatric major plant-host complex. Parasitoid strains may thus differ in their physiological compatibility with particular host species. Introduction of a parasitoid population can induce artificial allopatric speciation by forcing the parasitoid to locally adapt to its new host or host microhabitat. This scenario could induce the formation of adapted parasitoid races and over time even distinct species. The failure or success of a parasitoid introduction can thus depend on the physiological compatibility between the introduced parasitoid and local host population (Potting, 1996). Potting *et al*. (1997) reported the existence of *C. flavipes* strains based on differences in physiological compatibility between local parasitoid and host populations.

Laboratory investigations indicated that *C. flavipes* performed differently in various stem borer species and at different temperatures (Mbapila, 1997). Among many other factors that limit the distribution of *C. flavipes*, temperature is the most important. This study was carried out to investigate whether variability in terms of reproductive success existed in two different geographic populations of *C. flavipes* and the possible effect of temperature on the developmental time of *C. flavipes*.

MATERIALS AND METHODS

Insect collection and rearing

Chilo partellus larvae and pupae were collected from Melkasa Agricultural Research Center (MARC) experimental field (Cp Mel) and Ziway farmers' fields (Cp Ziw). Maize and sorghum plants, which showed signs of stem borer infestation, were dissected using a knife and larvae of *C. partellus* were collected. These larvae were given a fresh piece of maize or sorghum stem and kept in a plastic container (20 × 10 × 6 cm) covered with fine mesh screen, labeled and taken to the entomology laboratory of MARC. In the laboratory, the larvae collected from both areas were kept separately in a petri dish of 9 cm diameter and 2 cm depth and given fresh pieces of maize stem. Cocoons of *C. flavipes* were collected from MARC experimental field and Ziway farmers' fields.

The collected larvae of the two geographic populations were reared separately on sections of maize stem of approximately 6 cm long. In all experiments, each stem was changed every two days. The pupae were kept in a clean 30 × 30 × 30 cm perspex sleeve cages. Glossy paper stripes (10 × 45 cm) were each folded longitudinally and placed diagonally in the cages as an ovipostion substrate. Two cotton balls soaked with 20% sugar-water solution were placed in small vials (2.3 × 4.4 cm) and put at the corners of the cage to serve as a feeding source for the moths. The glossy papers were removed daily and checked for egg masses.

Glossy papers with mass of eggs were cut into smaller sections and kept in an incubator adjusted to 25 ± 1°C and was observed daily till the formation of black head. New glossy papers were placed in the cages to replace the removed ones. Enough sections of the papers containing approximately 300 black head stage of the same egg batches were placed in rearing plastic containers (20 × 10 × 6 cm) containing young seedlings of maize as food for the first instar *C. partellus* larvae. The plastic containers were covered with tight fitting lids with fine mesh screen lid to prevent the newly hatched larvae from escaping. The first three instars were fed on young leaves for 2-3 weeks. The later instars were fed on maize stems.

Fifteen day old larvae (4^{th} instar) were used for the experiments. New pupae were daily removed from the rearing petri dishes and separated by the date of collection to give the approximate age of the pupae. Mature pupae were then placed in the oviposition cages within a day or two before the predicted moth emergence date. The process of rearing was repeated for a number of

generations for the two geographic populations (Cp Mel and Cp Ziw) until the end of the experiments.

Cocoons of *C. flavipes* from Melkasa (Cf Mel) and Ziway (Cf Ziw) were kept separately in a 20 × 20 × 20 cm perspex sleeve cage. As soon as the cocoons turned to black color, two cotton balls soaked with 20% honey-water solution were placed in small vials (2.3 × 4.4 cm) and put in the corners of the cage to serve as food source for the emerging wasps.

Fourth instar larvae of *C. partellus* were exposed to one day old-mated female of *C. flavipes* using a hand stinging method described in Overholt (1994). Two or three hours before stinging, the female wasp was placed under incandescent light to activate it for stinging in the cage. Each host larva was held using soft forceps and offered individually to a female parasitoid. Oviposition of *C. flavipes* females was noticed as the larvae reacted violently when the ovipositor of the parasitoid was inserted. Generally, the whole interaction between the wasp and its host larvae lasted for few seconds. The stinging process continued until the parasitoids lost interest in oviposition or enough number of host larvae were stung. After oviposition each parasitized larva was removed from the cage and kept on a petri dish and given maize stem.

The stem was changed every two days for the stung larvae so that fungal infection was minimized. The parasitized *C. partellus* larvae were daily observed for cocoon formation. The cocoons were removed and transferred to the perspex sleeve cage for adult emergence. The rearing process continued up to the end of the experiments.

Suitability test

Fourth instar larvae of the two *C. partellus* population (Cp Mel and Cp Ziw) were exposed to a day old, mated adult females of the two *C. flavipes* population (Cf Mel and Cf Ziw) using a hand stinging method described in Overholt (1994). Each stung larva was placed separately in a petri dish (9 cm diameter and 2 cm deep). The test was replicated four times. In each replication ten parasitized *C. partellus* larvae were considered. A total of 160 insects from all larvae-parasitoid combination were placed in an incubator set at 28°C. The experiment was conducted in a completely randomized design (CRD). The treatments were Cf Ziw Cp Ziw, Cf Mel Cp Mel, Cf Ziw Cp Mel and Cf Mel Cp Ziw. Observations were made daily until cocoon formation, host death or pupation. Cocoons from each petri dish were transferred into glass vials covered with nylon organdy to avoid

escape of adult wasps.

Date of exposure, date of cocoon formation, date of adult emergence, number of larvae dead inside the host, number of progeny and sex of progeny were recorded. One day after cocoon formation, the stung larvae were dissected using a dissecting blade and the dead *C. flavipes* larvae inside the host were examined and recorded using the microscope. The progenies were sexed using antennal length and their number recorded.

Developmental time

One day old mated females of Cf Mel and Cf Ziw were used to parasitize Cp Mel and Cp Ziw. The experiment was repeated four times. In each replication ten parasitized *C. partellus* larvae were used. A total of 160 insects from all larvae-parasitoid combination were placed in an incubator set at 20°C, 25°C, 28°C and 30°C. The experiments were carried out in a complete randomized design (CRD) in factorial arrangements. The treatments were Cf Ziw Cp Ziw, Cf Mel Cp Mel, Cf Ziw Cp Mel and Cf Mel Cp Ziw (insects population factor), and different temperatures (20°C, 25°C, 28°C and 30°C). In each temperature regime, maize stem was changed once every two days until cocoon formation or death of the larvae. Cocoons from each petri dish were transferred into glass vial covered with nylon organdy to avoid escape of adult wasps. Date of exposure, date of cocoon formation and date of adult emergence were recorded.

Data were transformed using logarithmic transformation and analyzed using SPSS software following the procedure of General Linear Model (GLM). Where ANOVA was significant, means were separated using Tukey's studentized range test (HSD). Transformed data were used for reporting back.

RESULTS

Suitability test

Population interaction was significant with respect to the number of dead larvae inside the host (F_3=18.39, P<0.0001), number of dead cocoons (F_3=40.85, P<0.0001) and total progeny (F_3=26.03, P<0.0001). However, population interactions were not significant with respect to percent female progeny per host (F_3=0.544, P=0.653). The analysis of variance on the number of dead larvae inside the host, number of dead cocoons, total progeny per host and percent of female progeny per host are shown in Table 1.

Table 1 Analysis of variance for number of dead larvae inside the host, number of dead cocoon, total progeny per host and percent female progeny.

Variable	Source	DF	SS	MS	F	P
Number of dead larvae inside the host	Pop	3	119.319	39.773	18.388	<0.0001
Number of dead cocoon	Pop	3	1946.019	648.673	40.846	<0.0001
Total progeny	Pop	3	6557.919	2185.973	26.025	<0.0001
Percent female	Pop	3	317.660	105.887	0.544	0.653

Pop = population interaction

Comparison of the four-population interactions for mean number of dead larvae inside the host, dead cocoon and total progeny is given in Table 2. The result showed that when both the parasitoid and the host were from the same location, dead larvae inside the host and dead cocoon were significantly lower and the total progeny was significantly higher.

Table 2 Mean (±SE) number of dead larvae inside its host, dead cocoons and total emerged progeny under variable population interactions.

Population interaction	Dependent variable		
	Dead Cf larvae inside Cp	Dead Cf cocoon	Total Cf progeny
Cf Ziw Cp Ziw	0.35 ± 0.1a	1.27±0.23a	33.37±1.1a
Cf Mel Cp Mel	0.77 ± 2.38ab	2.65 ± 0.67a	35.87±2.1a
Cf Ziw Cp Mel	1.52 ± 0.27b	7.62 ± 0.84b	24.05±1.37b
Cf Mel Cp Ziw	2.62 ± 0.27c	9.77 ± 0.57b	20.37±0.94b

Means followed by the same letter(s) within a column are not significantly different from each other at 5% (HSD).
Cf Ziw Cp Ziw = *C. flavipes* from Ziway reared on *C. partellus* from Ziway
Cf Mel Cp Mel = *C. flavipes* from Melkasa reared on *C. partellus* from Ziway
Cf Ziw Cp Mel = *C. flavipes* from Ziway reared on *C. partellus* from Melkasa
Cf Mel Cp Ziw = *C. flavipes* from Melkasa reared on *C. partellus* from Ziway

Developmental time

Egg to adult developmental time was significantly affected by temperature (F_3=9027.4, P<0.0001), population interaction (F_3=89.04, P<0.0001) and the interaction of temperature and population interaction (F_3=52.39, P<0.0001). The analysis of variance for the developmental time is given in Table 3.

Table 3 Analysis of variance for egg to adult developmental time.

Source	DF	Type III SS	MS	F value	P
Temp	3	15907.680	5302.560	9027.481	< 0.0001
Pop	3	156.892	52.297	89.035	< 0.0001
Temp ∗ Pop	9	276.952	30.772	52.389	< 0.0001

Temp = temperature, Pop = population interaction, Temp ∗ Pop = interaction of temperature and population interaction.

Comparison of the mean number of days taken for egg to adult developmental time of population interactions within a temperature and effects of temperature on number of days from egg to adult developmental

time (mean ± SE) of population interactions is given in Table 4. Regardless of the type of populations (both Cp and Cf) used, days taken from egg to adult were inversely proportional to temperature. Population differences in terms of days taken from egg to adult were seen at all tested temperatures.

Table 4 Effect of population interaction and temperatures on mean developmental time (mean ± SE) from egg to adult.

	Temperatures			
Population interaction	20°C	25°C	28°C	30°C
Cf Mel Cp Mel	30.25 ± 0.2Aa	19.02 ± 0.07Ab	17.55 ± 0.25Ac	16.20 ± 0.07Ad
Cf Mel Cp Ziw	30.15 ± 0.17Aa	19.75 ± 0.07Bb	17.30 ± 0.07Ac	16.13 ± 0.06Ad
Cf Ziw Cp Ziw	27.7 ± 0.94Ba	18.50 ± 0.1Ab	16.20 ± 0.08Bc	16.13 ± 0.06Ad
Cf Ziw Cp Mel	27.03 ± 0.15Ca	19.02 ± 0.07Ab	17.05 ± 0.03Ac	17.18 ± 0.06Bc

Means followed by the same letter(s) within a column (uppercase letter) and rows (lowercase letter) are not significantly different from each other at 5% (HSD).
Cf Mel Cp Mel = *C. flavipes* from Melkasa reared on *C. partellus* from Melkasa
Cf Mel Cp Ziw = *C. flavipes* from Melkasa reared on *C. partellus* from Ziway
Cf Ziw Cp Ziw = *C. flavipes* from Ziway reared on *C. partellus* from Ziway
Cf Ziw Cp Mel = *C. flavipes* from Ziway reared on *C. partellus* from Melkasa

The regression equation for egg to adult developmental time was Y= 52.90-1.27X where Y represents the developmental time and X represents the temperature in °C. The R^2 and P values were 0.87 and 0.001, respectively (Fig. 1). The regression equation showed that egg to adult development time was significantly negatively correlated with temperature.

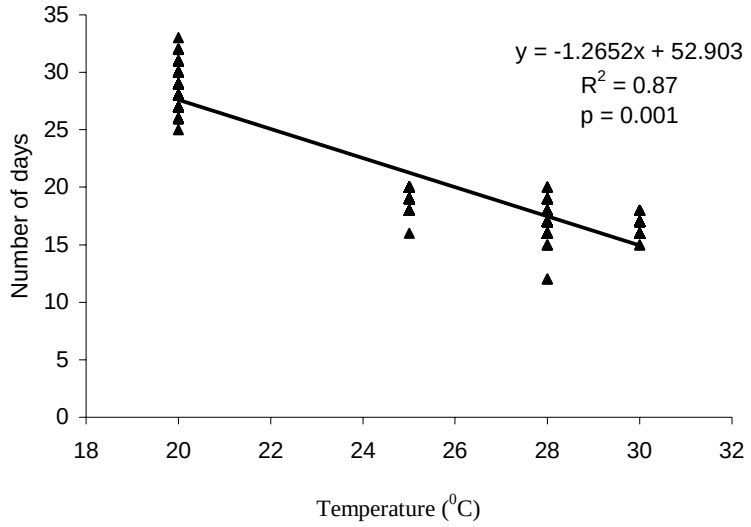

Fig. 1. Regression of egg to adult developmental time (days) (dependent variable) as a function of temperature (independent variable).

DISCUSSION

In the suitability test, the two geographic populations of *C. flavipes* showed variability in terms of reproductive success when the host insects were different in geographical location. Both geographic populations showed superiority on the *C. partellus* population of the same geographic location than a *C. partellus* from different geographic location. The highest total progeny and lowest dead cocoon recorded when Cf Ziw reared on Cp Ziw and Cf Mel reared on Cp Mel indicated that the two *C. flavipes* populations were best when the host was from similar geographical area.

Potting *et al*. (1997) also reported the occurrence of variation in terms of total progeny production among different geographical populations of *C. flavipes* that differed in the plant host-complex they were obtained from, but there was no significant difference in sex ratio of emerged progeny between the populations. The current work also found variation in total progeny between *C. flavipes* collected from Ziway and *C. flavipes* collected from Melkasa when the hosts were from different geographical location. But, in the current work the two populations of *C. flavipes* did not differ in host plant complex they were obtained from, they only differed in geographic location. The two geographical areas have different climatic property, Melkasa represents a dry hot area while Ziway is a humid hot area and these differences in climatic property may have contributed to the variation.

Emana Getu (2001) reported variability in mean number of dead larvae inside the host and dead cocoon among five different geographical populations of *C. flavipes* in Ethiopia which were reared on *C. partellus* from Melkasa. The current study also confirmed variability in mean number of dead larvae inside the host, dead cocoon and total progeny among *C. flavipes*/*C. partellus* population interactions tested. However, there was no significant difference in percent female produced, which agreed with the findings of Potting *et al*. (1997) and Emana Getu (2001).

Difference in physiological compatibility between different populations of *C. flavipes* may reflect difference in efficacy of the parasitoid population, but it may also reflect differences in immune response between different populations of *C. partellus*. However, since *C. flavipes* is introduced recently, intraspecific variation is not expected. Therefore, the most likely reason would be a difference in immune response of the hosts. The higher number of dead larvae inside the host for *C. flavipes* from Melkasa reared on *C. partellus* from Ziway and low number of dead larvae inside the host for *C. flavipes* from Ziway reared on *C. partellus* from Ziway, indicated a

difference in immune response of *C. partellus* from Ziway when the parasitoids were from different geographic locations.

Cotesiae sessamiae, a closer species to *C. flavipes*, was unable to develop in *B. fusca* populations of coastal area of Kenya because their eggs were encapsulated, but they successfully developed in other populations of *B. fusca* in Kenya. *Cotesia flavipes* was also unable to develop in most of *B. fusca* populations, but it could develop in some populations (Cugala and Omwega, 2001; Emana Getu, 2001; Matama *et al*., 2001). This indicated variation in virulence between parasitoid populations and variation in encapsulation ability between host populations.

In studying the developmental time of the two populations of *C. flavipes*, it was observed that the two populations of *C. flavipes* had different developmental times at different temperature. Even within the same temperature, they showed different developmental time when the hosts were from different geographical locations.

Many authors have reported that the developmental time of insects is dependent on the temperature of their environment and have determined the optimum temperature requirement of many insects (Vincent *et al*., 1997; James and Ying-Hong, 1998; Kirk *et al*., 1998). In the current study, the egg to adult developmental time of the two populations of *C. flavipes* was inversely related to the temperature regimes they were exposed to, irrespective of the host population they were reared on. The longest was at 20°C and the shortest at 30°C. This finding agreed with most of the works done on the effect of temperature on the developmental time of *C. flavipes* (Overholt *et al*., 1994; Mbapila, 1997; Emana Getu, 2001).

In the current experiment, the significance of the interaction of the population interaction and temperature in the developmental time of *C. flavipes* indicated that developmental time of *C. flavipes* varied with temperatures and in different geographic populations of *C. partellus*. Emana Getu (2001) also studied the developmental time of Indian and Pakistan populations under different conditions than used in the current study and suggested that the developmental time of *C. flavipes* varied with temperature, relative humidity and population.

The establishment and efficiency of parasitoids depend on the population increase which is the function of length of generation time, survival and fecundity (Rosh, 1990). If the two populations of *C. flavipes* tested are to be released in a cooler area which has a temperature of less than or equal to

20°C, population growth will be very slow which may end with low efficiency of biological control.

At 20°C, Cf Ziw had the shortest egg to adult developmental time than Cf Mel, which suggests its better adaptation to cooler areas. The longest developmental time of Cf Ziw on Cp Mel at 30°C than the other interactions at this temperature, the longest developmental time of Cf Mel reared on Cp Ziw at 25°C than the other interactions at this temperature and the shortest developmental time of Cf Ziw at 28°C than other interactions at this temperature suggested that temperature acted differently for different population interactions. Therefore, it may be useful to select populations based on the climate where they are intended for release as biological control agent.

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