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Factors influencing brood sex ratio in the egg parasitoid *Trissolcus basalis* (Woll.) (Hymenoptera: Scelionidae)*

ABSTRACT

This study examines the sex ratio response of the egg parasitoid *Trissolcus basalis* (Woll.) to both the presence of other conspecific females and to their traces when ovipositing on *Nezara viridula* (L.) egg masses. In both conditions *T. basalis* females lay a higher sex ratio (proportion of males) qualitatively agreeing with the local mate competition (LMC) theory. The ability of *T. basalis* to shift its sex ratio could be achieved by decreasing the number of laid eggs per wasp, which would increase the proportion of male eggs due to the sequence effect, i.e. "male-first strategy", and by direct oviposition of a higher proportion of male eggs. In all likelihood the external marking pheromone left by previous females provides the stimulus for sex ratio adjustment to the following females.

Key words: arrhenotoky, local mate competition, *Nezara viridula*, egg mass.

INTRODUCTION

Many parasitoid *Hymenoptera* are known to reproduce parthenogenetically by using arrhenotoky, i.e. females arise from fertilized and males from unfertilized eggs. Fisher's principle (1930) predicts that a mother gains equal fitness increments from the production of a son and a daughter in panmictic mating and in the absence of any discriminating interaction of environmental heterogeneity and fitness for the two sexes. Non-panmictic mating, argued by HAMILTON (1967), is one of the most common violations of Fisher's theory. Hamilton's theory, well known as Local Mate Competition (LMC), assumed a structured population in which groups of females could exploit a host patch for reproduction, and the progeny mates among themselves prior to dispersal. Under these LMC conditions, the optimal sex ratio is described by the equation $(n-1)(2n-1)/n(4n-1)$ where n is the number of females colonizing the host patch (HAMILTON, 1979). So when n is very large (outbreak populations)

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the sex ratio produced should be near 0.5, while when $n=1$ (completely inbred populations) the sex ratio approaches zero, i.e. a female produces only enough sons to mate all her daughters on the host patch. According to Hamilton's theory, gregarious and solitary parasitoids ovipositing on aggregated hosts do produce female biased sex ratio. Conversely, solitary parasitoids on isolated host produce equal sex ratios (STRAND, 1988b). Moreover, shifting from an unbiased sex ratio towards a female biased sex ratio was recently predicted even in populations where not all mating is at the emergence (NUNNEY & LUCK, 1988).

This paper reports experiments with *Trissolcus basalis* (Woll.), a solitary egg parasitoid of several Pentatomid species (JONES, 1988), which has been used mainly as a biological control agent of the Southern Green Sting Bug (SGSB), *Nezara viridula* (L.), in several countries (CLAUSEN, 1978). *N. viridula* egg masses can vary greatly in size, from 44 to 134 eggs with a mean of 91 ± 16.53 ($n=260$) (COLAZZA *et al.*, 1991). Many female scelionids fight for possession of the same host egg mass, but this behaviour is more restricted to species which attack small egg masses (WAAGE, 1982). So, large egg masses, like those of *N. viridula*, in the field may be exploited by more than one female. Unlike some other scelionids, *T. basalis* tolerates the presence of other ovipositing females on the same egg mass both in field (COLAZZA, unpubl.) and laboratory conditions (JONES & SIEGLAFF, 1991). The probability of multiple *T. basalis* females ovipositing at the same time on a *N. viridula* egg mass is not rare. In fact, during collection of pentatomid egg masses it was possible to observe a few egg masses with 2 and 3 females ovipositing simultaneously (COLAZZA, unpubl.). It could be common for a female wasp to encounter host egg masses already completely or partially exploited by other wasps. Large egg masses may exceed the daily fecundity of the wasps (WAAGE, 1982). The fecundity of newly emerged *T. basalis* females is on average about 62 ovarian eggs (MATTIACCI *et al.*, 1991), which is less than the average number of eggs/egg mass.

The biology of *T. basalis* satisfies these conditions and would influence the sex ratio qualitatively in agreement with the LMC theory. Previous studies on *T. basalis* have shown sex ratio variation, when the wasp attacks egg masses of different sizes and at different encounter rates. This fits LMC theory (COLAZZA *et al.*, 1991). It is reported here that isolated *T. basalis* females respond both to the presence of other conspecific females and their traces by adjusting the sex ratio as predicted by the LMC theory.

MATERIALS AND METHODS

HOST AND PARASITOID

N. viridula and *T. basalis* colonies were obtained from field collections and reared in a bioclimatic chamber ($24 \pm 1^\circ\text{C}$; R.H. $70 \pm 5\%$; L16:D8) following the methodology described by BIN *et al.* (1993). *T. basalis* females were paired at emergence with males for 24 hours to allow for mating. They were then isolated individually and used when they were 2-3 days old without oviposition experience. The experiments were run at room conditions ($20\text{-}22^\circ\text{C}$; R.H. 30-40%) in a circular arena (1.5 cm diameter, 0.5 cm height) covered with a cover glass and illuminated with low intensity light. *N. viridula* host eggs, laid on paper strips, were used when no more than 1 day old. All observations were recorded with a VCR (Panasonic NV-FS100), using a video camera (JVC KY-M280E) fitted with a 55 mm lens (Micro NIKKOR F/1:3.5) located directly above the arena.

EXPERIMENTS

The first experiment was set up to investigate whether shifts in sex ratio occur when *T. basalis* females encounter already-parasitized host eggs of different parasitized percentage; 8 females were placed at 30 min intervals on egg masses of 64 host eggs, permitting each one female to parasitize only 8 eggs. The first female encountered an un-exploited egg mass, while the following ones were in contact with hosts at increasing parasitism ratio: 0.13; 0.25; 0.38; 0.5; 0.63; 0.75 and 0.88. No superparasitism was permitted: if a female adopted an oviposition posture on an already parasitized host, she was delicately pushed off with forceps. This treatment was repeated 8 times (8 females/8 egg masses).

The second experiment was designed to verify whether *T. basalis* females modify their sex ratio due to direct contact with other conspecific wasps. 2, 4 or 8 female wasps were placed at the same time on mass of 64 host eggs. Respectively 32, 16 and 8 ovipositions were allowed per wasps and no superparasitism was permitted to take place. To better follow each wasp during the experiment, about 24-h prior the the experiment all the females were anaesthetised on a cold table (Peltier effect, Labco) at about -2°C and marked with non-toxic and odourless colours. The experiment was repeated 5 times (2, 4 or 8 females/5 egg masses).

In all the experiments the oviposition behaviour was recorded until all eggs were attacked and parasitized. Only marked eggs were considered as parasitized (COLAZZA *et al.*, 1991). Subsequently the video tapes were analy-

zed to obtain behavioural information and the oviposition sequence. Parasitized host egg masses were kept in a bioclimatic chamber until the pupal stage was reached (6-7 days), then they were separated individually in vials. Adults were sexed at emergence.

STATISTICAL ANALYSIS

Sex ratios (expressed as male percentage) were analyzed by variance analysis (ANOVA) after square root arcsin transformed data. Tukey's test was used to separate different means. Regression analyses were performed to determine how the host parasitism ratio affected the sex ratio of the wasps.

RESULTS

CONTACT WITH ALREADY PARASITIZED HOST EGG MASS

The mean sex ratio produced by individual *T. basalis* females on *N. viridula* egg mass at different ratios of un-parasitized and parasitized eggs by conspecific wasps are reported in fig. 1. Sex ratio produced by *T. basalis* females was strongly influenced when exploiting an egg mass at an increasing parasi-

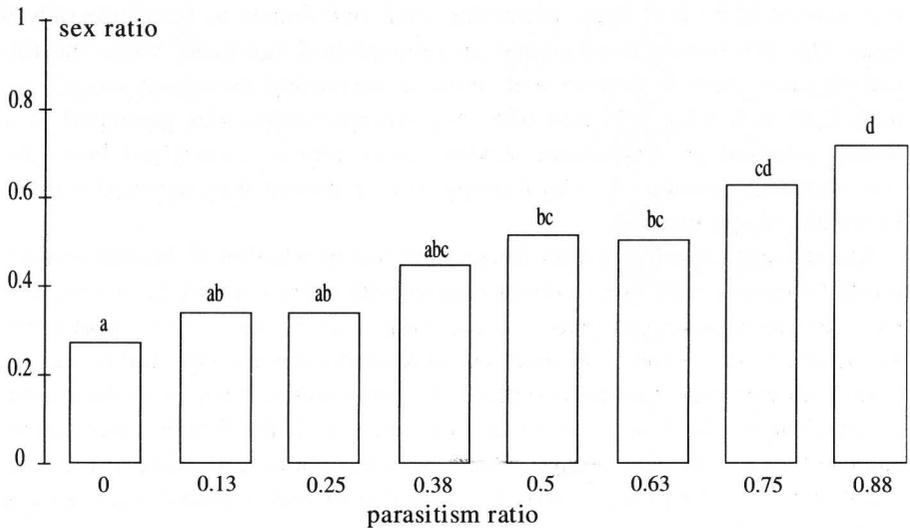


Fig. 1. Mean sex ratio (proportion of males) of *T. basalis* females when ovipositing on host egg masses at increasing parasitism ratios (proportion of parasitized eggs). Bars beneath the same letter do not differ significantly at the 5% level.

tism ratio ($F=4.68$; $df=7.56$; $P<0.001$; ANOVA), and was described by the regression: $y=0.26+0.53x$; $r=0.68$; $P<0.01$. The shift in sex ratio seems to be due to an increase in the probability of laying a male egg which indicates a direct response by the females to the parasitism ratio.

CONTACT WITH CONSPECIFIC WASPS

The mean sex ratios produced by *T. basalis* females ovipositing in groups of two, four or eight on *N. viridula* egg masses with 64 eggs each are reported in table I. There is a significant change in the mean sex ratio produced by the females in response to their density which increased from 0.0957 (two wasps) to 0.2044 (four wasps) and 0.2668 (eight wasps), as predicted by the

Females	n	sex ratio	
2	10	0.0957 \pm 0.0014	a
4	20	0.2044 \pm 0.0030	b
8	40	0.2668 \pm 0.0092	c

Table I. Variation in the average sex ratio (\pm SD) produced by *T. basalis* females when ovipositing on egg masses with 64 eggs at densities of 2, 4 and 8 wasps per egg mass. Values followed by the same letter do not differ significantly at the 5% level.

LMC theory ($F=7.23$; $df=2.67$; $P<0.01$; ANOVA). Figure 2 shows the sequence in which the females lay sons and daughters. The sequences of sex allocation seems similar, leading to the result that in these cases the shifting in sex ratio was due to a decrease in the number of eggs laid per female, and consequently an increase in the proportion of male eggs.

DISCUSSION

Several parasitoids are known to exert changes in the sex ratio, in accordance with the LMC model, in response to the number of mothers present. These either include solitary or gregarious parasitoids of eggs, larvae, pupae and oothecae (KING, 1993). Several examples are reported for egg parasitoids, females of *Trissolcus grandis* (VIKTOROV & KOCHETOVA, 1973) and *Telenomus remus* (VAN WELZEN & WAAGE, 1987) detect the presence of previous females using chemical trace(s), but *Telenomus heliothidis* do not (STRAND, 1988a). Changes in the sex ratio of *T. basalis* and *T. heliothidis* are inversely related to encounter rates with parasitized hosts (COLAZZA *et al.*, 1991; STRAND, 1988a).

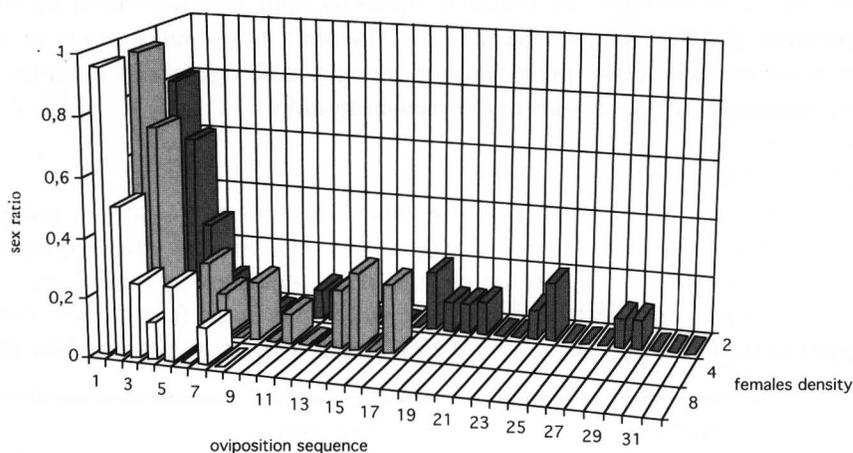


Fig. 2. Sequence of *T. basalis* progeny allocation on egg masses with 64 eggs at densities of 1, 2 and 4 wasps per egg mass. Each bar represents the mean sex ratio (proportion of males) per egg.

VAN WELZEN & WAAGE (1987) suggested three possible mechanisms to explain how a wasp can perceive the presence of conspecifics and, consequently, adjust its sex ratio: 1. the number of contacts, both visual and physical, with other wasps; 2. the frequency of encounters with already parasitized hosts; 3. the earlier presence and/or the concentration of trace odour(s), e.g. chemical markers, left by conspecifics. WAAGE & LANE (1984) proposed a simple but efficient behavioural mechanism adopted by female wasps to change the sex ratio without changing the pattern of sex allocation: females tend to lay proportionally more sons early in an oviposition bout, the so called "male-first strategy", so that as the proportion of total eggs parasitized by the following females decreases, the sex ratio of these wasps increases.

This study shows that *T. basalis* females respond both to the direct contacts with conspecific wasps and to the trace odours by increasing the proportion of sons they produce. The increase in *T. basalis* sex ratio due to the presence of conspecifics can be explained by a "male-first strategy", i.e. increased probability of laying a son independent of sequence. Instead, when *T. basalis* females encounter a host egg mass already exploited by conspecific wasps, they produce significantly more male eggs especially when more than the 50% of the eggs in the egg mass have been parasitized. In general exter-

nal marking pheromone(s) is considered an adequate cue for making adjustments in the sex ratio only if the wasp can distinguish between hosts containing their own offspring and those containing the offspring of conspecific females (VAN DEN DIJKEN & WAAGE, 1987; STRAND 1988b). Nevertheless, for naive females (i.e. females which have never oviposited), by definition, the first parasitized host egg mass can contain the offspring of different females only, thus, it may be adaptive to adjust the brood sex ratio. In fact, recent experiments have shown that naive *T. basalis* females are well able to discriminate between unparasitized and parasitized hosts (COLAZZA *et al.*, submitted). Finally, recent papers (CHASSIN & BOULÉTREAU, 1991; WAJNBERG 1993, 1994 in press.) have shown that both sex ratio determination and sex-sequence pattern seem to be under a strong genetic control, so that these traits can be considered as potential targets for genetic improvement in haplodiploid bio-control agents.

RIASSUNTO

I FATTORI CHE INFLUENZANO LA SEX RATIO DELLA PROGENIE DEL PARASSITOIDE OOFAGO

TRISSOLCUS BASALIS (WOLL.) (HYMENOPTERA: SCELIONIDAE)

In questo studio sono state esaminate le variazioni nella sex ratio del parassitoide oofago *Trissolcus basalis* (Woll.) mentre ovidepone su una ovatura di *Nezara viridula* (L.) in risposta sia alla presenza di femmine appartenenti alla stessa specie che alle loro sostanze marcant. In entrambi i casi le femmine del *T. basalis* hanno prodotto una sex ratio maggiore (espressa come proporzione dei maschi) in accordo con quanto previsto dalla teoria della competizione localizzata per l'accoppiamento, local mate competition (LMC). La capacità delle femmine del parassitoide di modificare la loro sex ratio è dovuta sia alla diminuzione del numero di uova parassitizzate per ciascuna femmina, con il conseguente aumento del numero di uova maschili (effetto sequenza); che ad un diretto aumento del numero di uova che daranno maschi. Con tutta probabilità il feromone di marcatura esterno depositato dalla prima femmina ovideponente fornisce lo stimolo alle femmine successive per modificare la loro sex ratio.

Parole chiave: riproduzione arrenotoca; competizione localizzata per l'accoppiamento, *Nezara viridula*, ovatura.

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REFERENCES

- BIN F., VINSON S.B., STRAND M.R., COLAZZA S., JONES W.A., 1993 - Source of an egg kairomone for *Trissolcus basal*s, a parasitoid of *Nezara viridula*. *Physiol. Entomol.*, 18: 7-15.
- CHASSAIN C., BOULÉTREAU M., 1991 - Genetic variability in quantitative traits of host exploitation in *Trichogramma* (Hymenoptera: Trichogrammatidae). *Genetica*, 83: 195-202.
- CLAUSEN C.P., 1978 - Introduced parasites and predators of arthropd pests and weeds: a world review. Agric. Handb. No. 480, Agric. Res. Service, US Dep. Agric.
- COLAZZA S., VINSON S.B., LI T.Y., BIN F., 1991 - Sex ratio Strategies of the egg parasitoid *Trissolcus basal*s (Woll.) (Hymenoptera: Scelionidae): influence of the host egg patch size. *Redia* (appendice), 74: 279-286.
- COLAZZA S., ROSI M.C., SEBASTIANI P., URSINI A.M., 1994 - Host discrimination in the egg parasitoid *Trissolcus basal*s (Woll.) (Hymenoptera: Scelionidae). (Submitted).
- FISHER R.A., 1930 - The genetical theory of natural selection. Oxford University Press, Oxford.
- HAMILTON W.D., 1967 - Extraordinary sex ratios. *Science*, 156: 477-488.
- HAMILTON W.D., 1979 - Wingless and fighting males in fig wasps and other insects. In: Blum M.S., Blum N.A. (eds) Reproductive competition and sexual selection in insects. Academic Press, New York, pp 167-220.
- JONES W.A., 1988 - World review of the parasitoids of the Southern Green Sting Bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). *Ann. Entomol. Soc. Am.*, 81: 262-273.
- JONES W.A., SIEGLAFF D.H., 1991 - Competitive behaviour of adults of three species of pentatomids egg parasitoids and its effects on reproductive success. In Wajnberg E. & Vinson S.B. (eds) *Trichogramma* and other egg parasitoids. Les colloques de l'I.N.R.A., 56: 33-36.
- KING B.H., 1993 - Sex ratio manipulation by parasitoid wasps. In: Wrensch D.L., Ebbert M. (eds) *Evolution and diversity of sex ratio in insects and mites*. Chapman and Hall, New York, pp 418- 441.
- MATTIACCI L., COLAZZA S., BIN F., 1991 - Influenza del regime alimentare sulla fecondità, longevità e fertilità del parassitoide oofago *Trissolcus basal*s (Woll.) (Hymenoptera: Scelionidae). *Atti XVI Congr. naz. ital. Ent.*, Bari, Martina Franca, 519-526.
- NUNNEY L., LUCK R.F., 1988 - Factors influencing the optimum sex ratio in a structured population. *Theor. Popul. Biol.*, 33: 1-30.
- STRAND M.R., 1988a - Variable sex ratio strategy of *Telenomus beliothidis* (Hymenoptera: Scelionidae): adaptation to host and conspecific density. *Oecologia*, 77: 219-224.
- STRAND M.R., 1988b - Adaptive patterns of progeny and sex allocation by parasitic Hymenoptera. In Gupta V. (ed) *Advance in parasitic Hymenoptera research*, E.J. Brill, Amsterdam, pp 293-312.
- VAN DEN DIJKEN M.J., WAAGE J.K., 1987 - Self and conspecific superparasitism by the egg parasitoid *Trichogramma evanescens*. *Entomol. exp. appl.*, 43: 183-192.
- VAN WELZEN C.R.L., WAAGE J.K., 1987 - Adaptive responses to local mate competition by the parasitoid, *Telenomus remus*. *Behav. Ecol. Sociobiol.*, 21: 359-365.
- VIKTOROV G.A., KOCHETOVA N.I., 1973 - The role of trace pheromones in regulating sex ratio in *Trissolcus grandis* (Hymenoptera, Scelionidae). *Zb. Obsch. Biol.*, 34: 559-562.
- WAAGE J.K., 1982 - Sib mating and sex ratio in scelionid wasps. *Ecol. Entomol.*, 7: 103-112.

- WAAGE J.K., LANE J.A., 1984 - The reproductive strategy of parasitic wasps. II. Sex allocation and local mate competition in *Trichogramma evanescens*. *J. Animal. Ecol.*, 53: 417-426.
- WAJNBERG E., 1993 - Genetic variation in sex allocation in a parasitic wasps: variation in sex pattern within sequence of oviposition. *Entomol. exp. appl.*, 69: 221-229.
- WAJNBERG E., 1994 - Genetic aspects of *Trichogramma* reproductive strategy. In: Wajnberg E., Hassan S.A. (eds) Biological control with egg parasitoids, (in press.).