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Influence of belated copulation on the fecundity and progeny sex ratio of *Binodoxys indicus* (Subba Rao and Sharma) (Hymenoptera: Braconidae)

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ABSTRACT: Belated copulation has been observed as one of the major factors that influence the fecundity and progeny sex ratio in parasitic wasps. A young (0-12h old) female mated with 0-12h old male, *Binodoxys indicus* produces 131.6±8.08 progeny within 4 days of life. However, older female mated with older male (both 96-108 h old) yields only 89.0±12.54 progeny. The progeny sex ratio (proportion of males) is female-biased (0.36±.03) when the parents were younger (0-12h old) but is male-biased (0.59±0.06) when the parents were older (96-108h old). Therefore, it implies that most of the daughters are produced during earlier phase of reproduction. The analysis of the data shows that only maternal age at copulation influences the total births significantly while both maternal as well as paternal age during copulation influence the progeny sex ratio. The implication of these results is discussed with reference to the maximisation of yield of total births as well as the female births for mass propagation and field release of the parasitoids in biocontrol programmes.

KEY WORDS: *Aphis gossypii*, *Binodoxys indicus*, biocontrol, parasitoid, progeny sex ratio

Binodoxys indicus (Subba Rao and Sharma) (Hymenoptera: Braconidae: Aphidiinae), a polyphagous aphid parasitoid, is distributed largely in the tropical and subtropical belt of India. It was observed to parasitize 10 species of aphids, *viz. Aphis citricola* van der Goot, *A. craccivora* Koch, *A. fabae* Theobald, *A. gossypii* Glover, *A. nasturtii* Kaltenbach, *A. nerii* Boyer de Fonscolombe, *Hysteroneura setariae* (Thomas), *Lipaphis erysimi* (Kaltenbach), *Myzus persicae* (Sulzer) and *Toxoptera aurantii* (Boyer De Fonscolombe) on more than 20 food plants in the terai belt (foot of Himalayas) of north eastern Uttar Pradesh (Singh *et al.*, 1999). Singh and Agarwala (1992) and Singh and Rao (1995) have demonstrated successful biological control of *A. craccivora* and *A. gossypii* by *B. indicus* in certain agro-ecosystems.

Like other aphid parasitoids, *B. indicus* reproduces arrhenotokously - sons develop parthenogenetically by unfertilised eggs while daughters develop by fertilised diploid eggs. Arrhenotokous reproduction by *B. indicus* provides a mechanism by which the mothers are able to adjust the sex of eggs by regulating sperm access to eggs at oviposition. As the basic necessity in biological control programmes using parasitoids is the mass propagation of maximum quantity of high quality females, it is necessary to know the factors that could maximise the production of females. Several factors have been recognised that influence the progeny production as well as their sex ratio in parasitic wasps (King, 1987; Singh and Pandey, 1997). In this investigation, attempts have been made to test the hypothesis whether belated copulation and oviposition influence fecundity, female birth and progeny sex ratio of *B. indicus*.

MATERIAL AND METHODS

Binodoxys indicus was reared on *A. gossypii* on pigeonpea (*Cajanus cajan* Millsp.) seedlings in rearing cages. For the experiments, third instar nymphs of *A. gossypii* were used. The female parasitoids employed for the experiments were those that emerged from aphids, which had been parasitized as third instar to nullify any possible effect of host-size. Possible nine reciprocal crosses of male and female parasitoids of varying age-classes, namely, 0-12, 48-60 and 96-108h were allowed. Before mating, the adults were fed with solution of honey (30%), honeydew (30%) and water (40%). For mating tests, a male was introduced into a 5ml glass vial containing a female parasitoid. The females that had not been seen in copula within 15 minutes of exposure were excluded as virgin females produce only sons.

The fed females of varying age-classes were allowed to mate with 0-12, 48-60, or 96-108 h old males and introduced into the cages (45 X 45 X 60cm) having potted, young pigeonpea seedlings (4-5 leaves) harbouring ca. 100 third instar aphid nymphs. After 24h, the females were withdrawn from all the cages and transferred to another cage with a fresh group of hosts as mentioned above. This act was repeated for their first 4 days of life. All the exposed hosts along with the seedlings were allowed to develop at 22°C, 14h photoperiod and 70-80 per cent relative humidity inside environmental chamber. After mummification, the mummies were kept in 100ml glass vials having moist absorbent paper at the bottom and placed in BOD incubators at the same temperature, photoperiod and humidity. When adults emerged from the mummies, they were sexed and counted.

Primary sex ratio (sexes of the eggs laid) cannot easily be estimated for endoparasitoids. Therefore, in this study, only secondary sex ratios were estimated. Statistical analyses were conducted using 2-way analysis of variance (ANOVA) and regression. Data of progeny sex ratio were arcsine transformed before analysis.

RESULTS AND DISCUSSION

Total and female births

The fecundity of the parasitoid decreased with belated copulation and oviposition and ranged from 131.6 ± 8.08 to 87.4 ± 9.91 births/female (Table 1). Females inseminated just after emergence by 0-12 h old male produced 25 and 33 per cent more progeny than 48-60 and 96-108 hold females, respectively.

Table 1. Influence of belated copulation on the fecundity of *B. indicus*

Maternal age (in hours)	Number of births per female		
	Paternal age (in hours)		
	0-12	48-60	96-108
0-12	131.6± 8.08	128.8± 4.43	121.6±13.53
48-60	105.0± 7.84	108.4±11.76	95.6± 9.20
96-108	98.8±16.52	87.4± 9.91	89.0±12.54
Regression equation	Y=128.2 - 8.2 X	Y=128.9 - 10.4 X	Y=118.4 - 8.2 X
Correlation coefficient (r*)	- 0.762	- 0.898	- 0.754

Values are expressed as mean ±SD; * All r-values are significant at P < 0.001

The corresponding figures were 19 and 47 per cent, and 26 and 37 per cent more for those females that were inseminated by 48-60 and 96-108 h old males, respectively. However, overall variation caused by maternal age was greater than that of paternal age at the time of copulation ($F_{mat-age}$=42.37; df=2,40; P < 0.001; $F_{pat-age}$=3.90; df=2,40; P < 0.05). Fecundity and maternal age at copulation were also negatively linearly correlated with significant correlation coefficients (Table 1). No such relation existed between fecundity and paternal age at copulation. Results shown in Table 2 display the total progeny produced by the parasitoid at varying paternal and maternal age-classes on successive days of oviposition after insemination. It illustrates that the female parasitoids maximally parasitized on the first day of their life after insemination and the rate of parasitism gradually decreased on the subsequent days of oviposition. All these relations were explained by linear equations, Y = a - bX with significant correlation coefficients (Table 2). The total number of progeny (21.07 births/female, data of paternal age pooled) produced by the freshly mated females on fourth day of oviposition was significantly less than those females mated on fourth day and oviposited on the same day since eclosion (28.87 birth/female, data of paternal age pooled).

Table 2. Effect of belated copulation on the progeny of *B. indicus* developed by eggs laid during subsequent days of oviposition

Paternal age (in hours)	Maternal age (in hours)	Number of progeny per female — Days of oviposition: 1st	2nd	3rd	4th	Regression equation
0-12	0-12	43.2±3.0	35.6±3.0	30.0±4.1	22.8±5.2	Y = 0.114+0.104X r = 0.882*
	48-60	33.8±3.4	30.0±4.9	24.4±2.2	16.8±3.3	Y = 0.142+0.116X r = 0.859*
	96-108	31.8±7.8	27.6±3.3	23.6±4.4	15.8±4.9	Y = 0.153+0.114X r = 0.891*
48-60	0-12	41.8±2.3	38.2±2.4	21.8±2.4	21.0±4.2	Y = 0.110+0.132X r = 0.919*
	48-60	33.2±6.1	29.4±2.6	27.8±5.5	18.0±3.5	Y = 0.158+0.116X r = 0.841*
	96-108	27.6±2.0	25.6±3.3	21.6±3.8	12.6±2.6	Y = 0.183+0.142X r = 0.860*
96-108	0-12	39.0±4.5	35.2±5.7	28.0±2.8	19.4±3.2	Y = 0.129+0.142X r = 0.913*
	48-60	30.4±4.6	26.2±2.6	24.6±3.6	14.4±2.3	Y = 0.195+0.146X r = 0.848*
	96-108	27.2±4.0	24.0±3.8	21.6±3.4	16.2±2.3	Y = 0.213+0.153X r = 0.833*

Values are expressed as mean±SD; *All r-values are significant at P < 0.001

Belated copulation and oviposition always decreased the female birth of *B. indicus*, which ranged from 84.4±6.12 to 37.2±9.88 female births/ female (Table 3). Females mated just after emergence by 0-12 h old male produced 39 and 71 per cent more female progeny than 48-60 h and 96-108 h old females, respectively. These figures are 23 and 81 per cent, and 50 and 76 per cent more for those females that were mated by 48-60 h and 96-108 h old males, respectively. However, overall variation in the female births caused by maternal age was greater than that of paternal age at the time of copulation ($F_{mat\text{-}age}$ =119.75; df=2,40; P < 0.001; $F_{pat\text{-}age}$ = 33.12; df=2,40; P < 0.001). The female births and parental age at copulation were also negatively linearly correlated with significant correlation coefficients (Table 3). Decrease in fecundity values as well as female progeny with age seems to be a general feature among aphid parasitoids (Pandey *et al.*, 1983; Srivastava and Singh, 1995; Pandey and Singh, 1998).

Table 3. Influence of belated copulation and oviposition on the female progeny production of *B. indicus*

Maternal age (in hours)	Number of female progeny per female			Regression Analysis		
	Paternal age (in hours)					
	0-12	48-60	96-108	a	b	r*
0-12	84.4±6.12	75.2±3.08	65.2±5.68	84.53	- 4.80	- 0.999
48-60	61.0±5.34	58.8±6.58	43.4±5.03	63.20	- 4.40	- 0.918
96-108	49.4±5.27	41.4±4.72	37.2±9.88	48.77	- 3.05	- 0.984
Intercept (a)	82.43	75.37	62.20			
Slope (b)	- 8.75	- 8.45	- 7.00			
Correlation coefficient (r*)	- 0.981	- 0.999	- 0.952			

Values are expressed as mean ±SD; * All r-values are significant at P < 0.001

Progeny sex ratio

Progeny sex ratio of *B. indicus* was significantly higher when the females were copulated late by the males of varying age-classes (Table 4). Regression between maternal and paternal age at copulation and progeny sex ratio gave linear relationships. ANOVA shows that the variation in progeny sex ratio is influenced greatly by maternal age than paternal age ($F_{mat\text{-}age}$=44.58, df=2,40, P < 0.001; $F_{pat\text{-}age}$= 34.80, df=2,40, P<0.001). Regression of progeny sex ratio on the successive days of oviposition after insemination established the positive linear correlation between these variables. All the correlation coefficients were significant at P < 0.01 (Table 5). The intercepts of the curve is considerably more for the females which were mated with older males, implying that the initial progeny sex ratio is greater when male mates were older. Similarly, the progeny sex ratio of *B. indicus* continuously increased on subsequent day of oviposition either calculated since adult eclosion or since insemination (Table 6). The slope of regression equation is slightly higher for the females, which were mated with freshly emerged males, implying that the rate of increase in progeny sex ratio was greater when male mates were younger (Table 5).

Table 4. Influence of belated copulation on the progeny sex ratio of *B. indicus*

Maternal age (in hours)	Number of female progeny per female			Regression Analysis		
	Paternal age (in hours)					
	0-12	48-60	96-108	a	b	r*
0-12	0.36±0.03	0.42±0.02	0.46±0.03	0.364	0.025	0.857
48-60	0.42±0.04	0.44±0.03	0.55±0.04	0.405	0.032	0.806
96-108	0.50±0.04	0.52±0.02	0.59±0.06	0.490	0.023	0.704
Intercept (a)	0.358	0.408	0.462			
Slope (b)	0.034	0.027	0.030			
Correlation coefficient (r*)	0.877	0.852	0.736			

Values are expressed as mean±SD; *All r-values are significant at P < 0.001

Table 5. Effect of belated copulation on the sex ratio of the progeny of *B. indicus* developed by eggs laid during subsequent days of oviposition

Paternal age (in hours)	Maternal age (in hours)	Number of progeny per female				Regression equation
		Days of oviposition				
		1st	2nd	3rd	4th	
0-12	0-12	0.2946 ±0.0484	0.3534 ±0.0367	0.4143 ±0.0268	0.4300 ±0.0368	Y = 0.114+0.104X r = 0.882*
	48-60	0.3602 ±0.0292	0.4133 ±0.0362	0.4573 ±0.0502	0.4953 ±0.0735	Y = 0.142+0.116X r = 0.859*
	96-108	0.4327 ±0.0417	0.4516 ±0.0551	0.5386 ±0.0470	0.6304 ±0.0345	Y = 0.153+0.114X r = 0.891*
48-60	0-12	0.3325 ±0.0702	0.3881 ±0.0302	0.4795 ±0.0481	0.5568 ±0.0457	Y = 0.110+0.132X r = 0.919*
	48-60	0.3916 ±0.0395	0.4428 ±0.0387	0.4470 ±0.0534	0.5131 ±0.0360	Y = 0.158+0.116X r = 0.841*
	96-108	0.4487 ±0.0104	0.5257 ±0.0428	0.5740 ±0.0401	0.6012 ±0.0394	Y = 0.183+0.142X r = 0.860*
96-108	0-12	0.3639 ±0.0665	0.4508 ±0.0314	0.5159 ±0.0519	0.5993 ±0.0322	Y = 0.129+0.142X r = 0.913*
	48-60	0.4909 ±0.0454	0.5347 ±0.0387	0.5697 ±0.0414	0.6403 ±0.0623	Y = 0.195+0.146X r = 0.848*
	96-108	0.5317 ±0.0367	0.5704 ±0.0415	0.6120 ±0.0828	0.6737 ±0.0897	Y = 0.213+0.153X r = 0.833*

Values are expressed as mean±SD; *All r-values are significant at P < 0.001

Regression of progeny sex ratio (Y) on total progeny (X) resulted in significant negative linear correlation coefficients when all age groups of the parents were pooled (Y = 0.7532 – 0.0026 X, r= -0.647, P < 0.0.01, Fig. 1).

Results shown in Table 5 illustrate that the progeny sex ratio of the parents of all age-classes continuously increased on subsequent days of oviposition, namely, more female progenies were produced early in their life. Such increase in progeny sex ratio may result from sperm depletion (Nadel and Luck, 1985), or from reduced sperm motility or viability (Pandey *et al.*, 1983). The parasitoid species where sperm depletion occurs, are usually polyandrous and readily remate and resume production of more female progeny in the later part of their life (Gordh, 1976). Since the female aphidiines are monandrous, it seems quite logical that the amount of sperm transferred in single mating would be sufficient to fertilise the same proportion of eggs throughout the life span

Table 6. Daily sex ratio of the progeny of individual *B. indicus* female parasitising *A. gossypii*

Day of oviposition after eclosion	Progeny sex ratio			Progeny sex ratio (data pooled)
	Paternal age (in hours)			
	0-12	48-60	96-108	
1	0.2946±0.0484 (5)	0.3325±0.0702 (5)	0.3640±0.066 (5)	0.3304±0.0648 (15)
2	0.3535±0.0368 (5)	0.3881±0.0303 (5)	0.4509±0.0314 (5)	0.3975±0.0517 (15)
3	0.3873±0.0389 (10)	0.4356±0.0622 (10)	0.5035±0.0479 (10)	0.4421±0.0688 (30)
4	0.4217±0.0356 (10)	0.4999±0.0722 (10)	0.5671±0.0478 (10)	0.4962±0.0798 (30)
5	0.4450±0.0455 (10)	0.4479±0.0363 (10)	0.5508±0.0420 (10)	0.4812±0.0640 (30)
6	0.4735±0.0655 (10)	0.5194±0.0379 (10)	0.6054±0.0621 (10)	0.5328±0.0779 (30)
7	0.5386±0.0470 (5)	0.5740±0.0402 (5)	0.6121±0.0828 (5)	0.5749±0.0634 (15)
8	0.6304±0.0346 (5)	0.6013±0.0395 (5)	0.6737±0.0897 (5)	0.6351±0.0635 (15)
Pooled	0.4431±0.1061 (60)	0.4748±0.0914 (60)	0.5409±0.0989 (60)	
Regression equation	Y=0.2525+0.0423X	Y=0.3206+0.0350X	Y=0.3661+0.0388X	Y=0.3107+0.0390X
Correlation coefficient (r*)	0.978	0.948	0.962	0.981

Values are expressed as mean±SD. Values in parentheses express the number of parental females. * All r-values are significant at P < 0.001.

of females (Srivastava and Singh, 1995). Therefore, decrease of the female progeny due to sperm depletion in case of aphidiines may be ruled out. The second factor, namely, less sperm viability and or motility may affect the progeny sex ratio. Ageing of the parasitoid is supposed to affect the inheritance of maternally and/or paternally inherited factors that determine the sex of the progeny (Skinner, 1992).

The lower progeny sex ratio resulting from the first few days of oviposition, irrespective of female age has also been observed for other aphid parasitoids (Singh and Pandey, 1997). However, *Aphidius colemani* Viereck and *Ephedrus cerasicola* Starý showed no consistent variation in progeny sex ratio with change in maternal age (Hofsvang and Hagvar, 1975a, b). The progeny sex ratio of parents who were copulated later (after 4 days) was maximum.

In the present study the progeny sex ratio significantly decreased with increase of total births. It means whenever progeny production lessened either due to ageing, lower adult survival, delayed oviposition, and other intrinsic and extrinsic factors (Singh and Pandey, 1997); progeny sex ratio increased.

Significant variation in sex ratio in the progenies of the parasitoids was observed in the field population as well as in the laboratory (Singh and Pandey, 1997). Therefore, it is important to understand the behaviour of the parasitoid regarding the placement of haploid and diploid eggs in the hosts as it potentially affects the success or failure of released parasitoids (Waage, 1986). The maximisation of female progeny in mass-rearing programmes also requires the knowledge of such factors that favour female biased population. The results presented herein furnish an insight into the optimum age of the parasitoid *B. indicus* (less than 2 days) for maximising the female progeny in the population both for mass rearing and field releases.

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