Sex allocation of three solitary ectoparasitic
wasp species on bean weevil larvae:
sex ratio change with host quality and local mate competition

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Abstract — Sex ratio manipulation by ovipositing females was surveyed in 3 solitary ecto-parasitic wasp species, *Dinarmus basalis* (Pteromalidae), *Anisopteromalus calandrae* (Pteromalidae), and *Heterospilus prosopidis* (Braconidae), that parasitize azuki bean weevil (*Callosobruchus chinensis* (L) (Coleoptera: Buruchidae)) larvae within azuki beans (*Vigna angularis*). Variables were local mate competition (LMC) and host quality (HQ). We used host age as a measure of host quality (from 9- to 16-day-old hosts), changed the number of ovipositing females to control the level of local mate competition (1 female and 10 females), and examined oviposition patterns of the wasps. The offspring sex ratios (proportion of females) of the 3 wasp species respond qualitatively same to HQ and LMC. The common qualitative tendency among the 3 species is an increase of sex ratios increase with host age. In the process of changing the sex ratio (9- 13-day-old) 3 wasp species respond only to HQ. In the hosts that end development in size (14-16-day-old) wasps respond to LMC. The response of sex ratio change to LMC in the old host age-classes are different among the 3 species. In the situation that there exists LMC (10 females) sex ratios are the same among the 3 wasps. However, the sex ratios in no LMC (single female) are heterogeneous among the 3 wasps.

Sex ratio selection has received much attention from both theoretical and empirical biologists (e.g., King 1987, 1988, 1989; Hamilton 1967, 1979; Charnov 1982; Karlin & Lesserd, 1986; Werren 1980, 1983). Parasitoid wasps are good organisms for sex-ratio studies, because their haplodiploid sex determination provides a mechanism by which females can manipulate offspring sex ratio. Unfertilized eggs develop into sons, and fertilized eggs develop into daughters.

Several factors are known to affect manipulation of sex ratio by ovipositing female wasps.

Many parasitoid wasps are known to adjust sex ratio in response to either local mate competition (LMC) or host quality (HQ). Basic models that incorporate an evolutionary view point have been developed for both sex ratio manipulation with local mate competition (Hamilton 1967, 1979; Taylor & Bulmer 1980) and variability in quality of hosts (Charnov 1979; Werren 1984; Werren & Simbolotti 1989).

LMC models assume that mating is local. When only 1 female oviposits in a patch, all the male offspring emerging from that patch will be brothers. In this situation, selection is predicted

to favor mothers that produce only enough sons to inseminate all locally available females. In contrast, when multiple females oviposit in a patch, not all the emerging males will be brothers. In this situation, a mother can increase the chance that one of her sons will inseminate available females, by producing an increased proportion of sons. Also, with multiple females in a patch, the advantage to a mother of producing additional daughters is reduced. The model has since been modified to fit various organisms and various local mate competition (LMC) models were generated (e.g., Hamilton 1979; Frank 1985; Herre 1985; Stubblefield & Seger 1990; Suzuki & Iwasa 1981; Werren 1987).

The HQ models predict how mothers should manipulate sex ratio in response to host quality (Charnov 1979; Charnov et al. 1981). The models qualitatively predict that the mother should oviposit a greater proportion of the sex that has larger relative fitness for a given host size (Charnov 1979). Werren (1984), Werren and Simbolotti (1989) and Ikawa et al., (1993) modified the HQ model and incorporated partial dispersal in pre-mating.

Many hymenoptera species exhibit variation in sex ratio in response to environmental factors other than local mate and host quality (Flanders 1939; King 1987). An unknown portion of this variability is due to facultative control of brood sex ratios (Wilkes, 1965; Holmes 1972; Wylie 1973; Werren 1980). The physical mechanisms underlying this control are not understood. Unfortunately, comparative experimental analysis of sex ratios among several wasp species has lagged behind its theoretical development.

In this paper, we examine how host quality (using weight correlated with host age) and local mate competition affect the offspring sex allocation of 3 solitary ectoparasitic wasp species, Dinarmus basalis (Pteromalidae), Anisopteromalus calandrae (Pteromalidae), and Heterospilus prosopidis (Braconidae). All of these species parasitize azuki bean weevil Callosobruchus chinensis (L) (Coleoptera: Bruchidae) larvae and pupae within the azuki bean (Vigna angularis). We also compare the responses of these species to the same levels of host quality (HQ) and local mate competition (LMC).

Materials and Methods

The experiments were conducted in a growth cabinet controlled at 30℃, 70% r.h. and 24L. We used 3 species of parasitic wasps, Dinarmus basalis (Pteromalidae), Anisopteromalus calandrae (Pteromalidae), and Heterospilus prosopidis (Braconidae). These wasps are solitary ectoparasites on larvae and pupae of several grain and bean weevils. We used the azuki bean weevils Callosobruchus chinensis (Coleoptera: Bruchidae) that infest azuki beans (Vigna angularis) as the host species in these experiments.

Hosts of same age class were introduced into a Petri dish (Falcon No. 1009, 100 mm dia 15 mm depth). Host age of Callosobruchus chinensis was defined the day after oviposition (oviposition period is about 1 h). The experiments had 8 different levels of host age (from 9-to 16-day-old) and 2 levels of local mate competition (1 ovipositing female and 10 ovipositing females per dish). We offered 40 hosts (8 beans each infested with 5 hosts and some uninfested beans) in single-wasp-experiments, and 400 hosts (80 beans each infested with 5 hosts) in 10-wasp-experiments. The host density per wasp was the same in each wasp density. We denoted the experiments as Db, Ac and Hp for D. basalis, A. calandrae and H. prosopidis, respectively. We also coded the experiments with the LMC level, e.g., Db/1 for 1 ovipositing female wasp of D.basalis, Db/10 for 10 ovipositing female wasps of D. basalis, and so on. For the experiments with 1 female, each host age had between 2 and 10 replicates (usually 5). Experiments with 10 females had only 1 or 2 replicates.

Inseminated female parasitoids from stock culture were isolated for about 24 h without hosts and then were separately introduced into a dish for 24 h. The female wasps searched for hosts inside the beans and oviposited eggs in the hosts immediately after being introduced. After a 24 h oviposition period, the female wasps were removed from the dishes. The dishes were kept 2 weeks in the growth cabinet until eclosion of the wasp offspring.

Results

Host quality

Figure 1 shows the relationship between host ages and host fresh weights. There was significant difference among host age-classes ($F_{7,72}=59.068$, $P<0.0001$). The developmental stage of the weevils in the age classes from 14- to 16-day-old are pre-pupa or pupa (Moriguchi, 1992), so it can be supposed that weight growth stops in these age classes. According to the prior information of the developing stage of the weevil (personal observation), the mean weights of the 14- to 16-day-old classes were compared.

There was no significant weight difference among the 3 age classes (14-, 15-, 16-day-old) ($F_{2,72}=3.025$, $P<0.0548$). The mean weight of 14-16-day old hosts was about 7 mg (sd 1.6116 mg). The host weight increases up to 14-days of age and then stops. Thus, host quality increases with the age classes from 9- to 13-day and the quality saturates in the 14-, 15- and 16-day classes.

Offspring sex ratios

The emergence sex ratios (proportions of females) for each host age and each level of local mate competition for the 3 wasp species
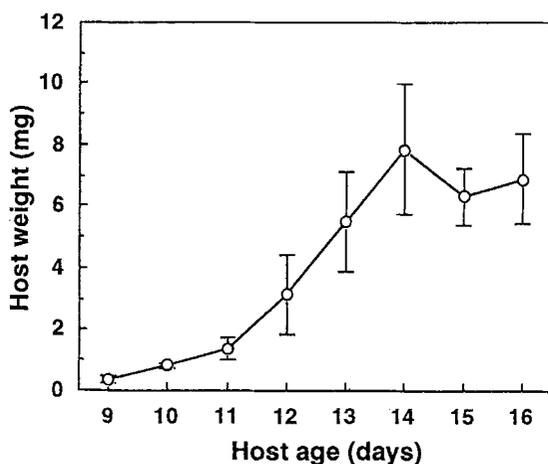

Fig. 1 Host age and host weight relationships. The vertical bars show 1 standard deviation. Ten replicates of each age class were done.
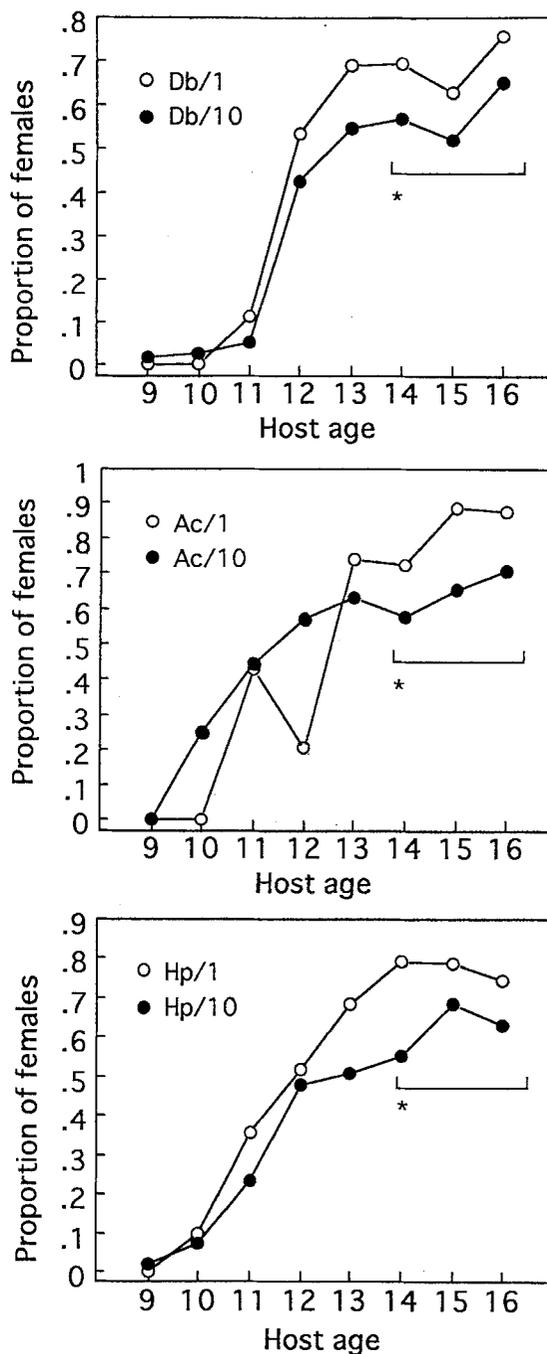

Fig. 2 Sex ratios of emerging offspring in the 3 wasp species. The sex ratios in the host age-classes from 14- to 16-day-old are pooled for comparing the ratios between the 2 LMC levels. Asterisks indicate that there is a significant difference between the 2 LMC levels at 5% error level.

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are shown in Figure 2. We cannot know individual variations of the emergence sex ratios in the experiments that contain 10 ovipositing females. Therefore, even though we could evaluate individual variations of sex ratios in the clutches of each female in the experiments that contained only 1 ovipositing female, we focused on the population sex ratio by pooling the replicates in the single female experiments. We define proportion of females in the offspring as "sex ratio".

The sex ratio of offspring increased with increasing host age in both LMC levels of all 3 wasp species (Fig. 2). We knew that the weight of the hosts grow in the 9- to 13-days age class and saturates in the age between 14 and 16 days. Therefore, we tried to conduct a planned comparison of the sex ratio with different levels of LMC and HQ in each wasp species as follows. First, we followed our prior knowledge of saturation of host weight in the 14- 16-day-old age class, we analyzed whether the sex ratios of emerging offspring among the 3 host age classes (14-, 15- and 16-day-old) of each LMC level are different or not. If the sex ratios among the age classes did not differ, we pooled the data, and the sex ratios generated by the pooled data were compared between the 2 LMC levels. Secondly, we compared the sex ratios of emerging offspring between the 2 LMC levels for each wasp species in each age class from 9- to 13-day. The comparisons were conducted by a G-test of independence ((male offspring, female offspring)×(two wasp densities)) (Sokal and Rohlf, 1981). When there were cells in which the number is less than 5 in the 2×2 table, we conducted a Fisher's exact test (Sokal and Rohlf, 1981).

There were no statistically significant differences among the 3 host age-classes (14-, 15- and 16-day-old) in the LMC levels of the 3 species ($G=1.748$, $df=2$, $P<0.4173$ in Db/1; $G=5.469$, $df=2$, $P<0.0636$ in Db/10; $G=3.115$, $df=2$, $P<0.1948$ in Ac/1; $G=1.527$, $df=2$, $P<0.466$ in Ac/10; $G=0.299$, $df=2$, $P<0.858$ in Hp/1; $G=4.529$, $df=2$, $P<0.104$ in Hp/10, respectively). In the host age-classes from 14- to 16-day, therefore, the data were pooled in each LMC level. In the age classes, there were statistically significant differences of the sex ratios

Table 1. G-test of independence of wasp offspring that emerged between the 2 wasp densities.
Db/1 vs. Db/10

Host age (days)	Experiment	Male offspring	Female offspring	G	F
9	Db/1	12	0	*	0.999
	Db/10	64	1		
10	Db/1	14	0	*	0.999
	Db/10	37	1		
11	Db/1	31	4	1.606	0.205
	Db/10	113	6		
12	Db/1	19	22	1.565	0.211
	Db//10	86	64		
13	Db/1	17	38	3.498	0.062
	Db/10	77	94		
14-16	Db/1	48	103	4.988	0.025
	Db/10	201	279		

*Fisher's exact test

Ac/1 vs. Ac/10

Host age (days)	Experiment	Male offspring	Female offspring	G	P
9	Ac/1	1	0	*	0.999
	Ac/10	6	0		
10	Ac/1	1	0	*	0.444
	Ac/10	3	1		
11	Ac/1	4	3	*	0.999
	Ac/10	5	4		
12	Ac/1	4	1	*	0.293
	Ac/10	3	4		
13	Ac/1	8	17	0.17	0.680
	Ac/10	13	22		
14-16	Ac/1	11	65	11.847	0.001
	Ac/10	44	77		

*Fisher's exact test

Hp/1 vs. Hp/10

Host age (days)	Experiment	Male offspring	Female offspring	G	P
9	Hp/1	6	0	*	0.999
	Hp/10	46	1		
10	Hp/1	9	1	*	0.999
	Hp/10	38	3		
11	Hp/1	9	5	0.934	0.334
	Hp/10	66	20		
12	Hp/1	15	16	0.116	0.734
	Hp/10	56	52		
13	Hp/1	11	24	3.449	0.063
	Hp/10	55	57		
14-16	Hp/1	28	98	10.920	0.001
	Hp/10	143	234		

*Fisher's exact test

between Db/1 and Db/10 (G-4.988, df=1, P<0.025), between Ac/1 and Ac/10 (G= 11.847, df=1, P<0.001), and between Hp/1 and Hp/10 (G=10.920, df=1, P<0.001) (Table 1). In all of the species sex ratio was lower in the 10-waps experiment than in the single-wasp experiment.

Comparisons of sex ratios among the species with the same LMC level in the 14-, 15-, 16-day-old host ages were conducted. In the single-wasp experiments the sex ratios were 0.682 (Db/1), 0.855 (Ac/1) and 0.708 (Hp/1), respectively, and varied among the wasp species (G=25.120, df=2, P<0.001). In the 10-wasp experiments the sex ratios were 0.581 (Db/10), 0.636 (Ac/10) and 0.620 (Hp/1), respectively, and did not vary among the wasp species (G=2.007, df=2, P<0.366).

The comparisons of the sex ratios of emerging offspring between the 2 levels of LMC in each age class from 9- to 13-day were conducted for each wasp species. Since all comparisons were designed independently, the type I error level was set at 0.05. In all species and in all host age-classes there were no statistically significant difference of the sex ratio between the 2 LMC levels (Table 1). Kendall's rank correlations are statistically significant between host age (9- 13-day-old hosts) and sex ratios in the pooled data of 2 LMC of each host age-class in the 3 wasps (t=1.00, P=0.0143 in Db, t=0.8, P=0.05 in Ac, and t=0.8, P=0.05 in Hp).

In summary, in young host age classes (9- 13-day-old) there was no response to LMC levels. Wasps responded only to HQ. In old host age-classes (14- 16-day-old) the wasps responded to LMC. The response to LMC (10 females) is identical among the 3 species, but the sex ratio without LMC (1 female) is different among the 3 species in old hosts.

Discussion

Age or weight of the host is a good predictor of quality for the 3 wasp species, even though there is a possibility that host quality may change with different host stages (Charnov, et al., 1981; Moriguchi, 1992; Nishimura, 1993).

Emerging offsprings from older or larger hosts have larger body size than those from younger or smaller hosts. If the relative fitness via daughters to via sons changes with increasing host age, then the sex ratio of offspring should be controlled by the mother according to the host age (Charnov, 1979). The sex ratio of offspring should also be controlled by the mother according to the local number of ovipositing females (Hamilton, 1967).

In this work we controlled 2 factors host quality (HQ) and local mate competition (LMC), to survey the change of offspring sex ratio of the 3 solitary wasps species. When we observe the sex ratio of emerging offspring wasps, the sex ratio change among host age-classes and between 2 LMC levels might be generated by differential sex-specific mortality from the egg to adult. There is no positive evidence of differential sex-specific mortality among host age-classes in the 3 wasp species (Charnov, et al., 1981; Charnov, 1982). Moriguchi (1992) reported that there is no differential sex-specific mortality in *A. calandrae*. In *D. basalis* there is also no differential sex-specific mortality (Nishimura, in ms, but also see Fujii and Khim 1990).

Qualitative offspring sex ratio response of the 3 wasp species to HQ and LMC is the same. The sex ratio change may be due to mother control. The common qualitative tendency among the 3 wasp species is that the sex ratios increase with host age. While changing the sex ratio (9- 13-day-old hosts), 3 wasp species respond only to HQ. In the hosts that end development in size (14- 16-day-old hosts), wasps respond to LMC.

Sex ratio response to LMC in old host age-classes differed among the 3 wasp species; sex ratios were the same among the 3 wasps if LMC (10 wasps) was present, but differed if it was not (1 wasp). HQ and LMC are the factors that make change the offspring sex ratio, but in the range of host size in which sex ratio is sensitive to HQ, there is no effect on LMC. The LMC is effective when there is no effect on HQ. Another notable point is that the reaction of sex ratio change to LMC differs among the 3 wasps. The variation of sex ratio in the single-wasp situation in old-age hosts indicates how

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biological information is important for understanding the facultative sex ratio of parasitoid wasps.

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