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Intraspecific Variation of P2 Value in a Coccinellid Beetle, *Harmonia axyridis*

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Abstract — Intraspecific variation in P2 value in a coccinellid beetle (*Harmonia axyridis*) was investigated. The analytical method by Parker et al. (1990) predicts that sperm-flushing displacement in the spermatheca may exist in the sperm utilization pattern of this species. Long duration of sperm transfer in the second copulation resulted in high fertilization success of the second male. Large male body size itself did not have an advantage in flushing efficiency of the previously stored sperm. However, through long duration of sperm transfer and larger ejaculate, males with large body size gain high fertilization success.

Since Parker's review (1970), many studies on P2 value have been conducted (Smith 1984; Redley 1989). However, most studies dealt with the average "species-specific" P2 value. Intraspecific P2 variation began to attract the attention of researchers only recently (Lewis & Austad 1990; Simmons & Parker 1992). Intraspecific P2 variation is discussed through 2 perspectives: 1) variation caused by the difference in copulation duration and mating interval (and other proximate factors). This approach attempts to verify the actual mechanisms of how sperm is used to fertilize eggs; 2) adaptational aspects of P2 variation, which results from competition for high fertilization success among males.

In this study, I tried to address both perspectives in a coccinellid beelte, *Harmonia axyridis*. To determine the type of sperm displacement, I applied the method of Parker et al. (1990) to present P2 data. I then scored several size characters of males and females and examined their relationship to P2 values. Even though nothing is known about P2 value and its relation to characters, there have been some studies with other species. Large body size contributes to efficient sperm transfer in *Scatophaga stercoraria* (Simmons & Parker 1992) and larger penis size may displace stored sperm more efficiently in *Ischnura graellsii* (Cordero & Miller 1992). A previous study examined P2 values in relation to only a single character (elytral length in *Tribolium castaneum*, Lewis & Austad 1990). Measurement of multiple size characters can allow control of indirect effects from correlated characters. Multiple regression was used for this purpose.

Materials and Methods

I measured P2 values of *H. axyridis* by using genetic polymorphism of elytra color. Elytral color polymorphism is controlled by 4 alleles at a single locus. The linear dominant-recessive relationship is as follows: *conspicua*>*spectabilis*>*axyridis*>*succinea* (Tan & Li 1934).

In April and May, 1992, I collected overwintering adults from a suburb of Nagoya City, Aichi, Japan. Adults were reared in the laboratory and fed on freeze-dried honeybee larvae under 16L-8D and 25℃ conditions. I obtained virgin males and females from the eggs laid by the adults. I could determine the genotype of the virgin adults from the phenotype of their parents and siblings.

Percentage of hatched eggs attributable to first and second male's sperm was determined by sequentially mating females of *succinea* (most recessive) homo genotype, first with a male that had no *succinea* allele and then with a *succinea* homo male.

Each male and female pair was confined in a plastic cup (60 ml). Just after the termination of the first copulation, the first male was elimanated and a second male was introduced. After the second copulation, females were isolated. Times of onset and termination of copulations were recorded. Clutch size, duration from the termination of the second copulation to oviposition and duration between sequential clutches were recorded as well. Under these mating conditions, *succinea* homo are offspring of the second male, and other genotypes are the first male's offspring.

Determination of Sperm Competition Type

The method of Parker et al. (1990) costructs *a priori* conjectural models of sperm utilization patterns and examines the fitness of the observed P2 values to the models. This aims to deduce the main mechanisms of currently known sperm precedence patterns (sperm mixing and sperm displacement) from an experimental set of P2 value. If observed value does not fit the model, then the sperm utilization pattern of that model is ruled out for the species. Three models were examined: the raffle model, the mixing displacement model and the non-mixing displacement model. In the 2 displacement (mixing and non-mixing) models, the common assumptions are that 1) the female sperm storage organ has a finite capacity that is filled with sperm from the first male, and 2) one-for-one displacement of stored fluid from the sperm storage organ takes place at the second copulation. In addition, it is assumed that there is instantaneous sperm mixing in the mixing displacement model and no mixing until the completion of second copulation in the non-mixing displacement model. The expected P2 should then fit the regression.

$-\ln(1-P2)=zt/S$ for the mixing displacement model (1)

$P2=zt/S$ for the non-mixing displacement model (2)

where S is capacity of sperm storage, z is the constant number of displaced sperm in unit time and t is the second copulation duration. In the raffle model, sperm from both males enter the sperm storage. No displacement occurs.

$$1/P2=r\,(S_1/S_2)+1 \tag{3}$$

is the general equation for this type of sperm utilization pattern, where S_1 and S_2 are number of sperm ejaculated by the first and second males, respectively, and r represents the ratio of the probabilities with which each sperm enters the sperm store.

In *H. axyridis*, copulation can be divided into 2 parts according to male behavior (Obata 1987). During the first part, the male transfers spermatophore materials. During the latter, sperm is transferred. In this study, sperm transfer duration was therefore substituted for the number of sperm ejaculated in equations S_1 and S_2.

Factors Influencing P2 Values

In order to test the relationship of size characters to P2 value, I measured the following characters: body length, body width, penis length and penis width of the males, and female body length and width. In addition to size characters, mating characters (duration of spermatophore and sperm transfer in 2 copulations and the mating interval) were measured as well. These measurements were used for multivariate analysis.

Results

Table 1 shows P2 value of eggs laid in sequential clutches. There was no significant difference among sequential clutches (Kluskal-Wallis H=1.809, P=0.613). There was no significant relationship between P2 value (Y) and the duration from termination of second copulation to oviposition (X) (first clutch: Y=48.37+1.62X, r=0.242, F=1.44, df=1,23, P=0.243, second clutch: Y=46.99+0.27X,

Table 1. P2 values of eggs in sequential clutches

	1st clutch	2nd clutch	3rd clutch	4th clutch	Total
N	25	25	21	17	25
Mean	54.5	48.6	52.6	61.7	48.1
S.D.	30.6	27.7	32.0	21.3	16.6

Numerals are arcsin-square-root transformed P2 values

r=0.047, F=0.05, df=1,23, P=0.823, third clutch: Y=45.36+0.91X, r=0.146, F=0.412, df=1,19, P=0.529, fourth clutch: Y=54.60+0.78X, r=0.229, F=0.721, df=1,13, P=0.411). Therefore, P2 value calculated from all clutches combined was used for analysis.

There were several significant positive correlations between body size characters of the second male and sperm transfer duration in the second copulation (body length: r=0.522, F=8.222, df=1,23, P=0.009, (body width: r=0.498, F=7.243, df=1,23, P=0.013,) penis width: r=0.254, F=1.513, df=1,23, P=0.232), while no significant correlation was detected between first male size characters and sperm transfer duration (body length: r=0.144, F=0.098, df=1,23, P=0.751, body width: r=0.256, F=1.473, df=1,23, P=0.238, penis length: r=0.130, F=0.364, df=1,23, P=0.553, penis width: r=0.208, F=0.945, df=1,23, P=0.342).

Fitting the Conjectural Models

Figures 1, 2, and 3 show the application of P2 value data to the raffle and displacement models, respectively. The observation of S_1/S_2 on 1/P2 was not significant (Y=1.815+0.463X, r=0.108, F=0.271, df=1,23, P=0.608), however, the intercept was not different from the expected value of +1.0 from equation (3) (F=0.495, df=1,23, P=0.489). Displacement models showed better fit to the data. For the mixing displacement model (equation 1), the regression of −ln (1−P2) on second sperm tranfer duration was significant (Y=−0.514+0.018X, r=0.584, F=11.911, df=1,23, P=0.002). Intercept did not differ from 0, the expected value (F=1.177, df=1,23, P=0.289). For non-mixing displacement model (equation 2), regression of P2 value on the second sperm transfer duration showed a signifi-

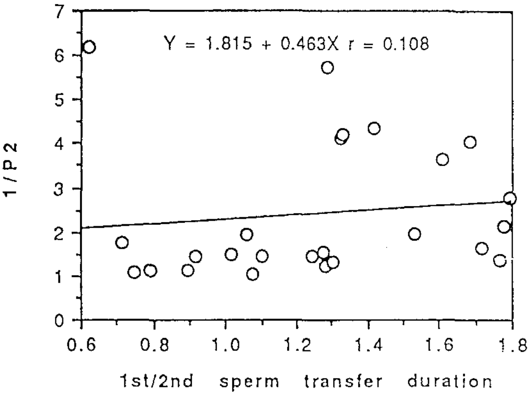

Fig. 1. Fitting of P2 value into the raffle model (Parker et al. 1990). Y-axis shows inverse of P2 value, and X-axis shows the ratrio of sperm transfer duration in the second copulation to that of the first copulation.

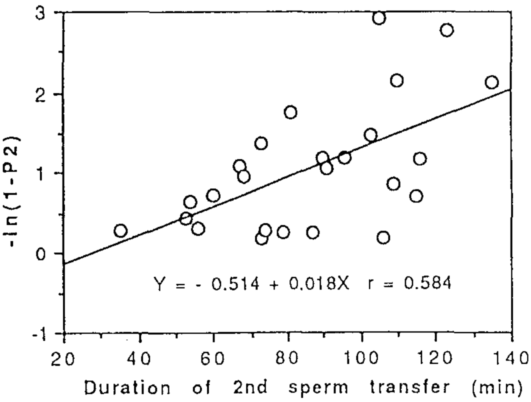

Fig. 2. Fitting of P2 value into the mixing displacement model (Parker et al. 1990). Y-axis shows −ln of proportion of eggs in the clutch that the first male fertilized. X-axis shows duration of second sperm transfer.

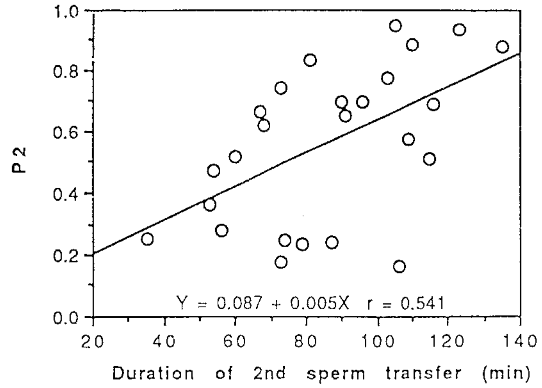

Fig. 3. Fitting of P2 value into the non-mixing displacement model (Parker et al. 1990). Y-axis shows P2 value, and X-axis shows duration of second sperm transfer.

cant relationship (Y=0.087+0.005X, r=0.541, F=9.534, df=1,23, P=0.005). Intercept was not different from the expected value of 0 (F=0.300, df=1,23, P=0.589).

Variables Explaining P2 Values

Table 2 shows simple correlation coefficients of P2 value on each character. Three characters, i.e., secondary sperm transfer duration, mating interval and body length of the second male, showed significant relationships with P2. Multiple regression with stepwise variable selection was applied. F-value of 2.0 was set as a criteria for entrance and removal of variables. This procedure revealed that only the second sperm transfer duration had an important effect on P2 value (r=0.574, F=9.796, df=1,23, P=0.005). No other variables had F-value above 2.0.

Discussion

The results indicate that the sperm utilization pattern of the raffle model is probably not representive for this species. Observed P2 data fit well to both displacement type models. Female spermatheca in this species is cuticulated, with a finite capacity. This morphology indicates that sperm flushing displacement can be easily achieved. The results from data analysis are consistent with the structural information of the female genital organ.

The rate of sperm mixing within the female sperm store is the critical difference between the 2 models. The assumptions of the models are both extreme: instantaneous mixing or complete absence of mixing until the completion of copulation. These do not seem to be realistic;

Table 2. Correlation coefficients between characters and P2 value (sample size: N=25)

Character	Coefficient	F-value	Probability
1st copulation			
Duration of spermatophore material	0.040	0.037	NS
Duration of sperm transfer	0.354	3.302	NS
Male body length	0.109	0.251	NS
Male body width	0.108	0.247	NS
Male penis length	0.170	0.622	NS
Male penis width	0.047	0.046	NS
2nd copulation			
Duration of spermatophore material	0.020	0.009	NS
Duration of sperm transfer	0.547	9.796	P<0.01
Male body length	0.415	4.584	P<0.05
Male body width	0.364	3.358	NS
Male penis length	0.329	2.672	NS
Male penis width	0.239	1.338	NS
Mating interval	0.416	4.819	P<0.05
Female body length	0.004	0.000	NS
Female body width	0.027	0.017	NS

mixing must occure, and an intermediate between the 2 extremes is likely. Mixing rate varies among species. Fast rates were reported from orthopteran species, *Grylloides supplicans* and *Gryllus bimaculatus* (Sakaluk 1986; Simmons 1987) and a cockroach, *Diploptera punctata* (Woodhead 1985). In these species random sperm mixing occures before the first clutch is laid. Six or seven days until completion of sperm mixing was reported in a species of damselfly, *Mnais pruinosa pruinosa* (Siva-Jothy & Tsubaki, 1989). Intermediate mixing was reported in a dragonfly, *Erythemis simplicollis* (McVey & Smittle 1984).

In this study, there was no significant difference among P2 value of sequential clutches. Nor was there any relation between P2 and duration from the termination of the second copulation to oviposition. This may indicate that a rather fast process of sperm mixing takes place.

Simple regressions demonstrated a positive correlation between P2 value and second sperm transfer duration, mating interval and body length of second male. On the other hand, multiple regressions indicated that only second sperm transfer duration had a significant relationship with P2. A highly significant correlation between second male body length and second sperm transfer duration is probably attributable to this, suggesting that the relation between P2 and second male body length was an indirect effect from the second sperm transfer duration.

Obata (1987) showed that 2 successive matings of the same *H. axyridis* male with virgin females resulted in significantly shorter sperm transfer duration at the second mating, suggesting there may be a substantial cost of copulation. Thus, even though a large body in itself does not contribute to sperm precedence, longer sperm transfer, i.e., greater amount of ejaculate from larger males, may result in high fertilization success.

Second male body size correlated with sperm transfer duration while the first did not. This may be because males may be able to discern if females have mated or not, and smaller males transfer less sperm to non-virgin females. In some species, male ability to discriminant between virgin and non-virgin females has been reported (Suter 1990; Cordero & Miller 1992). However, the reason for smaller males transferring less sperm when the female has not been mated remains unknown.

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