Reproductive isolation between populations showing different aggression in a subsocial spider mite, *Schizotetranychus miscanthi* Saito (Acari: Tetranychidae)

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Abstract

We studied the pattern of reproductive isolation between two groups of the subsocial spider mite species, *Schizotetranychus miscanthi*. *S. miscanthi* can be distinguished into two groups by differences in male-to-male aggressiveness, one has high and the other has low male-aggression. Habitats of these two groups are parapatric, and are not easily distinguished. We carried out cross experiments between populations taking the geographical factors into consideration. Results indicated that strong reproductive isolation acts on these different groups. Therefore, we concluded that there is little gene flow between the two groups, even when they are distributed in close proximity.

Key words: Tetranychidae, subsociality, antagonism, speciation, compatibility

INTRODUCTION

In order to analyze the evolutionary causes and/or maintenance mechanisms of polymorphism observed in a species, it is first necessary to know whether that phenomenon occurs within or between gene pools.

There are a number of species groups containing several sibling species in the Tetranychidae. For example, the *mori* and *celarius* species groups were first recognized through the observation of reproductive isolation between “species” (Saito and Takahashi, 1982; Gotoh, 1986; Gotoh and Tokioka, 1996; Osakabe and Komazaki, 1996). In Tetranychidae, limited dispersal capacity and restricted habitat requirements may enhance the process of adaptation to local conditions. As a consequence, reproductive isolation may arise between distant populations exposed to different selection pressures (Mayr, 1963; Baur and Baur, 1992). This would partly explain the complex nature of species groups in Tetranychids.

In the present paper, we carried out cross experiments between two groups of *Schizotetranychus miscanthi* Saito showing different intensities of male aggression. This subsocial spider mite, which is a member of the *celarius* species group, lives gregariously in woven nests on the leaf undersurface of Japanese pampas grass (*Miscanthus sinen-*

sis Anderss., Gramineae). Males counterattack against predators and also fight among themselves to establish their harem (Saito, 1990). Furthermore, there is clinal variation in male antagonism along winter harshness (Saito, 1995). It was recently revealed that two groups can be discriminated by differences in male aggressiveness, the relative length of male leg I and the severity of winter of the distributing locality that the spiders have adapted to (Saito, 1995; Saito and Sahara, 1999).

In order to find out what factors divide *S. miscanthi* into these two groups, and how male-to-male aggressiveness correlates with such a disruption, it is first necessary to establish whether or not the two groups share the same gene pool. We thus carried out cross experiments between two groups showing different intensities of male aggression.

MATERIALS AND METHODS

Sample collection. *S. miscanthi* is distributed over all the large islands of Japan except Hokkaido. In most regions, populations with highly aggressive males (we hereafter call this group the “high aggression group”) distribute in lowland or southern regions, and populations of the “low-aggression group” in highland or northern regions (Saito, 1995; Saito and Sahara, 1999). The possibility that reproductive isolation is a function of distance be-

Fig. 1. Localities where populations of *Schizotetranychus miscanthi* were collected. Filled circles show populations used in this study. TSZ, FKM, TBK and UNZ were collected on 29 September, 1998. ASH and KBK were collected on 14 March, 1999.

tween populations (Mayr, 1963; Prakash, 1972; Baur and Baur, 1992) forces us to consider geographical factors. To do so we thus selected three small regional blocks with altitudinal gradations steep enough to collect populations with different male aggression (Fig. 1) and where at least two different aggression groups had been collected by Saito and Sahara (1999). Two *S. miscanthi* populations, one from high and the other from low altitude, were collected from each block.

After sampling, mites were reared for at least two generations on detached leaves of their host plant (*M. sinensis*) under controlled conditions of $25 \pm 2^\circ\text{C}$, 40–70% R.H. and a photoperiod of 15L:9D. The laboratory cultures of most populations were initiated from more than 50 females collected randomly in the field, but the Unzen (UNZ) culture was established from only 20 females because of the small number of collected individuals.

Observation of morphology. Before conducting the cross experiments, we observed male morphology in order to determine which aggression group each population belonged to. The two groups discriminated by the relative length of male leg I (leg I/leg III) (for details, see Saito, 1990) corresponded to the two groups discriminated by intensity of

male aggression (Saito, 1995). Therefore, the relative length of leg I could be adopted as a criterion for ascertaining the aggression level of each population. The leg lengths (tibia+tarsus+genu+femur) of sample male specimens were measured using photo-micrographs and a computer (for details, see Saito, 1995), and the data analyzed by one-way ANOVA (model I) and multiple comparison test (Scheffe's test).

Crossing. We carried out cross experiments between populations. Since we could not simultaneously sample all populations, the experiments were carried out over two different periods: the first set was made on four populations (TSZ, FKM, UNZ and TBK) from Block A and B from 12 September to 25 December, 1998 (Table 1), and the second set was on ASH, KBK, UNZ and TBK populations from Blocks B and C from 7 March to 13 August, 1999 (Table 1). We termed the Tobuko and Unzen populations used in the first experiment as Tobuko1 and Unzen1, and those used in the second experiment as Tobuko2 and Unzen2.

S. miscanthi has a haplo-diploid (arrhenotokous) genetic system (Saito, 1994), so that females can reproduce male (haploid) offspring without mating. Therefore, reproductive compatibility should be

evaluated according to whether there are females among the offspring of crossed pairs. Furthermore, it can be said that the more compatible the inter-population cross, the closer the offspring sex ratio reaches to that of the within-population cross (control).

All experiments were conducted under conditions of $25 \pm 2^\circ\text{C}$, 40–70% R.H. and a photoperiod of 15L:9D. Leaf arenas were constructed using a piece of detached *M. sinensis* leaf (1.0×3.0 cm) placed on and surrounded by water-soaked cotton. A single teleiochrysalis stage female was picked up from each strain and introduced onto a leaf arena. After the female molted to the adult stage and constructed a nest, a male of a certain strain was introduced for mating. Fourteen days after mating, the number of eggs laid was recorded, and the female and male were removed from the experimental arena. Hatchability, survival rate from larva to adult and sex-ratio at maturity of these offspring were then observed daily.

Number of replicates (number of pairs tested) per cross combination are shown in Table 1. In this study, we lumped together offspring produced by all females of the same cross experiment for the calculations of hatchability (%), survival rate of immature (%), and sex ratio (%), while the mean number of eggs per female and its standard deviation (SD) for each cross were calculated based on the respective pairs.

In order to ensure normality, the values of hatchability, immature survival rate and sex-ratio of offspring were arcsine-root transformed. These transformed data were then used for analyses of ANOVA (model I) and MANOVA (model I), posteriori test for multiple comparisons (Scheffé's method) by use of the computer software, StatView (ver. 5, SAS Inc.) on a Macintosh computer (Apple Computer, Inc.)

RESULTS

Figure 2 shows the length of male legs I relative to legs III of the 6 populations observed in this study. There was a significant difference in the relative length of legs I between populations (ANOVA, $F_{5,46}=40.59$, $p<0.0001$). The relative length of male legs I in Unzen (UNZ) and Kubokawa (KBK) were significantly shorter than in Tsuyazaki (TSZ), Fukuma (FKM), Tobuko

Fig. 2. Difference in relative length of male leg (leg I/leg III) (for details, see Saito, 1990) which has been known to reflect male aggression intensity (Saito, 1995). There was a significant difference between populations by ANOVA, and they were categorized into two groups, longer leg I=high aggression and shorter leg I=low aggression group.

(TBK) and Ashizuri (ASH) (Scheffé's test, $p<0.0001$). We could thus determine that the populations of UNZ and KBK corresponded to the "low-aggression" group, and the other four populations to the "high-aggression" group.

Females mated with males of the same population (within-population cross=control) produced many female offspring: females accounted for 86–90% of eggs, though the 77% in the case of UNZ1×UNZ1 was an exception (Table 1). Those mating with males of the same aggression group also produced many female offspring (ca. 85%). However, females who mated with males of different aggression groups in the combinations of UNZ (female)×TBK (male), UNZ (female)×FKM (male) and vice versa produced no female offspring (Table 1).

Although ASH females mated with KBK males did not produce any female offspring, KBK females mated with ASH males did produce a few female offspring: three KBK females mated with ASH males each produced a single female offspring. These F_1 females could reproduce several (6 or 8) F_2 eggs which hatched normally regardless of fertilization, but the F_2 offspring could not develop into adults.

In the control, UNZ1 and UNZ2 had a different sex ratio, while there was no apparent difference

Table 1. Number of eggs laid during the first 14 days of the oviposition period, hatchability, survival rate of immatures and sex-ratio in F_1 progeny from crosses between populations

Cross type		Population crossed		No. of pairs	No. eggs average $\pm$ SD	Hatchability % (n)	Survival rate from larva to adult % (n)	Progeny sex ratio male % (n)
		♀	♂					
Between populations	Within block	TSZ	FKM	18	10.8 $\pm$ 3.8	93.8 (172)	94.5 (182)	14.5 (172)
		FKM	TSZ	13	11.2 $\pm$ 5.0	91.8 (146)	93.3 (134)	12.0 (125)
		TBK1	UNZ1 ^a	11	11.9 $\pm$ 5.8	93.1 (131)	80.3 (122)	100.0 (98)
		UNZ1	TBK1 ^a	12	8.6 $\pm$ 4.8	95.1 (103)	75.5 (98)	100.0 (74)
		ASH	KBK ^a	13	13.1 $\pm$ 3.8	88.8 (170)	61.6 (151)	100.0 (93)
		KBK	ASH ^a	11	10.5 $\pm$ 4.1	87.8 (115)	80.2 (101)	96.3 (81)
	Between blocks	TSZ	TBK1	12	14.9 $\pm$ 4.6	94.4 (179)	92.3 (169)	13.5 (156)
		TBK1	TSZ	15	10.9 $\pm$ 3.4	93.3 (163)	93.4 (152)	12.7 (142)
		FKM	UNZ1 ^a	13	13.8 $\pm$ 5.4	95.0 (180)	74.9 (171)	100.0 (128)
		UNZ1	FKM ^a	12	11.0 $\pm$ 3.7	97.7 (132)	76.7 (129)	100.0 (99)
		ASH	TBK2	12	14.8 $\pm$ 4.4	94.4 (178)	93.5 (168)	14.0 (157)
		TBK2	ASH	15	12.7 $\pm$ 5.0	95.3 (190)	90.6 (181)	12.2 (164)
		KBK	UNZ2	12	9.9 $\pm$ 3.0	92.4 (119)	81.8 (110)	17.8 (90)
		UNZ2	KBK	12	11.1 $\pm$ 4.4	91.7 (133)	86.9 (122)	16.0 (106)
	Within population	TSZ	TSZ	11	14.5 $\pm$ 5.1	91.3 (160)	93.2 (146)	12.5 (136)
		FKM	FKM	13	14.4 $\pm$ 4.9	94.1 (187)	91.5 (176)	12.4 (161)
		TBK1	TBK1	17	14.1 $\pm$ 4.0	94.2 (240)	94.2 (226)	12.7 (213)
		UNZ1	UNZ1	12	8.0 $\pm$ 3.9	86.5 (96)	100.0 (83)	26.5 (83)
		ASH	ASH	12	14.4 $\pm$ 5.1	98.8 (173)	87.7 (171)	15.3 (150)
		KBK	KBK	12	9.8 $\pm$ 3.7	96.6 (118)	80.7 (114)	15.2 (92)
		TBK2	TBK2	14	13.4 $\pm$ 4.8	98.4 (187)	92.9 (184)	15.8 (171)
		UNZ2	UNZ2	13	10.5 $\pm$ 3.6	91.9 (136)	92.0 (125)	16.5 (115)

^a Cross combinations indicate crosses between different aggression groups.

Table 2. Results of 2 way-ANOVA on sex ratio between inter- and intra-group crosses (G-category) and between inter- and intra-block crosses (D-category)

Source	d.f.	Sum of squares	Mean square	F-value	p
G-category	1	5.519	5.519	943.52	<0.0001
D-category	1	0.004	0.004	0.76	0.40
G*D	1	0.002	0.002	0.39	0.55
Residual	10	0.058	0.006		

between TBK1 and TBK2. The sex ratio of the UNZ1 control was higher than that of the UNZ2 control. We could not, however, understand why the control of UNZ1 and UNZ2 were different.

Table 2 shows the results of a two-way ANOVA on sex ratio between inter- and intra-group crosses (these two types of crosses are denoted hereafter as G-category) and between inter- and intra-block

crosses (denoted hereafter as D-category). No significant interaction (G-category $\times$ D-category, Table 2) indicated that the sex ratio was significantly different in G-category, while there was no difference in D-category (Table 2). In other words, the offspring sex ratio produced by pairs crossed between different aggression groups (D-category) significantly differed from that produced by pairs crossed within the same aggression groups (G-category). On the other hand, sex ratio was not influenced by differences in distance between crossed populations (D-category).

Because there was no prior hypothesis to predict from which parameters (no. of eggs, hatchability and immature survival) we could expect to detect differences, we adopted MANOVA. Table 3 shows that there was a significant difference in some parameters in the G-category, but not in the D-category. Thus we used Scheffé's test to analyze and detect which parameters were significantly differ-

Table 3. MANOVA for effects of inter- and intra-group crosses (G-category) and inter- and intra-block crosses (D-category) on no. eggs, hatchability and immature survival rate

Source	Wilk's Λ	d.f.	F -value	p
G-category	0.195	3, 8	11.033	0.0032
D-category	0.441	3, 8	3.384	0.0746
G & D	0.756	3, 8	0.859	0.5005

ent in the G-category. As a result, we found a significant difference in immature survival rate (Scheffé's test, $p=0.0002$), but not in number of eggs (Scheffé's test, $p=0.6078$) or hatchability (Scheffé's test, $p=0.9498$) in the G-category, i.e. between inter- and intra-group crosses.

DISCUSSION

The results of cross experiments clearly showed that reproductive isolation exists between several populations of the different aggression groups. Furthermore, it was revealed that populations belonging to the same aggression group are genetically very compatible even if distributed far apart from each other (the maximum distance between such populations reaches approximately 270 km). However, there was strong reproductive isolation between populations with different aggression levels even when distributed very closely (the minimum distance between them is approximately 8 km). We thus concluded that there is little gene flow between these two aggression groups, and they are regarded as two different species or at least sub-species.

Moreover, we found differences in the intensity of isolation between populations. For example, KBK females could reproduce female offspring when mated with ASH males. This means that there is a possibility of introgenesis occurring between these two populations having different aggression traits. Furthermore, as shown in Table 1 and the results of Scheffé's test on each parameter, the immature survival rate was different between intra- and inter-group crosses, and it was lower in the inter- than in the intra-group crosses. Low hatchability and a male-biased sex ratio in the inter-population cross were observed in *Amphitetranychus* (= *Tetranychus*) *quercivorus* Ehara et

Gotoh, and were caused by high mortality of fertilized eggs destined to become females (Gotoh and Takayama, 1992). In the same way, this low immature survival rate suggests that some eggs produced by inter-group pairs were fertilized (Sato et al., 2000). Although this variation in isolation intensity may reflect the history of separation of these two groups (Coyne and Orr, 1989), detailed discussions on this topic must await without first obtaining more precise data about the phylogenetic relationships between populations.

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