

Genetic Control of Albinism in *Panonychus osmanthi* EHARA et GOTOH (Acari: Tetranychidae)

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(Received 6 January 1997; Accepted 28 February 1997)

Three albino females were discovered in a field population of *Panonychus osmanthi*, the albinism being a result of spontaneous mutation. To determine the genetic control of the albinism, standard genetic crosses were conducted. Apparently the albinism was recessive to the normal pigmented trait and was under monogenic control. Albino mites with reddish eye-spots were obtained when pigmented hybrid females obtained from crosses between the albino and normal strains were either kept virgin or mated to males with various phenotypes. The eye-spots of albino females were not passed on to the next generation irrespective of the male phenotype. These results suggested that some pigment precursor possessed by pigmented females is responsible for the appearance of the eye-spots. Hybrid females resulted in a reduction in hatchability in F₂ eggs and a distortion of the female : male ratio. These phenomena might be explained in terms of the difference in mortality between haploid and diploid eggs: i.e., crosses between hybrid females and pure albino males without eye-spots resulted in the death of about 50% of both haploid and diploid eggs, whereas crosses between hybrid females and either normal males or eye-spotted albino males resulted in the death of about 50% of haploid eggs but did not cause the death of diploid eggs.

Key words: *Panonychus osmanthi*, albino, body color, eye-spot, lethality

INTRODUCTION

Albino mutations have been described in many arthropods, e.g., the migratory locust, *Locusta migratoria* (L.) (VERDIER, 1965; HASEGAWA and TANAKA, 1994), the desert locust, *Schistocerca gregaria* (FORSK.) (HUNTER-JONES, 1957; DEARN, 1977), the migratory grasshopper, *Melanoplus sanguinipes* (FAB.) (CHAMBERLAIN, 1981), the burrowing mayfly, *Hexagenia bilineata* (SAY) (MCCAFFERTY and BLOODGOOD, 1985), the Pacific spider mite, *Tetranychus pacificus* MCGREGOR (VAN ZON and HELLE, 1966 a; HELLE and VAN ZON, 1966), and the two-spotted spider mite, *T. urticae* KOCH (BALLANTYNE, 1969). With the exception of the mayfly, whose genetic basis for albinism is unknown, all albino mutations are recessive to the normal pigmented individuals and follow a Mendelian pattern of inheritance.

Normal adults of *T. pacificus* and *T. urticae* are yellowish or yellowish green with two pairs of reddish eye-spots on the propodosoma, whereas pure albino adults are milky white and completely lack eye-spots. Albino adults of *T. pacificus*, which were obtained from pigmented hybrid females, have reddish eye-spots only in the anterior eyes, but not in the posterior eyes (VAN ZON and HELLE, 1966 a). The presence of eye-spots appeared to be based on a maternal cytoplasmic factor: only pigmented females of *T. pacificus* could transfer a pigment precursor for eye-spots to their offspring (VAN ZON and HELLE, 1966 a, b; HELLE

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and VAN ZON, 1967; BALLANTYNE, 1969). Furthermore, BALLANTYNE (1969) reported that the mortality of F₂ eggs deposited by virgin F₁ females, which were obtained from crosses between albino and normal mites, was extremely high in both spider mite species. He speculated that chromosomal rearrangements may underlie such incompatibilities, but the data supporting such a conclusion were not convincing.

Panonychus osmanthi EHARA et GOTOH, which superficially resembles *P. citri* (McGREGOR), was described as a new species by EHARA and GOTOH (1996). In October 1994, three albino females of this species were collected from the fortunes osmanthus, *Osmanthus* × *fortunei* CARRIER, at Ryugasaki, Ibaraki, Japan. Normal adults of *P. osmanthi* are reddish, whereas albino mites are greenish. During establishment of a culture of these mites in the laboratory, we observed that some albino mites had reddish eye-spots, but others did not. Furthermore, several crosses resulted in a conspicuous reduction in egg hatchability and an increase in the female : male ratio, as observed in *Tetranychus* spp. (BALLANTYNE, 1969). The present paper describes the genetic control of the albinism of the body, which is treated separately from the presence or absence of eye-spots, and the mode by which eye-spots are manifested in *P. osmanthi* by crossing the albino strain with a normal one. Thirdly, we discuss possible mechanisms of the reduction in F₂ egg hatchability and distortion of the F₂ sex ratio obtained from the above crosses.

MATERIALS AND METHODS

Mites. The albino strain used in this study was established by three female adults collected from the fortunes osmanthus in Ryugasaki (35°55'N, 140°10'E), Ibaraki, on October 18 (1 adult) and on October 28 (2 adults) of 1994. A normal strain originated from about 100 females collected at the same site in 1993 (KITASHIMA and GOTOH, 1995). The stock cultures were maintained at 25 ± 1°C and 16L–8D according to the methods of KITASHIMA and GOTOH (1995), and all experiments were carried out under the same conditions.

Crossing. Two types of crosses were made. First, reciprocal crosses between the albino and normal strains were carried out, and then backcrosses were performed to determine the genetic control of the albino trait over two generations. Males obtained from virgin F₁ females were also checked to confirm the ratio of albino to normal males in the F₂ generation, because *P. osmanthi* was arrhenotokous, i.e., unfertilized haploid eggs developed into males, whereas fertilized eggs gave rise to females. In a second experiment to determine the mode by which the eye-spots are manifested, an experiment identical to the first experiment was carried out over three generations, and males obtained from virgin F₂ females were also checked for body color and the presence of eye-spots.

Females in the teleiochrysalis stage were picked from stock cultures, and individually transferred onto a leaf disc (ca. 4 cm²) of the orange osman, *Osmanthus fragrans* var. *aurantiacus* MAKINO, together with a male adult chosen from the stock cultures according to the experimental scheme. Males were removed 2 days after the females matured. Each female was then allowed to lay eggs for 5 days and then removed. The offspring were checked daily until adulthood to score egg hatchability, survival of the immature stages, sex ratio, body color and presence of eye-spots. There were three phenotypes of mites: the "normal mite" (Fig. 1A), which was reddish with eye-spots, the "albino mite with eye-spots" (Fig. 1B), which was greenish with reddish eye-spots, and the "albino mite without eye-spots" (Fig. 1C). The normal mite was regarded as having the normal phenotype and the other two were regarded as having the albino phenotype.

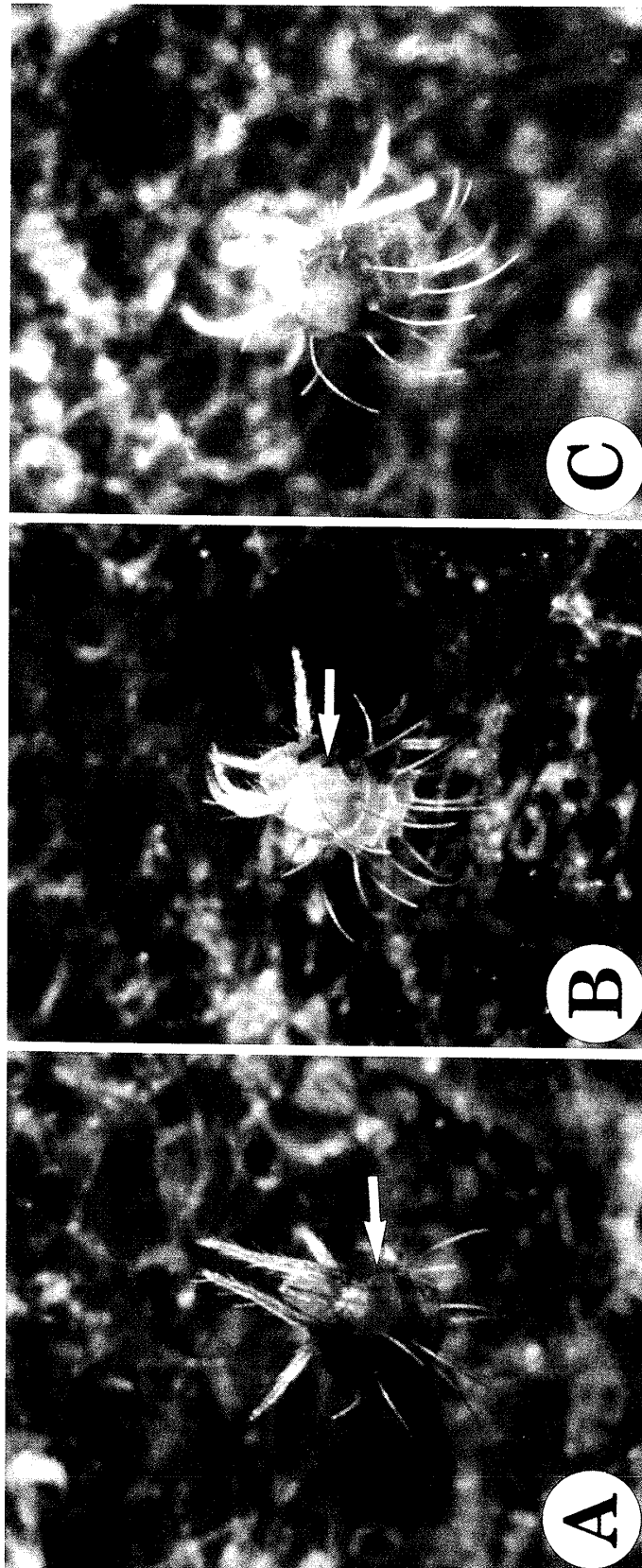


Fig. 1. Three phenotypes of *Panonychus osmanthi*. A: normal; B: albino with reddish eye-spots; C: albino without eye-spots. Arrows indicate eye-spots.

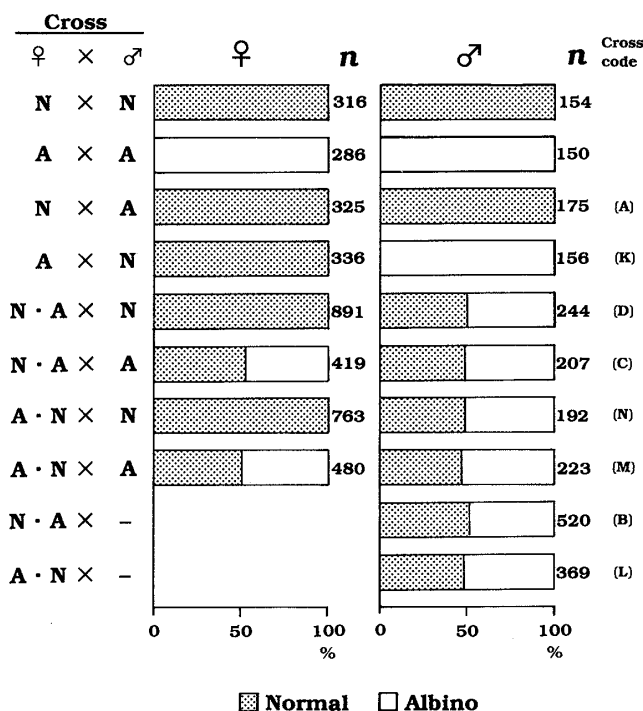


Fig. 2. Proportions of normal and albino individuals obtained from reciprocal crosses between the normal (N) and albino (A) strains and backcrosses in *P. osmanthi*. *n* shows the number of adults that emerged. Cross codes correspond to the codes appearing in Table 1, Fig. 3 and Appendix 1.

RESULTS AND DISCUSSION

Genetic control of albinism in body color

All F_1 hybrid females from the reciprocal crosses between the normal and albino strains showed the normal phenotype, as occurred in the crosses between normal adults, whereas crosses between albino adults produced only albino offspring (Fig. 2). The crosses between albino females and normal males resulted in a progeny of albino males and normal phenotype females because of haplo-diploidy (Fig. 2).

The F_1 females were backcrossed to either normal or albino males. If the albino trait is controlled by a single recessive gene, it would be expected that 50% of the male offspring from all crosses would be normal and the other 50% would be albino, whereas the female offspring from the crosses between F_1 females and normal males would be expected to be normal, and those from crosses between F_1 females and albino males would be expected to be 1 normal : 1 albino according to MENDEL's law of segregation. Unmated F_1 females would be expected to produce males with a normal : albino ratio of 1 : 1. The observed albino : normal ratios in the offspring obtained from all crosses and unmated F_1 females were not significantly different from the expected values (Fig. 2; χ^2 -test, $1.00 \geq p > 0.22$). Since reciprocal crosses gave a pigmented normal hybrid, the dominance of the normal trait is clear. The albinism in *P. osmanthi* is controlled by a single Mendelian unit, as shown in *T. pacificus* (VAN ZON and HELLE, 1966 a) and *T. urticae* (HELLE and VAN ZON, 1966).

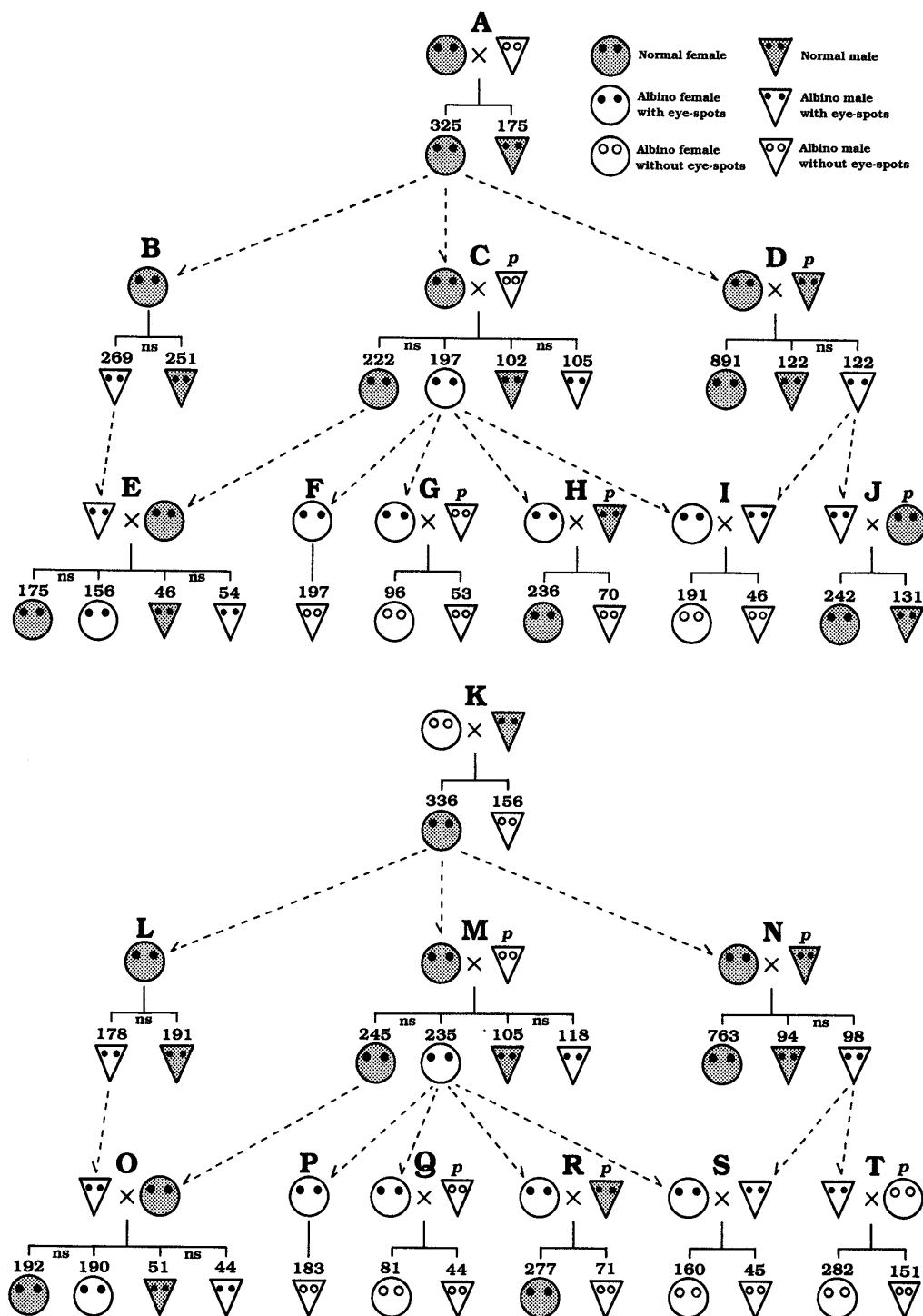


Fig. 3. Diagram of crossings between the normal and albino strains for analyzing the mode of appearance of reddish eye-spots in *P. osmanthi*. Capital letters on crosses indicate the cross codes, which correspond to the codes appearing in Table 1, Fig. 2 and Appendix 1. "p" on mite symbols indicates pure individuals obtained from the parental cultures. Numbers on mite symbols indicate the total number of adults emerged. A chi-square test was used for analysis of segregation in each sex (ns: not significant at $1.00 \geq p > 0.22$).

Mode of appearance of eye-spots

Two pairs of reddish eye-spots were observed in eye-spotted albino mites of *P. osmanthi* (cf. Fig. 1B). Albino mites with eye-spots appeared from reddish eggs deposited by hybrid females having the normal phenotype, but after hatching, immatures grew dull in body color and changed to greenish from reddish during the larval and nymphal stages. Albino mites without eye-spots appeared from colorless eggs and their juveniles were greenish. In the crosses of the pure albino females and pure normal males, colorless hybrid eggs were produced, but later, reddish eye-spots appeared only in the diploid eggs, which had been destined to become the normal phenotype.

Figure 3 shows the results of the appearance of eye-spots. In all cases, the appearance of eye-spotted albino mites was restricted either to crosses using pigmented females (C, D, E, M, N and O in Fig. 3), or to progenies from hybrid pigmented females which were kept as virgins (B and L in Fig. 3). In crosses between eye-spotted albino mites and between albino mites with and without eye-spots, the progenies lost the eye-spots (G, I, Q, S and T in Fig. 3). The facts that the father's pigmentation had no effect on the appearance of eye-spotted albino mites (H and R in Fig. 3) and that the eye-spots of the eye-spotted albino females were not passed on to males of the next generation (e.g., F and P in Fig. 3), suggest that the appearance of eye-spots is caused by a maternal effect. Since the mode of appearance of eye-spots in *P. osmanthi* is the same as that in *T. pacificus* (VAN ZON and HELLE, 1966 a), we reconfirm that the existence of some pigment precursor possessed by pigmented females is responsible for the appearance of eye-spots.

Reduction in hatchability and distortion of sex ratio in F₂

As shown in Appendix 1, crosses between the pure females (i.e., $+/+$ and w/w) and males with various genotypes resulted in normal offspring, high percentages of hatchability

Table 1. Comparison of observed and expected values in hatchabilities and female ratios of offspring obtained from crosses between normal and albino strains in *Panonychus osmanthi*

Cross code ¹	Observed		Expected		Chi-square value	Observed		Expected		Chi-square value
	Hatched	Un-hatched	Hatched	Un-hatched		Female	Male	Female	Male	
C	690	622	696	616	0.055 ^{ns}	419	207	426	218	0.088 ^{ns}
D	1,212	288	1,234	266	1.072 ^{ns}	891	244	891	249	0.040 ^{ns}
E	466	126	486	106	2.144 ^{ns}	331	100	356	99	0.265 ^{ns}
G	161	143	157	147	0.105 ^{ns}	96	53	95	48	0.130 ^{ns}
H	319	75	322	72	0.097 ^{ns}	236	70	242	66	0.165 ^{ns}
I	242	67	253	56	1.182 ^{ns}	191	46	182	50	0.322 ^{ns}
M	769	771	746	794	0.687 ^{ns}	480	223	457	234	0.726 ^{ns}
N	1,049	253	1,051	251	0.010 ^{ns}	767	192	768	196	0.029 ^{ns}
O	524	107	509	122	1.200 ^{ns}	382	95	370	95	0.039 ^{ns}
Q	144	130	133	141	0.820 ^{ns}	81	44	77	39	0.066 ^{ns}
R	377	115	397	95	2.472 ^{ns}	277	71	291	75	0.001 ^{ns}
S	223	36	209	50	2.696 ^{ns}	161	45	154	40	0.114 ^{ns}

¹ Cross codes correspond to the codes appearing in Figs. 2 and 3 and Appendix 1.

^{ns} Not significant at $0.98 > p > 0.10$.

(> 96%) and female-biased sex ratios (> 65%), but when hybrid females (+/*w*, *w*/+ and *w*/*w*) were crossed with males of different genotypes, hatchabilities were reduced and sex ratios in F₂ were distorted.

These phenomena may be analyzed in terms of the difference in mortality between haploid and diploid eggs: 50% of the haploid eggs laid by hybrid females which were kept as virgins and 50% of both haploid and diploid eggs laid by hybrid females which were mated with males (*w*) from the pure albino strain would die in the embryonic stage. In this case, the percentage of emerged females would be the same as that obtained in intra-strain crosses. On the other hand, in eggs deposited by F₁ hybrid females which mated with either males from the pure normal (+) or eye-spotted albino (*w*) strains, half of the haploid eggs would die, whereas all diploid eggs would survive. As a result, the percentage of total offspring that are females would be more than that in intra-strain crosses.

To test this hypothesis, the following assumptions were made: (1) the ratio of diploid eggs to total eggs (*d*) was 0.661, which was calculated from the sex ratios in intra-strain crosses of pure mites (Appendix 1); (2) the hatchability of diploid eggs when all diploid eggs would be viable (*hh*) was 0.972, which was calculated from values obtained from intra-strain crosses of pure mites (Appendix 1); (3) the hatchability when half of both haploid and diploid eggs was expected to die (*vh*) was 0.484–0.530, values which were obtained from virgin hybrid females (B, F, L and P in Appendix 1); (4) the survival rate (*s*) of immatures were the same as the observed values (Appendix 1); (5) the expected number of female and male adults could be approximated by (number of hatchlings in diploid eggs) × (survival rate) and (number of hatchlings in haploid eggs) × (survival rate), respectively.

For example, in cross code C, since the observed total number of eggs was 1,312 (Table 1), the number of hatchlings in diploid eggs would be 460 (1,312 × 0.661 (*d*) × 0.530 (*vh*)), and that in haploid eggs would be 236 (1,312 × 0.339 (1 – *d*) × 0.530 (*vh*)), and the number of female and male adults would be 425 (460 × 0.925 (*s*)) and 218 (236 × 0.925 (*s*)), respectively. Here, we assumed that 50% of both diploid and haploid eggs would not hatch. The same assumptions were made in cross codes C, G, M and Q, whereas in D, E, H, I, N, O, R and S, we assumed that 50% of haploid eggs would not hatch, but that all diploid eggs would hatch (Appendix 1).

As shown in Table 1, all of the observed values were very close to the expected ones (χ^2 -test, $0.98 > p > 0.10$). The results also show that a cytoplasmic factor of females (i.e., a maternal effect) was not related to the reduced hatchability and distorted sex ratio, because the same results were obtained from both hybrid females of “+/*w*” and “*w*/+” which had the cytoplasm of a normal mother and the cytoplasm of an albino mother, respectively. One explanation for the reduced hatchability and distorted sex ratio is that a lethal factor, which would be possessed by the pure albino strain, would be activated in hybrid females, but diploid or fertilized F₂ eggs would be saved from death only when they possessed a rescue factor obtained from pigmented males or eye-spotted albino males through some unknown mechanism. The details of the lethal factor and rescue factor have not been identified. BALLANTYNE (1969), who conducted studies on two mutant strains of *T. pacificus*, reported that such distortion may be based on chromosomal rearrangements. Our hypothesis differs from his hypothesis in the genetic mechanism that causes a reduction in hatchability and distortion of sex ratio, but our detailed data presented here should be useful as a first step in the understanding of these phenomena.

ACKNOWLEDGEMENTS

We thank Dr. N. CHONAN, Ibaraki University, for encouragement.

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Appendix 1. Number of eggs laid during the first 5 days of oviposition, hatchability, survival rate of immatures and sex ratio in the F₁, F₂ and F₃ progenies obtained from crosses between the normal and albino strains of *Panonychus osmanthi*

Cross code ¹	Cross ²		<i>n</i> ³	Total no. of eggs/♀	Hatchability (%) ⁴	Survival rate (%) in immature stages ⁴	Sex ratio (%) (♀/(♀+♂)) ⁴
	Female	Male		Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
A	+/+	+	19	27.0±4.36	96.3±4.79 ^a	96.3±4.49	67.2±6.58 ^c
	<i>w/w</i>	<i>w</i>	18	25.9±4.63	98.2±4.25 ^a	95.3±7.32	65.8±5.59 ^c
	+/+	<i>w</i>	26	21.3±5.08	96.5±5.37 ^a	92.5±6.96	65.2±5.21 ^c
B	+/w (N)	—	50	20.6±4.19	53.0±15.15 ^c	95.2±6.84	0 ^d
C	+/w (N)	<i>w</i>	54	24.3±4.78	52.6±13.58 ^c	92.5±9.11	66.0±12.15 ^c
D	+/w (N)	+	60	25.0±5.93	80.8±7.03 ^b	92.4±8.87	79.8±7.57 ^a
E	+/w (N)	<u><i>w</i></u>	23	25.7±6.82	78.6±6.29 ^b	93.6±6.36	77.3±7.99 ^{ab}
F	<u><i>w/w</i></u> (N)	—	19	22.2±4.02	51.9±12.30 ^c	91.9±7.53	0 ^d
G	<u><i>w/w</i></u> (N)	<i>w</i>	14	21.7±5.04	53.0±11.69 ^c	91.5±19.20	64.7±9.62 ^c
H	<u><i>w/w</i></u> (N)	+	18	21.9±7.19	81.0±11.20 ^b	95.6±5.76	77.2±10.33 ^{ab}
I	<u><i>w/w</i></u> (N)	<u><i>w</i></u>	14	22.1±5.89	79.4±9.71 ^b	91.9±6.70	80.1±8.33 ^{ab}
J	+/+	<u><i>w</i></u>	19	21.8±4.50	97.6±3.56 ^a	92.4±8.70	64.8±8.67 ^c
K	<i>w/w</i>	+	26	21.8±4.33	96.0±5.35 ^a	91.2±9.63	68.4±6.70 ^{bc}
L	<i>w</i> /+ (A)	—	38	21.5±4.37	48.4±11.76 ^c	92.0±8.47	0 ^d
M	<i>w</i> /+ (A)	<i>w</i>	55	28.0±5.13	49.9±12.47 ^c	92.6±8.04	68.2±11.57 ^c
N	<i>w</i> /+ (A)	+	46	28.3±7.10	80.4±7.22 ^b	91.7±7.24	80.0±6.94 ^a
O	<i>w</i> /+ (A)	<u><i>w</i></u>	24	26.3±5.07	83.1±6.76 ^b	91.2±8.13	80.4±8.36 ^a
P	<u><i>w/w</i></u> (A)	—	19	21.3±4.78	48.7±10.66 ^c	91.6±9.24	0 ^d
Q	<u><i>w/w</i></u> (A)	<i>w</i>	11	24.9±3.86	52.5±16.50 ^c	87.2±10.87	64.9±12.90 ^c
R	<u><i>w/w</i></u> (A)	+	20	24.6±5.61	76.6±8.34 ^b	92.0±7.72	79.4±8.80 ^{ab}
S	<u><i>w/w</i></u> (A)	<u><i>w</i></u>	12	21.6±6.93	86.1±8.78 ^b	92.8±8.39	79.4±9.22 ^{ab}
T	<i>w/w</i>	<i>w</i>	19	25.0±3.92	97.8±3.74 ^a	93.1±6.56	65.2±5.97 ^c
<i>F</i> -ratio					93.659****	0.826 ^{ns}	319.604****

¹ Cross codes correspond with the codes appearing in Table 1 and Figs. 2 and 3.

² +: dominant gene (normal); *w*: recessive gene (albino). Mites underlined indicate albino with reddish eye-spot, and N and A in parentheses indicate cytoplasmic types of the normal and albino mites, respectively.

³ Number of pairs tested.

⁴ **** means differed significantly at $p < 0.0001$ (one way analysis of variance, ANOVA); ^{ns} not significant at $p > 0.05$. Means in hatchability and sex ratio columns followed by different letters are significantly different at $p < 0.05$ (TUKEY-HSD test). Arcsin-transformed values were used for analyzing the percentages of hatchability, survival rate in immature stages and sex ratio.